ALTERATIONS IN THE RENAL VASCULATURE DURING THE DEVELOPMENT OF HYPERTENSION

BY

JOHN S. SMEDA, B.Sc., M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

ALTERATIONS IN THE RENAL VASCULATURE DURING THE DEVELOPMENT OF HYPERTENSION

DOCTOR OF PHILOSOPHY
(Medical Sciences)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Alterations in the renal vasculature during the development of hypertension

AUTHOR: John S. Smeda, B.Sc. (Brock University)
M.Sc. (Brock University)

SUPERVISOR: Dr. J.B. Forrest

NUMBER OF PAGES: xxvii,428

Abstract

The alterations in renal vascular structure and function, and their role in the development and maintenance of hypertension were examined in Kyoto Wistar spontaneously hypertensive rats (SHR) and Wistar Kyoto normotensive controls (WKY). A study of the renal vascular bed in SHR with established hypertension and age matched normotensive WKY indicated that when the isolated kidney was perfused at a variety of flow rates, under maximally relaxed conditions, the renal vascular resistance (RVR) was similar between SHR and WKY. Consistent with the above finding, morphometric measurements of light and electron micrographs indicated that the lumen diameter of relaxed main renal, interlobar, arcuate and interlobular arteries, as well as the preglomerular arterioles was similar in SHR and WKY. The cross-sectional areas of total intima, endothelium, subendothelial space, internal elastic lamina and total adventitia, as well as the volume fraction of axons, nerve sheath cells, fibroblasts, collagen and fluid filled space within the adventitia were only modestly altered in SHR. However, with the exception of the preglomerular arterioles, the media of all the renal arterial classes of SHR exhibited an increase in smooth muscle cell (SMC) cross-sectional area and volume that was produced by SMC hypertrophy and/or hyperplasia, while the extracellular space surrounding the SMCs was increased in both arteriolar and pre-arteriolar vessels.

Based on these structural alterations, it was hypothesized that,

if the mass of the arterial media is increased, contraction from the adventitial side would tend to push the media of the thicker walled hypertensive vessel into the lumen to a greater degree than the thinner walled WKY vessel. Under relaxed conditions, the RVR would be expected to be similar between SHR and WKY; however, during contraction RVR should be elevated to a greater degree in SHR than WKY. To test the above hypothesis, pharmacological studies were undertaken. Consistent with the model, at maximal relaxation the RVR was similar in SHR and WKY, while contraction of the renal vascular bed with infused norepinephrine (NE), $BaCl_2$, angiotensin II, or by stimulating the periarterial nerves produced a larger elevation of RVR in SHR. Aside from a modest increase in NE sensitivity within the renal vasculature of WKY, the contractile sensitivity to the various agents was not altered when SHR and WKY were compared. These studies indicated that the nerve mediated contractile responses within the renal vasculature were mediated by $alpha_1$ and dopamine receptors, and the proportion of the maximal response attributed to each receptor was similar in SHR and WKY.

Similar alterations, but of lesser magnitude, as those present in SHR with established hypertension were found to occur in prehypertensive SHR. The RVR at maximal relaxation was similar to that present in WKY, while the lumen diameter of the main renal, interlobar and cortical arteries was not modified between the two groups. All renal arteries of prehypertensive SHR that were studied exhibited an increase in the cross-sectional area ratio of arterial wall (intima + media) in relation to the lumen, and an increased number of SMC layers within the media. Consistent with the proposed model, when the renal

vasculature of prehypertensive SHR was maximally contracted by infusing NE or by stimulating the periarterial nerves (under conditions where the presynaptic uptake of NE was blocked) the amplitude of RVR change was higher in prehypertensive SHR than WKY. As in SHR with established hypertension, the contractile sensitivity of the renal vasculature to NE was modestly increased in WKY.

To further test if such alterations occur independently of high blood pressure, hydralazine (an antihypertensive drug that crosses the placental barrier) was fed to female SHR prior to, and during, pregnancy, and subsequently to newborn rats from birth to 21 weeks of age. These animals were compared to similarly treated WKY and nontreated SHR and WKY controls. Treated SHR maintained normal blood pressure throughout the treatment period. The in utero and post-natal normalization of blood pressure in SHR had virtually no effect in altering the renal vascular wall thickness. Within most of the arteries studied, SHR with normalized blood pressure had similar cross-sectional quantities of media and SMC layers as were present in untreated SHR, and greater quantities than that present in either control or treated WKY. The withdrawal of hydralazine from 26 week old in utero and post-natally treated SHR resulted in the re-establishment of hypertension within two days of withdrawal to the levels that were present in control SHR.

These results suggest that the thickening of the renal vascular wall in SHR could be of etiological importance in the initiation and maintenance of high blood pressure in SHR, and that such changes are not a secondary modification produced by the elevation of blood pressure.

ACKNOWLEDGEMENTS

I would like to thank Dr. J. Forrest for his advice, encouragement and support throughout the investigation.

I would also like to thank Dr. R. Lee for his advice and hours of stimulating discussion throughout my Ph.D. studies.

A special thanks to Dr. E. Daniel for his advice, and an apology for the suffering endured reading my thesis during his Florida vacation.

I also thank Dr. R. Garfield for his comments and suggestions during my supervisory committee meetings.

Finally, I would like to thank Ms. K. Mearow, Ms. S. Seaman, and Ms. M. Baine for typing this document.

Chapter 1

The mechanisms initiating and maintaining hypertension in Kyoto Wistar spontaneously hypertensive rats

Table of Contents

Chapter 2

Structural and functional alterations in the renal vasculature during the development of hypertension in Kyoto Wistar spontaneously hypertensive rats

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABREVEATIONS

a- internal (lumen) radius of an artery

A_{II}- angiotensin II

A_c- the cross-sectional area of an arterial component

cAMP- cyclic adenosine monophosphate

ATP- adenosine triphosphate

A_w- the cross-sectional area of the arterial wall(intima+media)

b- external radius of artery (lumen+intima+media)

C^{14}-glucose- carbon 14 labelled glucose

CNS- central nervous system

CP- infusion pressure.contributed by catheter

CSA- cross-sectional area

DBH- dopamine beta hydroxylase

DEAE- diethylamino ethyl cellulose

5,6 DHT- 5,6 dihydroxytryptamine

D_I- arterial diameter measured between internal elastic lamina

DOCA- deoxycorticosterone acetate

E- epinephrine

E_c- elastic modulus of collagen

ED_{20}- agonist dose producing 20% of the maximal response

ED_{50}- agonist dose producing 50% of the maximal response

E_e- elastic modulus of elastin

EGTA- ethyleneglycol-bis-(beta-amino-ethy ether) N,N-tetra-acetic acid

E_{inc}- incramental elastic modulus

Em- membrane potential

ESM- incremental strain modulus

EV_v- relative error of morphometric measurement

F- flow

f_c- fraction of collagen fibers supporting wall stress

cGMP- cyclic guanosine triphosphate

GTP- guanosine triphosphate

H^3- tritium

5 HT- serotonin

5 HTP- 5 hydroxytryptophan

IEL- internal elastic lamina

$I_{i\ smc}$- morphometric grid line intersects with smooth muscle cells

ISO- isoproterenol

ISOZ- isoenzyme

I.U.- international units

Km- Michealis Menton constant (substrate concentration producing half V_{max})

LD- arterial lumen diameter

LD_{100}- lumen diameter at a transmural pressure of 100 mmHg

NCR- normotensive Wistar rats

NE- norepinephrine

H^3-NE- tritium labelled norepinephrine

NGFA- nerve growth factor antisera

NTS- nucleus tractus solitarius

6-OHDA- 6 hydroxydopamine

P- pressure

P_i- number of grid points touching an arterial subcomponent

$P_{i\ smc}$- number of grid points touching smooth muscle cells

P_T- total number of grid points touching all subcomponents of artery

P_{TG}-total number of test points on a morphometric grid

R- radius

R_L- long axis of arterial lumen cut in cross-section

R_o- arterial radius at a transmural pressure of 0 mmHg

R_s- short axis of arterial lumen cut in cross-section

RVR- renal vascular resistance

S- tangential wall stress

SHR- Kyoto Wistar spontaneously hypertensive rat

SHR_t- SHR treated with hydralazine

SHR_c- SHR controls

SHR-D- SHR in a developing phase of hypertension

SHR-E- SHR in a established phase of hypertension

SHR-P- prehypertensive SHR

spSHR- stroke prone SHR

SMC- smooth muscle cells

SNA- sympathetic nerve activity

SNS- sympathetic nervous system

TP- total pressure

TRH- thyrotropin release hormone

TTX- tetrodotoxin

V/S- volume to surface area ratio

VSMC- vascular smooth muscle cell

V_v- volume fraction

W_c- volume fraction of collagen in arterial wall

W_e- volume fraction of elastin in arterial wall

WKY- Kyoto Wistar normotensive control rats

WKY_t- WKY treated with hydralazine

WKY_c- WKY controls

z- the line length of multipurpose grid

Chapter 1

The mechanisms initiating and maintaining hypertension Kyoto Wistar Spontaneously hypertensive rats - a review of the literature

A. Introduction

1. Development of the Kyoto Wistar spontaneously hypertensive rat (SHR)

SHR and Kyoto Wistar normotensive control rats (WKY) were bred by Okamoto and Aori (1963). The SHR colony was initiated by mating a male Wistar rat exhibiting sustained hypertension (a systolic blood pressure (BP) > 150 mm Hg) to a normotensive female Wistar rat with an above average BP. By selective brother and sister matings, an F_3 generation was produced where a 100% incidence of hypertension occurred without experimental intervention within 15 weeks of age. Subsequent inbreeding lowered the age at which the onset of hypertension occurred. The F_{26} generation typified the average SHR colony in use today. A 100% incidence of hypertension was found to occur by 7 weeks of age, while after 11 weeks of age systolic BP was established at between 180-210 mm Hg (Okamoto et al., 1972). WKY derived from the same stock of Wistar rats typically have BPs between 115-140 mm Hg from 10 weeks of age to death (Okamoto et al., 1972).

One of the observations made by Okamoto et al (1974) was that SHR suffered a high incidence of death by cerebral infarction and/or aneurysms (stroke). Furthermore, offspring of SHR that died from stroke exhibited a higher incidence of stroke than offspring of SHR that had

died from other causes. By selectively breeding SHR from the F_{24} and F_{25} generation who were progeny of SHR in which one or both parents of the mating pair suffered stroke, a F_{27} generation was obtained where the incidence of death by stroke was increased from 32 to 77% in male SHR and from 15 to 58% in female SHR. Okamoto named this subpopulation of SHR stroke prone SHR (spSHR). spSHR were found to exhibit hypertension at an earlier age than SHR and attained higher maximal systolic BPs (240 vs 180-200 mm Hg). spSHR also had shorter life span and were highly susceptible to stroke when placed on a high salt diet (Sadoshima et al., 1981).

2. (a) A comparison of SHR to essential hypertension in humans

It has been suggested by Okamoto (Okamoto, 1969; Okamoto et al., 1972), as well as others (for a review see McGiff and Quilley, 1981; Folkow, 1982), that SHR represents the best animal model of essential hypertension in humans. Essential hypertension is defined as hypertension without a known organic cause. SHR meet this criterion. The blood pressure of SHR is above normal and the mechanisms maintaining hypertension in SHR are not agreed upon. However, essential hypertension is probably a mosaic of diseases with a common outcome, high blood pressure (Birkenhager and DeLeeuw, 1979). In view of this, the mechanisms involved in the etiology of hypertension in SHR (a colony started by one mating pair) are no more a global representation of all essential hypertension than the mechanisms producing hypertension in an individual essential hypertensive patient. Likely, SHR best represent a subdivision of essential hypertension.

As in most essential hypertensive subjects, SHR have a normal cardiac output and an elevated vascular resistance to flow (Folkow et al., 1973; Iriuchyima, 1983). A great deal of emphasis has been placed on the fact that the pathological observations made in SHR are similar to those observed in essential hypertension (Okamoto, 1969). For example, Erinoda et al (1972) stressed that the diffuse narrowing of the retinal arterioles observed in essential hypertensive patients is also observed in SHR. However, this latter alteration, as well as other pathological modifications, are also present in experimental forms of renal hypertension (Byrom, 1969) and are not specific to either essential or spontaneous hypertension.

Essential hypertensive patients have been divided into low and high plasma renin activity groups, normal and high plasma prolactin groups and groups that are and are not responsive to antihypertensive treatment by bromocryptine (Stumpe et al., 1977). In established hypertensive SHR, most researchers have observed a decreased plasma and kidney renin activity (Sen et al., 1972; Shiono and Sokabe, 1976; Antonaccio et al., 1979). Consistent with the latter finding, isolated blood perfused kidneys from SHR secrete less renin (Tobian, 1975) and exhibit a reduced juxtaglomerular granulation (Okamoto, 1969) over normotensive controls. Plasma prolactin levels are increased in SHR (Sowers et al., 1979) and bromocryptine treatment does reduce blood pressure in SHR with established hypertension (McMurtry et al., 1979).

Studies performed on essential hypertensive subjects have indicated the presence of hyperactive, normal (Birkenhager and DeLeeuw, 1979) and hypoactive (DeQuattro et al., 1976) sympathetic nervous

system. In young SHR at a stage where blood pressure is initially rising, the sympathetic nervous activity appears to be increased (Grobecker et al., 1975; Nakamura and Nakamura, 1977; Nagatso et al., 1978). Adult SHR, on the other hand, exhibit on average a normal sympathetic activity as measured using plasma dopamine beta hydroxylase determinations (Axelrod, 1976; Yamori, 1976: Nagatsu et al., 1977). However, select individual vascular beds, i.e., renal vasculature, may be subjected to above normal sympathetic activity in SHR (Judy et al., 1976). Essential hypertensive patients have been further divided as to the degree of sympathetic nerve activation present when such patients undergo a posture change (DeQuattro et al., 1976). In this regard, SHR have been shown to exhibit an exaggerated sympathetic nerve activity in response to environmental stimuli (Folkow et al., 1973).

A wide variety of drugs have been tested on essential hypertensive patients (for a review, see Scriabine, 1980). In virtually all hypertensive subjects one or some combination of drugs can be found that control hypertension. However, tests on individual drugs always produce a subpopulation of essential hypertensive subjects that either do not respond or are marginally affected by a particular antihypertensive therapy. In fact, it might be argued that the effectiveness ratio for various antihypertensive drugs could be used to characterize an individual essential hypertensive subject, not unlike agonist potency ratios are used to characterize membrane receptors (Furchgott, 1972).

Table 1a outlines various antihypertensive treatments that have been carried out on SHR where the animals have been treated orally from

Table 1a. The effects of antihypertensive treatment on SHR treated from a young age (all drugs were administered orally).

Drug	Treatment*	Age at which** treatment started and finished		Systolic BP (mm Hg) in SHR at end of experiment		Age (wks) of hypertension (150 mm Hg) onset		Reference
		Start	Finish	Control	Treated	Control	Treated	
Diuretic								
Hydrochloro-thiazide	25 mg/kg o.g.d.	6	13	205	190	7	7	Guidicelli et al. (1981)
Clopamide	15 mg/kg o.g.d.	6	13	205	180	7	7	Guidicelli et al. (1981)
Chloro-thiazide	15 mg/kg o.d., d.w.	8	70	190	150	unknown		Fries and Ragan (1976)
β Blockers								
Atenolol	200 mg/kg o.g.d.	6	20	200	150	7	16-20	Guidicelli et al. (1981)
Atenolol	45x2 mg/kg o.g.d.	5	12	200	170	unknown		Takeda et al. (1979)
Propranolol	100 mg/kg o.g.d.	6	20	200	175	7	9	Guidicelli et al. (1981)
Propranolol	15x2 mg/kg o.g.d.	5	17	185	170	7	8	Takeda et al. (1979)
Propranolol	5 mg/kg d., diet	5	12	210	170	hypertensive at 5wks		Vavra et al. (1973)
Acetbutolol	1 g/kg o.g.d.	6	20	200	205	7	8	Guidicelli et al. (1981)
Pindolol	100 mg/kg o.g.d.	6	20	200	215	7	10	Guidicelli et al. (1981)
Pindolol	15x2 mg/kg o.g.d.	5	12	200	180	unknown		Takeda et al. (1979)
Oxyprenolol	15x2 mg/kg o.g.d.	5	17	200	180	8	9	Takeda et al. (1979)
Labetalol	25 mg/kg o.g.d.	5	12	200	175	7	8	Takeda et al. (1979)
α Blockers								
Prazosin	20 mg/kg o.g.d.	6	13	190	175	7	8	Guidicelli et al. (1981)
Vasodilators								
Dihydralazine	25 mg/kg o.g.d.	6	20	195	147	8	NA	Guidicelli et al. (1981)
Hydralazine	1.5 mg/kg o.d.dw.	8	80	215	90	unknown	NA	Fries and Ragan (1976)

Table 1a. The effects of antihypertensive treatment on SHR treated from a young age (all drugs were administered orally). cont'd

Drug	Treatment[a]	Age at which[aa] treatment started and finished		Systolic BP (mm Hg) in SHR at end of experiment		Age (wks) of hypertension (150 mm Hg) onset		Reference
		Start	Finish	Control	Treated	Control	Treated	
Angiotensin Converting Enzyme Blockers								
MK 421	25 mg/kg o.g.d.	4	15	200	140	6	NA	Richer et al. (1982)
Captopril	unknown	6	20	210	140	7	NA	Guidicelli et al. (1981)
Captopril	100 mg/kg o.d.dw.	4	16	195	120	7	NA	Ferrone and Anatonaccio (1979)
Ca^{+2} Antagonist								
Nicardipine	100 mg/kg o.g.d.	6	13	207	190	7	7	Guidicelli et al. (1981)
Peripheral Adrenergic Blockers								
Guanethidine	1st 2 wks 15 mg/kg, then 60 mg/kg o.g.d.	2	29	192	155	8	24	Lee et al. (1982)
Reserpine	0.25 mg/kg o.d.dw.	8	70	190	190	unknown		Fries and Ragan (1976)
CNS-acting Adrenergic Blockers								
α-methadopa[***]	5-8 g/l dw.	4	16	182	172	unknown		Tomanek et al. (1979)
α-methadopa	2.5-5 g/l dw.	4	24	191	130	unknown		Sen and Bumpus (1979)

[a] o. - orally administered; g. - administered by gavage; dw. - administered via drinking water; d. - quantity of drug given daily

[aa] age in weeks.

[***] methyldopa

Table 1b. The effect of antihypertensive treatment on SHR with either developing or established hypertension (all drugs orally administered).

Drug	Treatment[a]	Age at which treatment started and finished		Systolic BP (mm Hg) in SHR at end of experiment		Reference
		Start	Finish	Control	Treated	
Diuretic						
Methyclor-thiazide	1.48 mg/kg o.d.dw.	24	28	163	172	Fries et al. (1981)
β Blocker						
Propranolol	1.5 mg/kg o.g.d.	21	24	186	147	Greenberg (1981)
Propranolol	6.0 mg/kg o.g.d.	21	24	186	129	Greenberg (1981)
Propranolol	1.6 mg i.m. +3.2 mg d.o.	24	+15 days	165	175	Formen and Molrow (1974)
α-Blocker						
Prazosin	0.5 mg/kg o.g.d.	22	+4 days	189	143	Dadkar et al. (1980)
Prazosin	3 mg/kg 1 dose, o.g.	42	+1 day	210	140	Lefevre-Borg et al. (1979)
Vasodilators						
Hydralazine	100 mg/l dw.	15	19	174	150	Kwan and Daniel (1982)
Hydralazine	80 mg/l dw.	24	26	175	121	Fries et al. (1981)
Angiotensin Converting Enzyme Blockers						
Captopril			+2	165[f]	140[f]	Ferrone et al. (1980)
Captopril	100 mg/kg o.g.d.	+8 days	+8 days	167	128	Richer et al. (1983)
Enalapril	25 mg/kg o.g.d.	+8 days	+8 days	167	124	Richer et al. (1983)

Table 1b. cont'd The effect of antihypertensive treatment on SHR with either developing or established hypertension (all drugs orally administered).

Drug	Treatment[a]	Age at which treatment started[**]	and finished[**]	Systolic BP (mm Hg) in SHR at end of experiment		Reference
Peripheral Adrenergic Blockers						
Guanethidine	60 mg/kg o.g.d.	12	17	190	178	Lee et al. (1982)
Guanethidine	45 mg/kg o.g.d.	unknown (250g)	+2	165#	140#	Ferrone et al. (1980)
Reserpine	3.2 mg/l dw.	24	28	173	164	Ferrone et al. (1981)##
Reserpine	3.2 mg/l dw.	24	28	174	175	Ferrone et al. (1981)##
CNS-acting Adrenergic Blockers						
α-methyldopa	2.5-5.0 mg/l, dw.	12	15	200	147	Erhart and Ferrario (1981)
α-methyldopa	100 mg/kg o.g.d.	21	24	186	131	Greenberg (1981)
α-methyldopa	25 mg/kg o.g.d.	21	24	186	162	Greenberg (1981)
α-methyldopa	3-5 g/l dw.	17-29	+6	182	140-160	Spech et al. (1980)
Clonidine	0.3 mg/kg diet, d.	unknown	+5	205	180	Saito (1981)
Clonidine	0.3 mg/kg diet, d.	14	19	212	191	Yamaida et al. (1979)
Clonidine	0.03 mg/kg o.g.d.	21	24	186	109	Greenberg (1981)

[a] o. - orally; g. - administered by gavage; dw. - administered via drinking water; d. - quantity of drug given daily.

[**] age in weeks, unless otherwise noted. # BP represents the mean of systolic-diastolic. ## 2 separate experiments.

a very young, usually prehypertensive stage up to an age where blood pressure might be expected to be at maximal levels. Table 1b outlines studies where antihypertensive therapy has been initiated in SHR with established hypertension. Fries (Fries and Ragan, 1976; Fries et al., 1981) in his experience with essential hypertensive patients states that diuretics such as chlorothiazide and peripheral adrenergic blocking agents such as reserpine are usually equally, if not more, effective in controlling blood pressure than hydralazine. In SHR, on the other hand, the oral antihypertensive treatment of young and adult SHR indicates that diuretics such as hydrochlorothiazide, chlorothiazide , methychlorothiazide and clopamide, as well as the adrenergic blocker reserpine, are marginally effective in controlling blood pressure (Filczewski and Bogucka, 1979; Fries et al., 1981; Guidicelli et al., 1981). Fries and Ragan (1976) in a long term study did manage to reduce blood pressure in SHR with chlorothiazide; however, it required an extremely long treatment period (62 weeks) to do so. During this period, many SHR died from old age without achieving normal blood pressure. The survivors did however exhibit a mean blood pressure that bordered on hypertension (BP of 150 mm Hg). Unlike the above drugs, hydralazine is extremely effective in preventing hypertension development when treatment is started in young SHR and is capable of quickly lowering blood pressure in SHR with developing and established hypertension (Fries and Ragan, 1976; Fries et al., 1981; Guidicelli et al., 1981; Kwan and Daniel, 1982). Other drugs that are particularly effective in preventing and lowering blood pressure in SHR are angiotensin I to II converting enzyme inhibitors such as MK 421 and

captopril (Ferrone and Antonaccio, 1979; Ferrone et al., 1980; Guidicelli et al., 1981; Richer et al., 1982).

Beilin et al (1980) compiled a study involving the effectiveness of various drugs on human hypertension. Of 370 previously untreated patients having mild, moderate and severe hypertension, 198 were placed on diuretics, 97 on beta blockers, 58 on α methyldopa 17 on one of guanethidine, bethanidine or debrisoquine. The ability of diuretics, methadopa and beta blockers to control each of a variety of severities of hypertension was equal and somewhat better than treatment with guanethidine, bethanidine or debrisoquine. In SHR the above drugs exhibit a mixed effectiveness or an inability to control blood pressure (see Tables 1 a and b). In the case of beta blockers, in a study performed by Guidicelli et al (1981) atenolol treatment of SHR from 6 to 20 weeks of age retarded hypertension development. At the end of the study, SHR exhibited threshold hypertensive levels (150 mm Hg). However, in other studies of similar design involving atenolol, propranolol, actebutolol, pindolol, oxyprenolol and labetalol, these drugs were marginally or totally ineffective in controlling blood pressure in SHR. In studies involving the oral administration of propranolol to SHR with established hypertension, some researchers have reported a further elevation of blood pressure after 15 days of treatment (Lefeure-Borg et al., 1979), while others, for example Greenberg (1981), report a sharp drop in blood pressure after 21 days of treatment. Central nervous system adrenergic blocking agents such as α methyldopa also exhibit a mixed effectiveness. Tomanek et al (1979) treated 4 week old SHR for 12 weeks by placing α methyldopa (5-8 g/l) in

the drinking water and found the drug marginally effective in controlling blood pressure. Sen and Bumpus (1979), on the other hand, treated 4 week old SHR for 20 weeks with α-methyldopa (2.5-5 g/l) and achieved normotension. Studies involving the treatment of adult SHR with the oral administration of α-methyldopa have also met with moderate (Spech et al., 1980) to good (Greenberg, 1981; Erhart and Ferrario, 1981) success. The treatment of adult SHR for 5 weeks by placing clonidine in the solid diet has proven marginally effective in controlling blood pressure (Yomaida et al., 1979; Saita, 1981), while Greenberg (1981) has shown that the administration of clonidine via oral gavage for 3 weeks is capable of producing normotension. The treatment of young (neonatal SHR) with guanethidine, a peripheral sympathetic nervous system blocking agent, produces an incomplete sympathectomy (Johnson and Macia, 1979). Such an antihypertensive treatment of SHR from 2 to 29 weeks of age by Lee et al (1983 d) delayed the onset of hypertension but did not prevent it from occurring. In young adult SHR, guanethidine treatment was found to be virtually ineffective in the case of a 5 week study performed by Lee et al (1983 d) and modestly effective in the case of another study carried out by Ferrone et al (1980). Other experiments involving the treatment of 6 week old SHR for 7 weeks with prazosin, an $alpha_1$ receptor blocking agent thought to inhibit the post-synaptic contractile responses of norepinephrine (Scriabine, 1980), produced a marginal effect on blood pressure (Guidicelli et al., 1981). On the other hand, acute studies involving the oral administration of prazosin up to 4 days (Dadkar et al., 1980) have demonstrated the presence of a hypotensive effect.

2. (b) Conclusion

SHR best define a subdivision of essential hypertensive patients that exhibit (1) a family history of hypertension, (2) hypertension associated with low or normal plasma renin, high plasma prolactin and an acute hypotensive response to bromocryptine, (3) a normal or perhaps a hyperactive basal sympathetic nervous system that exhibits hyper-reactivity to environmental stimuli, (4) hypertension that is responsive to antihypertensive therapy by hydralazine and angiotensin converting enzyme inhibitors and relatively unresponsive to diuretics, reserpine, guanethidine and modestly responsive or unresponsive to beta blocking agents.

3. The focus of research on SHR

There are a limited number of mechanisms that can produce an increase in the systemic blood pressure in an animal. Potentially, an elevation in blood pressure can be produced by an increased cardiac output, a decreased blood viscosity or an increase in the systemic resistance to flow. There is no convincing evidence that the viscosity of blood is altered in SHR. Studies performed on SHR ranging in age from prehypertensive to adult animals indicate that cardiac output is either unaltered or slightly decreased when compared to controls (Albrecht, 1974). Other studies of SHR demonstrate that although the mean arterial blood pressure of SHR is elevated, the venous blood pressure at a number of sites is low and not altered (Greenberg, 1981). In view of this, a representation of the total peripheral resistance to blood flow can be determined by dividing the mean arterial blood

pressure by the cardiac output. Studies by Albrecht (1974) indicate that an increase in total peripheral resistance slightly precedes and closely parallels the increase in blood pressure observed in SHR. The total peripheral resistance is determined by all the parallel coupled regional vascular resistances. Experiments carried out on SHR ranging in age from 8 to 25 weeks show that the proportional resistance contribution by the renal, mesenteric and hindlimb vasculatures, as well as a number of smaller systemic vascular beds, is not different between SHR and WKY (Gobia et al., 1974; Nishiyama et al., 1976; Ferrone et al., 1979). Iriuchiyima (1983), on the other hand, contends that the hindlimb vasculature exhibits proportionally a slightly higher resistance alteration in SHR than other systemic vascular beds.

Many factors thought to contribute to the cause of hypertension have been studied. Much of this research has centered around the possibility that vascular tissue hyper-responds in hypertension, either by virtue of neurogenic or myogenic alterations. Such a change could decrease lumen diameter and raise vascular resistance. Under *in vivo* conditions vascular resistance can be governed at a number of levels: (1) The sympathetic nervous system may be overactive. Such an alteration could deposit greater quantities of the neurotransmitter norepinephrine (NE) within the synaptic cleft and therefore produce a greater degree of vascular smooth muscle (VSM) contraction. This alteration could result from a defect in the central nervous system, or alternatively, the quantity of transmitter released per unit stimulus could be increased by a change in the release and reuptake mechanisms, modifications in the innervation density of VSM, or a change in the

neuromuscular synaptic anatomy. (2) Increased vascular resistance could be produced by an increase in the contractile reactivity of the tissue. In such instances structural elements withing the blood vessel wall could be modified to produce a thicker vascular wall and/or one that is less distensible. A thickened vascular wall could cause a structural obstruction of the lumen which could raise the vascular resistance to blood flow. Furthermore, if blood vessels of equal lumen diameters, but different wall thicknesses (under relaxed conditions), are compared the degree of luminal obstruction per degree of smooth muscle cell (SMC) shortening during contraction will be increased in the thicker walled vessel. A decrease in vessel wall distensibility would tend to produce a smaller vessel lumen in response to a given transmural pressure. Both the above alterations could produce an increase in vascular resistance. An increase in the contractile reactivity of VSM could also be produced by VSM hypersensitivity to the neurotransmitter NE. Such a modification could result from alterations in the excitation contraction coupling mechanisms. In this regard, modifications in (a) the density of receptors stimulated by NE, (b) the efficiency of receptor action, (c) in the mechanisms governing the membrane potential, (d) the cellular handling of Ca^{+2}, and (e) the SMC adenylate cyclase - cAMP - protein kinase system could alter the NE contractile sensitivity. Furthermore, even in the absence of nerve stimulation, a decrease in the membrane potential or an increase in the unbound levels of Ca^{+2} could increase the basal levels of SMC tone and raise vascular resistance. The possibility that these and other mechanisms contribute to the maintenance of hypertension will be discussed in the balance of the

literature review.

B. Alterations in the peripheral sympathetic nerve activity (SNA)

1. (a) Measurements of sympathetic nerve activity (SNA) in SHR

The activity of the sympathetic nervous system in SHR has been assessed by measuring plasma dopamine beta hydroxylase (DBH) and NE levels and by direct measurements of nerve firing frequencies of sympathetic nerves.

DBH is an enzyme that is present in sympathetic synaptic vesicles and is released with NE into the adventitia along with NE in response to sympathetic nerve stimulation (Axelrod, 1976; Yamori, 1976). Unlike NE, presynaptic uptake mechanisms do not remove DBH. Furthermore, the presence or absence of adrenal glands does not affect plasma DBH levels in response to stress, indicating that plasma levels of this enzyme primarily represent vascular SNA (Weinshilboum and Axelrod, 1971; Weinshilboum et al., 1971). At approximately the age that hypertension develops young SHR and spSHR exhibit high plasma DBH levels when compared to control animals (Grobecker et al., 1975; Nagatsu et al., 1976; Nakamura and Nakamura, 1977; Nagatsu et al., 1978). The elevated plasma DBH levels in young SHR can be partially normalized by cutting the preganglionic fibres leading to the coeliac ganglia (Nakamura and Nakamura, 1977), suggesting that the elevated plasma DBH levels are in fact representative of an elevated SNA. Unlike young animals, adult SHR and spSHR exhibit either unaltered or a slightly reduced plasma DBH activity compared to WKY (Axelrod, 1976; Yamori, 1976; Nagatsu et al., 1978). In conclusion, plasma DBH measurements

suggest that during a period of 4 to 10 weeks of age, SHR exhibit an elevated SNA which in adult animals is reduced to near normal levels.

Plasma levels of NE are not altered in SHR when compared to WKY (Grobecker et al., 1975; Nakamura and Nakamura, 1977). However, studies performed by Shomig et al (1978) indicate that plasma levels of NE, but not adrenalin or dopamine, are increased in spSHR over WKY at the ages of 5, 12 and 28 weeks. The above finding has been interpreted as indicating that both young and adult spSHR have an overactive sympathetic nervous system. However, it should be pointed out that alterations in the reuptake and degradation of NE, modifications in the synaptic cleft width anatomy, as well as changes in the adrenal secretion of NE, could modify the plasma levels of NE. Therefore, the presence or absence of alterations in plasma NE may not reflect altered sympathetic nerve activity.

Direct recordings from postganglionic splanchic and/or renal nerve bundles of anaesthetized SHR indicate that during established hypertension, SHR exhibit 2 to 3 times the firing activity present in WKY (Judy et al., 1976; Thoren and Ricksten, 1979). The increased SNA appears to be generalized, since the cervical sympathetic, greater and lesser splanchnic, splenic and renal nerves of SHR all exhibit at least a two fold elevation in nerve activity over WKY (Judy et al., 1976).

In studies performed by Judy and his colleagues direct measurements of renal nerve activity indicated that nerve firing is increased in SHR over WKY starting at 5 weeks of age, being approximately 3 times greater in SHR than WKY at 24 weeks of age. Such activity coincided with the maintenance of hypertension in SHR. In

studies where the blood pressure was lowered by the intravenous (i.v.) injection of the ganglionic blocking agent hexamethonium, or the central acting adrenergic blocker α-methyldopa, the decrease in blood pressure achieved was directly related to the degree of inhibition of renal nerve firing activity (Judy et al., 1976; Judy et al., 1978). The above studies suggest that SNA is elevated in young and also adult SHR, contrary to the findings involving the measurements of plasma DBH.

Studies performed by Judy et al (1976) demonstrate that an experimentally induced increase or decrease in the mean arterial pressure (by aortic clamping or hemorrhage) respectively, decreased and increased the renal nerve firing activity in SHR and WKY. When 12, 24 and 52 week old SHR were compared to age matched WKY, SHR always exhibited an elevated renal nerve firing activity over WKY, under conditions where the arterial pressure of the two groups was experimentally made equal. In 24 and 52 week old SHR, but not 12 week old animals, the rate of decrease in renal nerve activity per increase in arterial pressure was lower in SHR than in WKY. In WKY, irrespective of age, renal nerve activity could be totally inhibited by increasing the mean arterial pressure to 165 mm Hg. A total inhibition of renal nerve firing activity could not be achieved in SHR by elevating blood pressure.

The mechanisms responsible for the above alterations in the SNA with respect to modifications in arterial blood pressure are unknown. However, structural alterations in the arterial tissue surrounding the carotid body secondary to hypertension could dampen the pressure signal perceived by the baroreceptors. In this regard, experiments by Andersen

and Brown (1980) and Andersen et al (1980) indicate that aging in both SHR and WKY is associated with a reduced aortic wall distensibility coupled to an increase in baroreceptor sensitivity. In WKY, the latter two variables are matched so that the pressure threshold for baroreceptor function remains unaltered. However, in older SHR, the decrease in wall distensibility exceeds the increase in baroreceptor sensitivity, resulting in a reduced ability of baroreceptors to initiate vasodilation in response to elevated pressure. In view of this, the resetting of the baroreceptors could contribute to the maintenance of advanced hypertension in SHR by playing a permissive role, allowing the occurrence of hypertension despite the presence of high blood pressure. However, the SNA is also increased in SHR over WKY under experimental conditions where blood pressures of the two groups are equal, and therefore alternative mechanisms that increase SNA must also be present. These will be discussed in a later section (section C).

1. (b) <u>The effect of sympathectomy on blood pressure development in SHR</u>

Various studies have used chemical, immunological or surgical sympathectomy to study the role of the sympathetic nervous system in the development of high blood pressure in SHR. Sinaiko et al (1980) used peripheral injections of 6-hydroxydopamine (6-OHDA) into SHR, at days 1, 4 and 7 of neonatal life, followed by biweekly injections up to 6 weeks of age and weekly injections up to 12 weeks of age. Such treatment reduced heart and kidney levels of NE by 80 to 99%, while brain NE levels remained only slightly altered. Treated SHR exhibited a 50 mm Hg drop in mean arterial blood pressure. However, such treatment did not

produce normotension; male SHR still exhibited a mean arterial pressure of 155 mm Hg. Ikeda et al (1979) obtained comparable results in spSHR. Neonatal 6-OHDA treatment reduced blood pressure on average by 75 mm Hg, although sp SHR still maintained a hypertensive state (150-175 mm Hg) between the ages of 10 to 23 weeks. Mulvany et al (1981b),on the other hand, injected SHR bidaily from birth up to 3 weeks of age. Such treatment prevented the development of hypertension; at 24 weeks of age, SHR maintained a mean arterial pressure of 122 mm Hg.

In other experiments, neonatal SHR have been treated with nerve growth factor antisera (NGFA) to achieve sympathectomy. As with neonatal 6-OHDA treatment of SHR, such experiments have produced differing degrees of success in controlling blood pressure. In experiments performed by Oparil and her colleagues (Page and Oparil, 1978; Oparil and Cutilletta, 1980), immunosympathectomized SHR exhibited mean arterial blood pressures between 20 and 40 mm Hg below control SHR at ages ranging between 6 to 43 weeks. However, NGFA treated SHR still developed hypertension, exhibiting a mean systolic blood pressure between 155 to 168 mm Hg during the established phase of hypertension. Folkow et al (1972), on the other hand, did produce normotension in SHR through the use of immunosympathectomy. SHR treated neonatally with NGFA exhibited a mean systolic blood pressure of 139 mm Hg at 32 weeks of age, while sham treated SHR had blood pressures in excess of 200 mm Hg. NGFA treated Wistar rats exhibited only a marginal decrease in blood pressure when compared to nontreated controls (113 vs 139 mm Hg).

The differing degrees of hypotension produced by the various techniques could be due to differing degrees of sympathectomy. In the

case of sympathectomy produced by 6-OHDA, recovery of the nerves can be quite rapid. For example, in pithed rats sympathetic nerve evoked pressor responses to tyramine injection are completely abolished by the I.V. injection of 6-OHDA. However, complete recovery can occur in 4 days (Finch et al., 1972). Furthermore, large decreases in tissue NE content do not necessarily signify a complete sympathectomy. For example, the neonatal treatment of SHR with guanethidine partially destroys the sympathetic nervous system. The NE content of the heart and spleen of 10 to 13 week old SHR decreases by 76 to 80% compared to control values. Yet surprisingly, such animals still exhibit substantial pressor responses when the vasomotor centers of the brain are stimulated (Johnson and Macia, 1979).

Experiments performed by Johnson and Macia (1979) indicate that the presence of even a partially intact sympathetic nervous system may be sufficient to initiate a full complement of hypertension. In these experiments it was shown that neonatal sympathectomy produced by either guanethidine or NGFA resulted in only a 10 to 12 mm Hg decrease in the blood pressure of adult animals. Such a marginal change in blood pressure occurred in spite of the fact that sympathetic nerve pressor responses were reduced by 50% in each of the two treatments. However, when both treatments were combined a total abolition of nerve responses was achieved in adult animals. Such treatment reduced the mean arterial blood pressure of SHR from 179 to 129 mm Hg. These experiments suggest that the sympathetic nervous system may act as a trigger in initiating hypertension; i.e., partial sympathectomy, instead of proportionally decreasing blood pressure could act as a trigger and initiate a full

complement of hypertension while only a severe total sympathectomy is capable of eliminating high blood pressure.

Brody and his colleagues (Lais et al., 1975; Touw et al., 1980) have argued that the sympathetic nervous system is not involved in maintaining hypertension in SHR. These researchers did not observe a difference in the electrical firing activity of the lumbar ganglia when 6 to 7 month old anaesthetized SHR were compared to WKY. Surgical sympathectomy of these ganglia reduced the hindlimb vascular resistance of WKY to a greater extent than SHR, suggesting that the hindlimb vasculature of the former group of animals is under greater sympathetic tone. In other experiments, Doppler probes were implanted in 2 to 3 month old SHR and WKY. Subsequently, based on the determination of renal, mesenteric and hindlimb blood flow, as well as the mean arterial blood pressure, a representation of the change in vascular resistance in response to various treatments could be determined in conscious animals. The injection of hexamethonium, a drug which has been shown to decrease peripheral sympathetic activity (Judy et al., 1976), produced a proportionally similar decrease in the blood pressure in SHR as in WKY. Similarly, the proportional decrease of the vascular resistance in response to treatment was similar in SHR and WKY in all the above vascular beds. In view of this, it was concluded that the increased vascular resistance observed in SHR is not the result of an overactive sympathetic nervous system, but rather is an intrinsic property of the SHR vasculature.

However, the above experiments may not be accurate enough to measure the difference in vascular resistance resulting from an

alteration in the SNA. This can be demonstrated by the use of a hypothetical situation. In the above experiments, hexamethonium treatment produced a 20 to 40% drop in the maximal vascular resistance in the different vascular beds. If, for the sake of argument, the basal vascular resistance in the absence of nerve stimulation is set at 50 units for SHR and WKY, and it is hypothesized that an elevation in vascular resistance resulting from the presence of an active sympathetic nervous system is 30 units in SHR and 20 units in WKY, the proportional drop in the total vascular resistance that would result from a total inhibition of the sympathetic nervous system would translate to only 37% in SHR (i.e., 30/80) and 28% in WKY (i.e., 20/70). The experimental error present in the experiments performed by Touw et al (1980) is such that this magnitude of alteration would not be significantly different if SHR and WKY were compared. For example, after hexamethonium treatment, the decrease in renal vascular resistance in SHR was 1.8 times that present in WKY (about 2.5 times the magnitude of change present in the above example), yet such a large change was still not significant at $P < 0.05$. Furthermore, the magnitude of alteration in vascular resistance under conditions where the SNA is decreased may not be correlated to the decrease in SNA. Local autoregulatory responses, as well as other cardiovascular adaptations, could act to readjust vascular resistance. In view of this, experimental results obtained by Lais et al (1975) and Touw et al (1980) should be viewed with skepticism.

1. (c) Conclusion

Experiments involving the measurements of plasma DBH activity

indicate that young but not adult SHR and spSHR exhibit an overactive sympathetic nervous system. Generally, measurements of plasma levels of NE have produced ambiguous results. However, some studies involving young and adult spSHR indicate that plasma levels of NE are elevated from the onset of hypertension, suggesting that the SNA is elevated even in adult spSHR. Direct measurements of SNA primarily from renal nerves indicates the presence of hyperactivity in both young and adult SHR.

In hexamethonium, amethyldopa treated and untreated SHR, the level of blood pressure present is directly related to the frequency of renal nerve activity. These latter experiments suggest that the sympathetic nervous system in general, or alternatively, the elevation in the renal nerve activity specifically may play an important role in elevating blood pressure in SHR. In adult SHR, a resetting of baroreceptor function in a manner where an increase in arterial pressure produces a reduced deactivation of the sympathetic nervous system could also help to maintain hypertension in SHR.

Studies involving the use of sympathectomy to study the role of the sympathetic nervous system in maintaining hypertension have produced ambiguous results. In view of the fact that harsh neonatal sympathectomy can prevent the subsequent development of hypertension in SHR (Folkow et al., 1972 b; Johnson and Macia, 1979; Mulvany et al., 1981) while such treatment produces only a modest drop in blood pressure in WKY (Folkow et al., 1972; Mulvany et al., 1981b), it appears that an intact sympathetic nervous system is essential for the development of hypertension in SHR. However, it is not clear from these studies whether an elevation in SNA in SHR is responsible for maintaining an

elevated vascular resistance. Several inconsistencies exist. For example, various sympathectomy treatments that produce a substantial decrease in parameters thought to be a measure of peripheral sympathetic activity, produce only marginal decreases in the blood pressure of SHR. The above studies suggest that the sympathetic nervous system may provide some factor that is essential in promoting the development of mechanisms that initiate hypertension without directly being involved in the maintenance of hypertension. It could be possible that the total absence of the sympathetic nervous system may alter vascular development in a manner where a defect in vascular smooth muscle function responsible for the elevation in vascular resistance is not expressed. An example of how this might occur will be discussed in a later section.

C. The role of the central nervous system in the establishment and maintentance of hypertension in SHR

1. Background

There are a number of areas within the brain that are thought to play an important role in the control of blood pressure. One of these areas is the nucleus tractus solitarius (NTS) in the medulla oblongata. Experiments by Doba and Reis (1974) emphasize the importance of the NTS in this respect. Electrolytic lesions that obliterate the NTS raise the systolic, diastolic blood pressure of anaesthetized rats from 125/97 to 201/151 mm Hg. In such a condition the rats die of congestive heart failure and excessive pulmonary edema, secondary to hypertension.

Afferent fibers from the facial (VII), trigeminal (V), glossopharyngeal (IX) and vagal (X) nerves terminate at the NTS. Of

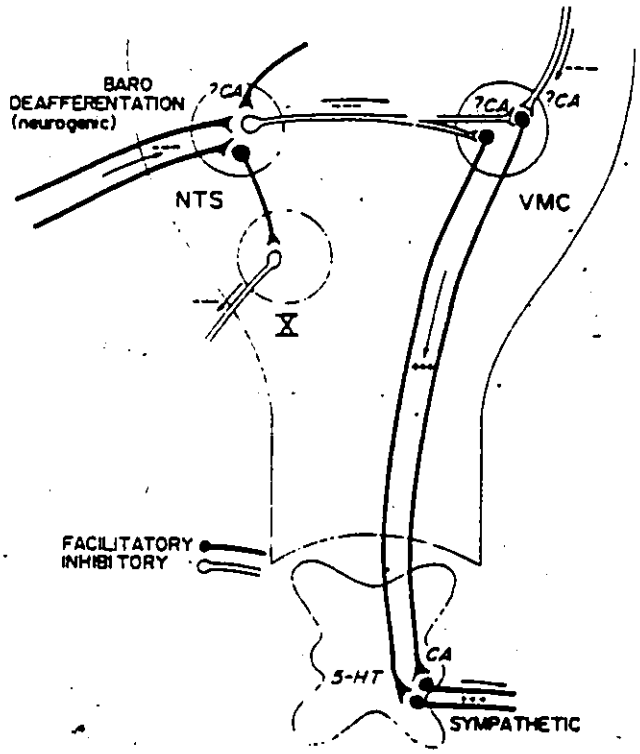
particular importance in blood pressure control, baroreceptor fibres that extend from the carotid sinus and the aorta as well as cardiopulmonary receptors which travel via the glossopharyngeal and vagal afferents synapse at the NTS (Zanberg et al., 1978; Abboud, 1982). Ergoreceptors and renal afferents have the ability to modulate NTS activity and the NTS also receives input from the supraoptic and paraventricular hypothalamic nulcei (Abboud, 1982).

Both catecholamine and serotonin containing pathways are thought to act as connections between the NTS and nerve cell bodies in the spinal cord that make contact with the sympathetic ganglia. Chalmers (1975, 1978) states that the spinal serotonin tracts originate from the B_1, B_2 and B_3 groups of nuclei in the brain, while the catecholamine tracts originate from the A_1 and A_2 regions of the brainstem. A short inhibitory catecholamine tract is thought to connect the NTS to the spinal catecholamine and serotonin tracts which are facilitory in nature.

Fig. 1 outlines a simplified and hypothetical model of the connections thought to be involved in the baroreflex responses. From the model presented, it can be seen that the destruction of the NTS or the surgical interruption of the baroreceptor afferents would eliminate the inhibitory influence on the spinal catecholamine and serotonin tracts, and in this manner raise peripheral sympathetic activity and blood pressure. An increase in baroreceptor nerve activity would stimulate NTS activity, stimulate vagal nerve firing and inhibit spinal catecholamine and serotonin nerve activity. However, even in this simple reflex, the NTS, vagal complex, as well as the vasomotor spinal

Fig. 1. A hypothetical model of the neuronal connections that are involved in the baroreflex response.

The destruction of the nucleus tractus solitarii (NTS) or the surgical interruption of the baroreceptor afferents would eliminate the inhibitory influence on the spinal catecholamine and serotonin tracts, and in this manner raise peripheral sympathetic activity and blood pressure (from Chalmers, 1975).


BARO
DEAFFERENTATION
(neurogenic)
?CA
?CA
?CA
NTS
VMC
X
FACILITATORY
INHIBITORY
5-HT
CA
SYMPATHETIC

serotonin and catecholamine tracts, can be further modulated by various other nerves arriving from more rostral regions of the brain (Chalmers, 1975, 1978; Abboud, 1982).

Attempts have been made to study the various regions of the brain and to ascertain their involvement in hypertension. Often the results of these studies have produced contradictions. This is largely due to the fact that the tools involved in these studies are limited in their specificity to pinpoint either the area or the mechanism of neuronal alteration present.

The importance of the CNS in maintaining hypertension in SHR was first demonstrated by Okamoto and his colleagues (Okamoto, 1969). The destruction of the spinal cord by pithing or by transecting the cord in the upper cervical area of 8 and 24 week old SHR and WKY produced a very large drop in blood pressure in SHR and a more modest reduction in WKY. After such treatment SHR and WKY exhibited similar blood pressures. Lesions of the posterior hypothalamus produced a more moderate drop in blood pressure that was greater in SHR than WKY. On the other hand, transection of the midbrain did not alter hypertension in SHR. These experiments suggested that the integrity of an area of the brain between the regions of the upper cervical cord and the midbrain was essential for the maintenance of hypertension.

2. (a) <u>Alterations in the central catecholamine tracts in SHR</u>

Brain catecholamine tracts appear to play a critical role in the establishment of hypertension in SHR. The injection of 6-OHDA into the ventricles of the brain of 6, 7 and 7.5 week old SHR depletes the brain and spinal cord of catecholamines (Kubo and Hashimoto, 1978). SHR

treated in such a manner maintain blood pressure in the upper normotensive range (140-150 mm Hg) for the length of the period of study, which has ranged between 5 to 12 weeks post treatment (Finch et al., 1972 a; Hausler et al., 1972; Kubo and Hashimoto, 1978). Although in the above studies WKY were not treated with 6-OHDA, studies by Kubo et al (1978) indicate that the intraventricular treatment of 5 week old normotensive Sprague Dawley rats with 6-OHDA did not alter their blood pressure. In view of this, it could be possible that the intraventricular treatment of rats with 6-OHDA does not produce a general hypotensive effect, but rather acts on the mechanism initiating hypertension in SHR.

Spinal injections of 6-OHDA into SHR deplete the spinal cord but not the brain of catecholamines (Kubo and Hashimoto, 1978). The above treatment of young and adult SHR or the intraventricular injection of 6-OHDA into SHR with established hypertension does not affect the progress or maintenance of hypertension (Finch et al., 1972; Kubo et al., 1978). This suggests that the upper brain but not the spinal catecholamine tracts are essential for the development of hypertension in SHR. However, once hypertension is established even the upper brain catecholamine tracts are not necessary in the maintenance of hypertension.

The specific catecholamine tracts thought to contribute to hypertension development in SHR are unknown and studies that have attempted to ascertain the particular areas involved have yielded conflicting results. Consistent with the finding that the intraventricular injection of 6-OHDA prevents hypertension development

in SHR, certain researchers have observed that the pressor areas of the brainstem and certain areas of the hypothalamus have increased NE content and an elevated activity of enzymes involved in NE synthesis in young but not old SHR.

Nakamura and Nakamura (1978 a, b) observed that 5 week old prehypertensive SHR have increased DBH activities (an enzyme involved in NE synthesis) in the locus coeruleus, the A_2 region of the brain and the intermediolateral area of the spinal cord when compared to WKY. These alterations were no longer significantly different when 25 week old SHR and WKY were compared. Since the electrical stimulation of the locus coeruleus in cats increases blood pressure, while both the A_2 and intermediolateral catecholamine tracts have been implicated in the control of sympathetic activity, the authors suggested that the above areas may be involved in promoting altered sympathetic activity in young SHR.

Nagaoka and Lovenberg (1977) found that DBH activity in the pons medulla region and hypothalamus was increased in 5 and 9 week old SHR, while 3 and 15 week old animals had decreased activity levels of this enzyme when compared to WKY. A similar pattern was observed for tyrosine hydroxylase (a key enzyme involved in NE synthesis) activity in the corpus striatum and hypothalamus. Versteeg et al (1976) sampled 27 brain nuclei; the most predominant alteration observed was an increase in the NE content in 11 week old SHR over WKY. Of particular importance, the locus coeruleus, NTS, A_1 and A_2 brain regions all exhibited higher NE and dopamine contents. Henk et al (1978) observed that 2 and 4 week old but not adult SHR had elevated adrenalin levels in

the A_1 region of the brainstem.

Palermo et al (1981) used the radioligands H^3-dihydro-alprenolol, H^3-clonidine and H^3-WB4101 to determine, respectively, the receptor densities of beta, $alpha_2$ and $alpha_1$ receptors in hypothalamic and brainstem homogenates. Eleven week old SHR exhibited a higher $alpha_1$ receptor density in brainstem extracts and a higher $alpha_2$ receptor density in hypothalamic extracts when compared to WKY. The binding affinity of each of the radioligands was not altered in hypertension. The interpretation of this results in terms of altered nerve function is difficult since postsynaptic alpha receptors could be involved in the transmission of nerve impulses, while presynaptic alpha receptors could inhibit catecholamine release.

Using another approach to study nerve function, Hayashi and Hakamura (1981) studied the rate of <u>in vivo</u> ^{14}C-glucose utilization within 100 different cerebral nuclei of 4 and 20 week old SHR and WKY. Of the 100 nuclei studied which included catecholamine containing cell bodies, glucose utilization was either increased or unaltered, but never decreased in SHR when compared to WKY.

The above experiments as a whole suggest that hypertension development in SHR is associated with an overactivity of the catecholamine containing nerves. However, all the evidence in favour of this phenomenon is indirect. Increases in the activity of enzymes involved in the synthesis of catecholamines or the catecholamine content of various brain cells may not be related to increased nerve activity. In fact, certain contradictions do exist. For example, Wijnen et al (1980) observed that the uptake of H^3-tyrosine, a substrate required in

the synthesis of NE, was increased in the medulla and hypothalamus of 3 week old SHR. This implied that the synthesis and release of NE may be increased in young SHR. To test this hypothesis, the synthesis of NE was blocked and the rate of depletion of NE from various brain areas was measured and used as an indicator of the neuronal release of NE, and the formation of H^3-NE from H^3-tyrosine was used to measure NE synthesis. The uptake of NE within the brain was found to be unaltered in SHR, as well, the depletion of NE after NE synthesis was blocked was similar when the A_1, A_2 and NTS regions of the brain were compared. Furthermore, certain hypothalamic nuclei of 3 week old SHR exhibited a reduced rate of NE depletion in spite of the fact that in other experiments this area was shown to exhibit increased tyrosine uptake.

2. (b) Alterations in the central serotonergic tracts in SHR

Buckingham et al (1976) found that the intraventricular injection of 5, 6 dihydroxytryptamine (5, 6 DHT) into 6 week old SHR depleted the brain and spinal cord of serotonin, and retarded the development of blood pressure of SHR for the 6 week period of study (at 12 weeks of age control SHR had a mean arterial BP of 160 mm Hg, while treated SHR had a BP of 140 mm Hg). The above treatment was ineffective in lowering blood pressure in SHR with established hypertension. These experiments suggested that spinal serotonin containing nerves are important in the development but not the maintenance of hypertension in SHR.

In experiments performed by Smith et al (1979), SHR and WKY were injected with an inhibitor of the enzyme aromatic L amino acid decarboxylase (Ro4-4602). The inhibitor used was capable of crossing

the blood-brain barrier and prevented the formation of serotonin within serotonergic nerves. Such treatment produced an accumulation of 5 hydroxytryptophan (5 HTP), a precursor of serotonin. Assays of 5 HTP thirty minutes after injection of Ro4-4602 into the rats was thought to give a representation of the serotonin synthesizing capability of serotonergic nerves. Using this assay, 4 week old prehypertensive SHR exhibited significantly increased 5 HTP levels over WKY in the pons medulla and spinal cord. However, at 8 weeks of age, when the systolic BP was 80 mm Hg above normal, these CNS areas were found to have unaltered serotonin synthesizing capabilities. The above study suggests that serotonin synthesis is increased in the CNS at a time when blood pressure is starting to elevate in SHR.

Some researchers have argued that the action of serotonergic nerves is increased due to a reduced serotonin uptake (Prina et al., 1981). This argument primarily stems from the fact that the platelets of SHR and some essential hypertensive subjects exhibit a reduced ability to take up serotonin (Bhargawa et al., 1979; Prina et al., 1981). Prina et al (1981) have suggested that this alteration could represent a genetic defect in the uptake system also present in serotonergic nerves. However, to my knowledge, no direct experimental evidence of such an alteration being present in the brain of SHR exists.

2. (c) Experiments involving the electrical stimulation of pressor areas in SHR and WKY

Various researchers have attempted to electrically stimulate various areas of the brain in an attempt to ascertain whether the pressor responses observed in SHR differ from those present in WKY. A

consistent observation is that electrical stimulation of the posterior hypothalamus evokes a greater increase in blood pressure in SHR than WKY. Such experiments have been performed in both young (Bunag and Takeda, 1979) and old (Bunag et al., 1976; Juskevich et al., 1978) SHR and WKY, under anaesthetized (Bunag et al., 1975; Juskevich et al., 1978; Takeda and Bunag, 1978; Bunag and Takeda, 1979) and awake conditions (Bunag et al., 1976), and under conditions where the blood pressure of SHR has been normalized by antihypertensive therapy (Juskevich et al., 1978).

The pressor effects produced by hypothalamic stimulation appear to be mediated through the sympathetic nervous system. Such stimulation produces and exaggerated sympathetic firing activity as indicated by direct recordings taken from the splanchnic and abdominal sympathetic nerves (Juskevich et al., 1978; Takea and Bunag, 1978; Bunag and Takeda, 1979). Furthermore, the injection of ganglionic blocking agents prevents the hypothalamic stimulation evoked pressor responses from occurring (Takeda and Bunag, 1978; Bunag and Takeda, 1979). In view of the above results it has been suggested that SHR have an altered nervous system where increases are mediated in part by nerve tracts that run in the area of the posterior hypothalamus.

Attempts to locate the particular nerve tracts involved have been unsuccessful. In cats, some nerve tracts present in the posterior hypothalamus appear to originate from cell bodies within the locus coeruleus. Within these animals electrical stimulation of the locus coeruleus produces pressor responses which are diminished by lesions of the posterior hypothalamus (Przuntek and Philippu, 1973). However,

Kawamura et al (1978) found that although WKY exhibited frequency related pressor responses when the locus coeruleus was stimulated, similar levels of stimulation in SHR produced either a small depression in blood pressure or, at high levels of stimulation, a rise in blood pressure that was less than that present in WKY. Further research is required to determine whether the nerve tracts of the locus coeruleus or other as yet unknown pressor connections are responsible in producing the hypothalamic evoked pressor responses.

2. (c) Conclusion

Central nervous system adrenergic and serotonergic nerves appear to play an important role in initiating hypertension in SHR. The injection of 6-OHDA or 5, 6 DHT into the brain ventricles of prehypertensive SHR depletes the brain and spinal cord of, respectively, catecholamines and serotonin and prevents the subsequent development of hypertension. The ventricular treatment of young normotensive Sprague Dawley rats with 6-OHDA does not alter blood pressure suggesting that 6-OHDA treatment does not produce a general hypotensive response.

Injection of 6-OHDA into the spinal cord of young and adult SHR or the intraventricular injection of 6-OHDA or 5, 6 DHT into the brain ventricles of SHR with established hypertension does not alter blood pressure. This suggests that (1) spinal catecholamine tracts are not required to initiate hypertension and (2) once hypertension is established, neither catecholamine or serotonin containing nerves are required to maintain hypertension. However, even at this stage of hypertension development, the central nervous system is still important in maintaining hypertension, since lesions of the cervical spinal cord

or spinal cord destruction by pithing does reduce the blood pressure of SHR to that present in similarly treated WKY.

The specific areas of the brain thought to be responsible for the initiation of hypertension are unknown. Furthermore, the techniques available to answer such questions are limited. In general, at a time when blood pressure first starts to elevate certain vasopressor areas of the brain of SHR exhibit an increased content of NE, and an increase in the activity of enzymes responsible for NE and serotonin synthesis. Certain brain areas of young adult animals also exhibit an increase in $alpha_1$ or $alpha_2$ receptor densities and an increase in glucose utilization. All the above experiments suggest that the initiation of hypertension may be associated with an overactivity of adrenergic and serotonergic nerves. However, this point is still controversial.

There is experimental evidence that suggests that lesions of the posterior hypothalamus produce a hypotensive effect that is greater in SHR than WKY, while the electrical stimulation of this area produces a greater sympathetic nerve evoked pressor response in SHR than WKY. The mechanisms involved in this response or its importance in hypertension are unknown.

D. Hormonal and neurohormonal alterations in SHR

1. Background

The initiation and the initial maintenance of hypertension in SHR cannot be explained by hormonal alteration. Ebihara (1972) performed experiments where 4 week old prehypertensive SHR and normotensive rats were parabiotically joined for a 12 week period. Such

a procedure involves the suturing of the scapular muscles and skin of an SHR to WKY producing a common peritoneal cavity and allowing the exchange of extracellular body fluid. It was observed that hypertension development in the SHR individual of the parabiotic pair did not alter the blood pressure of the normotensive control partner. On the other hand, other studies involving 2 kidney Goldblatt hypertensive rats, hypertension produced by partial renal ischemia (Schaectelin et al., 1963; Ebihara, 1972) and Dahl salt sensitive and resistant rats (Dahl et al., 1969) indicate that parabiotic combinations can either transfer hypertension to a normotensive partner or, in the case of Dahl salt sensitive rats, transfer a susceptibility factor to salt resistant rats permitting hypertension development upon salt loading. Studies involving SHR do, however, indicate that during advanced phases of hypertension CNS alterations in dopamine and/or peripheral alteration in prolactin and/or vasopressin could help maintain hypertension.

2. (a) Alterations in dopamine and prolactin in SHR

Alterations in central dopamine function, metabolism or the hypothalamic release of dopamine could play an important role in contributing to the initiation or maintenance of hypertension. The action of dopamine can be exerted through central pathways where dopamine is thought to decrease sympathetic outflow and peripheral resistance. Alternatively, a decreased release of dopamine from the hypothalamus could promote a hyper-secretion of prolactin from the pituitary. The latter hormone has the ability to potentiate NE contraction and act as an antidiuretic.

The injection of dopamine (10 µg) into the third ventricle of

rats produces a neurally mediated hypotensive response which reduces peripheral sympathetic activity by 35%, heart rate by 52% and produces a 30% drop in blood pressure (Baum and Shopshire, 1973). A similar reponse has been found in cats; Dutta et al (1975) observed a dose dependent decrease in blood pressure when dopamine or apomorphine (a dopamine receptor agonist) was injected into the brain ventricles. These effects were inhibited by the intraventricular injection of haloperidol (a dopamine receptor antagonist), atropine or scolopamine (muscarinic receptor blocking agents). The latter results suggest that the hypotensive effects exerted by the intraventricular injection of dopamine are mediated through cholinergic connections.

Horrobin and his colleagues have investigated the role of prolactin in controlling blood pressure. It was observed that the infusion of 50 µg/kg/hr prolactin into decerebrated rabbits for 4 hours increased the mean arterial blood pressure from 100 to 140 mm Hg (Horrobin et al., 1973). In other experiments involving isolated perfused rat mesenteric vascular beds, it was found that the presence of 50 ug/ml prolactin increased the maximal vascular resistance responses to NE and angiotensin II, respectiyely, by 60 and 40%. A comparable result was obtained in isometric tension studies of rat aortic strips. In the above studies the effects of prolactin could be inhibited by the presence of indomethacin, suggesting that the potentiation of contraction by prolactin is being mediated through prostaglandins (Manku et al., 1973; Mtabajei et al., 1976). It is unclear, however, whether the effects of prolactin discussed above are of physiological importance. The quantities of prolactin required to demonstrate NE

potentiation are much higher than the physiological quantities present in the circulation.

Prolactin is also thought to act at the level of the kidney to decrease the excretion of sodium and water. Burstyn (1976) found that the administration of 200 I.U. prolactin per day to rabbits for a 5 day period produced, respectively, a 45 and 41% reduction in sodium and urine excretion. Clinical studies by Horrobin (1971) also indicate that the daily injection of prolactin into humans produces sodium and water retention.

The importance of dopamine or prolactin alterations in hypertension is emphasized by the fact that bromocryptine, a dopamine receptor agonist, can produce normotension in a subpopulation of essential hypertensive patients with high prolactin levels (Stumpe et al., 1977). Other studies have shown that there is a significant correlation between the levels of plasma prolactin and the rise of diastolic blood pressure during pregnancy (Jenkins and Perry, 1978).

The role of dopamine and/or prolactin in the initiation and maintenance of hypertension in SHR has not been fully investigated. However, Sower et al (1979) found that plasma prolactin levels were elevated in 18 week old SHR over WKY. Within this study, it was observed that the pituitary prolactin responses to dopamine agonists and antagonists, as well as TRH, were normal when SHR were compared to WKY. This suggested that the hyperprolactinemia observed was produced by a decreased release of dopamine from the hypothalamus in SHR. In studies performed by McMurtry et al (1979) bromocryptine treatment of 18 to 24 month old SHR reduced plasma prolactin and the mean arterial blood

pressure of the animals from 178 to 129 mm Hg. However, in other experiments involving 13 to 17 week old SHR, bromocryptine treatment reduced plasma prolactin levels but did not alter blood pressure. Further experimentation is necessary to determine the role of dopamine and prolactin in elevating blood pressure in SHR. Based on the limited amount of expermental evidence available, it could be possible that long term hypertension alters the SHR physiology in a manner where blood pressure of old malignant hypertensive SHR but not younger SHR is susceptible to decreases in brain dopamine or increases in plasma prolactin. However, at present, this relationship is not clear.

2. (b) Alterations in vasopressin in SHR

Vasopressin is a neurohormone secreted from nerves present in the posterior pituitary. The plasma levels and the urinary excretion of vasopressin is modestly increased in 5 week old SHR with mild hypertension when compared to WKY. The onset of hypertension and aging is associated with a further increase in plasma vasopressin, while at malignant stages of advanced hypertension, SHR have approximately four times the plasma levels of vasopressin present in WKY (Crafton et al., 1978; Mohring, 1978; Mohring et al., 1978). During the beginning of established phases of hypertension, the injection of vasopressin antisera into SHR produces a modest 15 to 20 mm Hg drop in blood pressure. However, during malignant hypertension, such treatment can reduce blood pressure by up to 100 mm Hg (Mohring, 1978). The hypotensive effects produced by the injection of vasopressin antisera are transient lasting approximately 100 minutes. The re-establishment of hypertension is thought to reflect the replacement of antisera bound

vasopressin by freshly secreted hormone (Mohring, 1978).

Alterations in plasma vasopressin and the susceptibility of hypertension to vasopressin antisera are not limited to SHR. Mohring (1978) has demonstrated the presence of elevated plasma vasopressin in malignant stages of both DOCA/salt and one kidney Goldblatt hypertension in rats. As in SHR, vasopressin antisera is also capable of normalizing blood pressure in these hypertensive models during malignant hypertension. Mohring has suggested that increases in plasma vasopressin are the result of modifications secondary to high blood pressure.

Although vasopressin appears to play a role in maintaining high blood pressure during advanced hypertension in SHR, the mode of vasopressin action is not well understood. Although vasopressin is capable of directly contracting blood vessels in normal rats, the plasma levels of vasopressin are insufficient to produce vasoconstriction. Under normal physiological conditions, vasopressin is thought to act an an antidiuretic (1) increasing the water permeability within the collecting ducts and distal tubules of the kidney and (2) stimulating active sodium transport within the tubular epithelial cells, thus promoting sodium and water retention (Bisset and Jones, 1975). However, in the case of malignant hypertensive SHR, the injection of vasopressin antisera lowers blood pressure within 5 minutes, faster than the time predicted if vasopressin was acting only as an antidiuretic.

Mohring (1978) has suggested that vasopressin acts as a vasoconstrictor. In SHR with malignant hypertension, the plasma levels of vasopressin range between 3 to > 100 pg/ml. Since in normal rats the

threshold plasma levels of vasopressin required to produce vasopressor effects are in the range 25-30 pg/ml, a portion but not all of the SHR population has sufficient vasopressin to directly constrict arteries (Mohring, 1978). Mohring (1978), however, contends that during malignant hypertension in SHR there is up to a 1000 fold increase in the sensitivity to vasopressin contraction. The experimental evidence supporting this contention is indirect and is based on the fact that plasma vasopressin vs blood pressure dose response curves obtained by lowering plasma vasopressin levels in SHR (through the use of antisera) are shifted to the left of those obtained by elevating blood pressure via infusion of vasopressin into normotensive rats. Such a comparison can be criticized. Since two different methods were used to alter plasma vasopressin in WKY and SHR, a true comparison of alterations in sensitivity cannot be made.

Vasopressin could also act to potentiate sympathetic nerve evoked contraction. In this regard, subpressor doses of vasopressin have been shown to potentiate NE contraction of mesoappendix arteries and aortic strips (Altura et al., 1965; Altura and Hersy, 1967) and to potentiate sympathetic nerve evoked pressor responses (Cowley et al., 1974). In view of this, elevated levels of vasopressin during malignant hypertension, possibly in combination with other pre and postsynaptic modifications in the vasculature of hypertensive SHR, could produce an increase in vascular tone and resistance.

2. (c) Conclusion

In general, there is little evidence available that neurohormonal or hormonal alterations are involved in the initiation of

hypertension in SHR. Experiments involving the parabiotic combination of prehypertensive SHR and age matched Wistar controls indicate that the subsequent elevation of blood pressure in SHR does not affect the normotensive partner. However, studies by McMurtry et al (1979) indicate that plasma prolactin levels are elevated in SHR with established hypertension and bromocryptine treatment decreases both the plasma prolactin and the blood pressure of SHR. During advanced stages of hypertension, SHR also exhibit elevated levels of plasma vasopressin. During this stage of hypertension development, blood pressure can be lowered by injecting SHR with vasopressin antisera. This suggests that during late stages of hypertension alterations in central dopamine and/or peripheral prolactin or vasopressin may play a role in maintaining hypertension.

E. Alterations in the reaction of SHR to external stimuli

There are many reports in the literature that suggest that SHR may have a CNS that is altered in a manner where experimental stimuli are perceived or reacted to in an exaggerated manner in terms of pressor responses. Nakamura and Nakamura (1978 b) noted that when subjected to a 30 second immobilization stress, 20 to 40 week old SHR responded by elevating plasma DBH, NE and adrenalin to a greater degree than WKY controls. Both prehypertensive and adult SHR exhibit a higher degree of vagal suppression and increased heart rates over normotensive controls during defense responses to a variety of stressfuls stimuli (Folkow et al., 1973; Hallback and Folkow, 1974).

Many researchers believe that exaggerated pressor responses could help initiate or aggravate hypertension development in SHR.

Okamoto (1969) states that both prehypertensive (40 day) and young established hypertensive SHR (80 day) develop higher blood pressures when subjected to chronic visual, auditory, electrical or immobilization stresses. Folkow and his colleagues (Folkow et al., 1973; Hallback and Folkow, 1974) have argued that the hyper-responsiveness of the sympathetic nervous system to stress is produced by genetic alterations since such alterations are present in prehypertensive SHR. It was suggested that the elevations in blood pressure observed during such episodes could produce arterial structural adaptations that could permanently maintain hypertension. This theory will be discussed further in later sections.

F. Presynaptic Alterations in the Peripheral Sympathetic Nervous System in SHR

1. Background

Sympathetic axons innervating blood vessels contain many varicosities. The passage of an action potential results in an inward movement of Ca^{+2} at the varicosity which causes the migration of NE containing vesicles to the presynaptic junction where they fuse and release NE and other proteins. Once released into the synaptic cleft, the disposition of NE occurs in two manners. Presynaptically an active amine pump is present which can take up NE and internalize the transmitter. Once internalized, NE is either degraded or taken up into synaptic vesicles by another amine pump, permitting it to be released again during exocytosis. Alternately, NE can be taken up by vascular smooth muscle and deactivated. The de novo synthesis of NE takes place at the sympathetic terminal varicosity and within the sympathetic

ganglion cells. The quantity of NE containing vesicles in the varicosity is feed back controlled. A decrease in the vesicles within the varicosity accelerates vesicle formation in the neural cell body. The newly formed vesicles are subsequently transported down to the varicosity by active axonal transport (Vanhoutte, 1978).

There are a number of presynaptic factors that govern the magnitude of nerve evoked responses. Bevan (1977) has shown that there is a direct correlation between the density of innervation and the magnitude of contractile response in veins. The release of NE from the sympathetic varicosity is also controlled by a number of different presynaptic receptors (Westfall, 1977). Evidence is available indicating that alpha$_2$, dopamine, H_2-histamine, serotonin, adenosine and muscarinic receptors are present presynaptically. Stimulation of these receptors with agonists as well as the presence of prostaglandins E_1 and E_2 inhibit the synaptic release of NE, while alpha$_1$, angiotensin II and nicotinic receptors facilitate the release of NE (Westfall, 1977; McGrath and Shepherd, 1978; Shepherd et al., 1978; Verhoeghe et al., 1978; Zimmerman, 1978; Kawasaki et al., 1982; Ekas et al., 1983).

The distance between the presynaptic surface and the vascular smooth muscle is also an important determinant of response. Generally a narrow synaptic cleft will favor the accumulation of higher maximal NE concentrations within the cleft during nerve stimulation. Such an alteration also permits a greater presynaptic recovery of released NE and increases the effectiveness of presynaptic feedback inhibition of transmitter release (Bevan, 1977).

In view of the above discussion, it could be possible that

hypertension is associated with (1) alterations in the density of innervation, (2) alterations in the anatomy of the synaptic cleft, (3) alterations in the presynaptic control of NE release and uptake. These aspects will now be discussed.

2. (a) Alterations in nerve density and synaptic cleft anatomy

There is evidence in the literature suggesting that both young and adult SHR have blood vessels with increased nerve densities. Morphometric studies by Dr. Lee (personal communication, unpublished results) indicate that larger muscular mesenteric arteries in prehypertensive (3-4 week) SHR and smaller premucosal arteries of 10 to 12 week old SHR (Lee et al., 1983b) with established hypertension exhibit greater cross-sectional quantities of nerves (nerve sheath + axons) than age matched WKY. However, in each of the above age groups, arteries larger and smaller than those mentioned had an unaltered innervation density when SHR and WKY were compared. Fluorescent microscopy studies performed by Scott and Pang (1983a) indicate that from 2 weeks of age onward SHR exhibit an increase in the number of catecholamine fluorescent profiles around jejunal arteries when compared to WKY. In other studies performed by Saito and Lee (1982), the density of catecholamine fluorescence was increased in the cerebral vasculature of 24 week old SHR over WKY. Consistent with the above observation the incidence of granular vesicle containing nerve endings and the NE content of the blood vessels was elevated in SHR.

Scott and Pang (1983) studied the neural junction of jejunal arteries of SHR and WKY between the ages of 2 to 12 weeks. It was stated that the minimum cleft width between the nerve and SMCs was

unaltered in hypertension. However, data supporting this contention was not presented.

2. (b) Alterations in the release and uptake of NE by sympathetic nerves of SHR

Physiological and pharmacological investigations involving the release and uptake of NE in the renal vasculature of SHR have produced conflicting results. Work by Vanhoutte and his colleagues (Collis et al., 1980; Vanhoutte, 1981) involving 6 week old incipient hypertensive SHR indicates that the renal vascular reactivity and sensitivity to NE is not different than that present in WKY (discussed in a later section). However, stimulation of the renal nerves at frequencies up to 12 Hz evoked a much greater renal vascular resistance response in the isolated perfused kidneys of SHR than WKY. The increased response to nerve stimulation in SHR was associated with an elevated NE overflow (Vanhoutte, 1981). The inhibition of both the pre and postsynaptic NE uptake mechanisms did not alter the hyper-responsiveness observed in SHR during nerve stimulation. In view of this it was suggested that young SHR exhibit hyper-responsiveness during nerve stimulation due to an increase in the release of transmitter (Vanhoutte, 1981).

Vanhoutte and his colleagues (Collis and Vanhoutte, 1977; Vanhoutte, 1981) found that the renal vasculature of adult SHR exhibited an increased reactivity and sensitivity in response to the infusion of NE. However, in spite of the above alterations which favoured vascular hyper-responsiveness, periarterial nerve stimulation at frequencies up to 10 Hz produced similar vascular responses in SHR as in WKY. When the renal nerves were preloaded with H^3-NE, electrical stimulation was found

to produce both a smaller H^3-NE and total overflow in SHR than WKY (Vanhoutte, 1981). When the neuronal uptake of NE was blocked by cocaine such treatment potentiated nerve stimulated contractile responses in the SHR kidney to a much greater degree than WKY (Collis and Vanhoutte, 1977). It was concluded that although the adult renal vasculature of SHR is hyper-responsive to NE contraction, such a response is not exhibited during nerve evoked contraction due to a smaller release and a greater neuronal uptake of this amine (Vanhoutte, 1981).

Not all researchers agree with the above conclusion. Ekas et al (1983a, b) observed that the uptake of H^3-NE by the renal vasculature of isolated perfused kidneys of adult SHR and WKY was not different. However, the H^3-NE overflow during nerve stimulation (0.25 to 32 Hz) was 1.5 to 2.5 times that present in WKY. Nerve stimulation also produced a greater change in renal vascular resistance in SHR than WKY. In this study, it was concluded that the nerves of the renal vasculature of SHR have an unaltered uptake and exhibit an increased release of NE.

The isolated perfused mesenteric vasculature of SHR ranging in age from 14 to 21 weeks has been shown to exhibit hyper-responsiveness to both NE and periarterial nerve stimulation by two independent research groups (Kamikawa et al., 1980; Ekas and Lockhandwala, 1981; Kawasaki et al., 1982a; Ekas et al., 1983b). Both Ekas et al (1981, 1983b) and Kawasaki et al (1982a) have shown that periarterial nerve stimulation can evoke a greater overflow of preloaded H^3-NE in SHR. Work by Ekas et al (1981) has shown that the total uptake of H^3-NE by the mesenteric bed is equal in SHR over WKY. The elevated H^3-NE

overflow observed during nerve stimulation was present even under conditions where the presynaptic and postsynaptic NE reuptake systems were blocked by respectively cocaine and metanephrine. Both the above uptake blockers exhibited a proportionally similar inhibition of H^3-NE overflow in SHR and WKY. The above experiments indicate that the mesenteric arterial bed of SHR exhibits a similar pre and postsynaptic uptake of NE as that of WKY, although SHR are capable of neuronally releasing greater quantitites of NE in response to equal degrees of nerve stimulation.

Other researchers have argued that the neuronal uptake of NE in the mesenteric arteries is increased in SHR compared to WKY. Whall et al (1980) found the total wall, pre and postsynaptic uptake of H^3-NE was increased in intermediate sized mesenteric arteries (lumen diameter (LD) approximately 200 µm) of adult SHR over age matched WKY. Other studies by Webb and Vanhoutte (1981) indicate that helical strips of tail arteries taken from SHR, exhibited an increased total and cocaine sensitive H^3-NE uptake when compared to WKY. Consistent with the above observation, the inhibition of presynaptic uptake by cocaine, produced a greater potentiation of nerve evoked contraction in SHR than WKY.

2. (c) Altered presynaptic control of NE release

Various researchers have attempted to test the possibility that the elevated release of NE from the sympathetic nerve terminals of SHR could be produced by an alteration in the presynaptic control of transmitter release.

In studies performed by Ekas et al (1983b) it was observed that the release of H^3-NE during renal nerve stimulation was inhibited in a

dose dependent manner by tramazoline (2×10^{-9} to 2×10^{-7}M) an $alpha_2$ agonist and adenosine (0.3 to 10 µg/ml) in SHR and WKY. However, the studies indicated that in spite of the presence of an augmented release of H^3-NE in SHR and WKY, the threshold doses of tramazoline and adenosine inhibiting the release of H^3-NE were smaller, and the proportion of the total inhibition greater in SHR than WKY. In other experiments, the stimulation of $beta_2$ presynaptic receptors by salbutamol produced a proportionatelysimilar increase in H^3-NE release during nerve stimulation in SHR and WKY. It was concluded that the augmented release of NE from the renal sympathetic nerves of SHR was not due to a decrease in sensitivity or reactivity of presynaptic $alpha_2$ and adenosine (inhibitor) receptors, or an increase in the efficiency of $beta_2$ (facilitory) receptors.

In studies involving the isolated perfused mesenteric vasculature of adult SHR and WKY, Ekas et al (1981) also observed that the overflow of preloaded H^3-NE was elevated in SHR over WKY. However, in spite of this, the proportional inhibition of H^3-NE release by antagonist phentolamine was not altered when SHR and WKY were compared. These experiments were performed under conditions where the pre and or the postsynaptic uptake of NE was blocked respectively by cocaine and metanephrine. Studies performed by Kawasaki et al (1982a) indicate that the presynaptic $beta_2$ facilitation of NE release is augmented in the perfused mesenteric vasculature of adult SHR when compared to WKY. In these studies, it was observed that isoproterenol, a general beta agonist and salbutamol, a selective $beta_2$ agonist, potentiated pressor responses produced by periarterial nerve stimulation to a greater degree

in SHR than WKY. It was observed that release of preloaded H^3-NE was augmented in SHR during nerve stimulation and that isoproterenol proportionately increased the release of H^3-NE in SHR to a greater [redacted] than WKY. However, these alterations appear to be of pharmacological as opposed to physiological importance in altering nerve stimulated responses. The presence of propranolol (up to 5×10^{-7}M) a general beta receptor antagonist or practolol (5×10^{-6}M) a $beta_2$ selective antagonist did not alter nerve evoked contractile responses in either SHR or WKY. This indicates that although the presence of potent beta agonists such as isoproterenol or salbutamol increased nerve responses, the potentiation of transmitter release by the feedback stimulation of presynaptic beta receptors by NE is of marginal consequence in determining response. However, Kawasaki et al (1982a) speculated that a situation could exist where, for example, elevated circulatory levels of epinephrine (a finding observed by some but not all researchers) in SHR could produce the hyper-release of NE from the synaptic terminal.

In other experiments involving the isolated perfused mesenteric vasculature, Kawasaki et al (1982b) found that subpressor doses of A_{II} potentiated nerve contractile responses obtained for adult SHR two-fold over those obtained for WKY. Similar results were obtained under conditions where the presynaptic uptake of NE was blocked. In these experiments, the [redacted] portion of response obtained in the presence and absence of A_{II} was plotted as a function of A_{II} concentration (1 to 20 ng/ml). Dose response curves obtained for SHR were shifted to the left of those obtained for WKY. Since the A_{II} concentrations used could not potentiate NE contraction of the mesenteric vasculature, it was

suggested that the action of A_{II} was to stimulate presynaptic A_{II} receptors and facilitate NE release. This function was augmented in the mesenteric vasculature of SHR compared to WKY.

In experiments of analagous design to those of Kawasaki et al (1982b), Kamikawa et al (1980) observed that the proportional inhibition of nerve evoked contractile responses by adenosine (0.01 to 3 µg/ml) and ATP (0.1 to 10 µg/ml) were decreased in the mesenteric vasculature of adult SHR over WKY. These experiments were also performed in the presence and absence of neuronal NE uptake blockade. Since the nucleotide doses used had no effect on either basal vascular resistance or on NE induced contraction, it was concluded that the presynaptic inhibition of NE release by adenosine nucleotides was decreased in SHR over WKY.

2. (d) Conclusion

Experiments performed on both the cerebral and mesenteric vasculature indicate that, at least some classes of muscular arteries exhibit an increase in adrenergic innervation in SHR. In the mesenteric arteries of SHR this alteration is present in animals 2 weeks of age and older suggesting that such a modification may be important in initiating hypertension. The limited amount of information available indicates that the synaptic cleft width is not altered in the mesenteric arteries of SHR.

Perfusion studies of the whole mesenteric vascular bed of adult SHR and the renal vasculature of 6 week old SHR indicate that the neuronal release of NE during nerve stimulation is augmented in SHR while the reuptake of norepinephrine is unaltered. However, in studies

involving the renal vasculature of adult SHR, both a hyper-release and an unaltered neuronal uptake of NE and a decreased release and an increased uptake of NE have been proposed to occur in SHR. Studies of isolated segments of mesenteric and caudal arteries indicate that the neuronal uptake of NE is increased in adult SHR over WKY. In the studies involving mesenteric arteries, it is not clear whether the difference in results observed between isolated arterial segments and perfusion studies are the result of different study techniques or alternatively the result of differences in the properties of a particular arterial segment as opposed to an entire vascular bed.

Studies of the presynaptic control of transmitter release in SHR indicate that in the renal vasculature alpha$_2$ receptor stimulated inhibition and beta$_2$ stimulated facilitation of NE release is unaltered, while adenosine inhibition of NE release is augmented in SHR over WKY. In view of this, alterations in the feedback inhibition of NE release by NE or adenosine (of which the latter could be co-released with NE) cannot explain the augmented NE release observed in some experiments involving the renal vasculature of SHR. In the mesenteric vasculature, the presynaptic inhibition, of NE release by alpha receptors is unaltered in adult SHR. On the other hand, beta$_2$ and A_{II} stimulation of NE release is increased and the inhibition of NE release by adenosine nucleotides is decreased in adult SHR over WKY. Potentially, the feedback stimulation of presynaptic beta$_2$ receptors by released NE could facilitate the further release of NE. However, experiments have shown that the blockade of presynaptic (and postsynaptic) beta receptors with beta antagonists does not alter nerve evoked responses. The

physiological role of a decreased adenosine receptor efficiency in the mesenteric arteries of SHR is unknown. However, such an alteration could augment NE release within this vascular bed.

G. Postsynaptic Alterations in the Vascular Smooth Muscle Cells (VSMCs) of SHR

1. (a) Background

Norepinephrine (NE) released from sympathetic nerve varicosities binds to alpha and beta receptors on the vascular SMC surface (Bevan et al., 1980). It might be expected that such action would produce opposing effects since the occupation of alpha and beta receptors can respectively contract and relax the vasculature; however, with the exception of the rabbit facial vein (Pergram et al., 1976) and the coronary arteries (Bevan et al., 1980), such action virtually always produces alpha receptor mediated contraction. Very little is known of the molecular structure of the adrenergic receptors and the receptors have been characterized by the nature of the agonists and antagonists that bind to them.

The alpha and beta receptor concept was first proposed by Alquist (1948). It was found that smooth muscle (SMC) of the vasculature and genitourinary tract contracted when a number of catecholamines and related drugs were applied. The potency of contraction being epinephrine (E) > NE >> isoproterenol (ISO). Relaxation of the smooth muscle on the other hand was characterized by the following potency series: ISO >> E $\geq$ NE. Furthermore, ergot alkaloids and haloalkylamines were capable of blocking the former but not the latter response. On the basis of such observations, Alquist

(1948) hypothesized that two types of receptors were present in the tissue; an alpha receptor which produced SMC contraction and a beta receptor which relaxed the muscle (Alquist, 1948). Since that time, the alpha and beta receptor theory has been reinforced by the synthesis of agents that selectively act at one receptor and not the other. For example, phenylephrine acts as a selective alpha agonist while phentolamine selectively antagonizes alpha responses (Steer, 1977). At the beta receptor, ISO is a selective agonist while propranolol and dichloroisoproterenol are selective antagonists (Steer, 1977). In view of the discovery of such agents, Furchgott (1972) redefined the alpha and beta receptor. A beta receptor is one which mediates a response pharmacologically characterized by (1) (a) ISO > E > NE > phenylephrine or (b) ISO > NE > E > phenylephrine and (2) has a susceptibility to specific blockade by either propranolol or pronethalol at lower concentrations. An alpha receptor is one which mediates a response pharmacologically characterized by (1) a relative potency series in which E $\geq$ NE > phenylephrine > ISO, (2) is susceptible to specific blockade by phentolamine, dibenamine or phenoxybenzamine at relatively low concentrations (Furchgott, 1972). More recent work involving the synthesis of still more specific agonists and antagonists for the alpha and beta receptors has enabled researchers to subdivide them into alpha$_1$ and $_2$ (Jones and Michell, 1978; Lefkowitz and Hoffman, 1980; Weiner, 1980) and beta$_1$ and $_2$ (Weiner, 1980).

Many researchers have attempted to find a second messenger that is coupled to NE stimulation of the alpha receptor in VSM. Isolated experiments have suggested that decreases in cAMP or increases cGMP may

mediate the contractile responses of NE (see Steer, 1977; Jones and Michell, 1978 for review). However, more recent evidence indicates that many SMC preparations, including arteries fail to show alterations in cAMP in response to alpha stimulation (Jones and Mitchell, 1978), and increases in cGMP follow the contractile response and appear to be produced by an elevation in intracellular Ca^{+2} (Andersson et al., 1975; Goldberg et al., 1975; Steer, 1977). In view of the above contradictions, none of the above agents can uniquivocally be considered the second messenger for the alpha receptor. Alpha receptor stimulation in VSM does lead to two undisputed cellular actions. (1) With the exception of a minority of tissues which exhibit pharmaco-mechanical coupling (Somlyo and Somlyo, 1968), alpha receptor stimulation produces a change in the membrane potential. (2) There is an elevation in free intracellular Ca^{+2}.

Of all excitable tissues, SMCs exhibit the most variable electrical responses to transmitter stimulation. Microelectrode probes of VSM in physiological saline usually, but not always, indicate an electrically quiescent cell with a membrane potential near -60mV. NE stimulation of the cells evokes different responses in different types of vascular SMCs. To summarize these, activation can produce contraction with (1) no change in membrane potential of the VSM, i.e., pharmo-mechanical coupling (Somlyo, 1968; Droogmans et al., 1977), (2) a graded depolarization of the VSM without the production of an action potential (AP) (Von Loh and Bohr, 1973; Somlyo, 1975; Bulbring, 1979), (3) one or two APs followed by a depolarization of the membrane or (4) a train of spontaneous APs coupled to contraction (Droogmans et al.,

1977). Veins, on the other hand, are not electrically quiescent but in physiological saline undergo spontaneous APs (Golenhofen, 1975; Somlyo, 1975). The addition of NE increases the frequency of APs and contracts the tissue (Golenhofen, 1975; Somlyo, 1975). The variable electrical activity is further complicated by the fact that certain arteries such as the sheep carotid exhibit regional differences in their passive electrical properties (Mekata and Keatinge,1975 ; Keatinge, 1980).

Many vessels have the ability to exhibit multiple types of responses. The type of electrical response produced is determined by the membrane characteristics of the VSMC. For example, the rabbit ear artery has been shown to exhibit pharmaco-mechanical coupling, however, the addition of tetraethylamonium, a K^+ conductance blocker into the bathing media transforms this electrically quiescent artery to one that exhibits spontaneous action potentials (Droogmans et al., 1977). The application of NE (10^{-7}M) speeds up the rate of action potential production and produces a decrease in the baseline depolarization (Droogman et al., 1977). Essentially this treatment converts a pharmaco-mechanically coupled artery into one that is electro-mechanically coupled. In view of this, it could be possible that pathological modification in membrane composition could alter the type of electrical response produced by NE.

Alterations in membrane potentials in response to NE stimulation serve two important functions in the excitation-contraction coupling process. (1) Membrane depolarization by itself can open potential sensitive Ca^{+2} channels which can admit extracellular Ca^{+2}, raising the intracellular levels of this ion (Freeman and Daniel, 1973; Van Breeman

et al., 1980). (2) If action potentials are present in response to NE, these have been shown to be produced by an inward movement Ca^{+2} into VSM (Droogmans et al., 1977; Golenhofen, 1975). Thus, this provides a second route by which Ca^{+2} can enter the SMC. In addition to the above mechanisms there is evidence that indicates that NE can open receptor operated Ca^{+2} channels (Van Breeman et al., 1980)and most importantly release Ca^{+2} from intracellular source (Freeman and Daniel, 1973; Deth and Van Breeman, 1977; Van Breeman et al., 1980). In this regard, both the plasma membrane and the sarcoplasmic reticulum have been hypothesized to be sources for the internal Ca^{+2} store (Daniel et al., 1979; Somlyo et al., 1979).

In SMC, actin, myosin and tropomyosin structurally resemble their counterparts in striated muscle (Hartshorne and Gorecka, 1980). However, important differences between the two muscle types exists. (1) Troponin and Ca^{+2} binding protein that acts to regulate actinomyosin ATPase activity in striated muscle is absent in SMCs (Sobieszek and Bremel, 1975; Sobieszek and Small, 1976). (2) The regulatory apparatus through which Ca^{+2} acts to initiate contraction is most likely myosin linked in SMCs (Lehman et al., 1972; Sobieszek and Small, 1976) and is actin linked in striated muscle. (3) When actin + myosin from SMCs is combined it exhibits very low ATPase activity (0.3-3%) when compared with actinomyosin complexes from striated muscle (Barany, 1967). In striated muscle the high actinomyosin ATPase activity is inhibited by the addition of tropomyosin + troponin. The further addition of Ca^{+2} returns the ATPase activity to high levels. SMC actin + myosin combinations exhibit minimal ATPase activity, the addition of Ca^{+2} in

the absence of troponin + tropomyosin elevates the actinomyosin activity to maximal levels (Hartshorne, 1980).

In summary, any regulatory system proposed for SMCs must be myosin linked and capable in some manner of utilizing Ca^{+2} to activate an inactive actinomyosin. In this regard, the control of contraction in SMCs has not been fully elucidated. However, two theories on how contraction takes place exist.

The myosin phosphorylation theory

This theory has recently been reviewed by Hartshorne (1980). On excitation of the SMC, intracellular Ca^{+2} increases and a Ca^{+2} + calmodulin complex is formed (4 Ca^{+2}/1 calmodulin). This complex then interacts with another 105 K protein to form a tertiary complex which exhibits myosin light chain kinase ability (Dobrowska et al., 1978). This latter complex phosphorylates the light chains of myosin which enables myosin to form an actinomyosin complex that can undergo rapid cycles of cross bridging as long as Ca^{+2} is present (Hartshorne, 1980). When Ca^{+2} is removed intracellularly by sequestration or active transport through the membrane the myosin light chain kinase complex separates to free calmodulin (Hartshorne, 1980). Subsequently, phosphatases present at constant levels intracellularly are thought to dephosphorylate myosin allowing the separation of actin and myosin (Hartshorne, 1980; Frearson et al., 1976). Of additional interest, cAMP which increases during beta receptor mediated VSMC relaxation, stimulates myosin light chain kinase phosphorlation, deactivating the enzyme, preventing it from phosphorylating myosin (Adelstein et al., 1978).

The leiotonin theory

Leiotonin is a protein composed of two subunits with a MW of 80K and 18K (named leiotonin A and leiotonin C). The latter subunit exhibits properties quite similar to calmodulin and binds Ca^{+2}. Leiotonin in the presence of Ca^{+2}, tropomyosin and ATP cause a superpercipitation of actin + myosin from chicken gizzard and aorta (Mikawa et al., 1978; Hirata et al., 1980). Such a combination also exhibits Mg^{+2} actinomyosin ATPase activity. However, unlike the phosphorylation theory, leiotonin action is (1) actin not myosin linked, (2) has an absolute requirement for tropomyosin, (3) although some of the leiotonin preparations exhibit myosin phosphorylation the degree of ATPase activity and or percipitation is related to leiotonin concentrations not the degree of myosin phosphorylation (Ebashi et al., 1977; Hirata et al., 1977, 1980; Mikawa, 1979).

In summary, many of the results presented in the leiotonin theory are in direct conflict with the phosphorylation theory. A variety of researchers substantiate the myosin phosphorylation theory. However, only Ebashi and or his associates (Ebashi et al., 1977; Hirata et al., 1977; Mikawa, 1979; Hirata et al., 1980) find results consistent with the leiotonin theory. The phosphorylation theory provides a mechanism through which cAMP can produce relaxation while the leiotonin theory does not. In favour of the myosin phosphorylation theory ileum muscle skinned with staphylococcal alpha toxin increases tension and simultaneously exhibits myosin phosphorylation in response to $10^{-4}M$ Ca^{+2}. This indicates that myosin phosphorylation takes place in a relatively intact cell system (Cassidy et al., 1979).

1. (b) Conclusion

A number of alterations at the level of the SMC could be present that could alter both the passive and active tonal qualities of VSM. Hypertension could be associated with an increase in the density of postsynaptic alpha receptors or a decrease in the density of beta receptors. Such alterations could increase VSMC sensitivity and reactivity to NE contraction. Post receptor alterations in the excitation contraction apparatus could also exist that are conducive to the maintenance of hypertension. In this regard, the mechanisms coupling receptor stimulation to an elevation in intracellular Ca^{+2} could be altered. In hypertensive vessels, NE stimulation could be associated with a larger depolarization response which could admit greater than normal levels of Ca^{+2} through membrane potential and receptor operated channels. As well, the release of Ca^{+2} from an intracellular source in response to NE stimulation could be altered by virtue of either an increased quantity of Ca^{+2} storage sites, i.e. increased quantities of sacroplasmic reticulum and or plasma membrane, or alternatively by a more efficient coupling of the alpha receptor to the mechanisms initiating the release of Ca^{+2}.

Alterations in the VSMC plasma membrane could effect both the active as well as the passive properties of VSMC. The resting membrane potential could be altered to a more depolarized state. Such an alteration could increase the influx of Ca^{+2} and raise SMC tone. This could be produced by an altered permeability to electrolytes and or an altered electrogenic pump activity. The active efflux of Ca^{+2} from the VSMC could be altered which as well may alter the level SMC tone

present.

Alterations in (1) the quantity of contractile proteins (2) the ability of actin and myosin to interact (3) the ability of Ca^{+2} to interact with calmodulin and/or leiotonin C (4) the ability of calmodulin and other proteins to form myosin light chain kinase or the ability of leiotonin to interact with actin could alter the responsiveness of VSM to NE. Unfortunately, all the above possibilities have not been tested in SHR. What is known about such alterations will now be reviewed.

2. Alterations in the arterial contractile sensitivity and reactivity to NE and various other agonists in SHR

2. (a) Background

Various researchers have hypothesized that the excitation contraction coupling mechanisms of VSMC are altered in a manner conducive to the maintenance of hypertension. A great deal of research effort has been undertaken to answer two fundamental questions. (1) Do arteries from SHR initiate contraction at lower doses of contractile agonists? (2) Are arteries from SHR capable of generating greater tensions than normal arteries? If VSMC from SHR initiated contraction at lower doses of NE and are capable of greater tension development to a given dose of NE, such alterations would increase vascular resistance. To answer the above questions, pharmacological experiments involving tension studies of isolated arterial segments and perfusion studies involving isolated vascular beds have been undertaken.

In tension studies, a helical strip of arterial tissue or an arterial ring is cut from a segment of artery and connected to a tension

monitoring device. Using such a system a simple relationship between stimulus and response can be obtained. Tension development within the tissue, in response to an agonist is measured under conditions where the length or the lumen size of the tissue is stretched to produce optimal (maximal) responses to the agonist. In perfusion studies, a vascular bed is isolated and perfused with either blood or physiological saline under constant flow conditions. The infusion pressure of the perfusate is monitored and is used as an indicator of vascular resistance to flow. Using both the above methods agonist dose vs response curves can be made for various agonists. From these curves the arterial contractile sensitivity and reactivity of a agonist can be determined.

2. (b) The measurement of vascular contractile sensitivity to agonists

Sensitivity is determined by measuring the rightward or leftward shift of a dose response curve. Typically the agonist dose producing threshold responses (which is usually taken as meaning the dose producing 10% or 20% of the maximal response) or the ED_{50} value, (the agonist dose producing 50% maximal contraction) are used as indicators of contractile sensitivity. A decrease in the threshold or ED_{50} value indicates that the dose response curve has shifted to the left suggesting that an increase in sensitivity has taken place.

Decreases in the ED_{50} values obtained from NE dose vs contractile response curves have been interpreted as an indication that an alteration conducive to the maintenance of hypertension has taken place. Such an interpretation can be misleading. For example, an artery may exhibit greater NE sensitivity i.e. a lower ED_{50} value, when

compared to another artery, however the magnitude of contraction at the ED_{50} dose may be greater in the less sensitive artery. This is because the ED_{50} value represents an agonist dose producing a proportion of the maximal response and is independent of the magnitude of change. It is important to note that it is the magnitude of the contractile response produced by an artery at a given agonist dose that will ultimately determine vascular resistance.

2. (c) The measurement of vascular contractile reactivity to agonists

Typically, in arterial strip and ring studies, the degree of maximal tension developed in response to an agonist per equal length and width helical strips or equal vessel length arterial rings have been used as a measurement of reactivity. In perfusion studies, the amplitude of the vascular resistance change in response maximal contraction has been used as an indication of vascular reactivity.

The degree of reactivity present in an artery will depend on the blood vessel geometry as well as postsynaptic alterations in VSMC excitation-contraction. For example, a helical strip from a hypertensive artery may exhibit a greater cross-sectional area while arterial ring segments from hypertensive animals may have a thickened arterial media. Both such alterations could produce hyper-reactivity even in the absence of alterations in postsynaptic VSMC function. To compensate for reactivity alterations due to differences in blood vessel geometry some researchers have expressed maximal tension alterations as a proportion of the helical strip cross-sectional area. In arterial ring studies, Mulvany and his colleagues (for example, see Mulvany et

al., 1978) have combined tension studies with morphometric analysis. Hence the degree of contractile tension developed per unit blood vessel length can be expressed per medial wall thickness, total wall thickness and in some cases SMC cross-sectional area, such compensations will reflect contractile reactivity alterations at the level of the VSMC as apposed to reactivity alterations due to the presence of greater amounts of VSMC. In the case of perfusion studies, a thickened vascular wall will occlude the lumen, therefore raise the vascular resistance a greater degree in response to contraction. Using the above method of study, reactivity alterations could be caused by (1) modifications in blood vessel geometry, (2) changes in the number of open vascular pathways during contraction, (3) alterations in excitation-contraction coupling. It is not possible to differentiate between the above possible causes of reactivity alterations using the perfusion technique alone.

2. (d) The limitations of contractile studies involving the use of isolated arterial strips of tissue and the perfusion of isolated vascular beds

Tension studies involving isolated arterial strips of tissue

Advantages

1.) In studies involving helical strips (but not arterial rings), the tissue can be dissected in a manner where the longitudinal axis of the SMCs are oriented parallel to the axis of force generation.

2.) The helical strip length or the lumen diameter of arterial segments can be adjusted. This permits contraction to be studied at various tension loads.

3.) Both the active and passive wall mechanics of a descrete arterial segment can be analyzed (this aspect will be discussed in detail within a later section).

4.) The relationship of tension development and relaxation with respect to time can be studied.

5.) Tension development can be normalized to the cross-sectional area of a helical strip or the length and wall dimensions of an arterial segment.

6.) The medium surrounding the arterial segment can be controlled and manipulated.

7.) The properties of arterial segments at discrete points along the vascular bed can be studied and compared.

Disadvantages

1.) Studies involving helical strips are limited to larger elastic arteries since it is difficult to dissect such strips from small arteries. The properties of large elastic arteries could differ from small arteries which are thought to be involved in maintaining vascular resistance. Arterial ring segments of small arteries (LD=150 μm) have been studied (i.e. Mulvany et al., 1978, 1980, 1981a, b). However, neither arterial ring nor helical strip studies can predict how an entire vascular bed will react to a stimulus.

2.) The dissection of arterial segments could damage the tissue producing an alteration in its function. For example, in arteries with endothelial damage substance P will produce contraction responses as apposed to producing relaxation in intact arterial segments (Vanhoutte and Rimele, 1982).

3.) The dissection and isolation of vascular strips will eliminate neural and hormonal controls which may be important in determining responses to agonists such as NE under in vivo conditions.

4.) In multilayered arteries having two orientations of SMCs, it is impossible to dissect out helical strips in which the smooth muscle cells are all parallel to the axis of force generation.

Perfusion studies of entire vascular beds

Advantages

1.) The global effect of a stimulus on an entire vascular bed can be determined.

2.) Many of the neuronal and hormonal control mechanisms can be maintained in a more intact condition than in studies involving isolated arterial segments.

3.) When structural and functional alterations within a vascular bed are being assessed in terms of their importance in determining blood pressure, the measurement of vascular resistance provides a parameter that is a physiologically realistic determinant of blood pressure.

4.) A number of variables (i.e. perfusate composition, flow, pressure etc.) can be manipulated or controlled in experiments.

Disadvantages

1.) The exact site at which the stimulus is acting is unknown.

2.) The site of action of the stimulus may change with time.

3.) The relationship between stimulus and response is complex. Alterations in blood vessel wall components, vascular SMC function, the number of open blood flow pathways, as well as the degree of anastamosis between blood vessels within a vascular bed will all contribute in

determining the response.

4.) In most perfusion studies the vascular resistance of the arterial + venous vascular bed is measured. The degree and direction (i.e. contraction vs relaxation) of the response may be different in various arterial segments or between the arterial and venous vasculature. A situation could result where two apposing effects to a stimulus could cancel each other and produce little change in vascular resistance.

5.) The concentrations of an agonist could be different in areas proximal to the point of perfusate entry as apposed to more distal areas due to the uptake and degradation of the agonist or the presence of closed flow pathways.

2. (e) Alterations in the arterial contractile sensitivity and reactivity in SHR as determined by tension studies involving isolated arterial segments and perfusion studies of entire vascular beds

Table 2 a to c summarizes a random cross-section of the literature pertaining to sensitivity and reactivity alterations in response to (in most cases) NE contraction. These have been divided in terms of the type of preparation used (i.e. helical strip, arterial ring tension studies and perfusion studies) and the class of artery studied.

(i) Helical strip tension studies

Most helical strip studies (Table 2a) have involved the use of large arteries (with a lumen diameter > 500 µm). Studies performed on the aorta (Hallback et al., 1971; Shibata et al., 1973; Sutter and Ljung, 1977; Chaturvedi et al., 1978; Pang and Sutter, 1980; Asano et al., 1982) iliac (Swamy and Triggle, 1980a) and carotid (Swamy and

Table 2a. Helical strip tension studies; a (-) indicates a decrease, a (+) an increase and nc no change in sensitivity or reactivity.

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Thoracic aorta	helical st. tension	NA	NE (-)	- NCR, SHR-D; equal width and length stripe compared	Spector et al. (1969)
Thoracic aorta	helical st. tension	NE nc	NE nc	-NCR, SHR-E; equal width and length stripe compared -beta receptor blocked	Hallback et al. (1971)
Thoracic aorta	helical st. tension	A. NE nc K^+ nc B. NE nc K^+ nc	A. NE (-) K^+ (-) B. NE (-) K^+ (-)	-NCR; A. SHR-E; B. SHR-P; equal width and length -no adrenergic blockade during K^+ contraction	Shibata et al. (1971)
Aorta	helical st. tension	B. NE (-)	B. NE (-)(d) A. NE (-)(a) NE nc (b)	- A. WKY vs SHR-D; B. NCR vs SHR-E - (a) normal; (b) hydralazine; (c) force per helical strip cross-sectional area; (d) maximum force.	Sutter and Ljung (1977); Pang and Sutter (1980)
Thoracic Aorta	helical st. tension	NE (-)	NE (-)	-WKY, SHR-E; equal width and length stripe compared	Chaturvedi et al. (1978)
Thoracic Aorta	helical st. tension	A. NE nc (a) NE nc (b) B. NE nc (a) NE nc (b)	NA	-WKY; A. SHR-E; B. SHR-I -(a) normal; (b) plus propranolol -mg tension per equal length and equal width strip	Asano et al. (1982)
Femoral A.	helical st. tension	A. NE(+) (a) NE nc (b) B. NE nc (a) NE nc (b)	A. NE(+) (a) NE(+) (b) B. NE(+) (a) NE nc (b)	-same as above	Asano et al. (1982)
Mesenteric A.	helical st. tension	A. NE(-) (a) NE(-) (b) B. NE(-) (a) NE(-) (b)	A. NE nc (a) NE nc (b) NE(+) (a) NE(+) (b)	-same as above	Asano et al. (1982)

Table 2a. Helical strip tension studies; a (-) indicates a decrease, a (+) an increase and nc no change in sensitivity or reactivity. cont'd

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Iliac A.	helical st. tension	A. NE(-) K^+ nc B. NE(-) K^+(-)	A. NE(-) K^+ nc B. NE(-) K^+ nc	-WKY; A. SHR-E; B. SHR-P -equal width and length strips compared -adrenergic response not blocked during K^+ contraction	Swamy and Triggle (1980a)
Carotid A.	helical st. tension	A. NE(-) K^+(-) B. NE(-) K^+(-)	A. NE(-) K^+(-) NE(-) K^+(-)	-same as above	Swamy and Triggle (1980b)
Tail A.	helical st. tension	NE (+) (a) NE nc (b)	NE (-) (a) NE (-) (b)	-WKY and NCR, SHR-E; (a) normal; (b) + cocaine -equal length strips	Webb and Vanhoutte (1981a)
Femoral A.	helical st. tension	E nc K^+ (+)	NA	-S.D. control, SHR-E	Holloway and Bohr (1973)

WKY, Kyoto Wistar normotensive rats; SHR, Kyoto Wistar spontaneously hypertensive rats; NCR Wistar normotensive rats; SD, Sprague Dawley normotensive rats; SHR-E, SHR in an established phase of hypertension development, i.e., maximally high blood pressure; SHR-D, SHR in an intermediate stage of high blood pressure development, i.e., blood pressure is elevated over controls but below maximal levels; SHR-P, SHR in a prehypertensive stage of blood pressure development; spSHR, stroke prone SHR.

Sensitivity, a measure of the rightward or leftward shift of an agonist dose response curve as measured by doses of agonist producing threshold and/or 50% maximal response. Under sensitivity column, a (+) indicates a greater contractile sensitivity of an agonist in SHR over controls, i.e., a lower threshold or ED_{50} value. A (-) indicates higher threshold and ED_{50} value for an agonist in SHR over controls, i.e., lower contractile sensitivity; (nc) indicates no change in contractile sensitivity between SHR and controls.

Under the reactivity column, a (+) indicates an increased maximum tension response in either helical strip or arterial rings in SHR over WKY during maximal agonist contraction. The tension responses are normalized in different manners in relation to some blood vessel parameter. In perfusion studies, a (+) indicates an increase in the amplitude of vascular resistance change in SHR over controls in response to maximal agonist contraction. A (-) under the reactivity column indicates a decrease in reactivity in SHR compared to WKY; (nc) indicates no difference in reactivity of SHR compared to WKY.

All perfusion studies were performed under constant flow.

NE, norepinephrine; E, epinephrine; A_{II}, angiotensin II; Vaso, vasopressin; 5-HT, serotonin

SHR-I, SHR with incipient hypertension, i.e., blood pressure is slightly, but significantly, elevated over controls.

Table 2b. Arterial ring tension studies; a (-) indicates a decrease, a (+) an increase and nc no change in sensitivity or reactivity.

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Thoracic aorta	rings tension	NE nc	NE (-) K^+ (-)	-NCR, SHR-E; tension per equal vessel lengths -no adrenergic blockade during K^+ contraction -beta receptor blockade, no effect on NE response	Shibata et al. (1975)
Abdominal aorta	rings tension	NA	K^+ nc (a),(b) (c)	-WKY, SHR-E; active force per (a) vessel length, (b) media cross-section, (c) SMC cross section -no adrenergic blockade of K^+ contraction	Arner and Uvelius (1982)
Tail A.	rings tension	NE nc (a) NE (-) (b)	Ca^{+2}/NE (+) (c)	-WKY, SHR-E; (a) normal, (b) in the presence of 3 uM cocaine, (c) active force per vessel length, (d) active force per vessel length per medial thickness -Ca^{+2} reactivity is the maximum force developed in response to increasing Ca^{+2} in the presence of 10 uM NE	Mulvany et al. (1982)
Femoral A.	rings tension	NE nc (a), (b)	Ca^{+2}/NE nc (c) (d)	-same remarks as above reference	Mulvany et al. (1982)
Mesenteric A. (150 um)	rings tension		A. K^+ (+) (a) K^+ nc (b)(c) B. K^+ nc(a)(b) (c)	-WKY, A. SHR-E, B. SHR-I; (a) active force per vessel length; active force per vessel length per (b) wall thickness or (c) SMC cross section	Warshaw et al. (1979)
Mesenteric A. (190 um)	rings tension	NE nc (a) NE (+) (b)	NE (+) (a)	-WKY, SHR-E; (a) innervated; (b) denervated with 6-OHDA -equal length arterial rings studied	Whall et al. (1980)
Mesenteric A. (180 um)	rings tension		NE (+) (a) vs (c) (b) vs (d)	-(a) SHR-E normal; (b) SHR-E sympathectomized with 6-OHDA neonatally; (c) WKY normal; (d) WKY sympathectomized -equal length arterial rings studied	Mulvany et al. (1981 b)
Mesenteric A. (200 um)	rings tension	NA	NE (+) K^+ (+)	-WKY; SHR-E; tension per vessel length -K^+ contraction in presence of phentolamine	Whall et al. (1983)
Mesenteric A. (190 um)	rings tension	NA	NE and K^+ (+) (a) (b) (c) NE and K^+ nc (a) (b) (d)	-WKY, SHR-E; (a) nontreated; (b) WKY and SHR-E treated with hydralazine, chlorothiazide, reserpine in combination from 25 to 48 weeks of age -(c) active force per vessel length; (d) active force per vessel length per SMC cross-sectional area -NE plus K^+ contraction measured	Warshaw et al. (1980)
Mesenteric A. (150 um)	rings tension	NE nc	NE (+) (a) NE and K^+ nc (b) (c) (d)	-WKY, SHR-E; (a) active force per vessel length -(b) active force per vessel length per media thickness or (c) per wall thickness -(d) circumferential tension per SMC	Mulvany et al. (1978)

Table 2b. Arterial ring tension studies; a (-) indicates a decrease, a (+) an increase and nc no change in sensitivity or reactivity. cont'd

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Mesenteric A. (150 um)	rings tension	NE nc	NE (+) (a) NE and K^+ nc (b) (c) (d)	-WKY, SHR-E; (a) active force per vessel length -(b) active force per vessel length per media thickness or (c) per wall thickness -(d) circumferential tension per SMC	Mulvany et al. (1978)
Mesenteric A. (150 um)	rings tension	A. Ca^{+2}/NE (+) $\frac{1}{2}$ (a) Ca^{+2}/K^+ nc (b) NE (+) (a) K^+ nc (b) B. Ca^{+2}/NE (+) $\frac{1}{2}$ (a) Ca^{+2}/K^+ nc (b) NE (+) (a) K^+ nc (b)	A. NE and K^+ (+) (c) NE and K^+ nc (d) NE nc (e) B. NE and K (-) (c)(d) NE nc (a) K^+ nc (b)	-WKY $\frac{1}{2}$ A. SHR-E; B. SHR-P. -Ca^{+2} sensitivity measured as the ED_{50} value of Ca^{+2} dose response curve in the presence of 10 uM NE or 125 mM K^+ -(a) in the presence of 3 uM cocaine; (b) in the presence of 1 uM phentolamine; (c) active force per vessel length; (d) active force per vessel length per wall thickness; (e) in the absence of Ca^{+2}	Mulvany and Nyborg (1980)
Mesenteric A. (150 um)	rings tension	NE (+) (a)(b) (very small change in each case)	NE (+) (a)(b) (c)	-WKY, SHR-D-E (12-24 weeks); (a) nontreated; -(b) SHR vs WKY, both treated with antihypertensive drug filodipine from weaning, SHR normotensive (12 weeks); (c) SHR vs WKY, both sympathectomized neonatally with 6-OHDA, SHR normotensive (24 weeks) -NE dose response curves performed in the presence of 3 uM cocaine; equal length arterial rings studied	Mulvany et al. (1981 a)

WKY, Kyoto Wistar normotensive rats; SHR, Kyoto Wistar spontaneously hypertensive rats; NCR Wistar normotensive rats; SD, Sprague Dawley normotensive rats; SHR-E, SHR in an established phase of hypertension development, i.e., maximally high blood pressure; SHR-D, SHR in an intermediate stage of high blood pressure development, i.e., blood pressure is elevated over controls but below maximal levels; SHR-P, SHR in a prehypertensive stage of blood pressure development; spSHR, stroke prone SHR.

Sensitivity, a measure of the rightward or leftward shift of an agonist dose response curve as measured by doses of agonist producing threshold and/or 50% maximal response. Under sensitivity column, a (+) indicates a greater contractile sensitivity of an agonist in SHR over controls, i.e., a lower threshold or ED_{50} value. A (-) indicates higher threshold and ED_{50} value for an agonist in SHR over controls, i.e., lower contractile sensitivity; (nc) indicates no change in contractile sensitivity between SHR and controls.

Under the reactivity column, a (+) indicates an increased maximum tension response in either helical strip or arterial rings in SHR over WKY during maximal agonist contraction. The tension responses are normalized in different manners in relation to some blood vessel parameter. In perfusion studies, a (+) indicates an increase in the amplitude of vascular resistance change in SHR over controls in response to maximal agonist contraction. A (-) under the reactivity column indicates a decrease in reactivity in SHR compared to WKY; (nc) indicates no difference in reactivity of SHR compared to WKY.

All perfusion studies were performed under constant flow.

NE, norepinephrine; E, epinephrine; A_{II}, angiotensin II; Vaso, vasopressin; 5-HT, serotonin

SHR-I, SHR with incipient hypertension, i.e., blood pressure is slightly, but significantly, elevated over controls.

Table 2c. Vascular perfusion studies; a (-) indicates a decrease, a (+) indicates an increase and nc no change in sensitivity or reactivity.

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Hindlimb vasculature	perfusion PSS	NE nc	NE (+)	-NCR, SHR-E	Folkow et al. (1970)
Hindlimb vasculature	perfusion PSS	NE small (+)	NE (+)	-WKY, sp SHR-I	Dietz et al. (1978)
Hindlimb vasculature	perfusion PSS	NE (+)	NE (+)	-WKY, SHR-P	Lais and Brody (1978)
Hindlimb vasculature	perfusion PSS	NE (+) K^+ nc	NE (+) K^+ (+)	-WKY; spSHR, 5 weeks old -blood pressures not shown -no adrenergic blocking agents used during K^+ contraction	Shomig et al. (1978)
Hindlimb vasculature	perfusion PSS, blood		A. NE(+)(a)(b) 5HT(+)(a)(b) K^+(+)(a)(b) B. NE(+)(a)(b) 5HT(+)(a)(b) K^+(+)(a)(b)	-WKY; A. SHR-E; B. SHR-D -(a) blood perfused; (b) PSS -denervated vasculature; K^+ contraction tested with phentolamine	Cheng and Shibata (1980)
Hindquarter vasculature	perfusion blood	NE (+)	NA	-NCR, SHR-E -blood perfused	Lais and Brody (1975)
Hindlimb vasculature	perfusion PSS	NE nc 5HT nc	NE (+) 5HT nc	-NCR, SHR-E	Haeusler and Finch (1972)
Mesenteric bed	perfusion PSS	5HT (+)	5HT (+) NE (+)	-NCR, SHR-E	Haeusler and Finch (1972)
Mesenteric bed	perfusion PSS	NE(+)(a)(b)(c) K^+ (+)	NE(+)(a)(b) NE nc (c) K^+ (+) (a)	-NCR, SHR-E -(a) normal; (b) plus cocaine; (c) denervated -normal, cocaine-treated and denervated SHR were compared to non-treated NCR	Haeusler and Haefely (1970)
Mesenteric bed	perfusion PSS	NE nc 5HT nc	NE nc 5HT (+)	-SD controls; SHR-E -SD and SHR had low 5HT response	Hamilton (1975)
Renal vasculature	perfusion PSS	NE nc	NE (+)	-WKY, SHR-E	Folkow et al. (1971)
Renal vasculature	perfusion PSS	NE (+) 5HT (+) A_{II} (+)	NE (+) 5HT (+) A_{II} (+)	-WKY, SHR-E	Collis and Vanhoutte (1977)
Renal vasculature	perfusion PSS	NE(+)(a)(b)(c) A_{II}(+)(a)(b)(c) 5HT(+)(a)(b)(c) Vaso(+)(a)(b)(c)	NE(+)(a)(b)(c) A_{II}(+)(a)(b)(c) 5HT(+)(a)(b)(c) Vaso(+)(a)(b)(c)	-WKY: (a) spSHR-P; (b) spSHR-D; (c) spSHR-E	Barecek et al. (1980)

Table 2c. Vascular perfusion studies; a (-) indicates a decrease, a (+) indicates an increase and nc no change in sensitivity or reactivity. cont'd

Tissue	Preparation	Sensitivity	Reactivity	Remarks	References
Renal vasculature	perfusion PSS	NE nc	NE nc	-WKY, SHR-I-	Collis et al. (1980)
Renal vasculature	perfusion PSS	NE nc A_{II} nc	NE (+) A_{II} (+)	-WKY, SHR-E	Ekas et al. (1981 b)
Renal vasculature	perfusion blood	NA	NE (-) A_{II} (+)	-WKY, SHR-E -blood perfused	Fink and Brody (1979)

WKY, Kyoto Wistar normotensive rats; SHR, Kyoto Wistar spontaneously hypertensive rats; NCR Wistar normotensive rats; SD, Sprague Dawley normotensive rats; SHR-E, SHR in an established phase of hypertension development, i.e., maximally high blood pressure; SHR-D, SHR in an intermediate stage of high blood pressure development, i.e., blood pressure is elevated over controls but below maximal levels; SHR-P, SHR in a prehypertensive stage of blood pressure development; spSHR, stroke prone SHR.
Sensitivity, a measure of the rightward or leftward shift of an agonist dose response curve as measured by doses of agonist producing threshold and/or 50% maximal response. Under sensitivity column, a (+) indicates a greater contractile sensitivity of an agonist in SHR over controls, i.e., a lower threshold or ED_{50} value. A (-) indicates higher threshold and ED_{50} value for an agonist in SHR over controls, i.e., lower contractile sensitivity; (nc) indicates no change in contractile sensitivity between SHR and controls.
Under the reactivity column, a (+) indicates an increased maximum tension response in either helical strip or arterial rings in SHR over WKY during maximal agonist contraction. The tension responses are normalized in different manners in relation to some blood vessel parameter. In perfusion studies, a (+) indicates an increase in the amplitude of vascular resistance change in SHR over controls in response to maximal agonist contraction. A (-) under the reactivity column indicates a decrease in reactivity in SHR compared to WKY; (nc) indicates no difference in reactivity of SHR compared to WKY.
All perfusion studies were performed under constant flow.
NE, norepinephrine; E, epinephrine; A_{II}, angiotensin II; Vaso, vasopressin; 5-HT, serotonin
SHR-1, SHR with incipient hypertension, i.e., blood pressure is slightly, but significantly, elevated over controls.

Triggle, 1980b) artery indicate that the arterial sensitivity and reactivity to NE and K^+ contraction is either unchanged or reduced when SHR in a prehypertensive, developing or established phase of hypertension are compared to either WKY or normotensive Wistar rat (NCR) controls. Experiments involving the tail artery of SHR (Webb and Vanhoutte, 1981) in an established phase of hypertension indicate NE sensitivity is increased in the absence of cocaine but unaltered in its presence while reactivity of this artery to NE is reduced in SHR over both WKY and NCR controls. Both young and established hypertensive SHR have mesenteric arteries with a reduced contractile sensitivity to NE under normal conditions, and as well, under conditions where the beta receptor is blocked with propranolol (Asano et al., 1982). Within this latter study, no change NE reactivity was observed in SHR with established hypertension, however young hypertensive animals did exhibit an increased reactivity to NE contraction.

Studies involving the femoral artery of SHR in an established (Holloway and Bohr, 1973; Asano et al., 1982) and young hypertensive stage (Asano et al., 1982) indicate that in young SHR, NE sensitivity is unaltered when compared to WKY while in old SHR NE sensitivity is increased in the absence but not the presence of beta receptor blockade. Work by Holloway and Bohr (1973) has indicated that the femoral artery of adult SHR is slightly more sensitive to K^+ depolarization while epinephrine sensitivity is unaltered when these animals are compared to Sprague Dawley controls. The reactivity of the femoral artery to NE contraction is increased in SHR with established hypertension and in young hypertensive SHR in the absence but not the presence of

propranolol (Asano et al., 1982).

(ii) Summary

Studies involving helical strips of large arteries from SHR in a prehypertensive, developing and established phase of hypertension development have in general failed to support the contention that SHR vasculature is either hypersensitive or hyperreactive to NE contraction. Although there is some evidence of supersensitivity in arteries such as the tail and femoral artery of SHR (Webb and Vanhoutte, 1981; Asano et al., 1982) this alteration is present only at a certain age and under certain conditions. Most other studies indicate either no change or a decrease in NE contractile sensitivity exists (Spector et al., 1969; Hallback et al., 1971; Shibata et al., 1973; Sutter and Ljung, 1977; Chaturvedi et al., 1978; Pang and Sutter, 1980; Swamy and Triggle, 1980a, b; Asano et al., 1982). As well, the hypothesis that hypertension is associated with hyper-reactivity of the blood vessel helical strips is equally weak. Some evidence of hyper-reactivity is present in the mesenteric arteries of young SHR and the femoral artery of young and old SHR (Asano et al., 1982) however more often than not, studies involving other arteries indicate a decreased reactivity (Spector et al., 1969; Shibata et al., 1973; Sutter and Ljung, 1977; Chaturvedi et al., 1978; Swamy and Triggle, 1980b; Webb and Vanhoutte, 1981).

(iii) Arterial ring segment tension studies

Tension studies involving arterial rings are summarized in Table 2b. As in the helical strip studies, aortic rings from SHR in an established phase of hypertension exhibit no alteration in NE

contractile sensitivity (Shibata et al., 1973) and either a decreased (Shibata et al., 1973) or unaltered (Arner and Uvelius, 1982) contractile reactivity to both NE and K^+ when compared to WKY or NCR. The femoral and tail arteries of SHR in established hypertension exhibit either unchanged or decreased NE sensitivity in SHR over WKY (Mulvany et al., 1982).

Smaller mesenteric arteries with lumen diameters that average between 150 to 200 µm appear to be altered in a different manner than the larger arteries discussed above. Arterial ring studies of the above tissues indicate that in the absence of cocaine, innervated segments exhibit a marginal altered contractile sensitivity to NE when SHR and WKY are compared (Mulvany et al., 1978; Whall et al., 1980). However, if neuronal uptake is blocked with 3 µM cocaine (Mulvany and Nyborg, 1980; Mulvany et al., 1981a) or alternatively if the arteries are denervated with 6-OHDA (Whall et al., 1980), NE contractile sensitivity is increased in SHR over WKY.

It has been suggested by Mulvany and his colleagues that NE uptake by the adrenergic nerves in the adventitia of smaller mesenteric arteries is increased in SHR. The higher neuronal uptake of NE at the adrenergic synapse in SHR could decrease the effective NE concentration in the synaptic area and therefore decrease the degree of postsynaptic receptor stimulation. However, there is controversy as to whether the neuronal uptake of H^3-NE is increased in the mesenteric bed of SHR. Ekas and Lokhandwala (1981) have obtained results consistent with an unaltered neuronal uptake of NE, while Whall et al (1980) have observed an increased neuronal uptake in mesenteric arterial segments when SHR

and WKY were compared. Furthermore, the presence of cocaine does not potentiate NE sensitivity in all arteries. Both helical strip (Webb and Vanhoutte, 1981) and arterial ring (Mulvany et al., 1982) studies involving the tail artery of SHR and WKY have indicated that in the presence of cocaine, the NE sensitivity of this tissue is decreased in SHR as compared to WKY. Likewise, cocaine does not potentiate NE sensitivity in studies involving femoral artery rings (Mulvany et al., 1980).

When maximal tension development in response to NE stimulation is expressed per vessel length or equal length arterial segments are compared, small mesenteric arterial rings from SHR exhibit an increased reactivity over those obtained from WKY (Whall et al., 1980; Mulvany et al., 1981b; Whall et al., 1983). Likewise studies involving the same procedure and tissue have indicated the presence of hyper-reactivity to maximal K^+ (Warshaw et al., 1980; Whall et al., 1983) and NE plus K^+ (Mulvany and Lyborg, 1980) contraction in SHR. However, in these studies when the tension development per unit vessel length was further normalized to compensate for differences in medial thickness or alternatively if the response was normalized to SMC content, the reactivity observed in SHR was similar to that present in WKY (Mulvany et al., 1978; Warshaw et al., 1979; Mulvany and Nyborg, 1980; Warshaw et al., 1980). This strongly suggests that in small mesenteric arteries the increased reactivity observed in SHR is due to quantitative structural alterations such as increases in medial thickness and/or medial SMC content as apposed to functional post synaptic alteration at the level of the VSMC.

(iv) Summary

Arterial ring studies involving large arteries such as the aorta, femoral and tail artery indicate that in general NE sensitivity and reactivity is either unaltered or decreased in SHR over WKY. Although, small mesenteric arteries exhibit marginal differences in NE sensitivity when SHR and WKY are compared; the presence of cocaine (3 μM) or arterial denervation with 6-OHDA increases the NE sensitivity in SHR to a greater degree than WKY. In view of this, it has been suggested that the neuronal uptake of NE is increased in SHR over WKY and that the altered neuronal uptake decreases the effective NE levels coming in contact with the postsynaptic receptors to a greater degree in SHR than WKY. Such an alteration could hide the presence of an elevated NE sensitivity in SHR. However, there is controversy as to whether the neuronal uptake of NE is increased in the mesenteric arteries. Furthermore, in other tissues such as the tail artery, studies have indicated an elevated neuronal H^3-NE uptake in SHR, however here cocaine decreases NE sensitivity in SHR. This suggests that the action of cocaine is not the same in all arterial tissues and may be more complex than simply the inhibition of presynaptic NE uptake.

Maximal tension development per blood vessel length in response to NE, K^+ and NE + K^+ contraction is higher in the smaller mesenteric arteries of SHR when compared to controls. However if the SHR and WKY arteries are compensated for differing wall and medial thicknesses or are normalized to SMC content, reactivity is similar in SHR and WKY. This suggests that the elevated reactivity in SHR is being produced by structural alterations as apposed to altered excitation contraction

mechanisms at the level of the VSMC.

(v) Perfusion Studies

Perfusion studies (Table 2c) involving the hindlimb and mesenteric vasculature indicate that NE sensitivity is either unaltered (Folkow et al., 1970; Hausler and Finch, 1972; Hamilton, 1975) or increased (Haeusler and Haefely, 1970; Lais and Brody, 1975; Lais and Brody, 1978; Schomig et al., 1978) in SHR over WKY. In the renal vasculature most studies indicate that NE sensitivity is unaltered (Folkow et al., 1971; Collis et al., 1980; Fonteles and Jeske, 1980; Ekas et al., 1983b) while a minority of studies indicate that NE sensitivity is increased (Collis and Vanhoutte, 1977) in SHR and spSHR (Berecek et al., 1980). In virtually all perfusion studies involving the hindlimb, mesenteric and renal vasculature, SHR are found to exhibit an elevated vascular rectivity to NE and a wide variety of other contractile agents (McGregor and Smirk, 1968; Hausler and Haefely, 1970; Folkow et al., 1970; Folkow et al., 1971; Hausler and Finch, 1972; Collis and Vanhoutte, 1977; Deitz et al., 1978; Lais and Brody, 1978; Berecek et al., 1980; Cheng and Shibota, 1980; Ekas et al., 1983b). Unfortunately, using this experimental system, it is impossible to determine whether the increased reactivity is being produced by structural alterations and or alterations in nonstructural postsynaptic contractile mechanisms.

(vi) General discussion of tension and perfusion studies

A number of reasons can be postulated as to why helical strip studies in particular should yield differing results from studies involving arterial rings and perfusion. Helical strip studies typically

involve large arteries (i.e. aorta, femoral, carotid and tail artery). Such blood vessels in general exhibit modest sensitivity and reactivity alterations in SHR as indicated by arterial ring tension studies. Furthermore, the orientation of the SMCs in relation to the axis of contraction is a strong determinant as to the degree of tension a helical strip is capable of developing. Helical strips could be cut in a manner where the longitudinal axis of the SMCs are oriented at right angles to the axis of which tension is being monitored. The improper orientation of the SMCs could dampen the degree of tension development and mask the presence of altered reactivity when arterial strips from SHR and WKY are compared.

Another aspect of experimental protocol that could modify SMC sensitivity and reactivity to agonists is the sequence and number of agonists being tested on a particular tissue. Studies performed by Rapoport and Bevan (1983) involving histamine, KCl, NE, serotonin and papaverine indicate that the pretreatment of rabbit ear arteries with one contractile or vasodilator agent strongly effects both the sensitivity and maximal contractility of other agents. Such effects can last for as long as 5-6 hrs. In view of this, one can question both the ED_{50} and reactivity values from experiments where for example NE, serotonin, angiotensin II, vasopressin and or $BaCl_2$ contraction are tested on the same tissue (Collis and Vanhoutte, 1977; Berecek et al., 1980).

Perfusion studies within our laboratory (unpublished results) involving the infusion of NE into the mesenteric vasculature as well as studies by Ichijima (1969) on the in vivo abdominal vasculature of SHR

and WKY indicate that vascular beds do not contract evenly. Areas of hypercontraction and dilated segments exist. It could be possible that the variation in response between the various experiments previously discussed could be due to the testing of arterial segments of inherently different contractility.

3. (a) Alterations in receptor densities in SHR

There is some evidence to suggest that hypertension in SHR may be associated with alterations in the numbers of $alpha_1$, $alpha_2$ and beta receptors within tissues. Pettinger et al (1982) studied plasma membrane prepared from the entire kidney of SHR in an established phase of hypertension and of age matched WKY. $Alpha_1$ and $alpha_2$ receptor densities were determined by binding respectively, radioactive $alpha_1$ and $alpha_2$ antagonists H^3-prazosin and H^3-yohimbine to the membrane. Analysis of the specific binding through the use of Scatchard plots was used to determine the maximal numbers of receptors bound by the radioligands. It was observed that the density of $alpha_1$ and particularly $alpha_2$ receptors was increased in SHR over WKY. When SHR and WKY were placed on a high salt diet, a treatment that enhanced blood pressure development, $alpha_2$ receptor densities were found to increase in proportion to the level of blood pressure developed. If the alterations in receptor densities of the entire kidney of SHR are representative of the type of alterations present on the renal vasculature this could have the potential to produce NE contractile hyper-responsiveness in SHR kidneys.

In studies performed by Weiss et al (1983) $alpha_2$ receptor densities of the microsomal fraction of the tail artery, hypothalamus

and brain stem were determined by binding the alpha$_2$ agonist H^3-clonidine to the membrane. SHR in established hypertension were found to exhibit greater alpha$_2$ receptor densities in the plasma membrane fractions from the tail artery but not the hypothalamus or brainstem. Consistent with this observation EC_{50} values for alpha$_2$ agonists were lower in the tail arteries of SHR than WKY. Other studies by Agarawal and Daniel (1983) indicate that alpha$_1$ receptor densities and the binding affinities of both alpha$_1$ and alpha$_2$ receptors, determined by respectively binding H^3-prazosin and H^3-yohimbine to purified mesenteric artery membranes were not altered when adult SHR were compared to WKY. Alpha$_2$ receptor densities on the other hand were increased in SHR over WKY.

Work performed by Limas and Limas (1979) suggests that the density of beta receptors present in the aorta and inferior vena cava is decreased by approximately 40% in SHR over WKY at the ages of 5, 10 and 25 weeks. In this preparation, H^3-dihydroalprenolol binding to microsomal fractions was used to determine beta receptor density. The binding affinity, as measured by the dissociation constant for H^3-hydroalprenolol binding, was not altered between SHR and WKY at any of the above ages.

Most (Bhalla et al., 1980; Roberecht et al., 1981; Blumenthal et al., 1982) but not all (Limas and Limas, 1978) studies involving cardiac microsomal fractions indicate that SHR and WKY have similar beta receptor densities. These studies have involved rats sampled shortly after birth (Blumenthal et al., 1982) up to an age of 36 weeks (Bhalla et al., 1980). Of particular interest, in the three studies indicating

unaltered beta receptor densities, isoproterenol stimulation of the beta receptors of cardiac cell homogenates was found to produce a lesser activation of adenylate cyclase in SHR than WKY. The beta receptor is thought to be linked to adenylate cyclase via a GTP binding nucleotide regulatory protein (Rodbell, 1980, to be discussed in a later section). It could be possible that the nature of beta receptor coupling to adenylate cyclase and/or the properties of the regulatory protein are altered in SHR heart tissue and perhaps as well, arterial tissue. Such an alteration could lead to reduced cAMP levels during beta receptor stimulation in SHR which in turn might increase VSMC tone.

3. (b) Conclusion

There is evidence from a limited number of studies that plasma membrane fractions obtained from some arterial tissue of SHR contain unaltered $alpha_1$ increased $alpha_2$ and decreased beta receptor densities. If all the $alpha_2$ and beta receptors are coupled to respectively mechanisms of contraction and relaxation, increased $alpha_2$ and decreased beta receptor densities could have the potential to produce an elevation in the contractile response and a decrease in relaxation response to NE. However caution should be taken when making this interpretation. In the case of $alpha_2$ receptors, not all vascular beds exhibit $alpha_2$ mediated contractile responses (Schmitz et al., 1982). Furthermore, in the tail artery of SHR where an increase in $alpha_2$ receptor densities has been noted, there is an elevation in the contractile sensitivity to $alpha_2$ agonists when compared to WKY, however, SHR tail arteries exhibit reduced tension development in response to NE (Webb and Vanhoutte, 1981). In the case of the beta receptor, studies have shown that in SHR

and WKY beta blockade by the antagonist propranolol has very little effect on the contractile response produced by NE or for that matter nerve stimulation (Ekas et al., 1983b). In view of the above discussion, the physiological role of a decrease in postsynaptic arterial beta receptors or an increase in arterial alpha$_2$ receptors can be questioned.

4. (a) Alterations in Na^+, K^+ conductance in the VSMCs of SHR

Various researchers have attempted to determine whether water and electrolyte balance between the vascular smooth muscle and extracellular space are altered in SHR. Jones (1973, 1974a, b) failed to observe any alterations in the intracellular water (% wet wt and g H_2O/g dry wt) Na^+, K^+, Ca^{+2}, Mg^{+2} and Cl^- concentrations (mM) in the aorta, Na^+ and K^+ concentrations in the superior mesenteric and femoral arteries, and K^+, Na^+ and Cl^- concentrations in the plasma when SHR with established hypertension were compared to Wistar rats (NCR). In these experiments, the total levels of tissue electrolytes were determined using atomic absorption spectrophotometry. The intracellular electrolyte levels (mmoles/l cell H_2O) were calculated by determining the total tissue H_2O and electrolyte content and subtracting the H_2O and electrolyte levels contributed by the extracellular space. Hermsmeyer and his colleagues (Hermsmeyer et al., 1980; Hermsmeyer, 1981), using electron probe analysis, observed a lower intracellular Na^+ concentration (mM), but no change in the K^+ concentration when the caudal arteries of SHR were compared to WKY. Friedman and Friedman (1976) observed that the tail arteries of SHR, equilibrated in physiological saline at 37°C, exhibited a decreased Na^+ content

(mmoles/kg dry wt) and an unaltered K^+ content when compared to Carnsworth Farm normotensive controls. In these latter experiments, no mention is made as to whether the tissue electrolytes determined were compensated for the level of electrolytes present in the extracellular space.

In spite of the modest alterations in the steady state contents of electrolytes in the VSMCs of SHR, there is evidence available that suggests that defects in the membrane electrolyte permeability, as well as pumping activity, do exist. In the case of Na^+ and K^+, the prevailing hypothesis is that an increased permeability (promoting Na^+ influx and K^+ efflux down a concentration gradient) is counterbalanced by an elevated Na^+/K^+ pump activity (promoting Na^+ efflux and K^+ influx). The experimental evidence suggesting that such an alteration exists will now be discussed.

Studies performed by Jones (1973, 1974) indicated that, although the levels of aortic K^+ were unaltered in freshly excised tissue obtained from SHR when compared to NCR, the accumulation of internal K^+ ($[K^+]i$) in response to elevations in the external K^+ ($[K^+]o$) was higher in SHR than NCR when aortic tissues were incubated in organ baths. This suggested that the VSCMs of SHR have an increased ability to accumulate K^+. Using another approach, Hermsmeyer (1976 a) indicated that the level of $[K^+]o$ required to reduce the membrane potential to 0 mV (under conditions where the Na^+/K^+ pump is inactive) in caudal arteries was less in SHR than WKY. Since, at 0 mV membrane potential, the K^+ activity outside the cell should approximate that present inside, it was hypothesized that a lower K^+ activity existed in the caudal VSMCs.

In other experiments, Jones (1973, 1974a, b) observed that when the aorta, superior mesenteric and femoral arteries were preloaded with K^{32} and the efflux of K^+ studied, the loss of K^+ from the tissue with time was greater in SHR than NCR. The increased permeability appeared to be a general phenomenon. The efflux of Na^{24} at 2°C (temperature condition at which the Na^+/K^+ pump is inactive) and the efflux of Cl^{36} at 37°C from the preloaded aorta was also increased in SHR. The above studies predicted a reduced internal accumulation of K^+ and an increased accumulation of Na^+ (and Cl^-) in SHR VSMCs. However, since Jones failed to observe any alteration in the steady state cellular Na^+ and K^+ levels, it was hypothesized that an elevated Na^+/K^+ pump activity existed in the aorta.

Experiments performed by Friedman and Friedman (1976) also suggest that the permeability of Na^+ and K^+ is altered in the VSMCs of SHR. The tail arteries of adult SHR were placed in physiological saline with Li^+ substituted for Na^+ at 2°C (Na^+/K^+ pump deactivation). The loss of internal Na^+ and K^+ and the gain of Li^+ was found to be increased in SHR over NCR. In other experiments microelectrodes were used to measure K^+ efflux from the tail arteries. Under conditions where the Na^+/K^+ pump was inhibited (transfer to K^+-free physiological saline), the appearance of K^+ in the perfusate with time was increased in SHR. Since it was observed that the K^+ content of the SHR tail artery maintained in physiological saline at 37°C was normal while the Na^+ content was reduced, it was hypothesized that the increased Na^+ and K^+ permeability observed was counter-balanced by an enhanced Na^+/K^+ pump activity.

4. (b) Alteration in the Na^+/K^+ pump activity in SHR

Experiments performed by Jones (1973a, b), Friedman and Friedman (1976), Webb and Bohr (1979) and Hermsmeyer (1976 a) provide indirect evidence for the presence of an enhanced VSMC Na^+/K^+ pump activity in SHR. Jones (1973) preloaded aortas with Na^{24} at 37°C and subsequently placed the arteries in Na^+-free saline at 2°C. After an initial fast efflux of Na^{24} very little further Na^{24} loss occurred for up to 40 minutes. After 40 minutes at 2°C the Na^+/K^+ pump was reactivated by elevating the temperature to 37°C. An exponential loss of Na^{24} occurred over the next 20 minutes. The latter efflux was attributed to the active pumping of Na^{24} out of the aorta and was found to be greater in SHR than WKY. Friedman and Friedman (1976) obtained results consistent with those of Jones (1973). In these experiments the Na^+/K^+ pump was inhibited by placing tail arteries in K^+-free saline at 10°C for 10 hrs. Under these conditions the arteries became loaded with Na^+ and lost internal K^+. Reincubating the arteries in normal physiological saline at 37°C produced a rapid efflux of Na^+ that was thought to represent Na^+/K^+ pump activity. Using this protocol SHR tail arteries depleted their intracellular Na^+ to a much greater extent than NCR over a 3 hr incubation period at 37°C.

Webb and Bohr (1978, 1979) and Hermsmeyer (1976 a) studied what has been termed the K^+ relaxation phenomenon attributed to the Na^+/K^+ pump. If an artery is placed in K^+-free physiological saline, the Na^+/K^+ pump is inhibited presumably due to a lack of external K^+. This produces an accumulation of Na^+ within the cell. If normal or slightly above normal levels of K^+ are then reintroduced into the medium, the

Na^+/K^+ pump is reactivated and is further stimulated by the high internal Na^+. The large efflux of Na^+ (in relation to K^+ influx) promoted by the activation of the pump hyperpolarizes the VSMCs and produces a relaxation response. The relaxation response can be evoked in the presence of contractile agents such as NE or serotonin, or by elevating the external K^+ from 0 to 30 mM in the absence of vasoconstrictors. Ouabain inhibits such responses. It has been suggested that the degree of relaxation produced under the above protocol can be used as an indicator of Na^+/K^+ pump activity in VSMCs (Hermsmeyer, 1976; Webb and Bohr, 1978, 1979). Experiments by Webb and Bohr (1979) on helical strips of tail arteries indicate that the magnitude and duration of the relaxation response produced by the introduction of 15 mM K^+ into K^+-free medium in the presence of 10^{-7} µg/ml NE was increased in adult SHR. Hermsmeyer (1976 a) found that the maximal degree of transient hyperpolarization produced in the tail artery by introducing 30 mM K^+ into K^+-free medium was larger in SHR than WKY.

The above results have been interpreted as being consistent with the presence of a hyperactive Na^+/K^+ pump. However, it should be noted that such hyperactivity could also be produced by an increased influx of Na^+ during incubation in K^+-free saline or by an increased pump sensitivity to internal Na^+ in SHR, and therefore provides only indirect proof of an altered Na^+/K^+ pump.

In studies performed by Pamnani et al (1981), Rb^{86} uptake in intact tail arteries of SHR was used as an indicator of Na^+/K^+ pump activity. Using this technique, the tail arteries were preloaded with

Na^+ by incubating the tissues in K^+-free physiological saline at near 0^oC. Subsequently, the arteries were divided in half and the Rb^{86} uptake was measured in the presence and absence of ouabain. Since Rb^{86} is thought to substitute for K^+ in the Na^+/K^+ pump, the ouabain sensitive uptake was used to represent pump activity. It was observed that the tail arteries exhibited a greater ouabain sensitive and insensitive uptake of Rb^{86} than normotensive controls. Although this finding is consistent with the presence of an overactive Na^+/K^+ pump, an increase in Na^+ permeability or an increase in the sensitivity of the pump to internal Na^+ in SHR could also produce a hyperactive Na^+/K^+ pump.

4. (c) Conclusion

Experiments involving primarily the aorta and tail arteries of SHR indicate that the arterial VSMCs of SHR are more permeable to Na^+, K^+, Cl^-, and Li^+. In the case of Na^+ and K^+, this increased permeability might be expected to produce Na^+ gain and K^+ loss from the VSMC. There is indirect evidence that suggests that the Na^+/K^+ pump in VSMCs is hyperactive and either compensates or overcompensates for the increased passive Na^+ and K^+ permeabilities observed. The net result is either no change in the steady state electrolyte levels or, as some researchers have suggested, a modestly decreased Na^+ content.

The above findings, however, should be viewed with caution. In efflux experiments involving Na^{24}, K^{42} and Cl^{36} it is important that the efflux rates of the particular electrolyte being studied are measured under conditions where the intracellular levels of diffusable electrolyte are equal in the VSMCs of SHR and control animals. In

experiments performed by Jones (1973a, b, 1974) the concentration of virtually all the cellular electrolytes (mmole/l cell H_2O) were unaltered in the VSMCs of SHR when compared to NCR. However, within the cell, the levels of free electrolytes capable of diffusing could be different in SHR than NCR, thus altering the intra to extracellular electrolyte gradient and modifying the efflux rate of the electrolytes.

In experiments where the activity of the Na^+/K^+ pump has been assessed indirectly (i.e., K^+ relaxation phenomenon, Hermsmeyer, 1976; Webb and Bohr, 1978, 1979) and directly (i.e., Rb^{86} uptake, Pamnani et al., 1981), a hyperactivity of the Na^+/K^+ pump, such as that observed in the tail arteries of SHR, could be produced by the presence of higher levels of unbound intracellular Na^+ in SHR as compared to control vessels. The experimental protocols used could produce a situation where the levels of intracellular Na^+ are increased in SHR. For example, if the VSMCs of SHR are in fact more permeable to Na^+ than controls as has been suggested by Jones (1973a, b, 1974), then the preincubation of arterial tissues of SHR under conditions where the Na^+/K^+ pump is inactive (prior to the measurement of K^+ relaxation responses or Rb^{86} uptake) could result in a hyperloading of Na^+ into SHR as compared to WKY vessels. If such a situation occurred, the presence of an increased K^+ relaxation response or an elevated Rb^{86} uptake in the tail arteries of SHR would reflect the conditions of the experimental protocol as opposed to the true presence of an hyperactive Na^+/K^+ pump in the VSMCs of SHR.

5. (a) <u>Alterations in the membrane potential of the arterial SMCs of SHR</u>

At physiological temperatures the resting membrane potentials (Em) of the caudal (Hermsmeyer, 1976 a,b) and pulmonary artery (Kuriyama and Suzuki, 1978), as well as the mesenteric (Harder et al., 1981) and hepatic portal vein (Hermsmeyer, 1976 b), of SHR are similar to that present in WKY. Work by Kuriyama and Suzuki (1978) indicates that the passive electrical properties of the pulmonary artery and portal vein are also unaltered when SHR were compared to WKY. For example, the space constant (the distance at which an evoked electrotonic potential decays to 1/e, measured using a partitioned stimulation bath, Abe and Tomita (1968)), time constant (the time taken for an electrotonic potential to decay to 1/e), the maximum slope of the membrane potential change in response to K^+ induced depolarization, the change in membrane potential from normal (5.0 mM) to low (0.6 mM) K^+ were all unaltered in both types of blood vessels when SHR were compared to WKY. In the case of the portal vein which exhibits spontaneous spike activity, the frequency of action potential firing as well as the slope of depolarization for each action potential was similar in SHR and WKY. In spite of the lack of alteration in passive membrane properties within both blood vessels, the application of NE to both these tissues or PGE_2 to the portal vein produced a larger degree of membrane depolarization in SHR than WKY. Very little speculation was made as to the importance of such alteration in relation to hypertension.

In other experiments Hermsmeyer (1976 a, b) observed that the Em of SHR tail arteries was not different from that of WKY when the tissues were in physiological saline at 37°C. Consistent with the observation of Kuriyama and Suzuki (1978), NE (> 3 ng/ml) was found to produce a

greater depolarization in tail arteries of SHR than WKY. Other experiments performed by Hermsmeyer indicated that if the Na^+/K^+ pump is inactivated by performing the experiments at 16°C, the Em was less negative in SHR than in WKY. Experiments indicated that the proportion of the Em being maintained by the electrogenic pump activity was elevated in SHR over WKY (i.e., -12 mV vs -8 mV, respectively) indicating that the ratio of Na^+ extrusion to K^+ influx is increased in SHR. It was hypothesized that at 37°C the Em was maintained normal in SHR due to two opposing defects. An elevated electrogenic transport of Na^+ out of the VSMCs (favoring hyperpolarization) counterbalanced a less negative membrane potential that was present under conditions where the Na^+/K^+ pump was inactive. It was suggested that during NE stimulation the increased membrane permeability would be such that the electrogenic pump would no longer play an important role in maintaining the Em, allowing the establishment of a less negative membrane potential. In turn, such an alteration would increase the degree of contraction achieved by the artery.

Other studies by Abel and Hermsmeyer (1981) were undertaken to determine the importance of humoral factors in determining an increased sensitivity and decreased Em (at 16°C) in the tail arteries of SHR. The tail arteries from 2 week old SHR and WKY were transplanted into the anterior eye chamber of 12 to 14 week old SHR and WKY host animals. The eye chamber of rats is immuno-unreactive, allowing the transplants to remain viable for a 7 week period. After 7 weeks, the transplanted tail arteries were removed from the eye chamber. Microelectrode measurements of the Em at 16°C and pharmacological tension studies indicated that the

transplanted tail arteries took up the Em and NE sensitivity characteristics present in the tail arteries of the host animals. Both SHR and WKY transplants (in the SHR hosts) had Em and EC_{50} values that were comparable to those present in the tail artery of the SHR host and lower than similar transplants in the WKY hosts.

When the eye chamber of the host animal was sympathetically denervated by performing a superior cervical ganglionectomy prior to transplant, the host eye chamber was unable to confer the host animals Em (at 16°C) and EC_{50} characteristics to the transplant. The authors suggested that a trophic effect of the sympathetic nervous system is responsible for the altered membrane properties of VSMCs in SHR.

5. (b) Conclusion

Most analyses of electrolyte content of a variety of different arteries indicate that SHR arteries have either a normal electrolyte content or a slightly decreased content of Na^+. Surprisingly, a near normal electrolyte content is maintained in spite of the fact that the passive membrane permeability to Na^+, K^+ and a variety of other ions are increased in the VSMCs of SHR. It appears that Na^+ and K^+ levels are maintained at near normal levels in SHR by a hyperactive Na^+/K^+ pump which counterbalances the increased Na^+ and K^+ fluxes. Evidence supporting the presence of a hyperactive Na^+/K^+ pump in the VSMCs of SHR comes from a variety of sources. 1) Under certain experimental protocols an active Na^+ efflux from VSMCs can be demonstrated. Such an efflux is greater in SHR over normotensive controls. 2) Ouabain-sensitive Rb^{86} uptake (thought to represent Na^+/K^+ pump activity) is increased in arteries taken from SHR. 3) When arteries are

incubated in K^+-free media, the Na^+/K^+ pump is inhibited and Na^+ accumulates within the VSMCs. The reintroduction of K^+ reactivates the pump which actively transports Na^+ out of the VSMCs. A variety of researchers have hypothesized that the degree of hyperpolarization and/or relaxation observed in an artery after the reintroduction of K^+ is related to the activity of the Na^+/K^+ pump. In this regard, the degree of relaxation and hyperpolarization observed upon the reintroduction of K^+ is greater in SHR than normotensive controls, suggesting the presence of a hyperreactive Na^+/K^+ pump in SHR. 4) The degree of membrane potential supported by the Na^+/K^+ pump is greater in SHR over WKY, again suggesting the presence of a hyperactive pump.

Hermsmeyer and his colleagues (Hermsmeyer, 1976 a, b; Abel and Hermsmeyer, 1981) have shown that the tail arteries of SHR exhibit a decreased membrane potential at 16°C under conditions where the Na^+/K^+ is inactive. At 37°C this is counterbalanced by an increased active Na^+ efflux favoring hyperpolarization which results in the presence of an unaltered membrane potential when the tail arteries of SHR and WKY are compared. During NE contraction, the increases in membrane permeability are such that the Na^+/K^+ pump is no longer an important determinant of the membrane potential Thus, the tail arteries of SHR achieve a greater degree of depolarization during NE stimulation which in theory should increase contraction. Abel and Hermsmeyer (1981) suggest that the membrane defects observed in the tail arteries of SHR are conferred on the VSMCs through the sympathetic nervous system of SHR.

Hermsmeyer (1976 a) has hypothesized that vascular hyper-

reactivity to NE observed in many vascular beds within SHR could, in fact, be due to an increased ability of NE to depolarize (and contract) the VSMCs as opposed to structural alterations. However, it should be pointed out that this view can be contested. Ironically, the tail artery of SHR, although being more sensitive to NE contraction (i.e., exhibiting a lower threshold and ED_{50} values), exhibits a decreased maximal contractility in response to NE in some experiments (Webb and Vanhoutte, 1981 a; Webb et al., 1981 b). Interestingly, the tail artery of SHR in an established phase of hypertension has been shown by Cox (1981) not to develop a thickened vascular wall. Hence, at least within this case, it can be argued that the absence of reactivity is associated with the absence of structural alterations and it is possible that it is the latter alteration that is a primary determinant of vessel reactivity.

Recent work (Dr. D. Cheung, unpublished observations) indicates that the tail arteries of SHR have VSMCs with a decreased membrane potential and a decreased Na^+/K^+-ATPase activity. These alterations are consistent with the presence of an underactive Na^+/K^+ pump. Such an alteration is in fact more conducive to the maintenance of hypertension, since basal as well as active SMC tone would be increased by virtue of an increased depolarization. Furthermore, if the efflux of Ca^{+2} is linked to the influx of Na^+ in SMCs, as suggested by Blaustein (1977), a decrease in the Na^+ gradient would inhibit the transport of Ca^{+2} from the cell which would increase VSMC tone in SHR.

6. Alterations in the cable properties of VSMCs of SHR

The possibility that the spread of depolarization between VSMCs

is increased in arteries from SHR during NE contraction has not been extensively studied. Kuriyama and Suzuki (1978) provided evidence that the space constant (the distance at which an evoked electrotonic membrane potential decays to 1/e) and the time constant (the time taken for an electrotonic potential to decay to 1/e) were not different when the pulmonary artery and the portal vein of adult SHR and WKY were compared.

Studies by Holloway and Bohr (1973) have shown that the femoral artery of SHR (10 rats) in as established phase of hypertension exhibit spontaneous rythmic contractions which are virtually absent in the arteries of WKY (present in 2 out of 10 rats). The increased presence of spontaneous rythmic contractions in the femoral artery has also been shown to be present in DOCA/salt and 1 and 2 kidney Goldblatt forms of hypertension in rats (Bandick and Sparks, 1970; Holloway and Bohr, 1973). In SHR and the above hypertensive models the spontaneous contractions can be blocked by Ca^{+2} antagonists, suggesting that such phasic activity may be produced by spontaneous Ca^{+2}-evoked action potentials (Holloway and Bohr, 1973). It is of interest to note that the treatment of normally electrically quiescent carotid and tracheal tissue with K^{+} conductance blockers produces spontaneous contractile activity (Mekata, 1971; Kroegher and Stephens, 1975; Kannan and Daniel, 1978). In the above experiments, the occurrence of spontaneous activity has been shown to be associated with an increase in the membrane resisitance (Mekata, 1971; Kroegher and Stephens, 1975), and gap junction formation between smooth muscles (Kannan and Daniel, 1978). In experiments performed by Kroegher and Stephens (1975) an increase in the

length constant, a measure of the electrical conducting property of the tissue, was observed.

Although it still remains to be proven, if hypertension in SHR is associated with increases in VSMC gap junctions and increases in length constant, these alterations could produce a situation where a contractile impulse is propagated over a greater number of VSMCs. Since VSMCs are helically oriented around the long axis of vessels, such an alteration could promote a greater vessel contraction, in response to nerve stimulation.

7. (a) Alterations in VSMC Ca^{+2} handling in SHR

Alterations in the mechanisms that govern the levels of unbound intracellular Ca^{+2} can play an important role in determining the level of SMC tone. In this regard, an increased SMC permeability to Ca^{+2}, a decrease in the rate of Ca^{+2} efflux from the SMC, or a decreased ability of the SMC to sequester Ca^{+2} internally could (i) elevate basal level of SMC tone, (ii) have the potential to increase maximal tension responses during contraction, and (iii) decrease the ability of the artery to relax once the contractile stimulus has been removed.

Ca^{+2} binding to the plasma membrane also exerts an important membrane stabilization effect. Qualitative or quantitative alterations in such binding could effect permeability of the membrane to various electrolytes, which could alter VSMC function.

In studies performed by Webb and Bhalla (1976), differential ultracentrifugation was used to separate out a microsomal fraction (plasma membrane and sarcoplasmic reticulum) from the aorta of SHR in

established hypertension and WKY. Subsequently, this fraction was suspended in a manner that would form vesicles. The total accumulation of Ca^{+2} (within inside-out membrane vesicles) in the presence of ATP at varying concentrations of external Ca^{+2} was measured; however, no attempt was made to determine ATP-independent Ca^{+2} binding. It was observed that the amount of Ca^{+2} accumulated by the vesicles was less in SHR than in WKY. On the other hand, Ca^{+2}-dependant ATPase activity of the microsomal fraction, thought to represent the ATP cleaving portion of the Ca^{+2} pump, was increased in SHR. It was concluded that microsomal vesicles were either less leaky to Ca^{+2} and/or had a reduced active Ca^{+2} pump activity. It was suggested that the increased Ca^{+2}-dependant ATPase activity observed could be an attempt by the cell to compensate for the above defects.

Moore et al (1975) also observed a decrease in active Ca^{+2} uptake in aortic microsomal fractions. In this instance SHR were compared to Sprague-Dawley normotensive controls. The ATP-independant Ca^{+2} (Ca^{+2} binding) uptake by the microsomal vesicles was shown to be negligible. A sample of the preparation was subjected to sucrose density gradient separation; it was observed that the subfraction exhibiting maximal active Ca^{+2} uptake also exhibited maximal NADH oxidase activity, an enzyme thought to be present in the endoplasmic reticulum. On the other hand, microsomal subfractions demonstrating maximal 5' nucleotidase activity had a very low Ca^{+2} accumulating ability. In view of this, it was suggested that the active Ca^{+2} pump, whose activity is decreased in SHR, is present in the endoplasmic reticulum, and a decrease in pump activity in SHR may reflect a

decreased ability of the endoplasmic reticulum to sequester Ca^{+2}. Such an alteration could lead to an elevation in cellular Ca^{+2}.

Studies performed by Kwan and his colleagues (Kwan et al., 1979; Kwan and Daniel, 1981 a) are critical of previous findings that suggest that the Ca^{+2} pump is located within the sarcoplasmic reticulum. As discussed by Kwan and Daniel (1981 a) (i) the improper trimming of arterial tissue to remove non-muscle components, (ii) the use of non-specific and insufficient plasma membrane and endoplasmic reticulum enzyme markers and (iii) the ommision of sucrose density gradient centrifugation to separate plasma membrane from sarcoplasmic reticulum can lead to misleading results. Through the use of meticulous separation of non-arterial tissues from the mesenteric arteries in combination with differential centrifugation and sucrose density gradient separation, a plasma membrane fraction was isolated from mesenteric arteries by Kwan et al (1979). This fraction was characterized by the use of five enzyme markers and was estimated to be at least 75% pure plasma membrane (Kwan et al., 1979; Kwan and Daniel, 1981 a). Vesicles made from the fraction were found to exhibit a very high ability to accumulate Ca^{+2} in the presence of ATP. Kwan and his colleagues (Kwan et al., 1979; Kwan and Daniel, 1981 a) suggested that the active accumulation of Ca^{+2} by the microsomal fraction previously thought to be representative of the SMC sarcoplasmic reticulum was, in fact, due to contamination by plasma membrane. Consistent with this hypothesis, other fractions obtained from the sucrose gradient exhibit an ATP-dependant Ca^{+2} accumulating ability that is proportional to the activity of plasma membrane markers (5'-nucleotidase and alkaline

phosphatase) within the fraction (Kwan et al., 1979; Kwan et al., 1980 c).

The new plasma membrane isolation technique was subsequently applied to mesenteric arteries obtained from SHR and WKY (Kwan et al., 1980 a, c; Kwan and Daniel, 1981 a, b). Not only was a reduced ATP-dependant Ca^{+2} accumulating ability observed in SHR in a developing and established phase of hypertension, but also in SHR prior to hypertension development (Kwan et al., 1980 a). It was suggested that if the vesicles formed from the plasma membranes of SHR and WKY had an equal 'outside in' - 'inside out' distribution, then the reduced Ca^{+2} accumulating ability of the SHR plasma membrane could be of functional significance in maintaining an elevated level of Ca^{+2} within VSMCs. Furthermore, since such alterations were present prior to high blood pressure development they could be of etiological importance in establishing hypertension in SHR (Kwan et al., 1980 a).

Work by Kwan and his colleagues (Kwan et al., 1980 a, b, c) suggests that the binding of Ca^{+2} to the plasma membrane of mesenteric arteries is unaltered in SHR over WKY. It was shown repeatedly that arterial vesicle preparations from mesenteric arteries exhibited similar degrees of Ca^{+2} accumulation in the absence of ATP. In other experiments (Kwan et al., 1980 c) Ca^{+2} binding to the membrane of mesenteric arteries was studied in the presence of ATP and the Ca^{+2} ionophores X5374 and A23187. Ca^{+2} accumulation by membrane vesicles was unaltered in the presence of the two ionophores suggesting the the ability of the plasma membrane to bind Ca^{+2} in the presence of ATP is similar in both SHR and WKY. Unlike arteries, plasma membrane vesicles

prepared from veins of SHR were found to exhibit an elevated ATP-independant Ca^{+2} accumulation when compared to WKY (Kwan and Daniel, 1981 b).

To determine if a defect in the active Ca^{+2} pump (such as that observed in SHR) was important in maintaining other forms of hypertension, 1 and 2 kidney Goldblatt and DOCA/salt hypertensive rats were studied (Kwan et al., 1980 a, b, c). A decreased ability of plasma membrane vesicles to actively accumulate Ca^{+2} was observed in 1 kidney and 2 kidney Goldblatt hypertensive rats and in rats with DOCA/salt hypertension (Kwan et al., 1980 a, b). In 2 kidney Goldblatt hypertensive rats, the level of decreased Ca^{+2} pump activity was proportional to the level of blood pressure present (Kwan et al., 1980 b). In DOCA/salt hypertensive rats, the withdrawal of treatment produced a situation where 40% of the treated animals developed normotension while the balance maintained hypertension. It was observed that membrane vesicles obtained from the mesenteric arteries of the latter, but not the former, group demonstrated a reduced ability to actively accumulate Ca^{+2} (Kwan et al., 1980 a). The above findings suggest that a defect in the VSMC Ca^{+2} pump may be of common etiological importance in in the maintainence of various forms of hypertension.

Various researchers have noted that the presence of Ca^{+2} alters non-Ca^{+2} ionic permeability as well as functions attributed to the Na^{+}/K^{+} pump. Furthermore, the effects of Ca^{+2} appear to be different in the arteries of SHR as opposed to WKY. Jones (1974 a) noted that in the presence of Ca^{+2} (2.5 mM), the efflux of K^{42} from the preloaded aorta of SHR and WKY was very slow. The removal of Ca^{+2} from the incubation

media accelerated the initial efflux of K^{42} from SHR aortas to a greater degree than those of WKY. It was suggested the Ca^{+2} exerts a membrane stabilization effect and the removal of Ca^{+2} destabilizes the membrane of SHR aortas to a greater degree than WKY.

Ca^{+2} also has the ability to alter tension responses produced by K^{+} contraction of arteries. For example, Holloway and Bohr (1973) observed that the contractile response of femoral arteries produced by increasing external K^{+} from 4.7 to 40 mM was highly susceptible to the presence of Ca^{+2}. Femoral arteries from SHR exhibited a biphasic response. When compared to Sprague-Dawley controls, low levels of Ca^{+2} (1.6 to 4.1 mM) modestly inhibited K^{+} contraction in SHR arteries to a greater extent than controls; high levels of Ca^{+2} (4.1 to 11.6 mM) inhibited controls to a greater extent than SHR. Webb and Bohr (1980) obtained comparable results in the tail arteries of SHR and WKY. Ca^{+2} levels greater than 4 mM produced a greater inhibition of K^{+} contraction in WKY than in SHR. Within this study, it was shown that the presence of ouabain reversed the effects of Ca^{+2}. High levels of Ca^{+2} no longer inhibited but rather potentiated K^{+} contraction. Furthermore the magnitude of potentiation present was greater in SHR than in WKY.

The physiological significance of the presence of an altered Ca^{+2}-influenced inhibition of K^{+} contraction within SHR is unknown. However, such studies as well as others which, for example, indicate that SHR but not WKY arteries contract in the presence of La^{+3} (2.5 mM), Sr^{+2} (5 mM), ions which can block Ca^{+2} channels (Holloway and Bohr, 1973) suggest that a membrane defect in the handling of Ca^{+2} exists.

7. (b) Conclusion

Membrane vesicles prepared from microsomal or plasma membrane fractions exhibit a decreased active Ca^{+2} efflux, while Ca^{+2} binding and permeability are unaltered. A decrease in the active Ca^{+2} transport, regardless of whether it was present in the plasma membrane and/or sarcoplasmic reticulum, would increase the passive and active levels of intracellular Ca^{+2}. Such an alteration could i) increase basal tone, ii) increase contractile responses or iii) decrease relaxation responses, all of which could increase vascular resistance and help maintain hypertension. Work by Kwan et al (1980 a) has shown that a decrease in the Ca^{+2} pump activity of VSMCs is present before hypertension development, and also occurs in a variety of other forms of hypertension. This suggests that such an alteration may be of primary importance in establishing hypertension in SHR and perhaps other forms of hypertension.

Various studies also suggest the presence of a membrane defect in SHR that involves Ca^{+2} handling. For example, K^+ contraction is inhibited by Ca^{+2} to a lesser degree in SHR than WKY. In part, this phenomenon is dependant on Na^+/K^+ pump activity. SHR arteries also contract in the presence of La^{+3} and Sr^{+2}, while WKY arteries do not. Furthermore, Ca^{+2} removal increases K^+ permeability in SHR arteries to a greater degree than those of normotensive rats. The physiological significance of these latter alterations is unknown. The above experiments do, however, suggest that alterations in Ca^{+2} handling are present in the VSMCs of SHR.

8. Alterations in adenylate cyclase, cAMP and protein kinases in SHR

8. (a) Background

Increases in cellular 3',5' cAMP have the potential to relax VSMCs. There is a vast amount of literature available that has implicated cAMP with SMC relaxation. In arterial tissues, beta receptor- stimulated relaxation, as well as relaxation produced by isoflurane, halothane, dipyridamole, PGE_1, PGE_2, diazoxide and hydralazine, has in different arteries been associated with a rise in cellular cAMP (Kramer and Hardman, 1980). In the case of beta receptor stimulation by isoproterenol, the increases in cAMP are dose dependent and are inhibited by propranolol, a beta receptor antagonist (Triner et al., 1972). In the rabbit anterior mesenteric portal vein, there is a direct correlation between the increase in tissue cAMP and the percentage relaxation of NE contractions produced by the phosphodiesterase inhibitors RA 233, SC 2964 and papaverine (Collins and Sutter, 1975). Many other studies have indicated that the external application of dibutyryl cAMP, an analogue of 3', 5' cAMP, is capable of relaxing arterial tissues previously contracted with a variety of agents (Kramer and Hardman, 1980). Finally, cardiac membrane (Bhalla et al, 1980; Robberecht et al., 1981) and broken cell vascular muscle (Amer et al., 1974; Klenerova et al., 1975; Murthy et al., 1976) preparations exhibit an isoproterenol (beta agonist) stimulated, propranolol (beta antagonist) blocked increase in adenylate cylcase activity. These latter results suggest a positive link between beta receptor stimulation and the activity of the enzyme responsible for cAMP formation.

This area of study is not without controversy. In many vascular tissues there is a discrepancy between the degree of cAMP elevation and the degree of vascular relaxation. For example, in rabbit portal veins,

the phosphodiesterase inhibitor SC 2964 produces over a 7 fold increase in tissue cAMP levels and is capable of only modest relaxation of NE contraction. On the other hand, in the same tissue RA 233, another phosphodiesterase inhibitor, produces only a modest increase in cellular cAMP, yet can fully inhibit a maximal NE contractile response (Collins and Sutter, 1975). In some instances contraction is associated with an increase in arterial levels of cAMP (Kramer and Hardman, 1980). In the case of histamine contraction of bovine mesenteric arteries, the role of cAMP is particularly confusing. In this tissue, initially there is a small decrease in arterial cAMP 15 seconds after the application of histamine, followed by an increase in tension and cAMP. At maximal tension the arterial strips have dramatically elevated levels of cAMP. Yet in spite of this, the external application of cAMP to the tissue produces a relaxation response (Andersson, 1973).

In conclusion, although there is a vast amount of evidence which suggests that, particularly in the case of beta receptor stimulation, elevations in arterial cAMP levels are implicated in the relaxation of VSMCs, an increase in arterial cAMP levels does not always dictate a relaxation response.

A model of the membrane oriented events that are thought to occur during hormone stimulation and inhibition of adenylate cyclase have been outlined by Rodbell (1980). In the inactivated form, the receptor is thought to be bound to a nucleotide regulatory protein. In this complex, the receptor inhibits GTP binding to the regulatory protein. Hormone binding to the receptor triggers the release of inhibitory constraints on the regulatory protein, allowing its

interaction with GTP. This complex is thought to be capable of either stimulating or inhibiting the basal levels of 3', 5' cAMP formation from Mg^{+2} ATP by the catalytic unit. It was hypothesized that the type of action produced was governed by the type of receptor-regulatory complex formed. For example, beta receptors could be coupled to a stimulatory regulatory protein, while, on the other hand, hormones that decrease cellular levels of cAMP could act through inhibitory regulatory proteins. Both could share a common catalytic unit.

In addition to being controlled by the activation and deactivation of adenylate cyclase, cellular levels of cAMP are also controlled by phosphodiesterases. In VSMCs, 3', 5' cAMP is thought to be broken down to 5' AMP by two phosphodiesterase enzymes. One of these is characterized by a very low Km value and has a high affinity and specificity for cAMP. The other phosphodiesterase can breakdown both cAMP and cGMP, has a higher Km value (for cAMP breakdown), and exhibits an increased activity in the presence of Ca^{+2}-binding activator protein (Kramer and Hardman, 1980).

The actions of cAMP are exerted through the activation of protein kinases, which in the presence of ATP are capable of phosphorylating various substrates. In this regard, numerous researchers have demonstrated the presence of cAMP and cGMP-activated protein kinases in vascular smooth muscle (Sands et al., 1976; Allen, 1977; Kramer and Hardman, 1980). However, the subsequent action of phosphorylated substrates and the mechanisms through which they achieve arterial relaxation are not well understood.

Experiments have indicated that microsomal preparations from

canine and rat aortas (Bhalla et al., 1976; Allen, 1977), and rat mesenteric arteries (Kattenberg, 1981) are capable of incorporating P^{32} from ATP^{32} in the presence of cAMP and/or protein kinase. In some (Baudouin-Legros and Meyers, 1973; Bhalla et al., 1976; Webb and Bhalla, 1976; Fitzpatrick and Szentwanyi, 1977; Kattenberg, 1981), but not all instances (Allen, 1977), microsomal preparations from aorta have also exhibited an increase in Ca^{+2} binding and/or ATP-dependent Ca^{+2} accumulation when protein kinases and cAMP are added. In view of this, cAMP could effect relaxation by (i) stimulating the intracellular binding of Ca^{+2}, (ii) increasing the active transport of Ca^{+2} out of VSMCs or (iii) elevating the active sequestration of Ca^{+2} within the VSM. Alternatively, the presence of cAMP has been shown to stimulate the phosphorylation of myosin light chain kinase (Adelstein et al., 1978). Such action deactivates the kinase and prevents it from phosphorylating myosin, an action thought to be a prerequisite for SMC contraction (Hartshorne, 1980).

In view of the potential importance of cAMP in deterimining SMC tone, various researchers have attempted to determine whether hypertension in SHR was associated with a defect in VSMC mechanisms involved in the synthesis, breakdown and action of cAMP. In this regard, virtually all the work performed in this area has used the aorta as the test blood vessel.

8. (b) Alterations in VSMC cAMP levels in SHR

Studies involving the use of radioimmunological protein kinase binding assays to measure the blood vessel levels of cAMP predominantly indicate that the level of this nucleotide is reduced in a variety of

blood vessels freshly excised from SHR (Amer et al., 1974; Ramanathan and Shibata, 1974; Amer, 1979). Studies performed by Amer and his colleagues (Amer et al., 1974; Amer, 1979) indicate that in SHR with established hypertension, the levels of aortic cAMP (per wet tissue weight) are twice that found in Wistar rats (NCR). In studies performed by Sands et al (1976), it was observed that young adult SHR obtained from different distributors had differing degrees of hypertension. When SHR with modestly elevated blood pressure (150-160 mm Hg) and high blood pressure (> 180 mm Hg) were compared to WKY (120-130 mm Hg), the aortic levels of cAMP were decreased in proportion to the level of blood pressure present.

In studies performed by Ramanathan and Shibata (1974) the levels of cAMP present in the aorta, renal artery and portal vein were reduced in both prehypertensive (4 week) and established hypertensive (3 month) SHR when compared to either NCR or WKY controls. The presence of a reduced level of cAMP in the arteries of prehypertensive SHR suggested that such alterations are not secondary to high blood pressure development. This latter point is further confirmed by the fact that cultured aortic SMCs from SHR exhibit dramatically decreased levels of cAMP when compared to similar cultures initiated from WKY tissues (Sands et al., 1976). These latter results suggest that the mechanisms contributing to the decreased levels of cAMP observed in SHR could be of genetic origin.

8. (c) Alterations in VSMC adenylate cyclase

Various researchers have attempted to determine whether arterial homogenates of SHR have reduced basal and/or hormone stimulated

adenylate cyclase activity. Ramanathan and Shibata (1974) observed that the basal adenylate cyclase activity in SHR with established hypertension was elevated over that present in either WKY or NCR. However, most studies involving both young adult and established hypertensive SHR indicate that both the specific and total tissue activity of this enzyme are unaltered when SHR are compared to either WKY or NCR. Such studies have been carried out using the aorta (Amer et al., 1974; Triner et al., 1975; Amer, 1979; Bhalla and Sharma, 1982), mesenteric (Amer et al., 1974) and tail arteries (Triner et al., 1975) as test tissues.

Experiments where the catalytic subunit of adenylate cyclase has been stimulated by NaF have provided inconclusive results indicating a decreased (Amer et al., 1974; Amer, 1979; Bhalla and Sharma, 1982), increased (Triner et al., 1975) or unaltered (Ramanathan and Shibata, 1974) activity when the aortas of SHR were compared to WKY or NCR. In studies involving the tail artery (Bhalla and Sharma, 1982) NaF was found to produce a decreased stimulation of adenylate cyclase in SHR over WKY.

Most studies performed on mesenteric artery and aortic homogenates indicate that the ability of isoproterenol (a beta receptor agonist) to stimulate adenylate cyclase is reduced in SHR over WKY or NCR (Amer et al., 1974; Triner et al., 1975; Amer, 1979). However, in a study performed by Bhalla and Sharma (1982) isoproterenol, in the presence of GTP, stimulated adenylate cyclase equally in aortic homogenates obtained from SHR and WKY, while the same agonist exhibited a reduced ability to stimulate adenylate cyclase in the tail arteries of

SHR. Other studies involving glucagon, epinephrine (Ramanathan and Shibata, 1974) and GTP (Bhalla and Sharma, 1982) stimulation of aortic homogenates have failed to show any difference in the level of hormone stimulated adenylate cyclase activity when SHR were compared to WKY.

8. (d) Alterations in VSMC phosphodiesterase activity

Other studies have looked at the possibility that cAMP dependent phosphodiesterase activity may be increased in SHR. Using crude homogenates prepared from the aorta of developing hypertensive SHR, Triner et al (1975) observed no difference in the specific phosphodiesterase activity (activity/mg protein) when SHR were compared to WKY. Ramanathan and Shibata (1974), on the other hand, found that the specific activity of phosphodiesterase present in aorta, portal vein and renal artery homogenates was reduced in established hypertensive SHR over WKY and NCR.

In experiments performed by Amer and his colleagues (Amer et al., 1974; Amer, 1979), it was observed that the maximal aortic phosphodiesterase activity (per tissue weight) was increased in SHR over NCR. Using DEAE cellulose chromatography the aortic phosphodiesterases were separated into low and high Km forms of the enzyme (Amer, 1979). It was seen that in SHR a greater proportion of the total activity was contributed by the low Km form of the enzyme. This latter form of the enzyme is thought to play a particulary important physiological role in the breakdown of cAMP, since it is cAMP specific and is adapted to function at low cAMP levels (Sands et al., 1976). In view of this, Amer (1979) suggested that the low cAMP levels observed in aorta could be the result of an increase in the activity levels of low Km

phosphodiesterase.

Donnelly (1978) using DEAE cellulose chromatography separated out three phosphodiesterases from the aorta of adult SHR and WKY. The individual enzymes were found to hydrolyze specifically cAMP, cGMP and both nucleotides. Contrary to the findings of Amer (1979), the phosphodiesterase activity levels were not different between SHR and WKY. Within this study, a non protein phosphodiesterase activator was also isolated from the aorta of SHR and WKY. The quantitative amounts of this activator as well as its ability to stimulate phosphodiesterase were not altered between SHR and WKY. It was concluded that the decreased levels of cAMP present in the aorta was not due to altered phosphodiesterase activity.

Kramer and Hardman (1980) have hypothesized that phosphodiesterase activity may be under altered hormonal control in SHR. In this regard, insulin and certain plasma subfractions are capable of increasing phosphodiesterase activity, while thyroid hormones and aldosterone decrease activity (Wells and Hardman, 1977). However, it remains to be proven as to whether such mechanisms alter phosphodiesterase activity differently in the VSMCs of SHR as apposed to WKY.

8. (e) <u>Alterations in VSMC protein kinase in SHR</u>

Other research has explored the possibility that a defect in vascular cAMP activated protein kinase could exist in SHR. A qualitative or quantitative change in this enzyme could decrease its ability to be activated by cAMP and/or its ability to phosphorylate substrates. Such an alteration could inhibit VSMC relaxation and

potentially increase the vascular resistance.

Sands et al (1976) studied SHR with high (> 180 mm Hg) and moderate (150-160 mm Hg) hypertension. Homogenates of aortic segments from the former group exhibited an elevated basal protein kinase activity when the phosphorylation of endogenous substrate and added histone were measured in SHR. However, in this group of experiments, the protein kinase activity could not be elevated by the addition of cAMP to the homogenates. In SHR with moderate hypertension (150-160 mm Hg) both the basal and the cAMP dependent activation of protein kinase activity in aortic segments was similar in SHR and WKY. Homogenates of cultured aortic SMCs obtained from SHR, exhibited an increased basal protein kinase activity, while the cAMP dependent activation of this enzyme was similar when cultures from SHR and WKY were compared.

Coquil and Hamet (1980) failed to observe any alteration in the basal protein kinase activity when aortic homogenates from 5 week old prehypertensive and 18 week old adult, SHR were compared to WKY. In this study the cAMP activation of protein kinase was reduced in adult but not young SHR. On the other hand, Bhalla et al (1976) found a decrease in both the basal and cAMP dependent protein kinase activity when extracts obtained from adult SHR were compared to WKY.

Gupta et al (1982) also observed a decrease in the cAMP dependent specific protein kinase activity present in the aortic cytosol fraction when SHR with established hypertension were compared to WKY. When aortic homogenates were subjected to DEAE cellulose chromatography, two isozymes of protein kinase were eluted. Both isozymes had similar Km values in response to alterations in ATP, Mg^{+2} and cAMP. However,

Vmax (maximal activity, P^{32} transferred to histone per min per mg protein) was elevated in the isozyme first eluted from the column (ISOZ 1) over the following isozyme (ISOZ 2). It was found that the reduced cAMP dependent protein kinase activity of SHR aortas was due to 1) a slightly reduced Vmax in both ISOZ 1 and 2 and 2) a lower proportion of highly active ISOZ 1 to ISOZ 2.

8. (f) Conclusion

Studies performed on SHR with developing and established hypertension predominantly indicate that the basal levels of arterial cAMP are reduced in SHR over normotensive controls. The levels of aortic cAMP appear to be inversely related to the severity of hypertension present in adult SHR. Furthermore, decreased levels of cAMP have also been observed in arteries sampled from prehypertensive SHR and in cultured aortic SMCs from SHR. These latter findings suggest that if decreases in cAMP are in fact important in increasing SMC tone and that the mechanisms producing such alterations are likely of genetic origin and not a secondary adaptation to high blood pressure. Furthermore, since these alterations occur near the onset of hypertension in SHR, they could play an etiological role in elevating blood pressure.

Attempts to elucidate the mechanisms responsible for the decreased levels of cAMP that have been observed in SHR have produced confusing results. Most studies fail to observe a decrease in the basal (unstimulated) adenylate cyclase activity between SHR and WKY. Stimulation of the catalytic subunit of adenylate cyclase with NaF has produced results indicating an increased, decreased or unaltered

adenylate cyclase activity in SHR. Studies involving glucagon, epinephrine and GTP stimulation of aortic homogenates have also indicated similar activation in SHR as WKY. Most studies do, however, indicate that isoproterenol, a beta receptor agonist, exhibits a reduced ability to activate adenylate cyclase in aortic and mesenteric artery cell homogenates of SHR when compared to WKY. Such an alteration could be due to reduction in beta receptor density, as observed in SHR aortic membrane preparations (Limas and Limas, 1979) (discussed in the previous section). Alternatively, some as yet undiscovered alteration in the GTP binding regulatory protein could also exist. Although a defect in the mechanisms involved in the beta receptor mediated increases of arterial cAMP could explain why SHR arteries exhibit a decrease in the relaxation response to isoproterenol, it cannot adequately explain why basal levels of cAMP are decreased in SHR.

Other research has centered around the possibility that phosphodiesterase activity is increased in SHR arteries. However, experiments involving aortic homogenates obtained from SHR have demonstrated increased, decreased and unaltered phosphodiesterase activity when compared to controls.

Most, but not all, studies involving the measurement of cAMP dependent protein kinases indicate that aortas obtained from SHR exhibit a reduced ability to phosphorylate added histone substrate. This could be due to a decrease in Vmax activity and the proportional quantities of highly active ISOZ 1 within the aorta of SHR.

H. Alteration in the Structure of Blood Vessels in SHR

1. Background

Structural alterations can play an important role in increasing the vascular resistance to blood flow. The lumen diameter of blood vessels could be reduced in hypertension. Such an alteration could be produced by a number of mechanisms. The blood vessel wall could be thickened. If the vessel wall thickened inward towards the lumen, such encroachment could elevate vascular resistance even under conditions where the vasculature is relaxed. Alternatively, blood vessel wall thickening could occur towards the adventitia, leaving the lumen unaltered under relaxed conditions. In this instance, if the volume of the arterial media is conserved (as has been shown to be the case in mesenteric arteries, Lee et al., 1983 c), equal degrees of VSMC shortening would occlude the lumen of the thick walled vessel to a greater degree than that of a thin walled vessel under relaxed conditions. This feature of wall thickening is schematically illustrated in Fig. 38, and will be discussed later. Structural alterations in hypertension could also reduce the distensibility of the blood vessel wall. Under equal degrees of transmural pressure, a blood vessel with a reduced distensibility would maintain a functionally smaller lumen. The development of hypertension could also be associated with a reduction in the number of blood vessels within various vascular beds, thus reducing the number of pathways through which blood can flow.

2. (a) Alterations in the lumen diameter (LD) of blood vessels in SHR

Perfusion studies involving the entire systemic vasculature (Folkow et al., 1969), as well as the hindlimb vasculature (Folkow et al., 1970 a, b; Shomig et al., 1978), indicate that these vascular beds exhibit an elevated vascular resistance in SHR over WKY or NCR when they

are perfused with physiological saline at a constant flow, under relaxed conditions. Such an alteration takes place at an early age. Perfusion studies performed by Lais and Brody (1978) have shown that the hindlimb vascular resistance is elevated in 3 week old prehypertensive SHR. The increase in vascular resistance under maximally relaxed conditions is most easily explained by a decrease in the mean LD and/or a reduction in the number of blood vessels within a vascular bed.

The LD is not decreased in all the vascular beds of SHR. Studies performed by Lee et al (1983 a) and Hausler and Finch (1972) indicate that the mesenteric vascular resistance is unaltered between SHR and WKY when the isolated vascular bed is perfused with Krebs solution at moderate or low constant flow rates. In the case of Lee et al (1983 a), this finding was substantiated by the fact that morphometric analysis of the superior mesenteric artery, as well as the large and small mesenteric arteries sampled in areas prior to their entry into the intestine (premucosal) indicated that the lumen cross-sectional areas (maximally relaxed and compensated for sectioning angle) were similar in SHR and WKY.

Other studies involving the measurement of LD of SHR mesenteric arteries have produced mixed results. Mulvany and his colleagues used a myograph to stretch the lumen at a tension that would equate to a 100 mm Hg arterial pressure (LD_{100}). In similar aged SHR, the LD_{100} of premucosal mesenteric arteries was found to be either unaltered (Mulvany and Halpern, 1977; Warshaw et al., 1979; Warshaw et al., 1980) or reduced (Mulvany et al., 1978; Aalkjaer and Mulvaney, 1979). Hertel et al (1978) observed the mesenteric arteries using in vivo microscopy and

found that the small mesenteric arterioles of adult SHR exhibited a larger LD than those of WKY. In the latter study, it was suggested that the LD was expanded in SHR as a result of the presence of high blood pressure.

Every perfusion study performed on the isolated kidneys of SHR has indicated that the kidneys of both young (Collis et al., 1980) and adult (Folkow et al., 1971 a; Collis and Vanhoutte, 1977; Nagaoka et al., 1978; Fonteles and Jeske, 1980) SHR and WKY exhibit similar renal vascular resistance when they are perfused with physiological saline under relaxed conditions. However, in other experiments where the isolated renal vasculature of adult SHR was perfused with rat blood at constant flow rates above and below those present physiologically, adult SHR exhibited a higher renal vascular resistance than WKY (Tobian et al., 1975; Fink and Brody, 1979). In these instances it is impossible to ascertain whether the renal vasculature is relaxed. Even small degrees of vascular tone (produced by blood-born vasoconstrictor agents) when coupled to a thickened vascular wall could produce an elevation in vascular resistance. In one of the above studies, papavarine was used to relax the renal vascular bed (Tobian et al., 1975). SHR were still observed to maintain a higher renal vascular resistance over WKY; however, the absolute difference was small.

Morphometric studies performed by Pang and Scott (1981) on the relaxed main renal arteries of SHR, compensated for section angle, indicated that at 2 to 18 weeks of age SHR and WKY have similar LDs. Haeusler and Finch (1972) studied the vascular resistance of relaxed main renal artery segments. SHR with established hypertension and WKY

were found to display similar vascular resistances when perfused with Krebs at a constant flow.

In studies performed by Hsu et al (1982), a microsphere entrapment method was used to determine the LD of the preglomerular arterioles. It was found that under in vivo conditions SHR at 8 and 12 weeks of age had smaller afferent arteriolar diameters than WKY. It was suggested that the elevated renal vascular resistance observed in intact, anaesthetized SHR could be due in part to a reduction in arteriolar diameter. However, the infusion of hydralazine into SHR reduced the renal vascular resistance to normal, but did not alter the arteriolar diameter. This suggests that the reduced arteriolar diameters observed in the above study were not responsible for the elevation in vascular resistance that was observed.

Studies involving large elastic arteries, such as the aorta in SHR aged 4 to 18 weeks (Pang and Scott, 1981) and the femoral artery of SHR with established hypertension (Hansen et al., 1974), indicate that the LD is not different when SHR and WKY are compared. In vivo microscopy studies of the cremaster (Hutchins and Darnell, 1974; Bohlen et al., 1977; Bohlen and Lobach, 1978; Bohlen, 1979; Chen et al., 1981) and gracilis (Prewitt et al., 1982) muscles, and abdominal (Haack et al., 1980) vasculature of anaesthetized SHR and WKY indicated that the LDs of the intermediate to small resistance vessels (LD between 120 to 7 μm) is not decreased in SHR. In the above studies, the LD of the vascular beds was measured under conditions where the vasculatures were actively innervated (cremaster, gracilis, abdominal), denervated (cremaster, gracilis) and in the case of the gracilis muscle

vasculature, under conditions where the vascular bed was relaxed with sodium nitroprusside.

2. (b) Conclusion

Under maximally relaxed conditions, the total systemic vasculature of SHR exhibits an increase in vascular resistance when perfused with physiological saline under constant flow conditions. The increase in total systemic resistance could be produced by a decrease in LD or blood vessel numbers within the hindlimb vasculature of SHR. Studies performed on other vascular beds, such as the renal, mesenteric, cremaster and gracilis muscle, and abdominal vasculature, do not support the contention that the LD is decreased in relaxed blood vessels of SHR.

3. (a) Alterations in the vascular wall of SHR

Folkow and his colleagues (Folkow et al., 1970 a, b; 1973) suggested that wall thickening may play an important role in the maintenance of high blood pressure. It was hypothesized that such an alteration could exert two effects. A thickened vascular wall could encroach upon the lumen, blocking flow, and cause the artery to hyperreact to contraction. To test this hypothesis, Folkow and his colleagues, and subsequently other researchers, performed perfusion studies on isolated vascular beds. Initially, the vascular bed was relaxed with physiological saline and then contracted to maximal levels with NE or NE in combination with $BaCl_2$ plus vasopressin or serotonin. Such studies indicated that when the hindquarters (Folkow et al., 1970 a; Lais and Brody, 1978; Shomig et al., 1978), mesenteric vascular bed (Hausler and Finch, 1972) and renal vasculature (Folkow et al, 1971 a; Collis and Vanhoutte, 1977; Nagaoka et al., 1978) were contracted, dose

response curves for SHR diverge from those of WKY. The amplitude of response was higher in SHR than WKY or NCR at maximal contraction. This observation was used by Folkow et al (1973) and others as an indication that, on average, vascular wall thickening had taken place.

It should be pointed out that the above evidence provides indirect proof of the presence of a thickened vascular wall. Other researchers, such as Hermsmeyer (1976 a), have suggested that an increase in vascular reactivity in SHR, such as that observed by Folkow and his colleagues (Folkow et al., 1973), could be due to the presence of a decreased membrane potential during NE contraction of the VSMCs (discussed in a previous section). Daniel (1981) has suggested that a decrease in the active efflux of Ca^{+2} from VSMCs could also produce hypercontractility. Finally, maximal contractile responses of VSMCs to an agonist can be modified by the prior treatment of the tissue either with a vasodilant or by pre-contracting the tissue with other agents (Rapoport and Bevan, 1983). In view of this, other researchers have attempted to directly measure wall thickness by using histological or in vivo microscopy measurements.

Consistent with the results obtained from pharamacological studies, morphometric measurements on fixed, relaxed arteries and optic measurements of arterial tissue within live anaesthetized animals has indicated that during established hypertension the arterial blood vessel wall is thickened. However, such alterations are not uniformly distributed among all vascular beds. Furthermore, different sized arteries appear to be affected to different degrees.

Larger elastic arteries, such as the superior mesenteric (Lee et

al., 1983 a), main renal (Pang and Scott, 1981), femoral artery (Hansen et al., 1974), and in some cases the aorta (Pang and Scott, 1981), do not exhibit a thickened vascular wall in early established phases of hypertension. Vital microscopy studies performed on the cremaster (Bohlen and Lobach, 1978; Bohlen, 1979; Chen et al., 1981) and gracilis (Prewitt et al., 1982) muscle vasculature, measured under innervated, denervated (cremaster and gracilis) and maximally relaxed conditions (gracilis), indicate that the intermediate and small blood vessels of young (6-7 week) and old (16-18 week) SHR have wall thickness similar to that present in WKY.

Studies performed on the premucosal mesenteric arteries indicate that the vascular wall is thickened in SHR. Mulvany and his colleagues (Mulvany and Halpern, 1977; Mulvany et al., 1978; Warshaw et al., 1979, 1980; Aalkjaer and Mulvany, 1979) have carried out optical and histological measurements of both small and large mesenteric arteries stretched at a tension that is equivalent to an interluminal pressure of 100 mm Hg (LD_{100}). Studies performed on SHR with established hypertension have yielded similar results. Vessels having a LD_{100} of approximately 160 to 200 μm exhibited a thickened total vessel wall produced primarily be increased amounts of media. Within the media, the cross-sectional content of SMCs was increased in SHR. Morphometric measurements indicated that the increase in medial SMC content was produced by a greater number of SMCs per unit arterial length. In one study, however, smaller mesenteric blood vessels with an LD of approximately 150 μm at LD_{100} did not display a thickened vascular wall in SHR (Mulvany and Halpern, 1977), suggesting that a thickened vascular

wall may not be a consistent occurrence in smaller arteries.

In studies performed by Lee et al (1983 a, b), relaxed mesenteric arteries, that were perfusion fixed and compensated for histological sectioning angle, were compared between SHR and WKY at 28 and 12 weeks of age. It was observed that small and intermediate premucosal mesenteric arteries of SHR exhibited an increased wall thickness in both age groups. In 12 week old SHR, the wall was thickened in intermediate sized arteries as a result of an increase in the quantities of arterial intima, internal elastic lamina and SMC content. The arterial media was characterized by increased numbers of larger sized SMCs. In the case of smaller premucosal mesenteric arteries, wall thickening was produced by an increase in the quantities of intima, internal elastic lamina, greater quantities of similar sized SMCs and greater amounts of adventitia (Lee et al., 1983 b). In as yet unpublished data, Dr. Lee has shown that when the small mesenteric arteries of 12 week old SHR and WKY are subdivided , arteries less than 60 μm in diameter fail to display any indication of a thickened vascular wall in SHR over WKY (personal communication).

In other studies, Henrich et al (1978), using in vivo microscopy, found that the mesoappendix vasculature (a subdivision of the mesenteric bed) of young adult SHR exhibits wall thickening only in intermediate sized arteries. The wall thickness of terminal and preterminal arterioles (LD < 30 μm) was not altered in SHR when compared to WKY.

Unlike intermediate and small muscular arteries, the wall of the superior mesenteric artery is not thickened in 12 week old SHR (Lee et

al., 1983 a, b). On the other hand, the vascular wall of the superior mesenteric artery was thickened in 28 week old SHR when the animals had experienced long term hypertension.

The cerebral vascular bed of 30 week old SHR exhibits a thickened vascular wall primarily in smaller blood vessels with a LD < 80 μm (Norborg and Johansson, 1980). In this study the arteries were instillation fixed. The cross-sectional area of the media of contracted arteries was subsequently determined after which the length of the convoluted internal elastic laminae was measured. Subsequently, the radius and medial thickness was calculated in a manner where the artery was relaxed mathematically. Unfortunately, when such a technique is used to compensate for contraction, the radius is typically underestimated. This is due to the fact that the internal elastic lamina is shortened during contraction. Furthermore, the degree of shortening is less in SHR than WKY vessels (Lee et al., 1983 c). The above form of compensation would therefore tend to reduce the differences in the media to lumen ratios between SHR and WKY, suggesting that perhaps cerebral arteries with a LD larger than 80 μm could also display a thickened vascular wall in SHR.

Limas et al (1980) observed the presence of a thickened vascular wall in intrarenal vessels with a LD > 30 μm, and in the aorta of SHR. These alterations appeared to be a secondary alteration in hypertension occurring in SHR older than 10 weeks of age. However, in this study the arteries were contracted and no histometric method was used to try to relax the arteries. Furthermore, the arteries were not compensated for histological sectioning angles. In view of this, the above measurements

should be viewed with scepticism.

Various researchers feel that the arterial intima plays an important role in maintaining hypertension. Boyd (1980) has proposed that hypertension could cause endothelial damage. Arteries mechanically denuded of endothelium exhibit the intimal proliferation of SMCs, an increased deposition of subendothelial connective tissue and the subsequent regrowth of new endothelium. The newly formed intima is usually thicker than the original layer (Richardson et al., 1980). Such an alteration could increase vascular resistance.

Consistent with the above view, Lee et al (1983 b) observed that small and intermediate mesenteric arteries of SHR had elevated cross-sectional amounts of subendothelial space, while intermediate sized arteries also exhibited increased cross-sectional quantities of endothelium.

In studies performed on the aorta, subendothelial thickening has been reported in SHR ranging in age from 5 to 21 weeks (Stills, 1979; Haudenschild et al., 1980; Limas et al., 1980). The subendothelial space in the above studies was characterized by an increased accumulation of mucopolysaccharides (Limas et al., 1980), precipitated plasma proteins, collagen, elastin, monocytes, red blood cell fragments, SMCs (Haudenschild et al., 1980) and flocculent material reacting to fibrinogen antibodies (Stills, 1979). In other studies, Limas et al (1980) observed an increased accumulation of horseradish peroxidase in the subendothelial space of aortas excised from SHR, suggesting an increased endothelial permeability to the tracer. Haudenschild et al (1980) described SHR aortic endothelial cells as exhibiting a distorted

appearance and having an increased thickness. Unfortunately, in all the above studies the characteristics of both the endothelium and subendothelial space were visually assessed, and no true quantitative measurements were made. Therefore, the accuracy of such an assessment is strongly dependent on the ability of the various researchers to make an unbiased judgement.

The above alterations in the arterial intima of SHR have been assessed in terms of the ability of the intima to physically obstruct the lumen. Alternatively, the endothelium is very vasoactive. A wide variety of vasodilators, such as substance P, acetylcholine, bradykinin, histamine, hydralazine and thrombin exert their vasodilatory effects in part, or totally, through the endothelium (Vanhoutte et al., 1982). It could be possible that alterations associated with hypertension that damage the endothelium or alter its function could, in turn, reduce the vasodilatory action of blood born or nerve emitted vasodilators such as substance P. This, in turn, could increase SMC tone and vascular resistance.

3. (b) Conclusion

Perfusion studies performed by different researchers indicate that contraction of a variety of isolated vascular beds produces a greater amplitude of resistance change in SHR than in WKY or NCR. In some studies both receptor and non-receptor forms of contraction produce qualitatively the same type of reactivity alterations in SHR. It has been suggested that the elevation in vascular reactivity observed in perfusion studies involving SHR are caused by the presence of a thickened vascular wall which produces a hyperocclusion of the lumen

during contraction. However, the above forms of experiments provide only indirect proof of the presence of a thickened vascular wall. Other researchers have proposed that such hyper-reactivity can be produced by alterations in the membrane potential, Na^{+}/K^{+} pump or by alterations in the Ca^{+2} efflux from VSMCs in SHR.

In view of the above criticism, morphometric and in vivo microscopy studies have been performed in an attempt to provide direct evidence supporting the hypothesis that blood vessel wall thickening occurs in SHR. Such studies indicate that not all vascular beds are altered equally in SHR. For example, vital microscopy studies carried out on the gracilis and cremaster muscle vasculature demonstrate that blood vessel wall thickness is not increased in a variety of arterial classes when adult SHR are compared to WKY. In vascular beds that do display blood vessel wall thickening in SHR, all arterial classes are not altered equally. Although there are exceptions, generally larger elastic arteries, such as the superior mesenteric, main renal, femoral and in some but not all studies, the aorta, exhibit either a total absence or a marginal degree of vascular wall thickening in SHR. In addition, contrary to the belief of many researchers (i.e., Folkow et al., 1973), small blood vessels of SHR do not always display vessel wall hypertrophy. The arterial classes that are particularly affected are intermediate sized perarteriolar muscular arteries with an LD ranging between 200 to 60 μm. Wall thickening appears to be produced primarily by an increase in the cross-sectional quantities of medial, and in some cases, intimal components. Increased quantities of media are produced predominantly by an increase in the numbers of VSMCs. In some cases

these alterations are coupled to an increase in the size of the VSMCs. Intimal thickening, when it occurs, is associated with an accumulation of material within the subendothelial space. Various researchers have suggested that subendothelial thickening could be a secondary response due to endothelial cell injury as a result of high blood pressure. Quantitative studies have indicated that the cross-sectional quantities and the thickness of endothelial cells are increased in SHR. Such alterations could alter endothelial cell function. Since various vasodilators exert their action through endothelial cells, such changes could alter SMC tone and raise vascular resistance.

4. (a) Alterations in blood vessel connective tissues of SHR

Aside from contributing in a minor way to production of wall thickening, increases in vessel wall connective tissue content could decrease the distensibility of hypertensive arteries. Such alterations would favor a decreased LD under in vivo conditions.

Morphometric and biochemical studies have been undertaken to determine whether connective tissue metabolism is altered in SHR.

Morphometric measurements performed by Lee et al (1983 b) indicate that the cross-sectional quantities of elastin present within the internal elastic lamina and the arterial adventitia are increased in the large and small premucosal mesenteric arteries of 12 week old SHR over WKY. The smaller blood vessels of SHR were also characterized by an elevation in the adventitial quantities of fibroblasts but adventitial collagen remained unaltered. On the other hand, larger superior mesenteric arteries of 12 week old SHR and WKY exhibited similar cross-sectional quantities of medial and adventitial collagen

and elastin.

Pang and Scott (1981) morphometrically measured the volume fraction of collagen and elastin of both the abdominal aorta and main renal artery of SHR and WKY between the ages of 2 to 18 weeks. These parameters were not altered when SHR and WKY vessels were compared. However, the volume fraction represents only the proportion of the arterial wall occupied by a component and not the absolute quantity of that component. A situation could occur where the vascular wall is thickened in SHR, while the proportion of elastin and collagen remain unchanged. In this instance, the absolute amount of collagen and elastin could be increased in SHR, even though the volume fraction of the two components remains the same. However, in Pang and Scott's (1981) study, the thickness of the media plus intima were unaltered in the main renal artery and aorta of SHR. In view of this, it is unlikely that the absolute amounts of collagen or elastin were changed in SHR.

Biochemical analysis of arterial wall connective tissue contents and the enzymes involved in the synthesis of these components indicate that collagen biosynthesis is increased in SHR. Erhart and Ferrario (1981) studied both the collagen content and synthesis of the aortic intima and media excised between the subclavian and celiac branch points. Collagen synthesis as measured by the incorporation of C^{14}-proline into collagen per unit time was increased in adult SHR aortas. Within these experiments, the amounts of media present within the aorta was increased in SHR. Collagen content increased proportionally, producing an increase in the total amount of collagen present (mg collagen/vessel length), while the collagen concentration

(mg collagen/mg vessel wet wt) remained unaltered in the aorta of SHR.

In experiments performed by Iwatsuki et al (1977), collagen synthesis was increased in the aorta and mesenteric arteries of 5 month old SHR. In this study, the collagen concentration (g collagen per g tissue) was increased in both classes of arteries sampled from SHR. In the above experiments no mention was made as to whether the arterial adventitia was removed from the arteries. Therefore, it cannot be determined whether the increase in collagen synthesis observed takes place within the media plus intima, as opposed to the adventitia. Increases in the connective tissue content within the media and/or intima would exert a more dramatic effect in terms of decreasing arterial distensibility than similar alterations in the adventitia.

Studies by Sheridan et al (1979) indicate that the specific activity of lysyl oxidase is increased in the aorta of 12 week old SHR. This enzyme is involved in forming crosslinks between collagen or elastin. Such crosslinks are though to stiffen the collagen network and could potentially reduce arterial distenstibility. Furthermore, since extracellular collagen has been shown to have discrete attachment points to arterial SMCs at areas where myofilaments attach (Kimani, 1981), the increased crosslinkage of collagen may facilitate the transmission of the contractile force between the SMCs of arteries.

Alterations in collagen synthesis appear to take place at an early age in SHR. Oshima et al (1979) studied SHR between the ages of 7 and 34 weeks. Prolyl hydroxylase specific activity was found to be increased in the intima plus media of the aorta at 7 weeks of age, a time when blood pressure was starting to elevate. On the other hand, in

the mesenteric and heart tissue of SHR, the specific activity of this enzyme was increased only in SHR older than 17 weeks, long after hypertension was established.

Experiments performed by Ogawa and Ozaki (1978) emphasize the importance of collagen metabolism in hypertension. beta-aminopropropionitrile (BAPN), an inhibitor of lysyl oxidase, was found to prevent the development of hypertension in young SHR and to lower the blood pressure in older animals with established hypertension. Within these experiments, the exact mechanisms of BAPN action are unknown. In inhibiting collagen crosslinking, BAPN could increase the distensibility of the arterial vasculature allowing the maintenance of a larger lumen diameter. However, BAPN is also cytotoxic (Oakes, 1980) and is capable of reducing collagen levels in arteries (Sheridan et al., 1979). In view of this, it could be possible that BAPN exerts its action by decreasing collagen synthesis, as opposed to altering collagen crosslinking. Alternatively, the cytotoxic effects of BAPN could alter some other cellular function important in the maintenance of hypertension.

4. (b) <u>Alterations in the passive and active blood vessel wall mechanics of SHR</u>

Arterial distensibility has been measured in a number of different manners. When two arteries of equal dimensions are compared the more distensible artery will have a greater LD per any given transmural pressure. However, hypertensive and normal blood vessels are rarely the same dimensions. Alterations in the geometry of the blood vessel (i.e., lumen radius, wall thickness, etc.), even in the absence

of any alterations in the distensibility of the materials composing the artery, will have a significant effect on the way a blood vessel will expand (Burton, 1962). For example, if two arteries of similar material and wall thickness are compared, one having a smaller radius than the other, a similar transmural pressure will exert a greater tangential force per unit area in the larger artery, causing it to expand to a greater degree. With this in mind, various other calculations have been used to define the distensibility of the materials composing the artery.

The tangential wall stress (S) for a given transmural pressure P can be determined for an artery (Cox, 1979, 1981).

$$S=(a/(b-a))P \qquad (1)$$

In the above formula a and b are, respectively, the internal and external radii. S represents the tangential applied force per unit wall area.

The incremental strain modulus (Esm) has also been used to define the distensibility of arteries. Berry et al (1975) have defined Esm as the ratio of the increase in radius (ΔR) to the average radius during a pressure change (ΔP); ΔP is usually standardised at 20 mm Hg. When two arteries of equal dimensions are compared, the more distensible artery will exhibit a much greater change in radius in response to ΔP; Esm will therefore be increased.

The incremental elastic modulus (Einc) of a vessel is a representation of the incremental stress (applied force per unit area due to a pressure change, ΔP) to incremental strain (the degree of vessel deformity due to ΔP). A more distensible artery will exhibit a larger degree of deformity in response to an alteration in stress, hence

Einc will be low (Bergel, 1961). Einc can be determined from a radius vs pressure curve, for an artery (Cox, 1979, 1980).

$$Einc=(2a^2b^2/(b^2-a^2))(\Delta P / \Delta b) \qquad (2)$$

For a point (x, y) along the radius vs pressure curve, the internal and external vessel wall radii are determined (a and b, respectively). Using the points above and below (x, y), the slope of the curve at x, y is calculated. In the above formula, the slope at x, y equals $\Delta P/\Delta b$; hence, the Einc at x, y can be determined.

When two arteries are compared, typically Einc is calculated for each arterial radius of the radius vs pressure curve. Subsequently, a plot of Einc vs R/Ro is made. R/Ro is the radius used to calculate Einc divided by the radius present at 0 transmural pressure. Thus, the Einc of two arteries can be compared under standardized conditions.

In summary, a less distensible artery will exhibit a lower Esm and a higher Einc than a more distensible counterpart under standardized conditions. Under conditions of equal S (tangential applied force per unit wall area), a less distensible artery will exhibit a smaller LD. However, when two arteries are compared under equal transmural pressures, the presence of a distended lumen in one over the other, is suggestive, but not necessarily, indicative of the presence of increased arterial distensibility.

Greenwald and Berry (1978) studied the passive mechanical properties of the thoracic aorta of 6 week (incipient hypertensive) and 20 week old SHR and age matched WKY. Plots of the lumen diameter vs transluminal pressure indicated that young SHR exhibited a constantly smaller lumen diameter than WKY at any transmural pressure. The

characteristics of the radius vs pressure curves were such that the decrease in lumen radius in SHR was reflective of a structurally smaller artery, as opposed to an artery that is less distensible. At transmural pressures less than 150 mm Hg, Esm was higher in SHR aortas than those of WKY, suggesting that distensibility is larger in the aortas of young SHR than WKY. In the case of adult SHR, only the Einc of the aortas was measured. Einc was decreased in SHR over WKY at transmural pressures between 75-300 mm Hg, again suggesting the presence of an elevated distensibility in the aorta of SHR over WKY even during established hypertension.

Consistent with the passive wall characteristics that were observed, Berry et al (1978) found that SHR aortas exhibited a decrease in connective tissue content. It should, however, be mentioned that the above alteration is atypical; most studies performed on the aorta of SHR indicate that the content and, in some cases, the concentration of collagen are increased (e.g., Iwatsuki et al., 1977; Erhart and Ferrario, 1981). If the distensibility of the aorta is determined by the connective tissue content (which it may not be), such a result would indicate that aortic distensibility is more typically reduced in SHR.

Andersen et al (1980) studied the aorta from six groups of SHR ranging in age from 5 to 30 weeks. It was observed that the aortas of SHR older than 6 weeks of age maintained a smaller lumen (measured as the ratio of the LD to the LD present at 0 mm Hg, LD/LD_0) than WKY when subjected to a transmural pressure of 100 mm Hg. However, the tangential stress being exerted on the vessel wall was less in the aortas of SHR than WKY, in spite of the fact that both groups of

arteries were subjected to an equal transmural pressure (100 mm Hg). This was primarily due to the presence of a smaller lumen and a thickened vascular wall in the aorta of SHR. In other experiments the aortas of SHR and WKY were subjected to a transmural pressure corresponding to the systolic blood pressure of the rat from which the artery was excised. Since the systolic blood pressure was elevated in SHR older than 6 weeks of age, the aortas of these animals were subjected to higher transluminal pressures and tangential wall stress than the aortas of age matched WKY. It was observed that the value of LD/LD_0 was always smaller in SHR than WKY, in spite of the presence of an elevated transmural pressure in SHR aortas. Furthermore, the elastic modulus of the SHR aortas was elevated over that present in WKY at ages greater than 8 weeks. The above results suggested that the distensibility of the aorta was decreased in SHR older than 8 weeks of age.

Cox (1979, 1981) undertook an extensive study of the passive (0 mM Ca^{+2}, 2 mM EGTA) and active (K^+ contraction, 145 mM K^+) wall mechanics of the carotid and tail artery of SHR in an established phase of hypertension. Each of the above arteries exhibited different properties. At a transmural pressure of 0 mm Hg, the external diameter of the carotid arteries was greater in SHR than WKY , while the tail arteries had similar external diameters. Under passive conditions at transmural pressures greater than 50 mm Hg and under active conditions at pressures greater than 100 mm Hg, SHR carotid arteries maintained a smaller lumen diameter than WKY. In the case of the tail arteries, the passive distention of the arteries produced by increasing the

transluminal pressure was similar in SHR and WKY. However, under active conditions, at transmural pressures greater than 200 mm Hg, the external diameter of the SHR vessels was smaller than that of WKY. The active tension studies involving both the carotid and tail artery indicated that in SHR these arteries were capable of contracting to a greater degree at higher pressures and maintaining a smaller lumen diameter than those of WKY.

The above experiments suggest that both carotid and tail arteries of SHR display a reduced distensibility under active conditions, while under passive conditions distensibility is reduced only in the carotid arteries of SHR. However, the above measurements are governed by the geometric properties as well as the properties of the material composing the artery. When Cox (1979, 1981) replotted the changes in external diameter as a function of tangential wall stress, as opposed to transluminal pressure, both the carotid and tail artery of SHR under active or passive conditions exhibited a smaller external diameter per any given degree of wall stress. Thus, when equal degrees of tangential force per unit area are applied, even the tail arteries of SHR under passive conditions display a decreased distensibility.

An attempt was made by Cox (1979) to explain the mechanical properties of the carotid arteries of SHR in terms of collagen and elastin characteristics of the arteries. Earlier work on normal canine arteries indicated that the mechanical properties of the arteries can be described on the basis of a model in which the elastic modulus of a vessel (Einc) is determined by the elastic moduli of elastin and collagen (Ee and Ec), the relative amount of collagen and elastin (Wc

and We) and the fraction of collagen fibres supporting wall stress at a given strain or pressure (fc).

$$Einc = Ee\ We + fc\ Ec\ Wc$$

When the above model was applied to the carotid arteries of SHR and WKY the model did not fit the data. For example, if experimental values of Einc, We, Wc under conditions of known fc were used to compute Ee and subsequently Ec, the Ee values obtained for both SHR and WKY were above those normally though to be acceptable, while the Ec values obtained were within normal limits for SHR and below normal values in WKY. Furthermore, the decreased distensibility observed in the carotid and tail arteries of SHR could not be explained by alterations in collagen and/or elastin composition. In both arterial classes, the percentage composition of collagen and/or elastin was either normal or below normal in SHR (Cox, 1979, 1981). Within the carotid artery (Cox, 1979) of SHR, the collagen content (mg per arterial length) was increased slightly over WKY; however, this value was below that present in NCR which had a carotid artery of greater distensibility than that present in either WKY or SHR. In view of the above anomalies, Cox (1979) suggested that qualitative, as opposed to quantitative, differences in the type of collagen and elastin present or alterations in the crosslinkage of these proteins may account for the altered distensibility observed in SHR arteries. In this regard, BAPN, a collagen or elastin crosslink formation inhibitor, can prevent hypertension formation in young SHR (Ogawa and Ozaki, 1978). However, analysis of the amino acid composition of arterial collagen from SHR and WKY indicates that no remarkable differences exist between the two groups (Yamori and Ohta,

1979).

In other studies performed by Mulvany and his colleagues (Warshaw et al., 1975; Mulvany et al., 1977; Mulvany et al., 1978; Aalkjaer and Mulvany, 1979), a myograph was used to stretch premucosal small mesenteric arteries at varying tension. By measuring the internal circumference of the artery under these conditions, the LD and the circumferential wall force per unit arterial length were calculated. The above studies have been performed on mesenteric arteries with an effective lumen diameter (lumen diameter present at 100 mm Hg, LD_{100}) ranging from 130 to 250 μm, and have involved SHR ranging from 5 weeks (incipient hypertensive) to 50 weeks (established hypertensive) of age. All the above studies indicate that the change in the mesenteric artery LD (as a ratio of LD_{100} or 0.8 LD_{100}) in response to increases in circumferential tension (per arterial length) are similar in SHR and WKY under passive conditions. Other measurements of wall stiffness indicate that the Young's modulus (a relation of wall stress to deformity) under both active (K^+ contraction) and passive conditions, and the active half-time response (time taken to reach half-tension as a result of a decrease in circumference) are similar when mesenteric arteries from old (50 week) and young (6 week) SHR were compared to age matched WKY (Warshaw et al., 1979).

Other researchers have used perfusion studies of isolated vascular beds to determine whether an altered distensibility exists. Using this technique, vascular resistance, or some representation of it, has been measured at a variety of infusion pressures produced by elevating perfusion flow. Typically, vascular resistance decreases at

higher infusion pressures due to, in part, an expansion of the average lumen size of the vasculature. The change in vascular resistance per change in perfusion pressure or flow has been used as an indicator of vascular distensibility. This method has its limitations in that at low perfusion pressures part of the vascular bed could close down, decreasing the number of functionally open vessels present.

Studies involving total systemic and renal vasculature of adult SHR (Folkow et al., 1969; Folkow et al., 1971 a; Folkow, 1979), as well as the hindlimb vasculature of both adult and 3 week old prehypertensive SHR (Lais and Brody, 1978; Folkow, 1979), all indicate that vascular resistance decreases to a lesser extent in the face of higher perfusion pressures in SHR than WKY or NCR. In a recent study carried out by Folkow (1979), the relationship of vascular resistance to perfusion pressure was determined at different levels of NE contraction. At maximal relaxation up to contraction produced by 0.4 µg/ml, the decrease in vascular resistance in relation to increased transmural pressure was less in SHR than in NCR. The above studies suggest that the vascular beds of SHR exhibit either a decreased distensibility or a reduced ability to open in the face of elevated perfusion pressure when compared to WKY or NCR.

4. (c) Conclusion

There is experimental evidence supporting the hypothesis that the connective tissue content of SHR arteries is increased over that present in WKY. Such evidence resides primarily in biochemical analyses which have measured the activity of the enzymes involved in the synthesis of collagen, the rate of collagen synthesis, the collagen

content and, in some studies, the collagen concentrations of arteries. Most studies involving the aorta and some studies involving the mesenteric arteries indicate that parameters of collagen synthesis, content and, in some cases, concentration are increased in SHR over WKY. However, morphometric investigations of the volume fraction and cross-sectional area content of the connective tissue composition of the aorta, mesenteric and main renal arteries, and biochemical analyses of tail and carotid arteries indicate that only modest alterations in collagen and elastin composition exist between SHR and WKY.

Other researchers have argued that the connective tissues of SHR arteries are altered in a qualitative, rather than a quantitative, manner. There is some evidence supporting the above arguement. Decreases in arterial distensibility have been observed in spite of the absence of any large quantitative alterations in arterial connective tissue composition. Furthermore, the specific activity of lysyl oxidase, an enzyme which crosslinks collagen and elastin (increasing stiffness) is increased in the aorta of SHR, while the inhibition of the enzyme with BAPN can prevent hypertension development in young SHR or lower blood pressure in adult animals. However, amino acid analysis of SHR and WKY arterial collagen has failed to show the presence of any remarkable differences in composition between SHR and WKY.

Various investigators have attempted to determine whether arterial distensibility is altered in SHR. At a given luminal pressure, the degree of arterial distension present will depend on the geometric properties of the blood vessel as well as the properties of the materials composing the vascular wall. In view of this, analyses which

normalize arterial expansion to the degree of tangential (or circumferential) wall stress, and measurements of the incremental strain modulus or the incremental stress modulus have been used to more accurately define alterations in arterial distensibility that result from a change in the properties of the vascular wall. In this regard, studies performed on the tail and carotid arteries and some studies carried out on the aorta, indicate decreased arterial distensibility in SHR, while investigations involving mesenteric arteries indicate that arterial distensibility is unaltered when SHR and WKY are compared.

An alternative approach used by other researchers to measure arterial distensibility has been to perfuse various isolated, relaxed vascular beds at different flow-infusion pressure conditions. In these experiments, a higher perfusion pressure should expand the lumen and therefore reduce vascular resistance. Such studies carried out on a number of different vascular beds indicate that the rate of decrease in vascular resistance in relation to increases in perfusion pressure are less in the vascular beds of SHR than WKY. Although such an alteration is consistent with the presence of a reduced arterial distensibility in SHR, an increased vascular closure at lower perfusion pressures in SHR could also produce the same type of perfusion characteristics.

5. (a) Alterations in blood vessel density in SHR

A decrease in the number of blood vessels through which blood can flow can elevate systemic vascular resistance. In vivo microscopy investigations (vital microscopy) of the vascular beds of anaesthetized SHR and WKY have been undertaken to determine whether the number of functional and/or anatomical blood flow pathways are altered in SHR.

Using the above technique, Hutchins and Darnell (1974) made photographic collages of the cremaster muscle vascular bed of 5 to 6 week old anaesthetized prehypertensive SHR and age matched WKY. The individual arteries were categorized by their order of division extending from the capillaries. SHR were found to have 50% fewer precapillary arterioles (LD < 50 μm), and a 50% increase in the postcapillary venules. The length of the arteries was not significantly altered.

Chen et al (1981) used in vivo microscopy in conjunction with stereological techniques to determine the length and surface area per unit volume of the blood vessels present in the cremaster muscle. These parameters were used as a monitor of vascular density. It was found that a large proportion of the SHR vasculature could not be observed due to the fact that it was functionally closed to blood flow. Denervation or vasodilation (Na nitroprusside) increased the density of observable blood vessels. When the vascular bed was dilated, it was observed that 4 to 6 week old, incipient hypertensive SHR had anatomically fewer precapillaries (LD < 25 μm). Under actively innervated conditions and in the absence of vasodilation, the percentage of blood vessels closed to blood flow was three times greater in SHR than in WKY. Other investigations of similar design carried out by Bohlen and his colleagues have also substantiated the presence of an anatomical and/or functional decrease in the precapillary blood flow pathways in the cremaster muscle of 6 to 7 week, and 16 to 18 week old SHR (Bohlen and Lobach, 1978; Bohlen, 1979).

Alterations in blood vessel densities are not limited to the cremaster muscle vasculature. Prewitt et al (1982), using in vivo

microscopy and stereological techniques similar to those of Chen et al (1981), studied the gracilis vasculature of SHR and WKY that were 6 to 18 weeks of age. The experiments were performed under innervated, denervated or maximally relaxed conditions. SHR 6 weeks of age and older were found to have a decreased density of capillaries; SHR older than 12 weeks of age exhibited a greater functional closure of the vascular bed, while an anatomical decrease in arteriolar vessel density was observed only in 16 to 18 week old SHR.

Henrich et al (1978) studied the mesenteric vascular bed of the mesoappendix. The entire mesoappendix vascular bed of young adult SHR and WKY was photographed. The quantity and length of preterminal and terminal arterioles, two orders of postcapillary venules, postarteriole, mid and prevenule capillaries were determined. In addition, a distinct arteriovenous shunt consisting of very large capillaries was also quantitated. SHR displayed a 50 to 70% decrease in the number of terminal arterioles, first order venules and true capillaries. However, the number of arterial to venous shunt capillaries increased in SHR. All the various arteries, and capillaries and first order venules had decreased a length in SHR. In most cases SHR blood vessels exhibited a larger lumen than the corresponding WKY vessels. Vasodilation of the vascular bed increased the lumen diameter of SHR and WKY vessels equally, suggesting that the degree of vasoconstriction present in the mesoappendix vasculature is similar in SHR and WKY. To measure the overall effects of the above alterations, a hydraulic hindrance factor, thought to represent vascular resistance to flow, was calculated for capillary vessels. This calculation was based on the diameter, vessel

numbers and the length of vessels. It was found that in the true capillaries (non-shunt) the increase in capillary diameter in SHR more than compensated for the decrease in capillary numbers, and the hydraulic hinderance to flow was similar between SHR and WKY. On the other hand, arterial to venous shunt capillaries in SHR exhibited a reduced hydraulic hinderance, due to the fact that vessel numbers and the lumen diameters of the vessels were increased in SHR.

Consistent with the above observations, Haack et al (1980) found that SHR (5 to 10 weeks of age) had between 30 to 40 % fewer terminal arterioles in the peripheral abdominal vascular bed than either WKY or NCR. However, when a representation of vascular resistance was calculated, the values obtained for SHR were approximately one half that of WKY. This was due to the fact that the arterioles of SHR exhibit a larger lumen diameter, which again more than compensated for the decreased numbers of arteriolar vessels.

5. (b) Conclusion

Microscopy studies performed on the cremaster, gracilis, mesenteric and abdominal vascular beds of live, anaesthetized SHR, indicate the presence of both an increased functional closure of the vasculature, and an anatomical decrease in the numbers of smaller blood vessels in SHR over WKY. In the cremaster muscle, both the above alterations occur in prehypertensive and adult animals. The gracilis muscle of SHR, on the other hand, exhibits a decreased capillary density in prehypertensive SHR, while a decrease in anatomical numbers of arterioles and an increase in functional closure of the vasculature occur after the elevation of blood pressure. A decrease in capillary

and arteriolar densities has the potential to elevate blood pressure by restricting the number of pathways through which blood can flow. Furthermore, since both these alterations can take place prior to blood pressure elevation in SHR, such changes could be of potential importance in the etiology of hypertension development. However, studies involving the mesenteric and abdominal vasculatures indicate that the potential increase in the vascular resistance produced by a decrease in the density of arterioles or capillaries is often more than compensated for by an increase in the in vivo blood vessel lumen diameter within these vessels. Hence, within these regions, vascular resistance to blood flow may remain unaltered, or even decrease in SHR as compared to WKY.

I. Are Alterations in the Vascular Wall a Primary or Secondary Alteration in SHR

1. Background

Until recently, it has been a general conclusion that structural alterations, such as the presence of a thickened vascular wall in SHR as well as other forms of hypertension, was a consequence of high blood pressure. In Folkow's view (Folkow et al., 1973), hyperperception or reaction to environmental stimuli produces neuronal and hormonal induced elevations in blood pressure. Subsequently, the vascular wall was thought to adapt to the increased wall tension, producing a thickened vascular wall. It was suggested that wall thickening eventually encroached on the lumen and caused the artery to hyper-contract, leading to the presence of a permanently elevated blood pressure. There is experimental evidence that supports the above view. In experiments performed by Folkow et al (1971 b), a clamp was placed around the aorta

distal to the main renal arteries of 3 week old SHR. Such treatment permitted the maintenance of normotension in the hindquarters, but not in the vascular areas proximal to the clamp. After 6 to 16 weeks of such treatment, the isolated hindquarters were perfused with Tyrode's solution and compared to untreated SHR and NCR. The presence of an elevated vascular reactivity to maximal contraction was used as evidence for the presence of a thickened vascular wall. It was observed that aortic clamping prevented the occurrence of an elevated contractile reactivity in the hindlimb vasculature of SHR. Furthermore, the magnitude of reactivity present in the hindlimbs was proportional to the level of arterial blood pressure observed in the hindquarters.

The above observations are consistent with the fact that in Goldblatt forms of hypertension, the clamped hypotensive kidney does not exhibit vascular wall hypertrophy as is present in other hypertensive vascular beds (Byrom, 1969). Furthermore, in renal hypertensive rats structural alterations in the hindquarters occur after blood pressure elevation (Lundgren and Weiss, 1979), suggesting that they are secondary modifications in hypertension. Organ bath experiments involving rabbit ear arteries have demonstrated that DNA synthesis is increased in the VSMCs of the artery in proportion to the transluminal tension of the artery (Hume,1980). All the above studies are consistent with the hypothesis that an elevation in blood pressure can produce vascular wall thickening.

To further test the above hypothesis two types of experiments have been carried out to determine the etiological role of vessel wall hypertrophy in hypertension development. SHR have been studied in a

prehypertensive stage of blood pressure development to determine the types of structural alterations present. Alternatively, hypertension has been prevented by antihypertensive treatment and the vasculature has been subsequently measured and compared to control animals.

2. Studies performed on prehypertensive and incipient hypertensive SHR

2. (a) Large arteries of SHR

Andersen et al (1980) found that SHR developed a thickened vascular wall and a decreased lumen over WKY between the ages of 8 and 14 weeks. Blood pressure, on the other hand, was significantly elevated in SHR at 6 weeks. Within this study, wall thickness was measured in viable arteries under a distending pressure of 100 mm Hg. It was also shown that the aortic wall exhibits a decreased distensibility at 8 weeks of age. In view of this, the thicker vascular wall and decreased lumen observed at 100 mm Hg in SHR could be due to a decrease in the degree of vessel wall stretch as opposed to geometric alterations in the blood vessel. Pang and Scott (1981), on the other hand, did not observe a thickened intimal plus medial wall in the aorta or main renal artery at ages 2 to 18 weeks, when SHR were compared to WKY.

In morphometric studies involving the carotid artery, Gray (1982) has found that 12 to 24 hour old SHR exhibit a thickened carotid vascular wall, with an unaltered lumen diameter. It was suggested that blood vessel wall hypertrophy may be genetically determined in SHR. However, even at this early age, Gray's SHR already displayed an elevated blood pressure over WKY. Therefore, the possibility that such alterations are, in fact, an adaptation to high blood pressure cannot be

excluded.

In morphometric studies of the superior mesenteric artery of SHR, Lee and his colleagues failed to observe any significant alterations in lumen diameter or cross-sectional quantity of wall components in 3 to 5 week old prehypertensive (Lee et al., unpublished observations) and 10 to 12 week old established hypertensive SHR (Lee et al., 1983 a). However, as mentioned previously, 28 week old SHR did exhibit an elevated media to lumen ratio and an elevation in the cross-sectional area of adventitial and intimal plus media components when compared to age matched WKY (Lee et al., 1983 a).

2. (b) Intermediate and small arteries of SHR

Unlike larger elastic arteries, intermediate and small muscular arteries of SHR do show some evidence of altered structure prior to hypertension development. Quantitative morphological studies performed by Karr-Dullien et al (1981) indicate that arteries within the tail having an external diameter between 20 and 60 μm (but not smaller or larger arteries) exhibited an elevated media to lumen ratio in both newborn and 2 week old SHR placed on a normal or high salt diet. Unfortunately, in this investigation the blood pressure data for the animals was not shown. However, in an article directly preceding the above study, but dealing with another subject, the above authors indicated that blood pressure was elevated in 2 week old, but not newborn, SHR when compared to age matched WKY.

In studies performed by Lee et al (unpublished observations) involving 3 to 5 week old prehypertensive SHR, intermediate sized mesenteric arteries (LD approx. 130 μm) were found to have an unaltered

lumen diameter and an increased total cross-sectional wall area. In this instance, vessel wall thickening was produced by increased cross-sectional area quantities of endothelium, internal elastic laminae, SMCs, medial extracellular space and adventitia. Within the media, increased numbers of similar sized SMCs were present. Aside from a modest increase in subendothelial space, smaller premucosal arteries (LD approx. 55 μm) failed to exhibit any alterations in the cross-sectional area quantities of wall components.

In two studies carried out by Mulvany and his colleagues, one study involving 4 week old prehypertensive SHR, premucosal mesenteric arteries were found to have a wall thickness that was similar to that present in WKY (Mulvany and Nyborg, 1980). In another experiment (Warshaw et al., 1979), 6 week old SHR with incipient hypertension were found to exhibit a thickened vascular wall. In studies performed by Scott and Pang (1983 b), a thickening of the vascular wall in premucosal jejunal mesenteric arteries of SHR occurred between 4 and 8 weeks of age, between pre- and developing stages of hypertension.

Quantitative morphological studies by Nordborg and Johansson (1979) demonstrate that 15 day old SHR exhibited an elevated media to lumen radius ratio in muscular arteries from the mesenteric, renal and cerebral vascular bed. Renal and mesenteric arteries with an LD > 100 μm, and cerebral vascular arteries with an LD > 160 μm showed an elevation in the quantities of media to lumen. Very large cerebral, renal and mesenteric arteries were not altered when SHR were compared to WKY. Unfortunately, in the above study, 15 day old SHR already exhibited an elevated blood pressure, therefore it is impossible to say

whether such alteration preceeded hypertension. A peculiar observation made by Nordborg and Johansson (1979) was that after long term hypertension (200 days) only cerebral arteries with an LD < 40 µm exhibited an increased media to lumen radius, while in the mesenteric arteries, vessel wall thickening spread to both larger and smaller arteries. In the case of the renal arteries, adult SHR did not exhibit a thickened vascular wall when compared to WKY. No explanation was given as to why the structural changes present in 15 day old SHR were so different from the types of alterations present in the adults.

2. (c) Perfusion studies of the SHR vasculature

Various researchers have used isolated organ perfusion techniques to determine whether reactivity alterations thought to reflect structural adaptation are present in prehypertensive SHR. Lais and Brody (1978) observed that the isolated hindlimb of 3 week old SHR hyper-reacts to NE contraction when compared to WKY. Since qualitatively the same response could be produced by $BaCl_2$, a non-receptor mediated form of contraction, it is unlikely that the hyper-reactivity observed was due to an altered adrenergic receptor efficiency. In other studies by Touw et al (1980), the renal vasculature, but not the mesenteric or hindlimb vasculature, was found to react in an exaggerated manner in response to NE in 8 week old incipient hypertensive SHR when compared to WKY. On the other hand, Collis et al (1980) failed to observe any reactivity alterations to infused NE when the renal vasculatures of 6 week old incipient hypertensive SHR and age matched WKY were compared.

3. The effect of antihypertensive treatment on structural adaptation

3. (a) Large arteries of SHR

In studies performed by Andersen and Brown (1980), hypertension was prevented from occurring by treating SHR with a combination of hydrochlorothiazide, reserpine and hydralazine from 5 to 30 weeks of age. Such treatment lowered the blood pressure of SHR to that present in untreated WKY. However, treated WKY had a slightly lower blood pressures than that present in the above groups. When wall thickness was measured, treated SHR and untreated WKY exhibited similar wall thickness, while treated WKY had thinner walls than the above groups. Data showing the wall thickness of untreated SHR was not presented. However, other work by Andersen et al (1980) has shown that wall thickening occurs after 8 weeks of age in incipient hypertensive SHR. In view of this, it appears that the presence of a thickened vascular wall in the aorta of SHR could be an alteration secondary to hypertension.

3. (b) Intermediate and small arteries of SHR

In a study carried out by Mulvany et al (1981b), 6-OHDA was used to chemically sympathectomize both SHR and WKY from birth. This was achieved by injecting 6-OHDA into the animals every second day from birth to 3 weeks of age. At 24 weeks of age, SHR were normotensive (MAP, 103 mm Hg), but had a small, but significantly, elevated blood pressure over WKY (MAP, 95 mm Hg). Both sympathectomized and control SHR had approximately a 21% thicker vascular wall than, respectively, treated and control WKY. It was concluded that wall hypertrophy was due to intrinsic differences in the properties of SHR vessels and was not a

secondary alteration with respect to blood pressure.

Scott and Pang (1983 b) injected 2 day old SHR and WKY with capsaicin. Such treatment reduced the mean blood pressure of both 12 week old SHR and WKY to a similar degree (35 mm Hg and 29 mm Hg, respectively) over untreated SHR and WKY controls. In spite of exhibiting a lower blood pressure, both treated SHR and WKY displayed a thicker premucosal jejunal vascular wall than their untreated control counterparts. Furthermore, treated SHR and untreated WKY had similar blood pressures (101 ± 5 vs 91 ± 5 mm Hg, respectively); however, the vascular wall of treated SHR was still 55% thicker than that present in untreated WKY. It was concluded that vessel wall hypertrophy in the jejunal arteries of SHR is being produced by some factor other than high blood pressure.

Other studies involving the effect of antihypertensive treatment on structural adaptation provide a less clear picture as to the importance of high blood pressure in SHR. Mulvany et al (1981a) used felodipine to reduce the blood pressure of SHR from 6 to 12 weeks of age. In this instance, treated SHR and WKY had similar wall thicknesses. However, examination of the data indicates that antihypertensive treatment did not decrease wall thickness in SHR, but rather the wall thickness was increased in WKY. Furthermore, nontreated control SHR used in this experiment were, in fact, normotensive (MAP 121 mm Hg) at 12 weeks of age. In addition, felodipine produced only a marginal decline in blood pressure (i.e., 10 mm Hg), and the difference in blood pressure between treated SHR and WKY, as opposed to control SHR and WKY, was not particularly different (16 mm Hg vs 26 mm Hg;

respectively).

3. (c) Perfusion studies of the SHR vasculature

Folkow et al (1972) immunosympathectomized both SHR and NCR from birth with nerve growth factor antisera. At 32 weeks of age, sympathectomized SHR and NCR and control SHR and NCR had, respectively, the following average blood pressures, 139, 113, 210 and 139 mm Hg. Sympathectomy reduced the BP of SHR to normal, however, treated SHR still had significantly higher blood pressures than treated WKY. Experiments involving the isolated perfused hindlimb vasculature indicated that sympathectomized SHR with normal blood pressure still exhibited a higher level of maximal contractile reactivity than either normal or sympathectomized WKY. However, the reactivity observed was lower than that present in normal SHR. Thus it appeared that a severe lowering of blood pressure did partially, but not totally, attenuate the response thought to be produced by structural adaptations of the vascular wall.

In a study performed by Weiss and Lundgren (1978), SHR were treated from weaning to 10 months of age with one of metroprolol, hydralazine, propranolol and hydralazine plus guanethidine in combination. All the above drugs maintained the blood pressure at normal levels that were slightly higher than those of untreated WKY. Using a perfusion system analagous to that of Folkow et al (1972), treatment was found to lower the maximal contractile reactivity of the isolated perfused hindlimb of SHR. However, treated SHR still exhibited a higher vascular reactivity than untreated WKY, more so than would be expected based on the differences in blood pressure between the two

groups.

Both Folkow et al (1972) and Weiss and Lundgren (1978) have suggested that SHR resistance vessels may, for genetic reasons, be more prone to adapt to elevated pressure loads than normotensive animals; i.e., a very small elevation in blood pressure may produce a large structural alteration. However, in Folkow et als (1972) study, immunosympathectomized SHR had exactly the same MAP as untreated WKY, yet hindlimb reactivity was still higher in the former group.

4. Conclusion

There is no doubt that high blood pressure is capable of producing vessel wall hypertrophy. The hindlimbs of SHR can be protected from structural adaptation by clamping the terminal aorta and producing regional hypotension. Furthermore, under in vivo conditions, parameters that suggest an elevation in blood pressure are elevated in proportion of the degree of wall tension. In addition, in large arteries of SHR, such as the aorta, if wall thickening is observed, it occurs after the elevation of blood pressure and can be reversed by antihypertensive treatment. There is, however, experimental evidence that hypertrophy of the vessel wall, particularly in intermediate sized muscular arteries of SHR, can occur independently of hypertension. Morphometric studies of vascular beds from SHR indicate that vascular wall hypertrophy is present prior to the elevation of blood pressure, or alternatively occurs at the first sign of high blood pressure development. Similarly, neonatal antihypertensive treatment of SHR by sympathectomizing the animals with nerve growth factor antisera or by treating SHR neonatally with capsaicin lowers blood pressure, but does

not prevent the occurrence of vessel wall hypertrophy in the mesenteric arteries. All the above studies suggest that some factor other than high blood pressure is capable of inducing vessel wall hypertrophy.

Data obtained from perfusion studies involving isolated vascular beds is in part consistent with the above observations obtained from morphometric studies. In some instances, such as experiments by Lais and Brody (1978), an increase in vascular contractile reactivity has been observed in 3 week old prehypertensive SHR. Other perfusion studies involving the hindlimb vasculature of SHR treated from weaning to adult stages with antihypertensive drugs, or alternatively, immunosympathectomized SHR, have indicated that the normalization of blood pressure partially, but not totally, reduces the elevated maximal contractile reactivity observed in SHR. However, in all these studies it has been assumed that the differences in reactivity observed in response to contraction are totally produced by structural alteration. This may not be the case. Functional alterations in SHR arteries could result from the treatment. For example, denervation of arteries could alter sensitivity of the vascular bed to NE contraction, while long term treatment with antihypertensive drugs could lead to the accumulation of the drug in the tissue, which, in turn, could alter SMC contraction. Furthermore, as discussed previously, there are researchers that have suggested that the presence of mechanisms other than wall thickening could produce hyper-reactivity in SHR vasculature (e.g., Hermsmeyer, 1976 a; Daniel, 1981). Therefore, the morphometric evidence substantiating the presence of a thickened vascular wall is more substantial than the indirect evidence obtained from perfusion studies.

J. Overall Summary and Conclusions

SHR best define a subdivision of essential hypertensive patients that exhibit 1) a family history of hypertension 2) hypertension associated with low or normal plasma renin levels 3) a normal or hyperactive basal sympathetic nervous system that hyper-reacts to environmental stimuli and 4) hypertension that is responsive to antihypertensive therapy by hydralazine and angiotensin converting enzyme blockers and relatively unresponsive to diuretics, reserpine or guanethidine. Hypertension in SHR is initiated and maintained by an elevation in total peripheral resistance within the animals. Research in this area has centered around the possibility that the elevation in vascular resistance is produced by neurogenic and/or vascular myogenic alterations.

1.) What is the role of the sympathetic nervous system (SNS) in initiating and maintaining hypertension in SHR?

The SNS is important in the initiation of hypertension in SHR. Studies involving the measurement of plasma dopamine beta hydroxylase (DBH) an indicator of sympathetic nerve activity (SNA) and direct recordings from renal nerve bundles indicate that SNA begins to elevate in SHR when blood pressure starts to rise, while neonatal sympathectomy (6-hydroxydopamine (6-OHDA) growth factor antisera + guanethidine treatment) can prevent hypertension development.

There is controversy as to whether an overactive SNS maintains hypertension in adult SHR. Adult SHR and WKY have similar levels of DBH in the plasma. Furthermore, severe chemical or immunological

perturbations of the SNS produce only a modest drop in the blood pressure of SHR during the established phase of hypertension. However, direct measurements of renal and abdominal SNA have indicated that the firing frequency is increased in SHR. Studies performed by Judy et al (1976, 1978) have shown that the level of renal nerve activity present in SHR and WKY is directly related to the blood pressure present within the animals. In these experiments, manipulations which raised and lowered the blood pressure of the animals were associated with, respectively, an increase and decrease in renal nerve activity.

A possible explanation of the above conflicting results could be that the overall (average) SNA in adult SHR and WKY is similar; however, the SNA may be selectively elevated in the renal vasculature. Such a focused change could facilitate the maintenance of hypertension. Consistent with this view, renal denervation retards hypertension development in SHR.

The SNS could exert a trophic influence on the vasculature of SHR. Bevan (1975) has shown that sympathectomy can retard smooth muscle cell (SMC) replication in blood vessels. An overactive SNS in young SHR could accelerate SMC replication, causing the vascular wall to thicken and permanently maintain hypertension in SHR. On the other hand, Abel and Hermsmeyer (1981) have provided evidence that an intact SNS is essential for certain membrane defects to develop in the vascular SMCs of SHR (discussed later). Hermsmeyer (1976a) has suggested that when such alterations are present they promote increased responsiveness to norepinephrine (NE) which could elevate the vascular resistance. Although both the above theories can be critisized, they do suggest

mechanisms that could explain how the SNS could initiate hypertension in SHR without being involved in hypertension maintenance.

Other work has centered around the possibility that the quantity of vascular sympathetic innervation and the presynaptic control of NE release are altered in the SNS of SHR. Studies involving the cerebral and mesenteric vasculature indicate that certain classes of arteries in SHR exhibit hyperinnervation when compared with WKY. Perfusion studies performed on the mesenteric vasculature have indicated that the release of NE in response to nerve stimulation is increased while the reuptake of NE is decreased in SHR. In these studies it was shown that the inhibition of NE release mediated by presynaptic adenosine receptors is decreased in SHR while the presynaptic facilitation of NE release by $beta_2$ and A_{II} receptors is increased in SHR. The augmented release of NE in response to nerve stimulation, and the decreased presynaptic inhibition of NE release by adenosine (which could be co-released with NE) could elevate the mesenteric vascular resistance in SHR. However, it is not clear whether such alterations are present in other vascular beds. For example, in the renal vasculature of SHR, both an augmented and decreased neuronal release of NE has been observed while the presynaptic control of NE release favors a decrease in NE levels within the synapse in SHR.

2.) <u>What is the role of the central nervous system (CNS) in the initiation and maintenance of hypertension in SHR?</u>

The presence of an intact CNS is essential for hypertensionn development in SHR. The injection of 6-OHDA or 5, 6 dihydroxytryptamine (5, 6 DHT) into the brain ventricles of young SHR depletes the brain and

spinal cord of, respectively, catecholamines and serotonin and prevents the subsequent development of hypertension. However, the injection of 6-OHDA into the spinal cord or the injection of 6-OHDA or 5, 6 DHT into the third ventricle of adult SHR doesn't significantly alter blood pressure. The results indicate that intact brain catecholamine and serotonin tracts, but not spinal catecholamine tracts, are essential for hypertension to develop. However, once hypertension has been established, central catecholaminergic and serotonergic nerves aren't required to maintain hypertension. In adult SHR, experiments have indicated that lesions of the posterior hypothalamus produce a hypotensive effect that is greater in SHR than WKY while electrical field stimulation of this area increases the blood pressure in SHR to a greater degree than WKY.

A number of questions remain as to the role of the CNS in initiating and maintaining hypertension. The specific neuronal tracts thought to be involved in the initiation of hypertension are unknown. Furthermore, at present it is unknown whether alterations in CNS activity play a primary role in initiating hypertension or alternatively act as essential connections between other mechanisms that are primarily altered and in turn responsible for the establishment of hypertension.

3.) <u>What role do hormonal alterations play in the initiation and maintenance of hypertension in SHR?</u>

There is no evidence that hormonal alterations play a role in either initiating or maintaining hypertension during the early established phases in SHR. However, during later phases of established hypertension, SHR do exhibit elevated plasma levels of prolactin and

vasopressin. During this stage of hypertension development bromocryptine treatment (which lowers plasma prolactin) or the injection of vasopressin antisera lowers the blood pressure of SHR. It has been hypothesized that prolactin could act as an antidiuretic and as well potentiate NE contractile responses of vascular SMCs, while vasopressin in addition to acting as an antidiuretic could directly contract the vasculature. If the above actions of prolactin and vasopressin occurred in vivo in SHR, they could promote salt and water retention and increase vascular tone, thus aggrevating hypertension.

4.) What role do post synaptic alterations in vascular SMCs play in the initiation and maintenance of hypertension?

a.) Is the arterial sensitivity and reactivity in response to NE contraction altered in SHR?

A great deal of research has been performed to determine whether arteries from SHR 1) initiate contraction at lower doses of NE (increased NE sensitivity) and/or 2) are capable of generating greater maximal tensions that normal arteries (increased NE reactivity). The results obtained depend on the type of system used to study the arteries, the type of artery studied, the way the data was expressed and the conditions under which the experiments were performed. Studies involving dose-tension relationships using equal length helical strips of arteries have been performed using large elastic arteries (i.e., aorta, femoral and carotid artery). Most of these studies have indicated that NE contractile sensitivity and reactivity is either unchanged or decreased (i.e., higher ED_{50} values and lower maximal tension development) in SHR compared to WKY. Tension studies involving

equal length arterial rings of small muscular mesenteric arteries have indicated that maximal tension development (reactivity) in response to NE is greater in SHR than WKY. The increased reactivity observed in SHR in these experiments appeared to be produced primarily by the presence of a thickened vascular wall. In these studies, NE sensitivity was increased in the arterial segments sampled from SHR, but only under conditions where the neuronal uptake of NE was blocked with cocaine. Perfusion studies involving entire vascular beds indicate that at constant perfusion, maximal NE contraction almost always produces a larger amplitude of vascular resistance change in SHR than WKY, while NE sensitivity is either unaltered or increased in SHR compared to WKY.

In conclusion, there is evidence that vascular reactivity is increased the blood vessels of SHR; on the other hand, NE sensitivity is most often either unaltered or increased in SHR. In instances where the NE contractile sensitivity is increased, such an alteration could (in conjunction with an elevated reactivity) increase vascular resistance; however, such a finding is not consistently observed in the arteries of SHR.

b.) <u>Are the densities of arterial adrenergic receptors altered in SHR?</u>

There is evidence from a limited number of studies that plasma membrane or microsomal fractions obtained from the arterial tissues of SHR contain unaltered $alpha_1$, increased $alpha_2$ and decreased beta receptor densities. At present the physiological significance of such alterations are unknown. Under conditions of maximal nerve stimulation many vascular beds do not exhibit an $alpha_2$ receptor mediated

contractile response. Furthermore, the presence of beta receptor antagonists does not alter nerve mediated responses.

c.) Is the membrane potential altered in the vascular SMCs of SHR?

Experiments performed by Hermsmeyer (1976a) have indicated that under resting conditions at 37°C the membrane potential of vascular SMCs of the tail artery is equal in SHR and WKY. However, in SHR two apposing defects were found to maintain a normal membrane potential. The Na^+/K^+ pump was hyperactive resulting in the electrogenic portion of the membrane potential to be greater in SHR than WKY, while under conditions where the Na^+/K^+ pump was inhibited the non electrogenic portion of the membrane potential was more depolarized in SHR than WKY. Hermsmeyer (1976a) hypothesized that during NE contraction membrane permeability was increased in vascular SMCs to the extent where the Na^+/K^+ pump is no longer an important determinant of the membrane potential. Under such conditions contraction was governed by the nonelectrogenic portion of the membrane potential. Since this was less negative in SHR than WKY such an alteration would lead to an increase in NE contractile response in the vascular SMCs of SHR.

Other experiments performed by Abel and Hermsmeyer (1981) indicate that the membrane defects discussed above can be transmitted to normal WKY arteries by incubating them in the anterior eye chamber of SHR. However, if the eye chamber is sympathectomized, SHR loose their ability to alter the arteries transplanted from WKY. These experiments suggest that the SNS of SHR exerts a trophic influence altering the membrane properties of VSMCs.

d.) Is the Ca^{+2} pump altered in the vascular SMCs of SHR?

Studies have indicated that the activity of the ATP dependent Ca^{+2} pump responsible for lowering intracellular Ca^{+2} levels in vascular SMCs is reduced in SHR. Such an alteration could 1) increase the basal vascular tone of the VSMCs 2) increase contraction in response to agonists or 3) decrease relaxation responses. Kwan et al (1980a) have shown that such alterations present prior to hypertension development in SHR and are present in a variety of other forms of hypertension. This suggests that a decrease in the Ca^{+2} efflux from the vascular SMCs of SHR may be of primary importance in the establishment of hypertension in SHR and other forms of hypertension.

e.) Is the adenylate cyclase system altered in SHR?

Studies performed on the arteries of SHR in a prehypertensive, developing or established phase of hypertension development indicate that the basal levels of vascular SMC cAMP are reduced in SHR over normotensive controls. Such alterations are likely of genetic origin since they also occur in cultured aortic SMCs of SHR. Two other key alterations in the adenylate cyclase system exist in the vascular SMCs of SHR. Isoproterenol (but not NaFl or glucagon) stimulation of arterial homogenates stimulates adenylate cyclase to a lesser degree in SHR than WKY. This alteration could be due to a reduction in the beta receptor densities observed in the vascular SMC membranes of SHR. Finally, cAMP dependent protein kinases isolated from SHR exhibit a reduced ability to phosphorylate added histones. Since in most arteries increases in intracellular cAMP relax vascular SMCs, a decrease in cellular cAMP in conjunction with a reduced ability of cAMP to activate

protein kinases could increase SMC tone and raise vascular resistance.

f.) What role do structural alterations play in the maintenance of high blood pressure in SHR?

A variety of structural alterations have been observed in the vascular beds of SHR. Perfusion studies performed by Folkow and his colleagues (Folkow et al. 1969, 1970a, 1970b, 1973) have indicated that under relaxed conditions the total systemic and hindlimb vascular resistance are increased in SHR over normotensive rats. This suggests that the average lumen diameter of the vasculature and/or the density of the blood vessels is decreased in SHR. However, not all vascular beds are altered in the same manner. For example, morphometric and/or perfusion studies performed on the relaxed renal and mesenteric vasculature indicate that the lumen diameter and vascular resistance are similar in SHR and WKY while measurements made under in vivo conditions indicate that the lumen diameter of cremaster and gracilis muscle blood vessels are increased in SHR.

Studies performed on the cremaster and gracilis muscle as well as the abdominal and mesoappendix vasculature have indicated that the anatomical number of precapillary arterioles is decreased in SHR compared to WKY. In addition in vivo both the cremaster and gracilis muscle vasculature of SHR exhibit an increased function closure of the vasculature to blood flow. Both the above types of changes have been observed in prehypertensive as well as adult SHR suggesting that these alterations are not a secondary modification resulting from the presence of high blood pressure.

A variety of researchers have observed that the vascular wall is

thickened in SHR. The major site of alteration appears to be within the media of the blood vessels which in SHR exhibit a greater cross-sectional area of SMCs. Studies involving mesenteric arteries have indicated that the number of medial SMCs are increased in SHR. On the other hand, work by Owens et al (1981) involving the aorta has shown that in this blood vessel, the size but not the number of SMCs is increased in SHR.

The majority of evidence indicates that wall thickening in large elastic arteries such as the aorta and superior mesenteric artery occurs after hypertension development in SHR. Furthermore, studies involving the aorta have shown that this alteration can be prevented by antihypertensive therapy started from an early age. In small mesenteric arteries the thickening of the vascular wall occurs prior to hypertension and is not prevented from occurring when the blood pressure of SHR is normalized by neonatal sympathectomy or capsaicin treatment.

Some classes of arteries do not exhibit a thickened vascular wall in SHR. The vascular walls of the arteriolar and pre-arteriolar blood vessels of the cremaster and gracilis muscle vasculature are not thickened in SHR. In the mesenteric and mesoappendix vasculature, the presence of a thickened vascular wall disappears as the vasculature is traced towards the capillary.

The consequence of a thickened vascular wall is two fold. If the vascular wall is thickened towards the lumen (a situation which may occur in the hindlimb vascular bed) this could increase the vascular resistance. Secondly, a thickened vascular wall could cause an artery to hyper-react to contractile stimuli and thus further increase the

vascular resistance. Since in certain arteries such alterations take place just prior to hypertension development they could play a primary role in the initiation of hypertension.

Biochemical analysis performed predominantly on large elastic arteries have indicated that the synthesis and crosslinking of collagen as well as the collagen content (mg collagen/arterial length) but not usually the collagen concentration (mg collagen/dry weight) are increased in arteries sampled from SHR compared to WKY. Such alterations could decrease the distensibility of an artery causing it to maintain a smaller lumen diameter at a given transmural pressure and would facilitate the presence of an elevated vascular resistance. In this regard, mechanical studies performed on the aorta, tail and carotid arteries have shown that arterial distensibility is reduced in adult SHR compared to WKY. However, studies involving smaller mesenteric arteries have indicated that the alterations in lumen circumference in relation to tangential wall stress are similar in SHR and WKY.

The alterations in collagen content and/or synthesis in the arteries occurs after the elevation of blood pressure in SHR and the alteration in aortic distensibility present in SHR can be prevented by antihypertensive therapy. These results suggest that such alterations are a secondary modification that develop in SHR as a consequence of high blood pressure.

Chapter 2

Structural and functional alterations in the renal vasculature during the development of hypertension in Kyoto Wistar spontaneously hypertensive rats

Introduction

In view of the importance of the kidney in renal forms of hypertension many researchers have argued that in all forms of hypertension renal function must be reset in order for hypertension to develop (Tobian, 1974; Cowley, 1980; Guyton et al., 1981). According to Tobian (1974), hypertension will not develop unless renovascular resistance is increased, regardless of changes in resistance in other vascular beds, since a natriuretic response by the kidney will normalize blood pressure. Consistent with this view, renal vascular resistance (RVR) in SHR is increased (DiBona and Rios, 1978; Arendshorst and Beierwaltes, 1979; Hsu et al., 1982), and occurs at a very early age; at 8 weeks of age the RVR of conscious SHR is twice that of WKY (Hsu et al., 1982). This suggests that resetting of the renal vasculature may be of primary importance in establishing high blood pressure.

Although there is agreement that RVR is increased in SHR, the mechanisms producing such an alteration are in dispute. Some researchers have argued that there is an intrinsic defect in the kidney. In this regard, Kawabe et al (1978) found that blood pressure is dramatically elevated when the kidneys of SHR are transplanted into the

F_1 generation of a WKY-SHR cross (SHR x WKY). On the other hand, when the kidneys of either WKY or WKY x SHR were transplanted into the WKY x SHR, the blood pressure was not altered. Work by Folkow and his colleagues indicates that the isolated kidneys of SHR require a much higher perfusion pressure to maintain the same glomerular filtration rate as WKY under conditions where the vasculature is maximally relaxed (Folkow et al., 1977). Furthermore, during contraction by norepinephrine, the vascular resistance increases in SHR kidneys to a greater degree than in WKY (Folkow et al., 1971 a). All the above experiments suggest that the kidney of SHR has either a structural and/or a functional defect that could restrict blood flow through, and glomerular filtration in, the kidney, not unlike a clamp in Goldblatt forms of hypertension (Coleman et al., 1974).

Work by Vanhoutte and his colleagues (Collis and Vanhoutte, 1977; Vanhoutte, 1981) disputes the importance of intrinsic renal defects in the maintenance of hypertension in SHR. Although the renal vasculature of adult SHR exhibited an increased sensitivity and reactivity to norepinephrine contraction over WKY, when contraction was produced by stimulating the renal nerves, both SHR and WKY produced similar RVR responses (Collis and Vanhoutte, 1977). It was hypothesized that an increase in the rate of discharge of renal nerves in SHR or some external humoral influence, but not an intrinsic defect in the kidney, was responsible for elevating RVR. On the other hand, Ekas and his colleagues found that in isolated kidneys, the RVR responses were greater in SHR than WKY during equal degrees of nerve stimulation (Ekas et al., 1983 b).

Another point of view has been presented by Arendshorst and Beirewaltes (1979). In anaesthetized intact SHR, the site of increased RVR was found to be in preglomerular arteries. Clamping the aorta proximal to the renal arteries and thus reducing the renal arterial pressure to normal, reduced the RVR to values present in WKY. It was suggested that the elevated RVR in SHR was not produced by a fixed defect in the kidney, humoral factor, or a constant alteration in renal nerve activity or function, but rather, SHR exhibited an exaggerated autoregulatory response to an elevated mean arterial pressure. At high blood pressures the SHR kidney maintained a normal blood flow by elevating RVR. However, DiBona and Rios (1978), using similar techniques, found that normalizing the renal arterial blood pressure did not alter the elevated RVR in SHR.

Objectives, Rationale and Approach of the Present Study

Overall Objective

The principal aim of the present study was to investigate the intrinsic alterations in renal vascular structure and function in SHR, and to assess the role that these alterations play in the development and maintenance of hypertension.

Background

Folkow and his colleagues (Folkow et al., 1973) have suggested that vascular resistance in SHR increases because the vascular wall is thickened and encroaches upon the lumen. This hypothesis was based on indirect evidence obtained from perfusion studies of the isolated vascular beds of SHR. Such an alteration could elevate the vascular resistance even when the blood vessels are maximally relaxed, and cause a hyperocclusion of the lumen with a marked increase in resistance during contraction.

Various morphological studies have been undertaken to determine the nature of the structural alterations involved in producing a thickened vascular wall. Boyd (1980) suggested that during hypertension the endothelial cells of arteries were damaged and the replacement of new endothelium was associated with a thickening of the subendothelial space which encroached upon the lumen. Biochemical (Iwatsuki et al., 1977; Erhart and Ferrario, 1981) and morphometric (Lee et al., 1983 b) studies have indicated that collagen and/or elastin synthesis and content are increased in arteries sampled from SHR. Such alterations have the potential to 1) decrease the distensibility of an artery and

therefore cause the artery to maintain a smaller lumen at a given transmural pressure and or 2) thicken the vascular wall by increasing the cross-sectional area occupied by the internal and external elastic laminae and the medial extracellular space. Other researchers have argued that alterations in the intimal and medial extracellular space play a minor role in promoting blood vessel wall thickening in the arteries of SHR and have suggested that the primary change is in the quantity of smooth muscle cells (SMCs) within the arteries (Lee et al., 1983 b). In this regard, Owens and his colleagues (Owens et al., 1981), based on their studies of the aorta, have argued that the size but not the number of SMCs is increased in SHR. Lee et al (1983 b), on the other hand, have shown that increase in SMC number but not size is the predominant change which occurs in the mesenteric arteries of SHR.

Other research has focused on the adventitia of blood vessels. Saito and Lee (1982), Scott and Pang (1982) and Lee et al (1983 b) have shown that the quantity of sympathetic innervation within the adventitia is increased in both the cerebral and mesenteric vasculature of SHR. Increased innervation of arteries could increase the contractile response obtained during nerve stimulation and therefore maintain hypertension.

The structural studies that have been performed on the renal vasculature of SHR are limited in terms of the information that was obtained concerning the intrinsic structural defects present in the kidney. Mandal et al (1977) and Limas et al (1980, 1983) studied the intrarenal vessels of SHR. In these studies, instillation fixation was used to prepare the arteries for light and or electron microscopy. This

method of fixation contracts the renal arteries. Furthermore, in these studies compensation for section angle of arteries was not done. Although both studies concluded that vascular walls of the renal vessels were thickened and the lumen diameter was reduced, the results did not adequately support these conclusions. For example, in an extensive study performed by Limas et al (1983), the ratio of the blood vessel external to internal diameter (E/L) was measured in renal arteries having a lumen diameter of 30 to 50 µm and 50 to 100 µm. Since the arteries were in various states of contraction the range of overlap in E/L ratios between arteries sampled from SHR and WKY was so great that there was no statistically significant difference ($P<0.05$) between the groups. Their conclusion that the vascular wall was thickened in the renal vessels of SHR was based on the fact that SHR appeared to have more renal arteries with a larger E/L ratio than WKY. Studies performed by Mandal et al (1977) provide even more limited information since no measurements of arterial dimensions were made and their conclusions were based only on visual assessment.

In a study performed by Nordborg and Johansson (1979) an attempt was made to compensate for the degree of contraction present in the renal vasculature. The length of the internal elastic laminae (IEL) of contracted intrarenal vessels were measured and the dimensions of the renal arteries were calculated under a condition where the wall of the arteries was evenly distributed (mathematically) around a maximally expanded IEL. Using this form of compensation, the vascular wall of renal arteries having a lumen diameter between 100 and 150 µm was thickened in 15 day old, but not 200 day old SHR when compared to age

matched WKY. However, recent studies by Lee et al (1983 c) have criticised the histometric technique used by Nordborg and Johansson (1979) to compensate for contraction. In these studies, it was shown that the IEL of arteries shortens during contraction and does so to a greater degree in blood vessels sampled from SHR than those from WKY. Therefore, the method of correction used by Nordborg and Johansson (1979) underestimates the lumen diameter, and does so to a greater degree in contracted blood vessels of SHR than WKY.

In studies carried out by Pang and Scott (1981), the main renal artery of a variety of age groups of SHR and WKY was perfusion fixed under relaxed conditions. No differences in wall thickness or lumen diameter were observed when SHR and WKY were compared. Unfortunately, none of the intrarenal blood vessels were studied. The main renal artery which was studied is a large elastic artery that differs both in physiological function and morphological characteristics from the small muscular arteries thought to be important in the control of blood pressure. Therefore, the lack of alteration in this large vessel during hypertension may not be representative of other more distal portions of the renal vascular tree.

A. <u>A morphometric study of the renal vasculature of SHR in an established phase of hypertension</u>

In view of the inadequacy of information on the structural alterations in the renal vasculature associated with hypertension in SHR, the following study was undertaken.

The renal vasculature of SHR in an established phase of hypertension was perfusion fixed at maximal relaxation and processed for

light and electron microscopic examination. Morphometric measurements were performed on the renal vasculature extending from the main renal artery to the preglomerular arterioles and compared to similar measurements obtained from the kidneys of age matched WKY. The diameter of vessel lumen, the cross-sectional area of the arterial intima, media, adventitia and the major subcomponents of these fractions (endothelium, subendothelial space, IEL, SMCs, medial extracellular space, collagen, nerve axons, nerve sheath cells, fibroblasts and fluid filled space) were determined. The cross-sectional area of each of the major subcomponents was compensated for sectioning angle. The aims of this aspect of the study are outlined below.

General objective

To determine if structural alterations conducive to the maintenance of high blood pressure are present in the renal vasculature of SHR in an established phase of hypertension.

Specific questions

a) Are the lumen diameters of the relaxed renal arteries decreased in SHR?

b) Is the vascular wall thickened in the renal arteries of SHR?

c) If the blood vessel wall is thickened in SHR, what arterial components are responsible for producing such alterations?

d) Are all the arteries of SHR affected in a similar manner, or do structural alterations occur only in arteries of a certain size and or location?

e) Is there increased innervation of the renal arteries of SHR ?

B. A morphometric study of the renal vasculature of prehypertensive SHR

The morphometric analysis from the above study (A) indicated that the prearteriolar blood vessels of SHR in an established phase of hypertension were thickened primarily as a result of an increase in the cross-sectional area of medial extracellular space and the presence of SMC hypertrophy and or hyperplasia. The lumen diameter of the renal arteries was not altered when SHR and WKY were compared. This led to the question as to whether such changes are a secondary alteration resulting from the elevation of blood pressure or, alternatively, if such changes could be important in the initiation of hypertension in SHR. If the latter case is true, then wall thickening of the renal vasculature could precede the elevation in blood pressure observed in SHR. In view of this, morphometric analysis at the light microscopic level was performed on the renal vasculature of 4 to 5 week old prehypertensive SHR (one to two weeks prior to hypertension development) and age matched WKY. The aims of this study are outlined below.

General objective

To determine if blood vessel wall thickening precedes hypertension development in SHR.

Specific questions

a) If renal blood vessel wall thickening does occur prior to high blood pressure development in SHR, what layers are altered?

b) Are all classes of arteries affected equally, or is wall thickening initiated in a particular category of renal artery?

C. A physiological and pharmacological study of the isolated kidney of SHR in established and prehypertensive phases of hypertension development

Prehypertensive SHR (study B) were found to have similar alterations in the renal vasculature as those present in SHR with established hypertension. The lumen diameter of the renal arteries was not different when SHR and WKY were compared. However, the wall (intima + IEL + media) to lumen ratio was increased in every class of renal artery sampled from SHR. Further, nearly all the renal arteries sampled from prehypertensive SHR had greater numbers of SMC layers within the media. Quantitatively, the magnitude of wall thickening was smaller in prehypertensive SHR than in SHR with established hypertension. However, the fact that this alteration was present one to two weeks prior to hypertension development suggests that such changes could be important in the initiation of high blood pressure development. Based on the structural alterations observed in the morphometric studies, a model is presented hypothesizing that such changes would produce a situation where, at maximal relaxation, the RVR would be similar in SHR and WKY; however, equal degrees of SMC contraction would occlude the lumen of the SHR vessels, and therefore raise the RVR, to a greater degree than in WKY.

Subsequently, pharmacological and physiological experiments were carried out to see if the renal vasculature responded in a manner consistent with the proposed model. Alterations in RVR in response to norepinephrine (NE) contraction were determined. If structural changes are important in altering RVR response, then various types of infused vasoconstrictors as well as contraction by nerve stimulation should

raise the RVR to a greater degree in SHR than WKY (i.e., hyper-reactivity). Thus, alterations in RVR response to contraction by $BaCl_2$, KCl, angiotensin II (A_{II}) in adult SHR, and renal nerve stimulation in the presence and absence of blocked neuronal uptake system in old and young SHR were determined. Finally, receptor antagonists were used to determine the type of receptors involved in producing nerve stimulated contractile responses and the proportion of the response attributed to each receptor type. The aims of this aspect of the study are outlined below.

General objective

To determine if the isolated left kidney of established and prehypertensive SHR exhibits an unaltered RVR under maximally relaxed conditions and hyper-reactivity in response to contraction as predicted by the structural alterations observed in the morphometric study.

Specific questions

a) Under maximally relaxed conditions, are the RVRs of the isolated left kidneys of established and prehypertensive SHR similar to those of age matched WKY?

b) Does the renal vasculature of prehypertensive and established hypertensive SHR exhibit hyper-reactivity in response to infused NE?

c) If hyper-reactivity to NE is present, is it produced by geometric alterations in the vascular wall of SHR, or alternatively, are such alterations specific only to NE contraction?

d) Does the renal vasculature of established and prehypertensive SHR hyper-react in response to periarterial nerve stimulation?

e) What receptors are involved in mediating nerve stimulated contraction of the renal vasculature? Is the proportional response attributed to each receptor subtype different in SHR when compared to WKY?

f) Are the threshold or ED_{50} values for NE contraction of the renal vasculature altered in SHR when compared to WKY?

D. The effect of in utero and post natal normalization of blood pressure on the renal vascular structural changes in SHR

Until recently, it has been a general conclusion that blood vessel wall thickening in hypertension is a pathological alteration produced by high blood pressure (Folkow et al., 1973; Lundgren 1974; Weiss, 1974). The findings of the morphometric studies on the renal vasculature of prehypertensive SHR and age matched WKY in the present thesis are inconsistent with this view. Blood vessel wall thickening was found to take place in the renal arteries prior to increase in blood pressure, suggesting that such alterations are not secondary during development of hypertension. However, arguments have been made that there is in fact no prehypertensive phase in SHR, and that SHR are born with an elevated blood pressure which in some cases required special techniques to detect (Gray, 1982). It could be argued that such episodes of high blood pressure could establish renal vascular structural changes even before high blood pressure is detected.

To test this hypothesis, female SHR were treated with hydralazine and maintained normotensive prior to and during pregnancy. Since

hydralazine passes the placental barrier and is present in the fetal and maternal circulation in similar quantities (Liedholm et al., 1982), *in utero* the fetus should experience the same hypotensive effect as the mother. After birth, until sampling at 21 weeks, SHR were treated daily with quantities of hydralazine required to produce normotension in SHR with established hypertension. Tail cuff blood pressure measurements from 3 weeks of age to the time of sampling indicated that the treated rats were in fact normotensive. Subsequently, the renal vasculature was perfusion fixed and processed for studies involving light microscopy. The renal vascular dimensions of treated SHR and WKY were compared with those present in untreated control groups. The aims of this study are outline below.

General objective

To determine if renal blood vessel wall thickening can be prevented in SHR by the *in utero* and postnatal normalization of blood pressure through antihypertensive therapy.

Specfic questions

a) What structural alterations within the renal vasculature of SHR are pressure dependent and pressure independent?

b) Do certain size classes of renal arteries in SHR exhibit pressure dependent wall thickening?

c) What effect does hydralazine treatment have on the renal vasculature of WKY?

d) Can the *in utero* and postnatal normalization of blood pressure in SHR, by hydralazine treatment, permanently lower blood pressure in SHR?

Material and Methods

A. Animals Used

SHR were taken from a colony that is maintained at McMaster University. This colony was originally started by breeding animals obtained from Ayerst Laboratories (Montreal). WKY were either bought directly from Canadian Breeding Farm (Montreal) or taken from a WKY colony at McMaster University that was derived from Canadian Breeding Farm animals. To avoid complications produced by hormonal fluctuations during the oestrus cycle, only male animals were studied.

Two age groups were studied; SHR in an established phase of hypertension, most of which were 21 weeks of age, and prehypertensive SHR 4 to 6 weeks of age. Both these groups were compared to age matched control WKY.

B. Method of Blood Pressure Measurement

A tail cuff compression method was used to measure awake blood pressure of virtually all the animals studied. The rat was warmed in a special rat restrainer (Rat Holder Temperature Control Unit, Mark IV). Subsequently, an inflatable cuff was placed around the base of the tail while distal to this point the sensor of a pneumatic pulse transducer was place over the dorsal tail artery. The pulse and heart rate were recorded using a physiograph (DMP-4A). A sphygmomanometer (Programmed Electrosphygmomanometer, PE300) was used to inflate the cuff until the pulse disappeared. Upon deflating the cuff, the pressure at which the

pulse reappeared was taken as the systolic blood pressure of the rat. A minimum of three readings were recorded for each rat. All components of the blood pressure measuring device are manufactured by Narco Biosystems Inc., Houston, Texas.

A direct method of blood pressure measurement was used on a group of 4-5 week old prehypertensive SHR (n=10) and age matched controls (n=10). This was done to confirm that in this age group SHR were prehypertensive, as suggested by the tail cuff measurements. The rats were anaesthetized with sodium pentobarbitol (i.p. 60 mg/kg). Subsequently, the aorta at the junction of the femoral artery was catheterized (0.021 in I.D. X 0.036 in O.D. catheter tubing). The systolic and diastolic pulse pressures were recorded via a Statham P23Db pressure transducer connected to a Hewlett Packard 7702B recorder.

C. Perfusion System

The same perfusion system was used to measure perfusion pressure and flow and to calculate renal vascular resistance (RVR) and to construct dose response curves and perform electrical stimulation studies.

Rats were anaesthetized with sodium pentobarbitol (i.p. 50 mg/kg) and the abdominal cavity opened. The adrenal and spermatic artery of the left kidney, and the main renal artery of the right kidney and the mesenteric arteries were tied. In the case of adult and young rats, a catheter (0.036" I.D. X 0.050" O.D. and 0.021" I.D. X 0.036" O.D., respectively) was inserted into the aorta caudad to the renal-aortic junction of the left kidney, in a manner that ensured continuous blood flow through the kidney. The segment of the aorta

proximal to the left renal artery was clamped and the left kidney perfused by a syringe pump (Harvard) with modified Krebs solutions (143 mM Na^+, 127 mM Cl^-, 5.3 mM K^+, 2.25 mM Ca^{+2}, 0.892 mM Mg^{+2}, 25 mM HCO_3^-, 1.16 mM PO_4^{+2}, 58.7 mM glucose, 1.5% dextran) made isotonic to rat plasma (340 mOsm) and oxygenated with 5% CO_2 in O_2. The renal vein was then severed allowing a free outflow of the perfusate. This enabled the perfusate to flow only through the left kidney. A second catheter was connected to a pressure transducer (Statham P23Db) from a T-junction about 4 cm upstream on the perfusate catheter. This allowed the perfusion pressure to be recorded on a Hewlett Packard 770 2B recorder. Since the outflow pressure from the renal vein was zero, the renal vascular resistance (RVR) could be calculated using the following formula:

$$RVR=(TP-CP)F$$

TP = total infusion pressure of catheter + kidney (mm Hg)

CP = infusion pressure exerted by the catheter when not connected to kidney (mm Hg)

F = flow rate (ml/min).

D. Renal Vascular Resistance vs Flow Measurements

The RVR at various perfusion rates was studied under conditions where the vascular bed was maximally relaxed. Initial experiments were done where the left kidney was perfused with Krebs solution containing 1) sodium nitroprusside (10 mg/L), 2) isoproterenol (10 mg/L), or 3) EGTA (5 mM). In adult SHR and WKY none of these vasodilating agents produced further relaxation as indicated by the presence of a drop in RVR. Likewise, prehypertensive SHR and WKY also exhibited similar RVR

in the presence and absence of sodium nitroprusside (10 mg/L). In all subsequent experiments RVR was determined at flow rates of 0.41, 0.82, 2.04 and 4.10 ml/min in adult rats, and 0.041, 0.082, 0.204, 0.41, 0.82, 2.04 and 4.10 ml/min in young rats.

E. Preparation of Kidney Tissues for Light and Electron Microscopy

Krebs solution (23°C) was perfused through the left kidney at a flow rate of 0.82 ml/min in older rats and 0.082 ml/min in young rats for 15 minutes after which the vascular resistance of the kidney was calculated. The kidney was then processed for light and electron microscopy using the protocol outlined below (Lee et al., 1980, procedure IV).

1. The kidney was perfusion fixed for 40 min at a flow rate of 0.82 ml/min (adult rats) or 0.082 ml/min (young rats) with a solution of 2.5% glutaraldehyde, 1.86% sucrose, 0.063 M KPO_4 buffer, pH 7.4, 400 mOsm.

2. The renal vessels were then perfused for 20 min with 0.200 M KPO_4 buffer (pH 7.4, 400 mOsm).

3. The main renal and dorsal central interlobar arteries, as well as cortical tissue containing arcuate, interlobular arteries and preglomerular arterioles were dissected out of the kidney.

4. The tissues were postfixed for 45 min by immersion in buffered glutaraldehyde, then washed four times with wash buffer, using the solutions described in 1 and 2 respectively.

5. The specimens were further fixed for 2 hours with 1% OsO_4 in 0.05 M Na cacodylate, 0.78% NaCl, (pH 7.4, 400 mOsm).

6. Subsequently, the tissues were stained for 2 hours in 0.5% uranyl acetate dissolved in distilled water. During the 2 hour period the

solution was changed once.

7. Following staining, the arteries were dehydrated successively in 70%, 80%, 95% and 5 times in 100% concentrations of ethanol, for 20 min at each step.

8. After dehydration, the tissues were infiltrated with Spurr's resin for three days, the resin being changed once every day.

9. Finally, the specimens were embedded in Spurr's resin and hardened for 24 hours at 73°C.

This method of tissue processing has been shown to produce minimal volume alterations in rat aortic vascular smooth muscle cells (Lee et al., 1980). Morphometrically determined cell volumes obtained from transmission electron micrographs indicate that the mean cell volume decreases only 1.86% over prefixation cell volumes as determined using a Coulter counting method. Furthermore, the buffered glutaraldehyde fixative (used in step 1) does not alter SMC tone when aortic strips attached to an isotonic tension monitoring device are fixed (Lee et al., 1981).

For light microscope measurements, 1 μm thick sections of the renal arteries were cut in cross-section, stained with 1% azure II-methylene blue dye in 1% Na borate and mounted on glass slides. Thin sections (600-800 Å) of each artery were cut using a glass knife in the same plane of section and then mounted on 200 mesh, 3 mm diameter grids. The sections were then stained with a solution containing 0.080 M lead nitrate, 0.120 M sodium citrate, 0.160 M NaOH in CO_2-free distilled water, pH 12. The stained sections were examined in a Phillips EM 301 electron microscope. Examination of the micrographs indicated a lack of

convolution of the internal elastic lamina confirming that the vessels were fixed under relaxed conditions.

F. Morphometry

1. Photography

Using a light microscope each artery was photographed on 35 mm film at 25x, 100x, 200x or 400x magnification, depending on the size of the vessel. In the case of the main renal artery additional photographs were taken of the vessel wall at 400x magnification. 35 mm transmission electron micrographs of the entire arterial intima + media , or a maximum of 10 random frames per artery, were taken at 550x magnification for interlobar arteries, and at 720x and 1000x magnification for cortical arteries. The adventitia of all arteries was photographed at 2000x magnification, ten random frames being taken for each artery. In the case of the main renal artery, due to the large wall thickness, the intima and media were photographed separately at 1300x and 550x, respectively, ten frames of each layer being taken. Electron micrographs of arterioles were photographed at 550x, 720x or 1000x magnification depending on the size of the arteriole. All 35 mm photographs taken using the light and electron microscopes were printed on 8" x 10" paper after a further 9 times enlargement. Micrographs of a slide micrometer and an etched carbon coated grid were used to determine the final magnification of, respectively, light and electron micrographs.

2. Morphometric measurements

The true cross-sectional area of the lumen, intima + media and adventitia were determined from low magnification light micrographs of

the entire artery using a multipurpose grid. Fig. 2 shows the type of grid used, superimposed on a light micrograph of an artery, and the same artery with the long (R_L) and short (R_S) radii of the axes labelled.

The following formula was used to calculate the cross-sectional area of the intima + media, adventitia and lumen from light micrographs and to compensate these dimensions for section angle.

$$Ac = ((Pi \times A_T) / P_{TG}) \times (R_S / R_L) \qquad (1)$$

Ac = cross-sectional area of an arterial component

Pi = number of points hitting the arterial component

P_{TG} = total number of test points on the grid

R_S = short radius of lumen

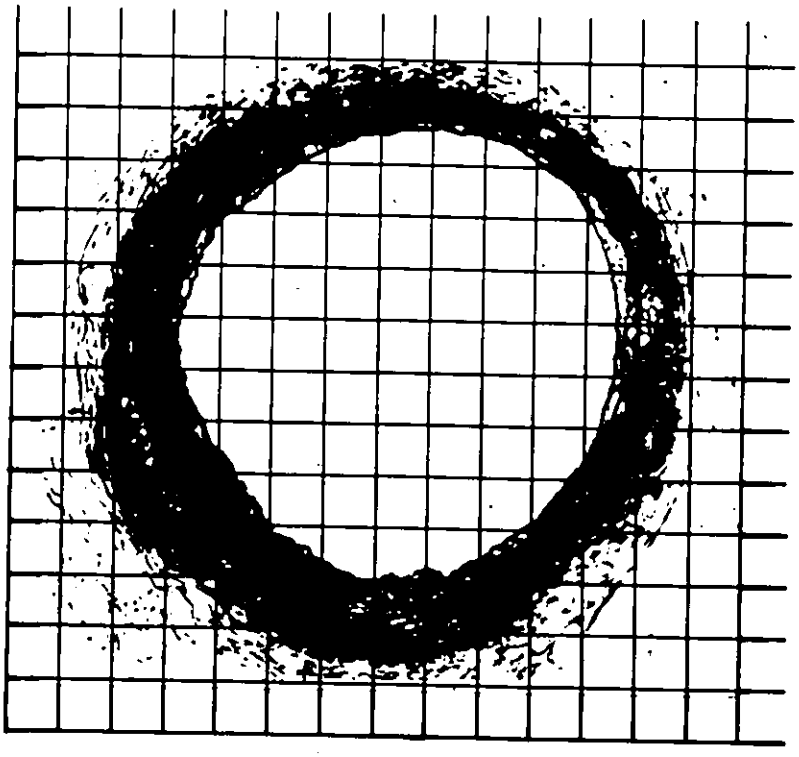
R_L = long radius of lumen

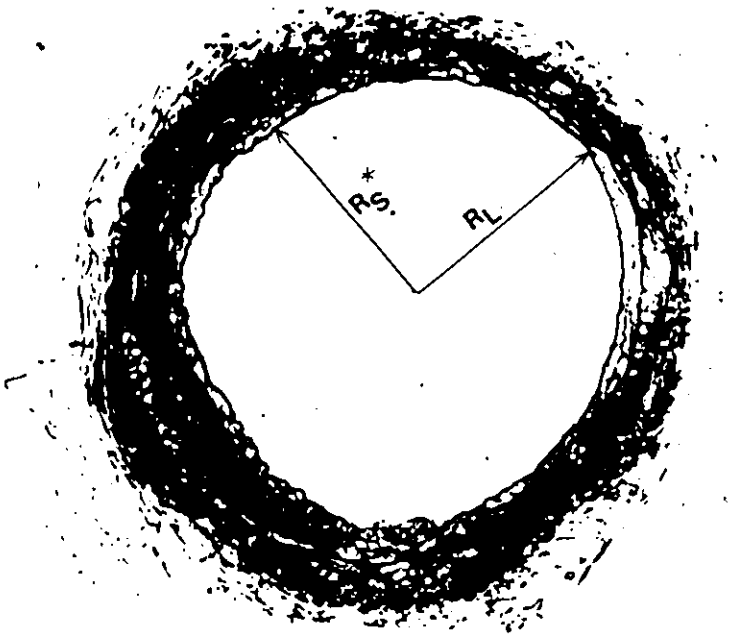
A_T = total area covered by grid.

Fig. 3a is an electron micrograph showing a cross-section of an arterial wall. The various subcomponents that were were measured are labelled. A multipurpose grid was used to calculate the cross-sectional area of the subcomponents of the intima, media and adventitia. Fig. 3b shows the type of grid used superimposed on an electron micrograph of arterial intima + media (upper figure) and adventitia (lower figure). In the case of the intima + media two lines were drawn across the artery perpendicular to the internal elastic lamina. The volume fraction of the endothelium, subendothelial space, internal elastic lamina, medial extracellular space, SMCs and the external elastic lamina were calculated. Within the adventitia, the volume fraction of adventitial collagen, fibroblasts, axons, nerve sheaths and fluid filled space were calculated. The following formula was used to calculate the volume

Fig. 2. The morphometric protocol used to determine the arterial dimensions from light micrographs.

Shown is a reduced light micrograph of a main renal artery sampled from a 21 week old SHR. A multipoint grid was superimposed on the micrograph. In the case of the main renal artery, the points (corners of squares) falling on the lumen and non-adventitial portions of the vascular wall, and in smaller arteries, the points falling on the adventitia, were determined. The average point counts per arterial component, as well as the long (R_L) and short (R_S) radial axes of the lumen (between the internal elastic lamina) were used to calculate the true cross-sectional area of an arterial component as described in the Methods.





* $R_S = 300\ \mu m$

Fig. 3a. Transmission electron micrograph of a small interlobar artery from WKY.

The micrograph shows the various layers of the arterial wall and the major subcomponents within each layer that were morphometrically measured. The micrograph is at approximately 9000 x magnification; the calibration bar within the blood vessel lumen = 1 μm. FS, fluid filled space; F, fibroblast; C, collagen; NS, nerve sheath; N, nerve axon; SMC, smooth muscle cell; I, internal elastic lamina; SE, subendothelial space; E, endothelium; L, lumen.

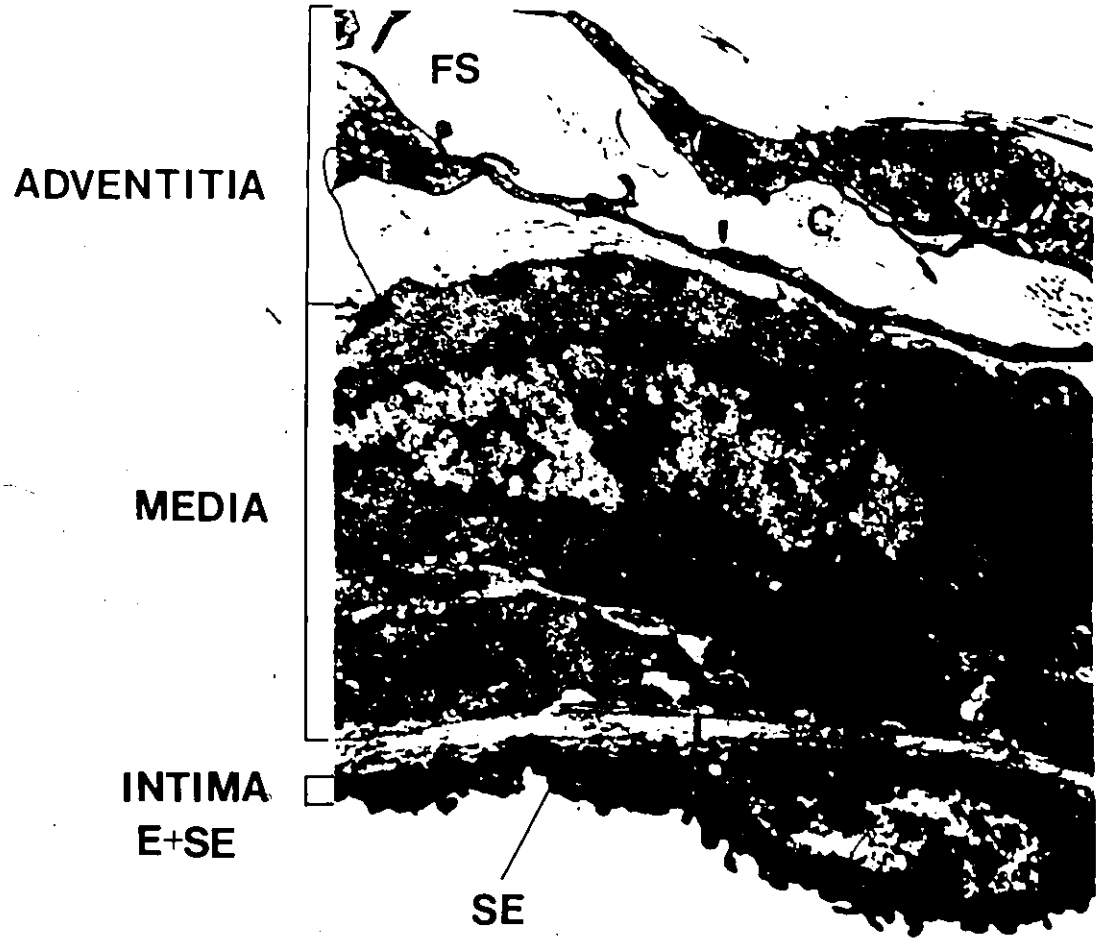
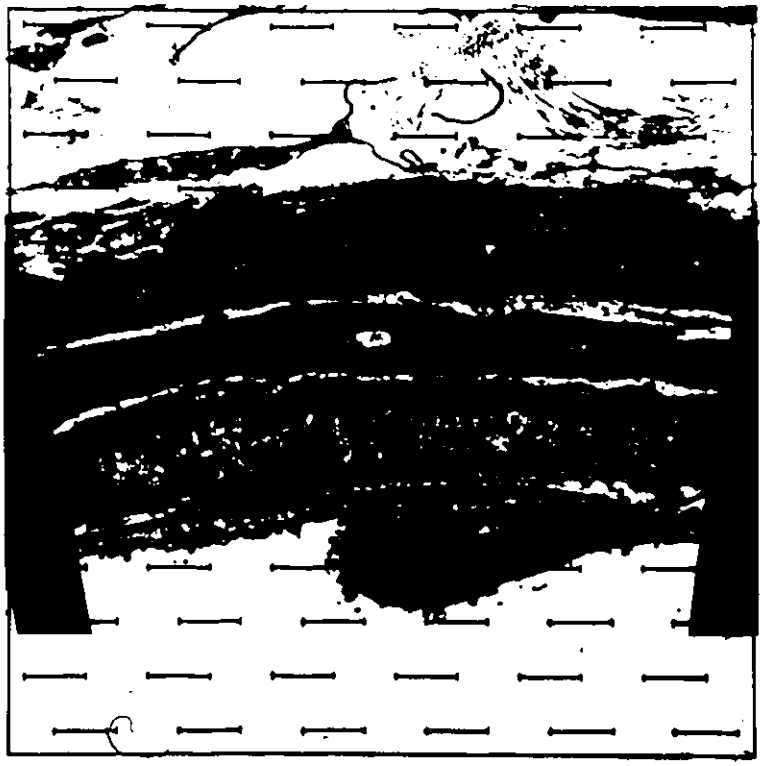

FS
ADVENTITIA
C
MEDIA
INTIMA
E+SE
SE
L

Fig. 3b. The morphometric protocol used to determine the cross-sectional area of the subcomponents of the arterial wall.

A multipurpose grid was superimposed on electron micrographs of non-adventitial (upper) and adventitial (lower) wall. The two figures shown are reductions of 8" x 10" electron micrographs, the actual grid size being 19 x 19.2 cm. The points (ends of lines) falling on individual adventitial and non-adventitial wall components were determined. In the case of the arterial media, the line intersects with arterial smooth muscle cells were also determined. The grid was then rotated 90° and the above measurements were again repeated for each micrograph. The point counts were used to determine the volume fraction of adventitia or non-adventitial wall that a particular component occupies as outlined in the Methods section. Since the total cross-sectional wall area could be determined from light micrographs of the same artery (see Fig. 2), the cross-sectional area of an arterial subcomponent could be calculated from the volume fractions. The point and intersect counts falling on smooth muscle were used to determine the volume to surface area ratio of SMCs, using the method described by Weibel and Bollender (1973).



fraction of each medial and adventitial component.

$$V_v = Pi / P_T \qquad (2)$$

where V_v = volume fraction of a subcomponent

Pi = points hitting an arterial subcomponent, i.e., SMC, IEL

P_T = total number of points hitting all the subcomponents of the adventitial or nonadventitial arterial wall.

The true cross-sectional area of the intima and media and the subcomponents of these areas can be calculated by multiplying the volume fraction of the nonadventitial wall occupied by these layers by the true cross-sectional area of the wall (A_w) obtained from formula 1;

e.g., $V_{v\ media} \times A_w$ = cross-sectional area of the media.

The mean volume to surface area ratio (V/S) was used to determine whether SMC hypertrophy had taken place within medial cross-sections. If individual SMC volume increases within the media, V/S should also increase. For this calculation, a multipurpose grid was used on electron micrographs of the media (Fig. 3b), the number of grid points and surface intersects with the medial SMCs were recorded. The grid was then rotated 90° and the count was repeated a second time. The following formula was used to calculate V/S.

$$V/S = (z \times Pi_{SMC}) / (4 \times Ii_{SMC}) \qquad (3)$$

where z = the line length of the test grid at each magnification

Pi_{SMC} = the points hitting SMCs in micrograph

Ii_{SMC} = the line intersects with the surface of SMCs

If SMC hyperplasia occurs within the media, the number of SMC layers within the media would increase. To test for this alteration, the SMC layers were counted in four quadrants spaced equally along the

arterial wall using phase contrast light microscopy. Hence, increases in medial SMC cross-sectional area were able to be distinguished in terms of SMC hypertrophy, hyperplasia, or both alterations.

3. Accuracy of the Morphometric Measurements

The accuracy of the determination of a subfraction depends on the volume fraction (V_v) occupied by the fraction and the total number of points (P_T) used to determine V_v. Weibel and Bollender (1973) define the relative error in the determination of V_v (EV_v) by the following formula:

$$EV_v = 0.6745 \sqrt{1-V_v / P_T \times V_v}$$

From the above formula it can be observed that the least accurate measurement will involve fractions with a small V_v determined using the lowest number of P_T. In the present study the rarest component observed was the nerve axon. The fewest determinations were performed on the dorsal-central interlobar artery (one per kidney) from 5 SHR vs 5 WKY. For these arteries the mean V_v of the axons was 0.027 (SHR) and 0.030 (WKY). Each individual artery was determined using a P_T of 3,360. The relative error of the mean for the determination of an individual artery with a V_v of 0.027 would be 6.9%, while the error for the mean determination for 5 arteries from 5 SHR would be 3.1%. The determination of all other components had much lower errors since the V_v of the components is greater. The measurements made on all other classes of arteries would have a smaller error since a larger number of arteries (cortical arteries) from a greater number of rats (main renal arteries) were sampled.

G. Dose Response Curves

All the dose response curves involving contractile agents were performed using the perfusion system outlined above (section C). The temperature of the kidney and the perfusate were kept at 37°C by performing the experiment within a temperature controlled plexiglass case. All experiments were carried out at a constant flow rate of 0.82 ml/min. This flow rate was chosen since it produced maximal infusion pressures that could be accurately recorded by the Statham 23 Db pressure transducer in response to the infusion of maximal levels of NE or $BaCl_2$. Oedema formation was monitored by comparing the wet weight of the perfused left kidney with that of the unperfused right kidney.

1. Norepinephrine (NE) concentration vs renal vascular resistance (RVR) dose response experiments

Experiments involving SHR with established hypertension and age matched WKY were performed in the presence of 10^{-5} M cocaine to block the neuronal uptake of NE. Cumulative dose response curves were obtained by infusing 0, 10^{-8}, 2×10^{-8}, 5×10^{-8}, 10^{-7}, 2×10^{-7}, 4×10^{-7}, 10^{-6}, 3×10^{-6}, and 10^{-5} M NE concentrations in Krebs solution, through the renal vasculature of the left kidney. Oxidation of NE in stock solutions was prevented by the presence of 0.1% ascorbic acid. An identical protocol was followed for the prehypertensive SHR and age matched WKY, except that the experiment was performed in the presence of 10^{-6} M cocaine and the 3×10^{-6} M dose of NE was omitted.

2. K^+ concentration vs RVR dose response experiments

K^+ induced depolarization occurs by (a) the release of NE from periarterial nerve terminals and (b) the depolarization of the vascular smooth muscle directly. In the present experiment, the effect of nerve

transmitter release was blocked postsynaptically by performing the experiment in the presence of 10^{-8} M prazosin + 10^{-6} M spiroperidol. These two agents, an $alpha_1$ and dopamine receptor antagonist, respectively, when used in combination, totally abolished the contractile response produced by maximal nerve stimulation (discussed in a later section). Dose response curves were obtained by infusing 5.3, 30, 40, 50, 60, 80 and 100 mM doses of KCl dissolved in Krebs solution containing these antagonists. The infusates were made iso-osmotic (340 mOsm) by decreasing the NaCl concentration in proportion to the elevation of KCl.

3. $BaCl_2$ concentration vs RVR, dose response curves

These experiments were performed in the presence of 10^{-8} M prazosin + 10^{-6} M spiroperidol. Cumulated dose response curves were produced by infusing 0, 1.46, 2.43, 4.87, 7.30, 9.73, 19.5, 34.1 and 48.7 mM concentrations of $BaCl_2$ mixed in a modified Krebs solution. The Krebs solution used was the same as that previously outlined (section C) except that the following were omitted: SO_4-2, PO_4-3, HCO_3-. The solution was buffered to pH 7.4 with 25 mM 3-[N-Morpholino] propanesulfonic acid (MOPS). The above adjustments were necessary to prevent Ba^{+2} from forming a precipitate. The osmolarity of the $BaCl_2$ infusates was adjusted to 340 mOsm by decreasing the NaCl concentration.

4. Angiotensin II contraction of the renal vasculature

The maximal amplitude of response to angiotensin II (A_{II}) was obtained by infusing 10^{-7} M A_{II} dissolved in Krebs containing 10^{-8} M prazosin + 10^{-6} M spiroperidol through the renal vasculature. To test that a maximal response had been obtained, 10^{-6} M A_{II} was infused after

the 10^{-7} M dose. In all cases no further contraction occurred.

5.Beta receptor relaxation of vasculature during NE contraction

To test the involvement of the beta adrenoreceptor during infused NE contraction, the renal vasculature was contracted maximally with 10^{-5} M NE in Krebs. Subsequently, 10^{-5} M NE in the presence of 10^{-7}, 10^{-6} or 10^{-5} M propranolol (a beta receptor antagonist) was infused through the kidney. The alterations in the renal vasculature resistance were noted.

H. Nerve Stimulation Studies

The same perfusion system as that described above for the dose/response experiments was used in the nerve stimulation studies. A pair of E2B Grass subdermal platinum electrodes were bent at 90^{o} placed in a clamp and adjusted so that the interelectrode distance was 2.5 mm. The electrodes were connected to a Grass SIU5 stimulus isolation unit coupled to a Grass S48 stimulator. Nerves enter the kidney with the main renal artery within the adventitia. The electrodes were positioned at the renal hilus on either side of the renal artery. The same set of electrodes were used in experiments involving SHR and WKY.

1.Stimulation frequency vs RVR response

The RVR was measured during stimulation at frequencies of 2, 4, 6, 8, 10, 12 and 14 Hz. The stimulus duration was 2 ms at a potential difference of 20 mV (21 weeks old SHR, WKY) or 60 V (4-6 week old SHR, WKY). A train of pulses was applied for a 2 min duration. Initially the kidney was perfused with Krebs alone and the RVR responses at each stimulation frequency plotted. These response curves were then duplicated in the presence of 10^{-5} M (21 week SHR, WKY) or 10^{-6} M (4-6

week SHR, WKY) cocaine. After an experiment was completed, 1 µg/ml tetrodotoxin (TTX) in Krebs was infused through the kidney for 4 min, followed by a 10 Hz train of pulses applied for 2 min. Since nerves, but not SMCs, contain TTX sensitive Na^+ channels, the presence of a contractile response in the presence of TTX would indicate a non-nerve mediated contractile response. Experiments exhibiting a TTX insensitive response were rejected. Oedema formation was monitored by comparing the wet weight of the perfused left kidney to that of the unperfused right kidney.

2. Receptors involved in nerve stimulated contraction

To determine the receptors involved in nerve stimulated contractile responses, the nerves entering the kidney of 23 week old SHR and WKY were stimulated, using a train of pulses of 10 Hz, ~2 ms, 60 V for 2 min. The alteration in RVR was noted. Receptor antagonists were then tested: (a) 10^{-7} and 10^{-6} M propranolol (a beta receptor antagonist), (b) 10^{-8} M prazosin (an $alpha_1$ antagonist), (c) 10^{-7} and 10^{-6} M yohimbine (an $alpha_2$ antagonist), (d) 10^{-7} M phentolamine (an $alpha_1$ and $alpha_2$ antagonist), (e) 10^{-6} M methysergide (a 5-HT receptor antagonist), and (f) 10^{-6} M spiroperidol (a dopamine receptor antagonist). Only spiroperidol (10^{-6} M) altered RVR in the presence of 10^{-8} M prazosin. All other antagonists either alone, or in combination, in the presence of 10^{-8} M prazosin failed to produce a further alteration in the RVR. On the basis of this finding the proportion of the total response that could be attributed to $alpha_1$ and dopamine receptors was calculated. The renal vasculatures of SHR and WKY were then stimulated electrically as described above, and RVR measured in the

presence of: (a) Krebs alone, (b) 10^{-7} M propranolol, (c) 10^{-8} M prazosin + 10^{-7} M propranolol, (d) 10^{-6} M spiroperidol + 10^{-8} M prazosin + 10^{-7} M propranolol, and the receptor fraction of the total response calculated.

I. Hydralazine Studies

SHR and WKY were obtained from the same sources as those used in the morphometric and pharmacological studies of the kidney. The perfusion system used was that described above (section C).

1. Treatment protocol

Preliminary experiments were performed to determine the concentration of hydralazine needed to reduce the blood pressure of SHR to that present in resting WKY. A concentration of 100 mg hydralazine per litre of drinking water produced normotension within one week in 21 week old SHR with established hypertension, prior to treatment. During the next 4 weeks of treatment, the drinking rate of the rats was monitored for 5 days/week using calibrated anti-spill water bottles. The amount of ingested drug was calculated to be 0.0145 ± 0.0013 mg/day/g rat. A protocol was then used where female SHR were treated with 100 mg/l hydralazine (in the drinking water) until blood pressure had returned to normal. A male SHR was then introduced into the cage to inseminate the female. Hydralazine crosses the placental barrier in humans and is present in the fetal circulation at comparable levels to that present in the mother's blood (Liedholm et al., 1982). Therefore, there is reason to believe that the fetal SHR is subjected to the same hypotensive effects of hydralazine as the mother. However, in humans

very little active hydralazine is present in the mother's milk. In view of this, newborn SHR were tube fed hydralazine at a concentration of 0.0169 mg/day/g rat. This was the highest rate of hydralazine ingestion observed in the drinking rate study discussed above. After weaning, the 4 week old SHR were separated from the mother and placed on a water supply of 100 mg/l hydralazine. In a separate room, WKY rats were treated in utero and postnatally with hydralazine in an identical manner to the SHR.

2. Sampling protocol

The blood pressure of hydralazine treated and untreated SHR and WKY was monitored using the tail cuff compression method, from 3 to 21 weeks of age. At 21 weeks of age the vasculature of the left kidney was perfusion fixed for light microscopy and analyzed morphometrically.

The fixation and morphometric methods used have been outlined in E and F of the Methods Section. In the case of measurements involving the lumen diameter, the cortical arteries were subdivided into arcuate and interlobular arteries. This was done primarily on the basis of the location of the arteries within the cortex and their direction, as outlined by Fourman and Moffat (1971). In the rat kidney the main renal artery divides into six to ten major branches termed interlobar arteries which radiate into the renal cortex via the pelvic mucosa. The renal cortex of the rat kidney can be divided into outer and inner zones. The outer cortex contains numerous glomeruli, proximal and distal convoluted tubules and the beginning of the collecting ducts. Interlobular arteries are present within this region along with preglomerular and efferent arterioles. Unlike arterioles, interlobular arteries are

closely associated with interlobular veins. These arteries radiate parallel to each other and perpendicular to the renal capsule. On average, an interlobular artery has a lumen diameter two to three times that present in arterioles. The inner cortex contains the terminal ends of the descending proximal convolute tubules, the thick ascending loops of Henle and collecting ducts. In the rat kidney this region has very few glomeruli and arterioles, but does contain an extensive network of capillaries. Arcuate arteries are associated with veins and travel parallel to the renal capsule between the border of the inner and outer cortex and perpendicular to the loops of Henle and the interlobular arteries. On average, an arcuate artery has a lumen diameter that is two to three times that present in interlobular arteries. Fig. 4 is a schematic diagram of the various renal arteries that were sampled.

3. Hydralazine withdrawal study

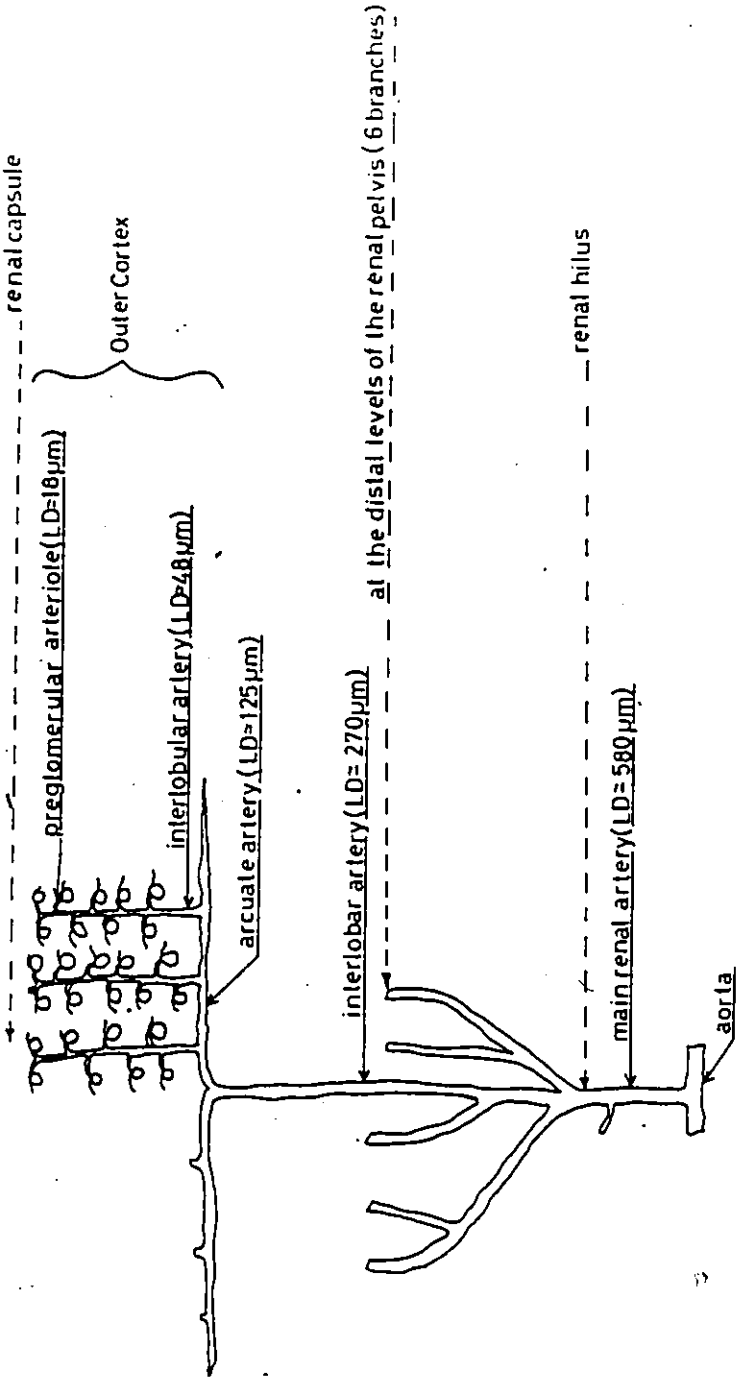
To study the long term effects of hydralazine treatment, two groups of rats were used. One group of SHR was treated in utero and postnatally as described above up to 26 weeks of age, after which the drug was withdrawn from the drinking water. The blood pressure prior to and after drug withdrawal was measured on a regular basis. The second group of rats studied were 21 weeks of age and in an established phase of hypertension. Hydralazine (100 mg/l) was introduced into the drinking water for a period of 286 days after which the drug was withdrawn. The blood pressure of the rats prior to, during and after drug treatment was monitored.

J. Statistical Analysis

An unpaired Student's t test was used to analyze statistical

Fig. 4. A schematic diagram of the preglomerular vascular bed of the rat.

The main renal artery enters the kidney at the hilus and divides into 6 to 10 branches which are termed interlobar arteries. The interlobar arteries follow the pelvic mucosa to the junction of the inner and outer renal cortex where they divide into arcuate arteries. Interlobular arteries radiate towards the renal capsule from the arcuate arteries. Numerous preglomerular arterioles arise at right angles from the interlobular arteries. (LD = lumen diameter).


renal capsule
Outer Cortex
preglomerular arteriole(LD=18µm)
interlobular artery(LD=48µm)
arcuate artery (LD=125µm)
interlobar artery(LD= 270µm)
at the distal levels of the renal pelvis (6 branches)
renal hilus
main renal artery(LD= 580µm)
aorta

differences between two groups (i.e., SHR vs WKY). In the case of the hydralazine treatment study, four treatment groups were compared (i.e., SHR and WKY treated with hydralazine, and untreated SHR and WKY). An analysis of variance (ANOVA) was used to test for significance among the four groups. Subsequently, an unpaired Student's t test was used to compare the individual groups. The means of two groups were considered significantly different at $P < 0.05$. All results are presented as means ± one standard error.

Results

A. Blood Pressure (BP) Profile of the SHR and WKY Colony

Fig. 5 shows the systolic blood pressure (tail cuff compression method) of male SHR and WKY aged 4 to 22 weeks. Three phases in the development of hypertension were observed. A prehypertensive phase, where the BP of SHR does not differ from WKY, occurred from birth to 6 weeks of age. In phase two the BP increased in the SHR to the age of 11 weeks (developing phase of hypertension). In the final phase (established hypertension) BP changes very little with age beyond 11 weeks. The present study deals with animals that were in the prehypertensive (Phase 1) and established (Phase 3) phases of hypertension.

B. Rats in an Established Phase of Hypertension Development

1. Renal vascular resistance (RVR) vs perfusion flow

Table 3 shows the physical characteristics of the SHR and WKY used in the perfusion study. The ages of SHR and WKY were similar. However, body weight, systolic blood pressure and the ratio of the kidney to body weight were significantly elevated in the SHR compared to WKY.

Fig. 6 shows the alterations in RVR in relation to perfusion flow. RVR was not elevated in SHR over WKY at flow rates of 0.41, 0.82, 2.04 and 4.10 ml/min when the renal vasculature was maximally relaxed.

Fig. 5. Blood pressure versus age profiles of the male SHR and WKY colony used within the study.

The systolic blood pressure was measured using the tail cuff compression method. The mean systolic blood pressure for each age ± one standard error measurement of the mean (SEM) and the level of significant difference between common ages (i.e., $0.005 = P<0.005$) is shown. In SHR and WKY used in the study, three phases of hypertension development can be observed. A prehypertensive phase is present in SHR which extends up to 6 weeks of age during which SHR and WKY have similar blood pressures. This is followed by a rapid rise in the blood pressure of SHR, termed the developing phase of hypertension. After 11 weeks of age, maximal hypertension is established and changes little with age.

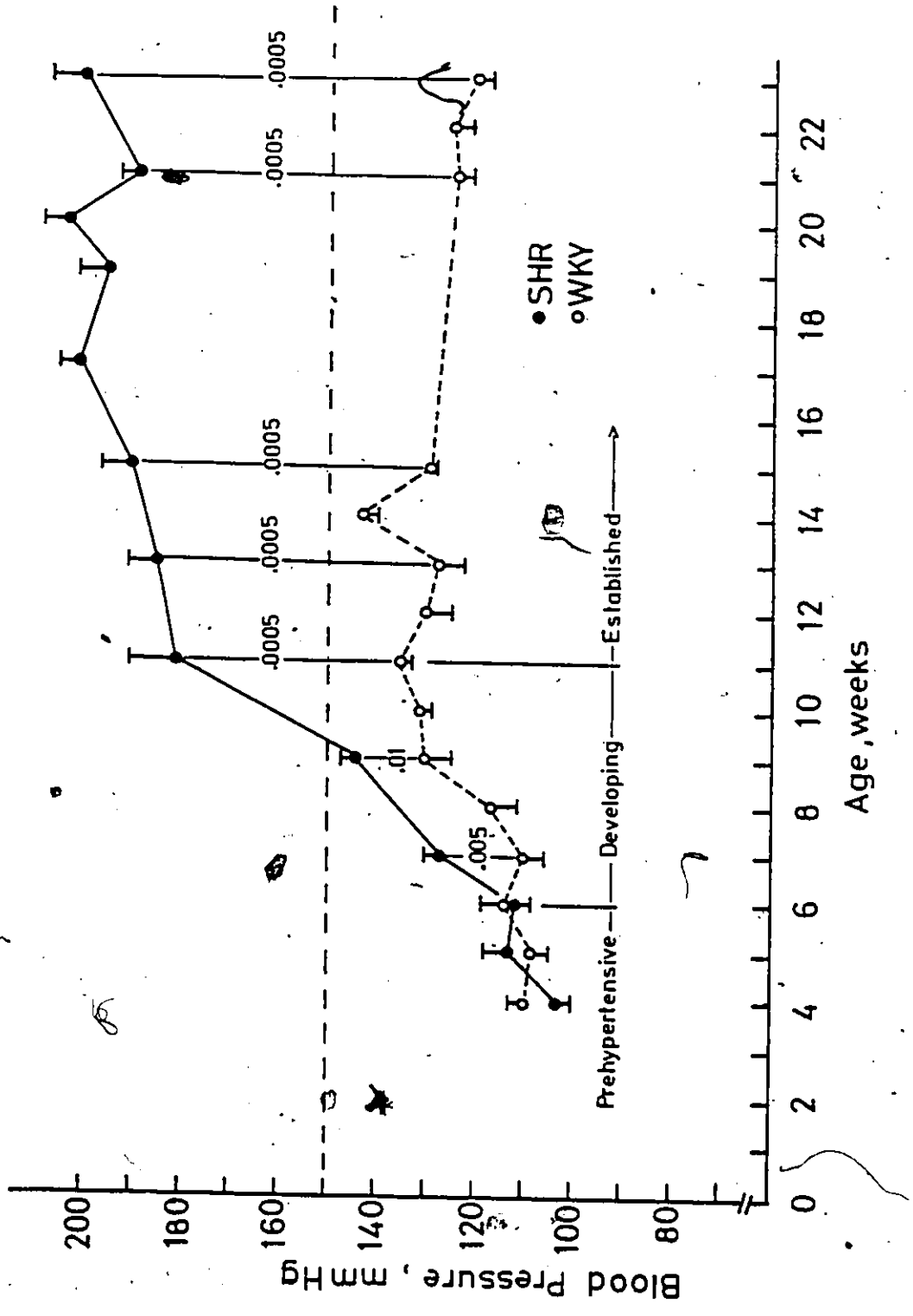
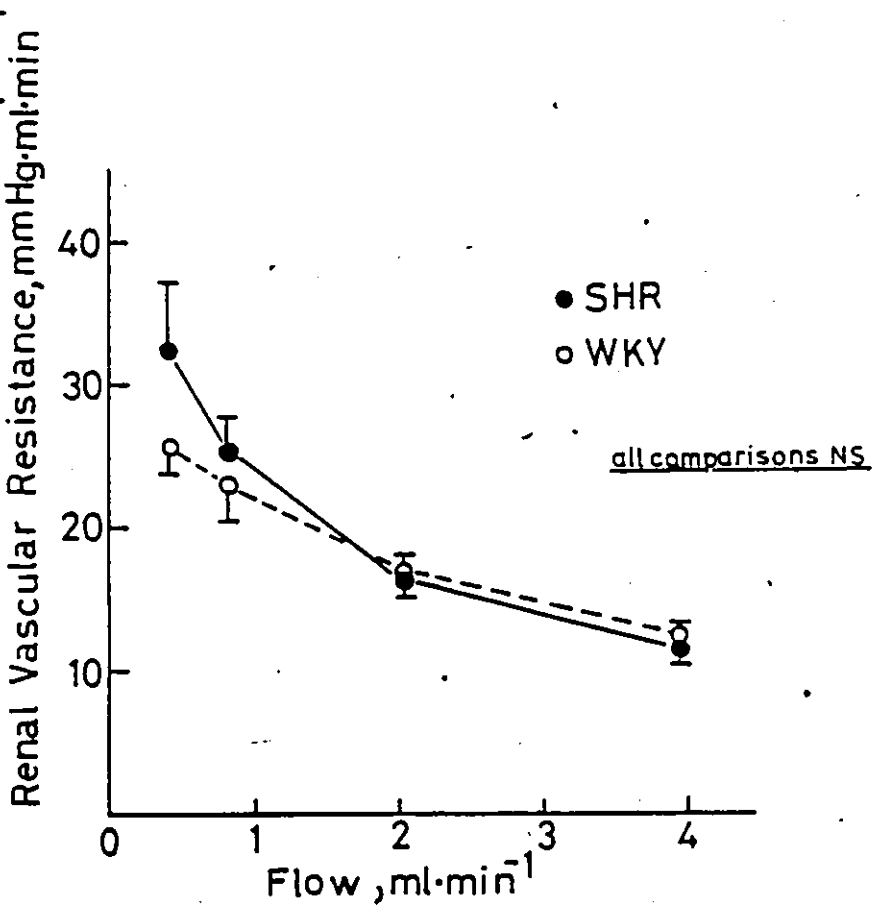

Blood Pressure, mmHg
200
180
160
140
120
100
80
0
2
4
6
8
10
12
14
16
18
20
22
Age, weeks
Prehypertensive
Developing
Established
.005
.01
.0005
.0005
.0005
.0005
.0005
•SHR
°WKY

Table 3. Physical characteristics of adult SHR and WKY used to determine vascular resistance at various perfusion flow rates.

	SHR n=5	WKY n=5	P
Age (wks)	21.2±.146	21.8±.626	NS
Wt (g)	367 ±6.62	332 ±5.39	0.005
BP (mmHg)	197 ±3.74	124 ±3.73	0.005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	4.08±.020	3.87±.074	0.05

Fig. 6. Perfusion flow versus renal vascular resistance in SHR in an established phase of hypertension and age matched WKY (mean ± SEM, ns ≙ $P>0.05$).

The renal vascular resistance of the isolated left kidney of 21 week old SHR and WKY was measured under conditions where the renal vasculature was relaxed by perfusing the kidney with Krebs solution.


Renal Vascular Resistance, mmHg·ml·min⁻¹
40
30
20
10
0
1
2
3
4
Flow, ml·min⁻¹
● SHR
○ WKY
all comparisons NS

The RVR did, however, decrease in both SHR and WKY at higher flow rates.

2. Morphometric analysis of the renal vasculature

Table 4 shows the physical characteristics of the rats used for the morphometric study of the renal vasculature. The age and body weight of SHR and WKY did not differ. However, the blood pressure and the kidney weight/g body weight was elevated in SHR over WKY. The RVR at a flow rate of 0.82 ml/min, was similar in SHR and WKY during maximal relaxation.

(a) Lumen diameter of renal vessels

Fig. 7 shows the lumen diameter of the renal vessels fixed in a relaxed state. Consistent with the lack of any altered renal vascular resistance, SHR and WKY exhibited no significant difference in the lumen diameter of the main renal, interlobar, arcuate-interlobular arteries and the preglomerular arterioles.

(b) Alterations in the wall components of the renal vessels

Although lumen diameter of the vessels studied was similar in SHR and WKY, structural alterations in the wall components did exist. These results are summarized in Table 5a and b. For this aspect of the study, the cortical arteries (arcuate-interlobular) were subdivided into divisions based on the arterial diameter between the internal elastic lamina (D_I).

The larger renal arteries exhibited increased cross-sectional area (CSA) quantities of intimal components. The main renal, interlobar arteries and the preglomerular arterioles of SHR were found to have greater CSA quantities of subendothelial space than WKY. The CSA of the

Table 4 . Physical characteristics of adult SHR and WKY used in the morphometric study of the renal vasculature

	SHR n=6	WKY n=10	
Age (wks)	21.3 ± .154	21.3 ± .411	NS
Wt (g)	352 ±15.5	334 ±3.38	NS
BP (mmHg)	194 ± 4.16	121 ±2.20	.005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	4.00± .087	3.53± .131	.05
Renal Vascular Resistance ($mmHg.ml^{-1}.min^{-1}$)	25.4 ± 1.97	22.7 ±1.64	NS

Fig. 7. Lumen diameters of the main renal, interlobar and arcuate-interlobular arteries, as well as the preglomerular arterioles of SHR in an established phase of hypertension and age matched WKY (mean ± SEM).

The renal arteries of 21 week old SHR and WKY were perfusion fixed under constant flow and relaxed conditions. All lumen diameters have been compensated for sectioning angle.

Lumen Diameter, μm

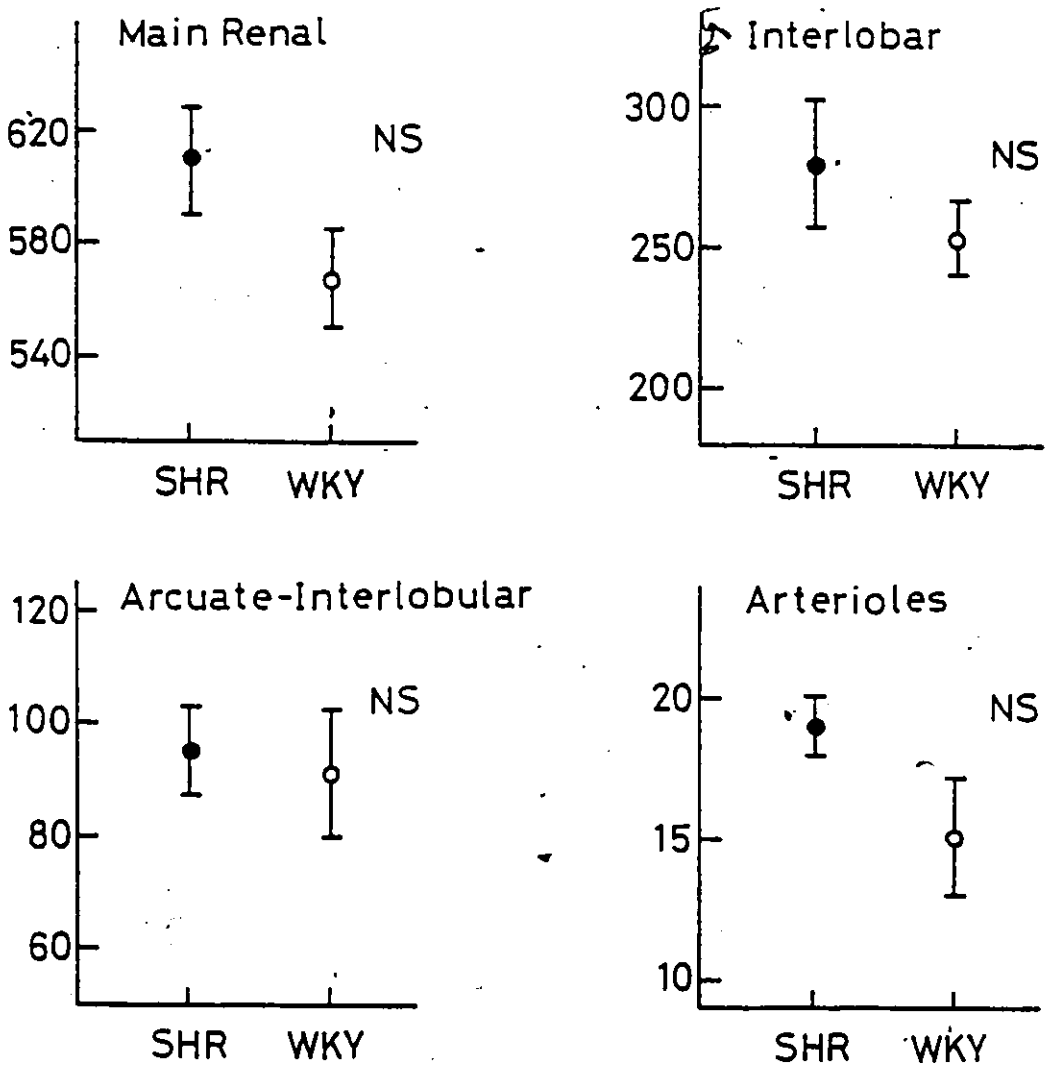


TABLE 5a. Morphometric analysis of the renal arterial subcomponents of adult SHR and WKY.
Intimal and medial components and the arterial adventitia are expressed in cross-sectional areas (μm^2, mean ± SEM).

				CORTICAL ARTERIES*				
		Main Renal Artery	Interlobar Artery	≥120	120–95	95–70	<70	Arterioles
INTIMA								
Endothelium	SHR	3082 ± 891	1430 ± 112	706 ± 54	488 ± 25	334 ± 16	168 ± 15	30 ± 4
	WKY	3036 ± 530	931 ± 53	622 ± 28	430 ± 36	305 ± 21	164 ± 18	25 ± 5
	P<	N S	.005	N S	N S	N S	N S	N S
Subendothelial Space	SHR	1343 ± 380	594 ± 36	273 ± 36	150 ± 14	62 ± 8	25 ± 5	10 ± 2
	WKY	861 ± 241	363 ± 81	197 ± 35	75 ± 14	48 ± 6	23 ± 4	7 ± 1
	P<	0.05	0.05	N S	N S	N S	N S	0.05
INTERNAL ELASTIC LAMINA	SHR	4238 ± 930	978 ± 147	328 ± 23	239 ± 17	128 ± 13	70 ± 9	
	WKY	2670 ± 732	578 ± 87	281 ± 18	178 ± 15	116 ± 7	68 ± 11	NA
	P<	0.01	0.05	N S	0.05	N S	N S	

*These arteries were divided into four categories, based on lumen diameter (μm), as measured between the internal elastic lamina.

TABLE 5b. Morphometric analysis of the renal arterial subcomponents of adult SHR and WKY. Intimal and medial components and the arterial adventitia are expressed in cross-sectional areas (μm^2, mean ± SEM).

				CORTICAL ARTERIES*				
		Main Renal Artery	Interlobar Artery	≥120	120–95	95–70	<70	Arterioles
MEDIA								
Smooth Muscle Cells	SHR	69258 ± 5412	21920 ± 2562	7311 ± 409	4307 ± 242	2748 ± 166	1151 ± 122	101 ± 14
	WKY	47843 ± 4924	14520 ± 555	5505 ± 330	2882 ± 184	2160 ± 183	884 ± 96	85 ± 15
	P<	0.01	0.01	0.01	0.05	0.05	N S	N S
Intercellular Space	SHR	48872 ± 4842	4862 ± 1126	1530 ± 121	1057 ± 110	527 ± 111	258 ± 46	19 ± 2
	WKY	31329 ± 2728	3844 ± 248	1008 ± 68	565 ± 68	352 ± 34	115 ± 13	11 ± 3
	P<	0.01	N S	0.01	0.01	0.05	0.01	0.05
EXTERNAL ELASTIC LAMINA	SHR	3882 ± 236	406 ± 32	166 ± 21	96 ± 11	66 ± 9	24 ± 4	
	WKY	2485 ± 199	217 ± 75	121 ± 14	95 ± 17	54 ± 9	17 ± 3	NA
	P<	0.005	0.05	N S	N S	N S	N S	
ADVENTITIA	SHR	68890 ± 8447	18000 ± 3325	8667 ± 972	7868 ± 775	6162 ± 534	2426 ± 368	
	WKY	70340 ± 6271	13362 ± 1592	9681 ± 1662	7739 ± 347	5114 ± 635	2003 ± 207	NA
	P<	N S	NS	N S	N S	N S	N S	

*These arteries were divided into four categories, based on lumen diameter (μm), as measured between the internal elastic lamina.

endothelium was increased in the interlobar arteries of SHR. The endothelium and subendothelial space were unaltered in the cortical arteries.

The CSA of the internal and external elastic lamina was increased in the main renal and interlobar arteries of SHR, but not in the cortical arteries. Both the internal and external elastic laminae were virtually absent in preglomerular arterioles.

The CSA of the media of SHR renal arteries was greatly increased during established hypertension. All renal arteries of SHR with a D_I greater than 70 µm had increased CSA quantities of SMCs. With the exception of the interlobar arteries, the medial intercellular space of all arteries was increased in SHR.

The CSA of the adventitia was unchanged in all arteries studied. Table 6 outlines the volume fractions of the adventitial sub-components. In SHR, the volume fraction of fibroblasts was elevated in the interlobar arteries and in cortical arteries with a D_I of less than 95 µm. The volume fraction of collagen, nerve axons, nerve sheath cells, and fluid filled space was not altered with hypertension.

Since the CSA of SMCs in the media of renal arteries is increased in SHR, an attempt was made to ascertain whether this was due to an increase in SMC number and/or volume. The results of this study are summarized in Table 7. Increased SMC volume would result in an increase in the SMC volume to surface area ratio (V/S). Such an analysis indicated that in SHR, the interlobar arteries and cortical arteries with a D_I greater than 70 µm exhibited an increase in the SMC V/S ratio. The main renal arteries, cortical arteries with a D_I less

TABLE 6. Morphometric analysis of the renal arterial adventitial subcomponents of adult SHR and WKY. Values are expressed as a volume fraction of the adventitia.

			CORTICAL ARTERIES*			
		Interlobar Artery	≥120	120–95	95–70	<70
ADVENTITIA VOLUME DENSITY (Vv)						
Fibroblasts	SHR	0.242 ± 0.021	0.217 ± 0.027	0.203 ± 0.025	0.200 ± 0.026	0.247 ± 0.020
	WKY	0.103 ± 0.015	0.115 ± 0.013	0.143 ± 0.023	0.143 ± 0.012	0.185 ± 0.020
	P<	0.001	N S	N S	0.05	0.05
Collagen	SHR	0.266 ± 0.021	0.170 ± 0.005	0.143 ± 0.018	0.137 ± 0.016	0.137 ± 0.020
	WKY	0.330 ± 0.034	0.202 ± 0.023	0.179 ± 0.018	0.185 ± 0.018	0.158 ± 0.012
	P<	N S	N S	N S	N S	N S
Nerve Axons	SHR	0.027 ± 0.008	0.016 ± 0.003	0.016 ± 0.004	0.010 ± 0.003	0.010 ± 0.002
	WKY	0.030 ± 0.005	0.015 ± 0.005	0.013 ± 0.003	0.012 ± 0.002	0.013 ± 0.003
	P<	N S	N S	N S	N S	N S
Nerve Sheath Cells	SHR	0.037 ± 0.015	0.027 ± 0.007	0.020 ± 0.003	0.007 ± 0.002	0.012 ± 0.004
	WKY	0.037 ± 0.008	0.019 ± 0.007	0.015 ± 0.005	0.008 ± 0.002	0.009 ± 0.002
	P<	N S	N S	N S	N S	N S
Fluid	SHR	0.394 ± 0.023	0.548 ± 0.039	0.597 ± 0.029	0.642 ± 0.037	0.584 ± 0.022
	WKY	0.438 ± 0.003	0.597 ± 0.036	0.642 ± 0.032	0.642 ± 0.022	0.626 ± 0.021
	P<	N S	N S	N S	N S	N S

*These arteries were divided into four categories, based on lumen diameter (μm), as measured between the internal elastic lamina.

TABLE 7. Renal arterial SMC surface to volume ratios and medial SMC layers in adult SHR and WKY.

				CORTICAL ARTERIES*				
		Main Renal Artery	Interlobar Artery	≥120	120-95	95-70	<70	Arterioles
Smooth Muscle Cell Volume/Surface Ratio	SHR	0.81 ± 0.05	1.25 ± 0.06	0.85 ± 0.02	0.74 ± 0.03	0.76 ± 0.06	0.59 ± 0.04	0.40 ± 0.04
	WKY	0.78 ± 0.06	0.81 ± 0.04	0.77 ± 0.03	0.62 ± 0.02	0.64 ± 0.03	0.58 ± 0.03	0.42 ± 0.05
	P=	NS	0.001	0.05	0.05	0.05	N S	N S
Smooth Muscle Cell Layers	SHR	5.73 ± 0.56	4.48 ± 0.137	3.51 ± 0.09	2.94 ± 0.12	2.49 ± 0.12	2.35 ± 0.13	1.29 ± 0.08
	WKY	4.98 ± 0.23	4.57 ± 0.092	2.89 ± 0.11	2.48 ± 0.08	2.27 ± 0.09	1.89 ± 0.07	1.25 ± 0.10
	P=	0.05	N S	0.05	0.05	N S	0.05	N S

*These vessels were divided into four categories based on lumen diameter (μm), as measured between the internal elastic lamina of the blood vessel.

than 70 µm and preglomerular arterioles showed no alterations in V/S ratios when SHR and WKY were compared.

If the increase in SMC CSA resulted from an increased number of SMCs then one would expect the number of SMC layers also to be increased. Such an analysis (Table 7) indicated that the main renal artery and cortical arteries with a D_I greater than 95 µm and less than 70 µm exhibited increases in the number of SMC layers. Interlobar arteries and cortical arteries with a D_I between 70 and 95 µm, and the preglomerular arterioles showed no alterations in the number of SMC layers.

Thus in SHR the interlobar and one class of cortical arteries (D_I 95-70 µm) showed increased SMC volumes without increased numbers (SMC hypertrophy), whereas the main renal artery and small cortical arteries (D_I < 70 µm) showed increased SMC numbers without change in individual cell volume (SMC hyperplasia). With the exception of arterioles, all the other renal arteries of SHR exhibited alterations in both SMC volume and number. Therefore, it appears that both processes play a role in increasing the CSA quantities of SMCs; the degree of involvement of SMC hypertrophy or hyperplasia varies depending on the size and location of the renal arteries.

3. <u>Summary of the findings of the morphometric study of the renal vascular changes of SHR in an established phase of hypertension</u>

(i) Consistent with the perfusion study previously outlined, the relaxed renal arteries of hypertensive rats did not exhibit a decreased lumen diameter over normotensive controls.

(ii) There was very little alteration in the CSA amounts of adventitia, intima or its sub-components when SHR and WKY were compared.

(iii) The most consistent alteration that occurred was a thickening of the media, particularly in hypertensive arteries with a D_I of greater than 70 um. This was produced by an increase in the CSA of the medial extracellular space coupled with either SMC hypertrophy, hyperplasia or a combination of both alterations.

(iv) Arterioles showed very little alteration in structural components when SHR were compared to WKY.

C. Rats in a Prehypertensive Phase of Hypertension Development

1. Renal vascular resistance vs perfusion flow

To determine whether (as in adult SHR) renal vascular perfusion with Krebs produced maximal relaxation in prehypertensive SHR, the renal vasculature of the left kidneys of 4 female, 4 to 5 week old SHR were perfused initially with Krebs, followed by Krebs plus 10 mg/l Na nitroprusside (a vasodilator). Finally, the Na nitroprusside was washed out by perfusing the kidney with Krebs. The RVR was compared under each of the three perfusion conditions at flow rates of 0.041, 0.082, 0.20, 0.41, 0.82 and 2.04 ml/min. RVR was similar under the three perfusion conditions at any given flow rate. This indicated that, as in adult SHR, perfusion of the kidney of 4-5 week old SHR with Krebs does maximally relax the renal vasculature.

In other experiments, the RVR of 5 (male) prehypertensive SHR

and 3 WKY were compared at a number of flow rates. Both groups of animals were between 4 and 5 weeks of age. Tail cuff blood pressure measurements indicated a systolic blood pressure of 108±6 mm Hg for SHR and 117±7 mm Hg for WKY (no significant difference, $P>0.05$). The body weight of the SHR group was slightly less than the WKY animals (44±0.7 g vs. 48±2 g, $P<0.05$). Fig. 8 outlines the results of the experiment. At flow rates of 0.041, 0.082, 0.41, 0.82, and 2.04 ml/min no significant difference in RVR existed between the two groups. At a flow rate of 4.10 ml/min, SHR did exhibit an increased RVR over WKY. It should be pointed out, however, that 4.10 ml/min is an extremely high perfusion flow rate. The very sharp drop in RVR observed in WKY between the flow rates of 2.04 and 4.10 ml/min compared to the relatively modest drop in RVR in SHR, suggests that at the higher flow rate, WKY may have developed a leaky vascular bed. This suggests that at flow rates that do not damage the renal vasculature, the resistance of the relaxed vasculature is similar when prehypertensive SHR and WKY are compared. Subsequent experiments involving prehypertensive SHR and WKY whose kidneys were perfused with Krebs at a flow of 0.82 ml/min also indicated that RVR was similar between prehypertensive SHR and age matched WKY (discussed in later sections).

2. Morphometric analysis

Table 8 summarizes the physical characteristics of the prehypertensive group of SHR and age matched controls that were used in the morphometric study of the renal vasculature. These animals were anaesthetized (60 mg/kg sodium pentobarbitol, i.p.) and the aorta at the junction of the femoral artery was catheterized for direct measurement

Fig. 8. Perfusion flow versus renal vascular resistance in prehypertensive SHR and age matched WKY (mean ± SEM).

The renal vascular resistance of the isolated left kidney of 4 to 5 week old SHR and WKY was measured under relaxed conditions at a variety of flow rates.

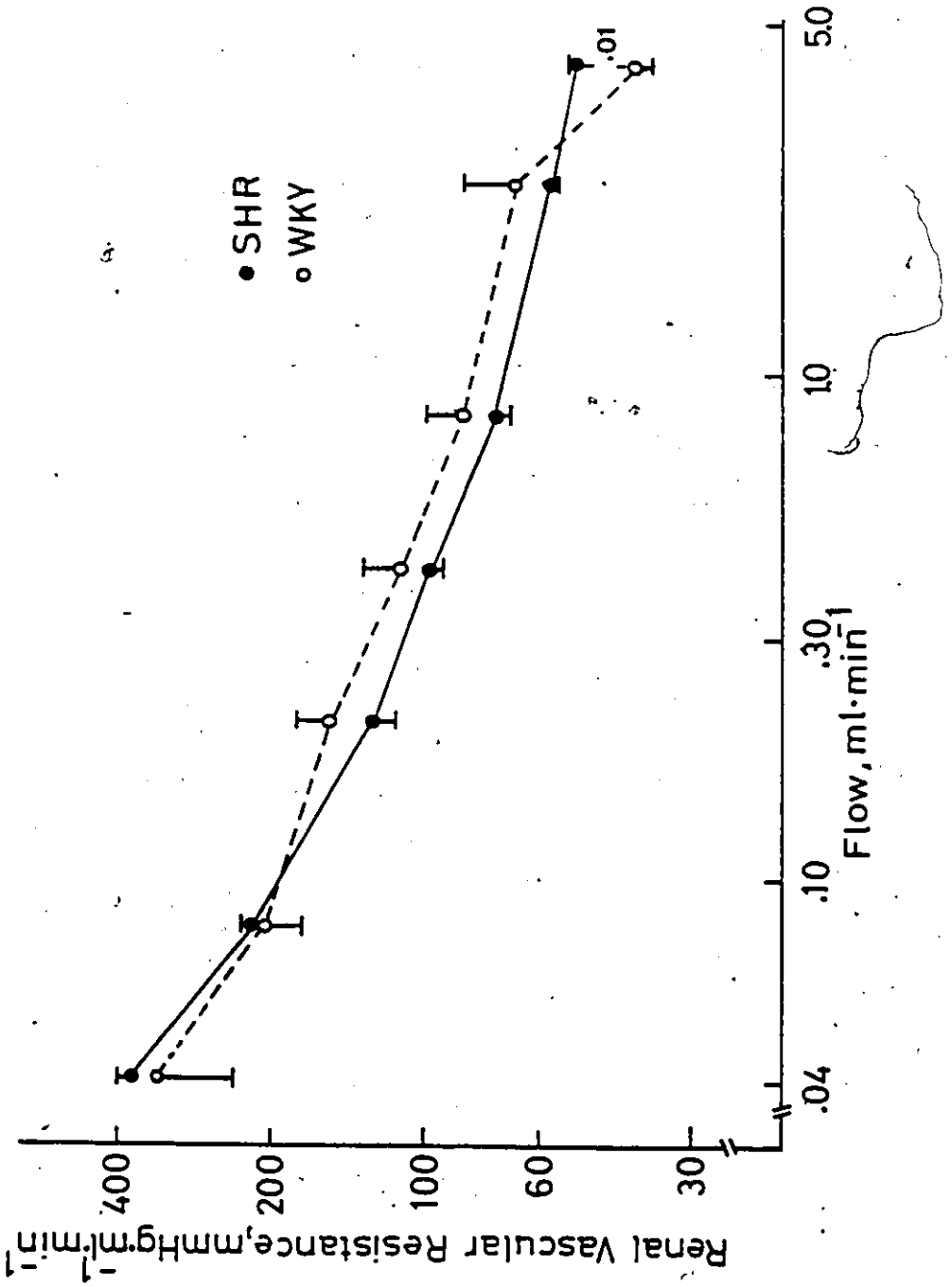

Renal Vascular Resistance, mmHg·ml·min⁻¹
400
200
100
60
30
.04
.10
.30
1.0
5.0
Flow, ml·min⁻¹
.01
• SHR
○ WKY

Table 8. Physical characteristics of prehypertensive SHR and WKY used in the morphometric study of the renal vascular bed.

	SHR (n=10)	WKY (n=10)	P
Age (wks)	4.0 ± .21	4.4 ± .16	NS
Wt (g)	47.9 ± 4.6	55.2 ± 3.8	NS
BP (mmHg)*			
systolic	80.4 ± 5.1	82.6 ± 6.0	NS
diastolic	61.1 ± 4.7	64.9 ± 5.4	NS
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	6.43 ± .32	5.38 ± .11	0.05
Kidney perfusion pressure (mmHg) at a flow rate of 0.082 ml/min	19.0 ± 1.52	13.7 ± 1.54	0.05

* direct measurement from the aorta

of systolic and diastolic blood pressure. Neither of the two measurements proved to be significantly different between SHR and WKY. However, systolic blood pressure obtained by direct measurement was lower than that obtained using the tail cuff compression method on nonanaesthetized rats. As in adult SHR, the kidney to body weight ratio was higher in prehypertensive SHR than WKY.

Initially, prior to fixation, the left kidney was perfused with Krebs at a flow rate of 0.082 ml/min. It was observed that at this flow rate, the infusion pressure was higher in SHR than WKY ($P<0.05$). This indicated that, at least at the one flow studied, renal vascular resistance was increased in SHR. This caused concern, since it suggested that either 1) the average lumen diameter was reduced in the renal vasculature of this group of prehypertensive SHR and/or 2) that the renal vasculature of such animals exhibited a reduced number of open vascular pathways.

(a) Lumen diameter of the renal vessels

Fig. 9 outlines the alterations in the lumen diameter of the renal vessels. As in older rats with established hypertension, the lumen diameter of the main renal, interlobar and arcuate-interlobular arteries was not significantly different between prehypertensive SHR and WKY. Thus the increased RVR in the relaxed vasculature of this groups of SHR could have been produced by either 1) a decrease in lumen diameter of postglomerular vessels and/or 2) a decreased number of blood vessels in preglomerular and/or postglomerular arteries and veins.

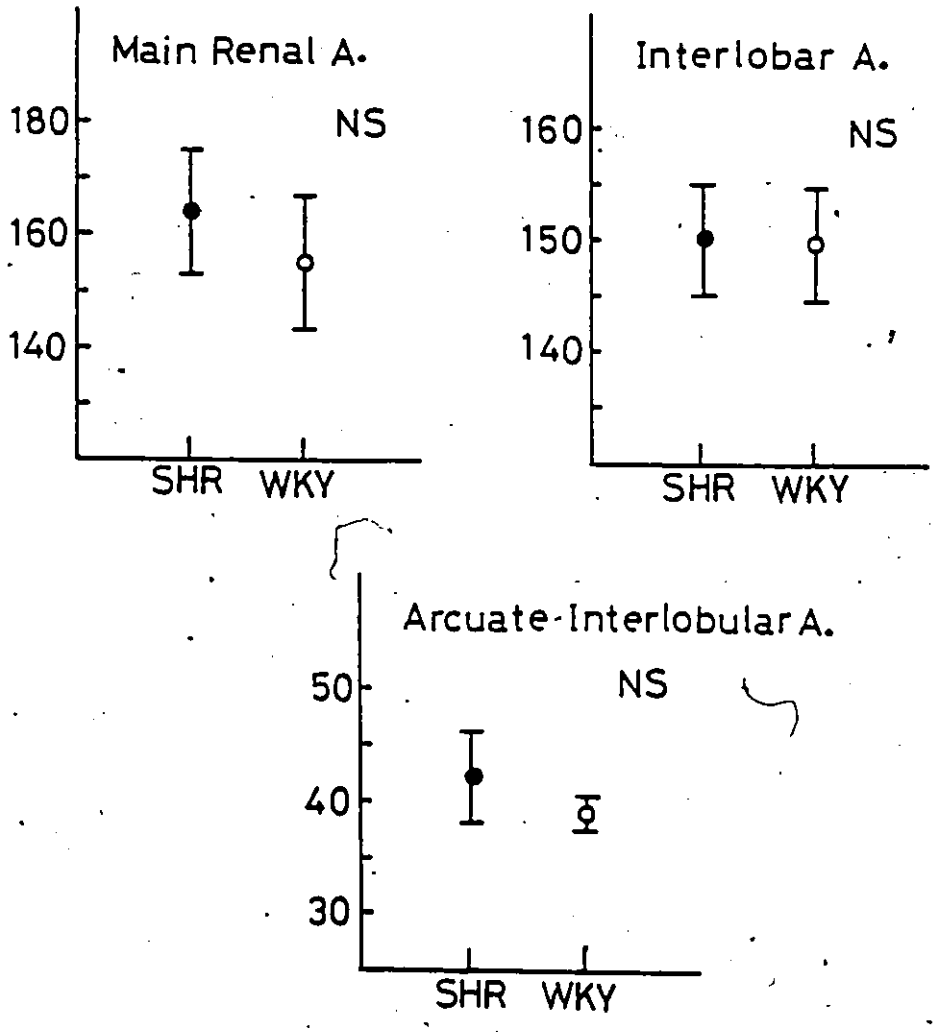
(b) Alterations in the wall components of the renal vessels

The structural alterations in the wall components of the renal

Fig. 9. Lumen diameters of the main renal, interlobar and arcuate-interlobular arteries of prehypertensive SHR and age matched WKY (mean ± SEM).

The renal arteries of 4 week old SHR and WKY were perfusion fixed at a constant flow rate in a relaxed condition. All lumen diameters have been compensated for sectioning angle.

Lumen Dia., μm


Main Renal A.
NS
180
160
140
SHR
WKY
Interlobar A.
NS
160
150
140
SHR
WKY
Arcuate-Interlobular A.
NS
50
40
30
SHR
WKY

vessels of prehypertensive SHR are outlined in Table 9. Prehypertensive SHR exhibited a significant increase in the CSA of intima in the main renal artery and in arcuate interlobular arteries with a D_I between 40 to 80 µm. These same classes of vessels also exhibited an increase in the CSA of media in SHR. As in older SHR with established hypertension, the CSA of adventitia was not greatly altered in prehypertensive SHR. When the CSA of the nonadventitial wall was normalized to the CSA of the lumen (i.e., the wall (intima + media) to lumen ratio), all the arterial groups showed an increase in this ratio in SHR compared to WKY. The number of SMC layers was increased in SHR compared to WKY in the interlobar arteries and in arcuate-interlobular arteries with a D_I greater than 80 µm and less than 60 µm.

3. Summary of the findings of the morphometric study of the renal vascular changes of SHR in a prehypertensive phase of hypertension development

(i) The renal vascular resistance was increased at maximal dilation in the prehypertensive SHR used in the morphometric study. This, however, was not a consistent finding. Perfusion studies at a variety of flow rates, as well as other studies that will be reported in later sections, indicate that the renal vascular resistance of kidneys perfused with Krebs is not altered when prehypertensive SHR are compared to age matched WKY.

(ii) The relaxed prearteriolar renal arteries from prehypertensive SHR did not exhibit a decrease in lumen diameter when compared to WKY. This suggests that the increased renal vascular

Table 9. Morphometric analysis of the renal arterial subcomponents of prehypertensive SHR and WKY.

		Main Renal Arteries	Interlobar Arteries	Arcuate - Interlobular Arteries			
				>80 um	80-60 um	60-40 um	40-20 um
Intima um^2	SHR	4417 ± 548	1849 ± 196	754 ± 86	443 ± 28	338 ± 26	164 ± 9.1
	WKY	2892 ± 399	1470 ± 144	632 ± 47	346 ± 48	192 ± 22	155 ± 12
	P<	0.05	NS	NS	0.05	0.005	NS
Media um^2	SHR	20874 ± 1717	7047 ± 860	2941 ± 259	1722 ± 83	1104 ± 51	560 ± 38
	WKY	16567 ± 1581	5919 ± 503	2473 ± 165	1415 ± 148	761 ± 68	499 ± 32
	P<	0.05	NS	NS	0.05	0.005	NS
Adventitia um^2	SHR	33261 ± 5705	6537 ± 751	3365 ± 215	2073 ± 107	1365 ± 72	665 ± 34
	WKY	37557 ± 8061	9610 ± 1581	3774 ± 390	2119 ± 83	1111 ± 121	730 ± 49
	P<	NS	NS	NS	NS	0.05	NS
Wall Lumen	SHR	.462 ± .025	.485 ± .015	.555 ± .023	.682 ± .021	.851 ± .030	1.31 ± 0.64
	WKY	.380 ± .019	.405 ± .015	.488 ± .030	.556 ± .035	.661 ± .031	.996 ± .056
	P<	0.01	0.005	0.01	0.005	0.005	0.005
SMC Layers	SHR	4.53 ± .173	3.75 ± .118	2.65 ± .142	2.06 ± .043	1.80 ± .053	1.48 ± .051
	WKY	4.30 ± .180	3.01 ± .117	2.36 ± .089	1.88 ± .158	1.38 ± .136	1.29 ± .061
	P<	NS	0.005	0.05	NS	0.005	0.05

resistance in this age group of animals was due to either a decrease in the lumen diameter of the postglomerular blood vessels and/or a decrease in the number of renal blood vessels in SHR compared to WKY.

(iii) In SHR, the main renal artery and intermediate sized arcuate-interlobular arteries exhibited increases in both intimal and medial CSA when compared to WKY.

(iv) All classes of renal arteries studied exhibited an increase in the wall (intima + media) to lumen ratio in SHR. Further, in SHR, virtually all the renal arteries were found to have increased numbers of SMC layers when compared to WKY.

(v) The above findings clearly indicate that blood vessel wall thickening occurs in SHR prior to the elevation of blood pressure.

D. Pharmacological Study of SHR in Established Hypertension

1. Norepinephrine (NE) concentration vs renal vascular resistance dose response curves

Table 10 shows the physical characteristics of the rats used in the NE contraction study. Neither the weight nor the age were significantly different between SHR and WKY. However, blood pressure and the kidney to body weight ratio was significantly elevated in SHR over WKY. When the experimental left kidney was weighed and compared to the unperfused right kidney, no significant difference in wet weights was observed, indicating little oedema formation in the experimental kidney.

Table 10. Physical characteristics of adult SHR and WKY used in the perfused norepinephrine, contraction study.

	SHR (n=6)	WKY (n=7)	P
Age (wks)	22.1±.747	22.9±.370	NS
Wt (g)	308 ±6.29	292 ±16.6	NS
BP (mmHg)	190 ±5.27	122 ±4.04	0.001
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	5.80±.174	5.20±.201	0.05
Kidney Wt (g)			
Perfused	1.76±.017	1.63±.090	NS
Unperfused	1.74±.080	1.45±.098	0.05
	NS	NS	

Fig. 10 shows the alterations in RVR in response to the infusion of NE. At maximal dilatation, the RVR was similar between SHR and WKY. Contracting the renal vasculature produced diverging curves, the RVR being higher in SHR over WKY animals at 2×10^{-7} M and at greater concentrations of NE.

2. The role of the beta receptors in norepinephrine contraction

To determine if the beta receptor plays an important role in the NE contractile response, the kidneys of 4 SHR (23.3 ± 1.03 weeks old, 318 ± 17.0 g, 169 ± 5.1 mm Hg blood pressure) were perfused with 10^{-5} M NE and subsequently with 10^{-5} M NE containing 10^{-7} and 10^{-6} M propranolol, a beta blocking agent. Fig. 11 summarizes the results of the experiment. The stimulation of beta receptors normally relaxes most arteries. However, blocking the beta receptors did not potentiate contraction in response to NE. The presence of 10^{-7} M propranolol, a beta receptor antagonist, did not alter the NE response significantly, while 10^{-6} M propranolol decreased the NE response substantially.

3. $BaCl_2$ concentration vs renal vascular resistance response curves

Table 11 shows the physical characteristics of the rats used in the $BaCl_2$ contraction study. The age and weight of SHR and WKY were not significantly different. The blood pressure and kidney to body weight ratio were elevated in SHR over WKY. After the conclusion of the experiment the perfused left kidneys were found to have similar weights to the unperfused right kidneys in both SHR and WKY.

Fig. 10. Alterations in renal vascular resistance in response to norepiniephrine contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The left kidney of 22 week old SHR and WKY was perfused with Krebs solution at a constant flow rate. Responses to cumulative doses of norepinephrine in the presence of 10^{-5} M cocaine were recorded.

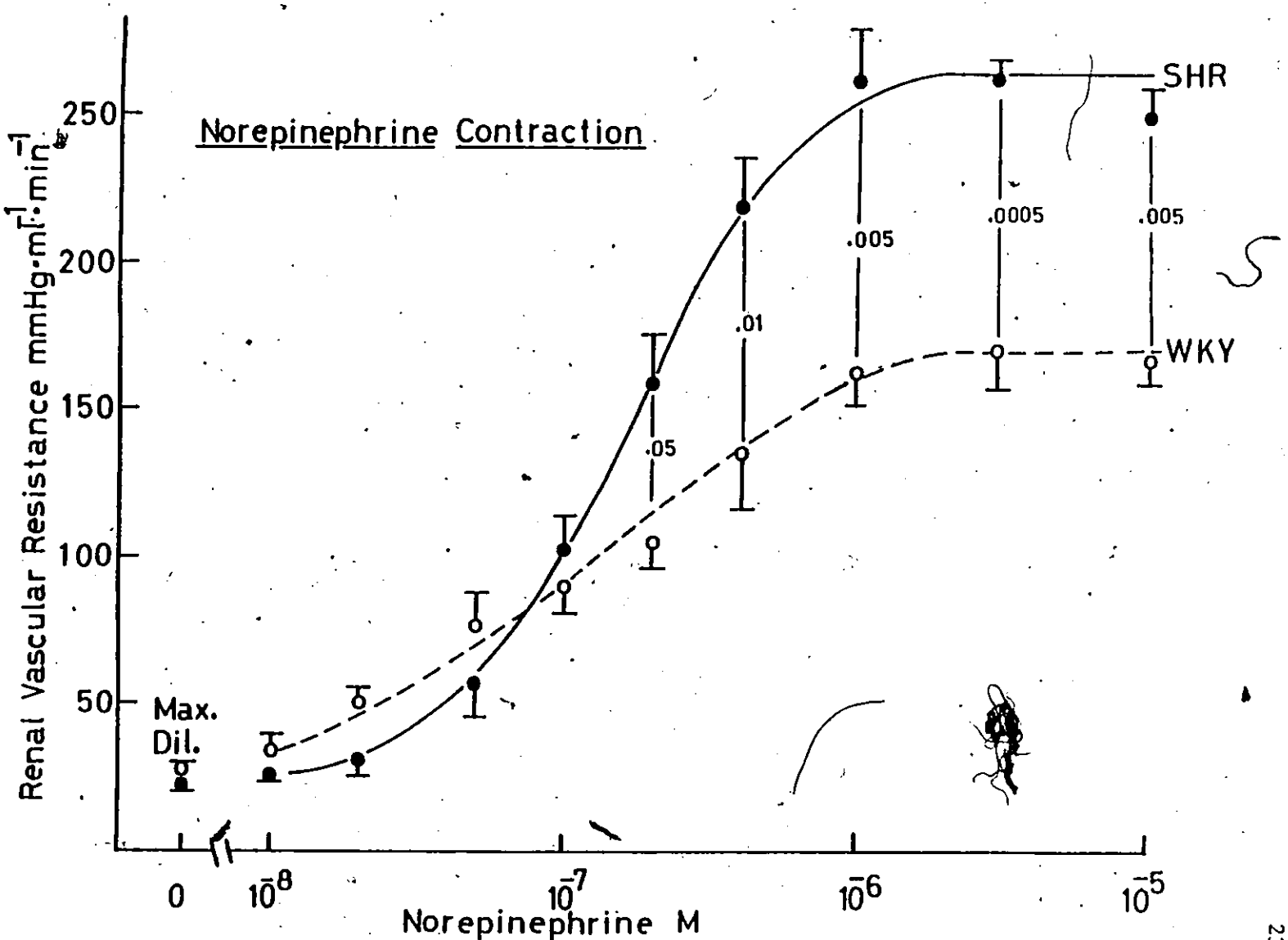

Norepinephrine Contraction
Renal Vascular Resistance mmHg·ml⁻¹·min⁻¹
250
200
150
100
50
Max.
Dil.
SHR
WKY
.005
.01
.05
.0005
.005
0
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Norepinephrine M

Fig. 11. The effect of blocking beta receptors (propranolol) on norephinephrine contraction of the renal vasculature of SHR with established hypertension (mean ± SEM).

The left kidney of 23 week old SHR was perfused at a constant flow. The renal vascular resistance was measured during Krebs perfusion after contraction with 10^{-5} M NE, and subsequently during contraction with 10^{-5} M NE in the presence of 10^{-7} and 10^{-6} M propranolol.

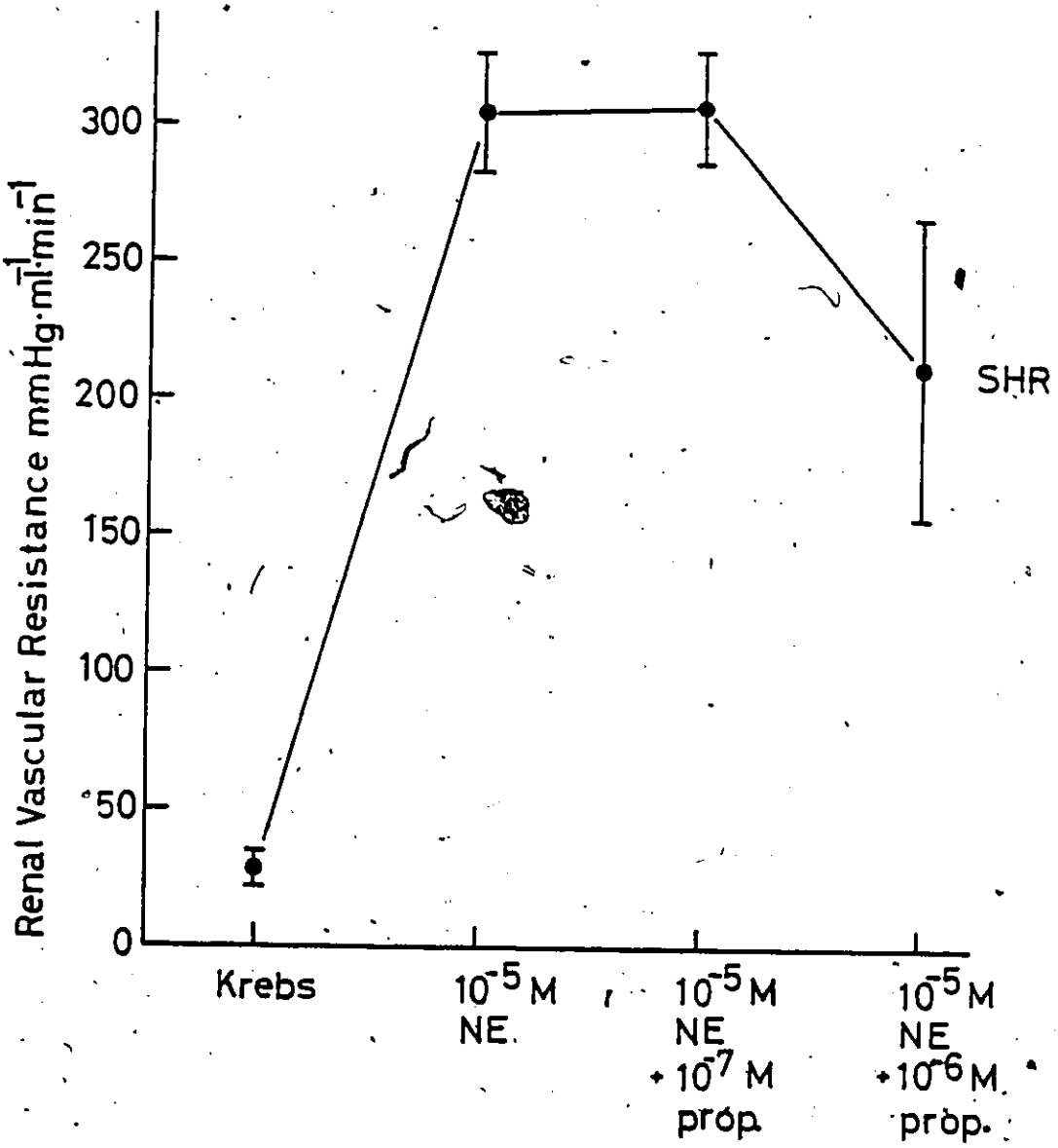

Renal Vascular Resistance mmHg·ml⁻¹·min⁻¹
300
250
200
150
100
50
0
SHR
Krebs
10⁻⁵ M NE.
10⁻⁵ M NE + 10⁻⁷ M prop.
10⁻⁵ M NE + 10⁻⁶ M prop.

Table 11. Physical characteristics of adult SHR and WKY used in the $BaCl_2$ contraction study.

	SHR (n=4)	WKY (n=4)	P
Age (wks)	31.5±.25	31.2±1.74	NS
Wt (g)	353±9.5	330±9.15	NS
BP (mmHg)	184±6.3	132±7.29	0.005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	5.16±.125	4.45±.177	0.01
Kidney Wt (g)			
Perfused	1.66±.107	1.49±.055	0.005
Unperfused	1.80±.131	1.59±.067	NS
	NS	NS	

Consistent with the results obtained in the NE study, at maximum dilation, the renal vascular resistance did not differ between SHR and WKY (Fig. 12). $BaCl_2$ contraction produced a higher amplitude of RVR change in SHR over WKY; significance at $P<0.05$ was observed at $BaCl_2$ levels equal to and greater than 19.5 mM (Fig. 12).

4. K^+ concentration vs renal vascular resistance dose response curves

The physical characteristics of the rats used in the K^+ contraction study are outlined in Table 12. No significant difference in age or body weight existed between SHR and WKY. The blood pressure and the kidney to body weight ratio were elevated in SHR over WKY. No differences in the kidney weights of perfused and unperfused kidneys were observed in the SHR and WKY.

Fig. 13 shows the alterations in RVR in relationship to K^+ concentrations. Unlike the results obtained in the NE and $BaCl_2$ contraction studies, there were no differences in the magnitude of contractile responses observed when SHR and WKY animals were compared. Initially, it was thought that the lack of altered reactivity could be due to the fact that K^+ is a weaker contractile agent than either NE or $BaCl_2$. Therefore, after K^+ contraction, the same kidneys were equilibrated with Krebs and contracted to maximal levels with angiotensin II (A_{II}). Although A_{II} is a very potent agonist (i.e., contraction occurs at very low doses), the degree of maximal contraction that can be evoked is only about 40 to 75% of the maximal response produced by K^+. When the kidneys were equilibrated with Krebs no

Fig. 12. Alterations in renal vascular resistance in response to $BaCl_2$ contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The left kidney of 31 week old SHR and WKY was perfused with modified Krebs solution at a constant flow. The Krebs solution was similar to that used in other experiments except that it contained no SO_4, PO_4 or HCO_3. These modifications were essential in order to prevent Ba^{+2} from precipitating from solution. Responses to cumulative doses of $BaCl_2$ were measured. The effects of norepinephrine released from the sympathetic nerves was blocked by including 10^{-8} M prazosin + 10^{-6} M spiroperidol in the Krebs solution. The Krebs solution was maintained at a constant osmolarity (340 mosm) by decreasing NaCl.

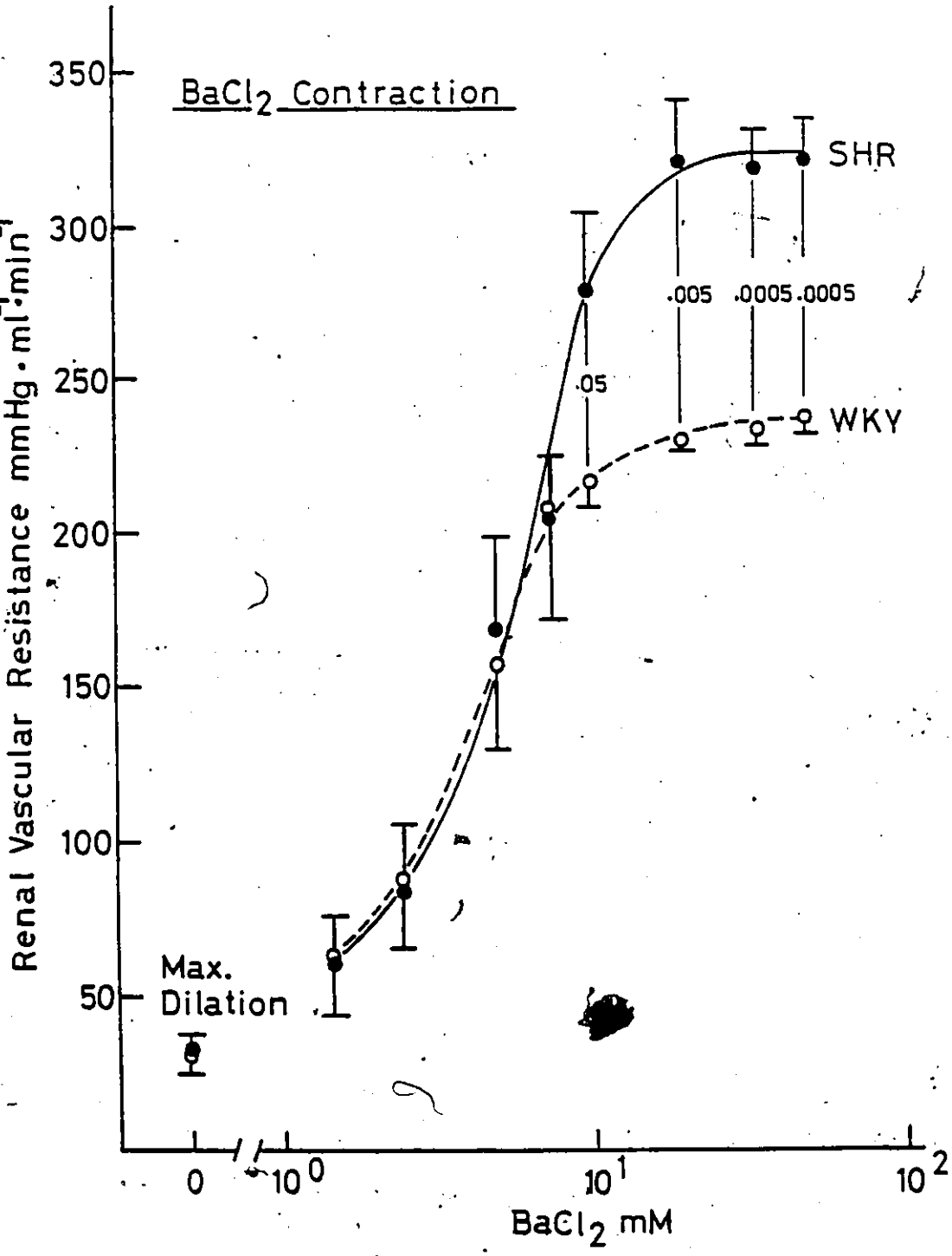
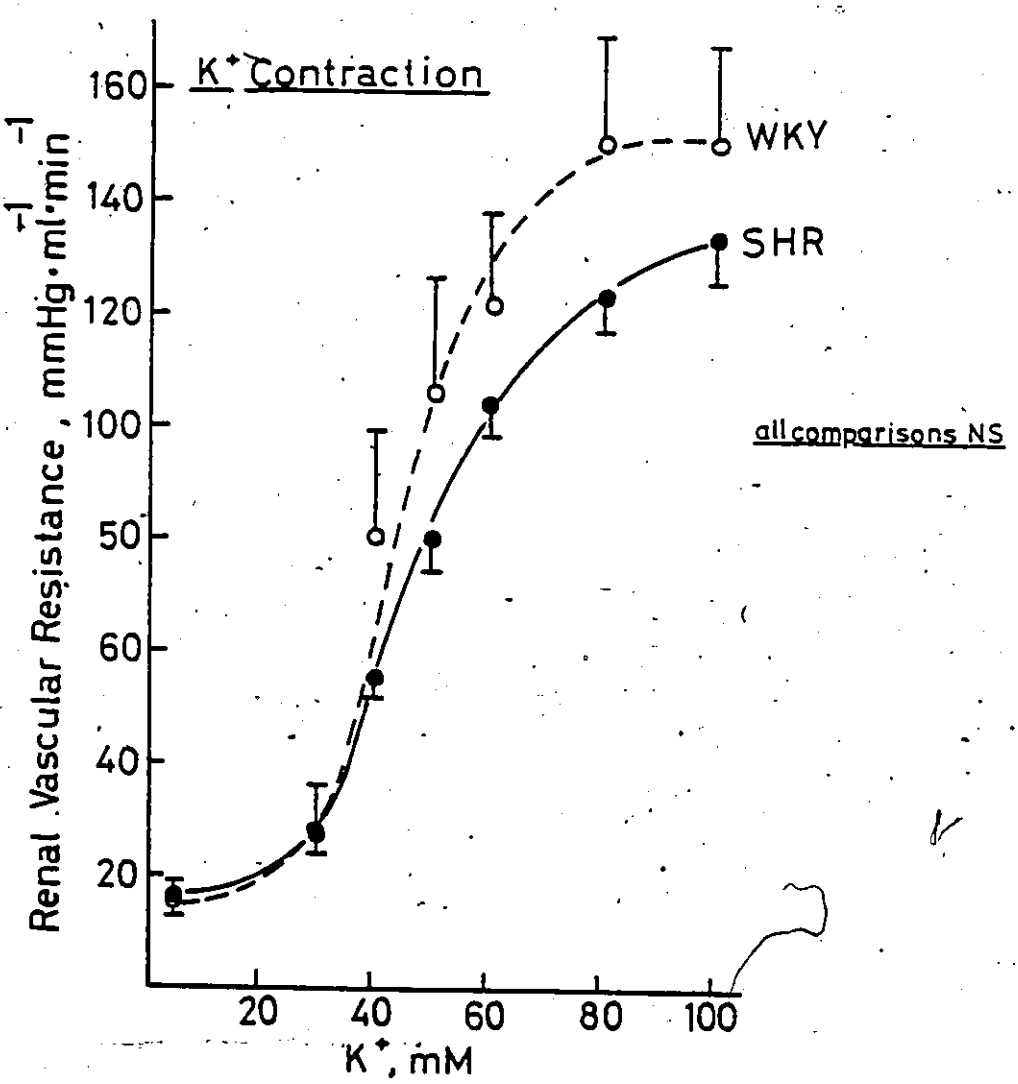

BaCl2 Contraction
Renal Vascular Resistance mmHg · ml-1 · min-1
350
300
250
200
150
100
50
SHR
WKY
.005
.0005
.0005
.05
Max.
Dilation
0
10 0
10 1
10 2
BaCl2 mM

Table 12. Physical characteristics of adult SHR and WKY used in the KCl and angiotensin II contraction studies.

	SHR (n=6)	WKY (n=6)	P
Age (wks)	29.5±1.26	29.4±1.60	NS
Wt (g)	338 ±16.8	335 ±6.64	NS
BP (mmHg)	183 ±5.10	132 ±4.24	0.0005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	4.91±.185	4.43±.113	0.01
Kidney Wt (g)			
Perfused	1.80±.131	1.49±.055	NS
Unperfused	1.66±.107	1.59±.067	0.05
	NS	NS	

Fig. 13. Alterations in renal vascular resistance in response to KCl contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The left kidney of 29 week old SHR and age matched WKY was perfused with Krebs solution at a constant flow. Responses to cumulative doses of KCl were recorded. The effects of norepinephrine released from the sympathetic nerves was blocked by including 10^{-8} M prazosin + 10^{-6} M spiroperidol in Krebs solution. The Krebs was maintained at a constant osmolarity (340 mosm) by decreasing NaCl.


K+ Contraction
Renal Vascular Resistance, mmHg·ml⁻¹·min⁻¹
160
140
120
100
50
60
40
20
WKY
SHR
all comparisons NS
20
40
60
80
100
K+, mM

differences in the basal resistance were observed. However, maximal contraction with 10^{-7} M A_{II} produced a higher RVR alteration in SHR over WKY (Fig. 14). A sub-population (4 SHR and 4 WKY) of animals [illegible]cted with K^+ and then A_{II} were subsequently equilibrated with PO_4^{-2}, SO_4^{-2}, HCO_3^--free, MOPS buffered isotonic Krebs and contracted with 48.7 mM $BaCl_2$ (Fig. 15). Again, the basal resistance was similar between SHR and WKY; however, $BaCl_2$ contraction produced a higher RVR response in SHR over WKY.

5. Alterations in the sensitivity to NE, $BaCl_2$ and K^+ contraction

To determine if the contractile sensitivity of various agents was altered, the dose response curves previously presented were replotted. Individual responses (RVR) were expressed as a percentage of the maximal change in RVR. Subsequently, the curves were analyzed for a right or leftward shift. The results of this study are shown in Fig. 16 to 18. Of the perfused agents only NE exhibited evidence of altered sensitivity (Fig. 16). At perfusate concentrations of 10^{-8}, 2×10^{-8} and 5×10^{-8} M, the renal vasculature of WKY was found to respond in a higher proportion of its maximum than SHR. SHR and WKY did not exhibit any differences in the sensitivity to $BaCl_2$ (Fig. 17) or K^+ (Fig. 18) contraction.

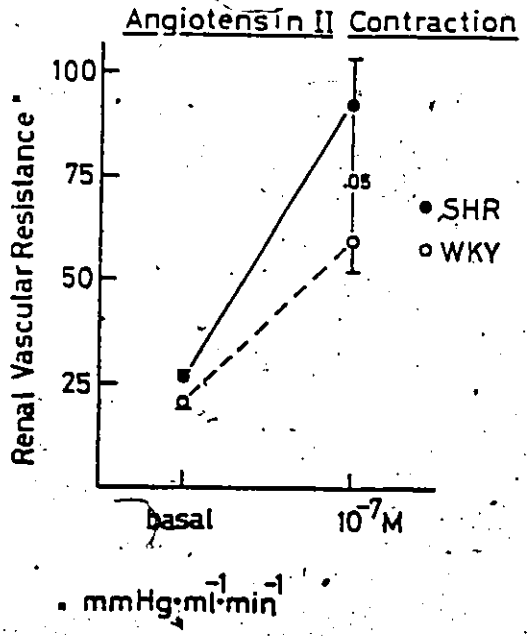
Table 13 summarizes the ED_{20} and ED_{50} values obtained for the various contractile agents. In the case of NE contraction, ED_{20}, but not ED_{50}, values were increased in SHR over WKY. ED_{20} and ED_{50} values for K^+ or $BaCl_2$ contraction were not altered with hypertension.

Fig. 14. Alterations in renal vascular resistance in reponse to angiotensin II contraction in SHR with established hypertension and age matched WKY previously contracted with KCl (mean ± SEM).

The conditions are the same as those outlined in Fig. 13. After contraction with KCl, the renal vasculature was relaxed by perfusing the kidney with Krebs solution. Subsequently, 10^{-7} M angiotensin II was infused and the renal vascular resistance was measured. The infusion of 10^{-6} M angiotensin II did not further elevate renal vascular resistance.

Fig. 15. Alterations in renal vascular resistance in response to $BaCl_2$ in SHR with established hypertension and age matched WKY previously contracted with KCl (mean ± SEM).

The conditions are the same as those outlined in Fig. 13. The renal vasculature was relaxed with modified Krebs solution and contracted maximally with $BaCl_2$ in the presence of 10^{-8} M prazosin + 10^{-6} M spiroperidol.


Angiotensin II Contraction
Renal Vascular Resistance
100
75
50
25
.05
SHR
WKY
basal
10^{-7}M
mmHg·ml^{-1}·min^{-1}

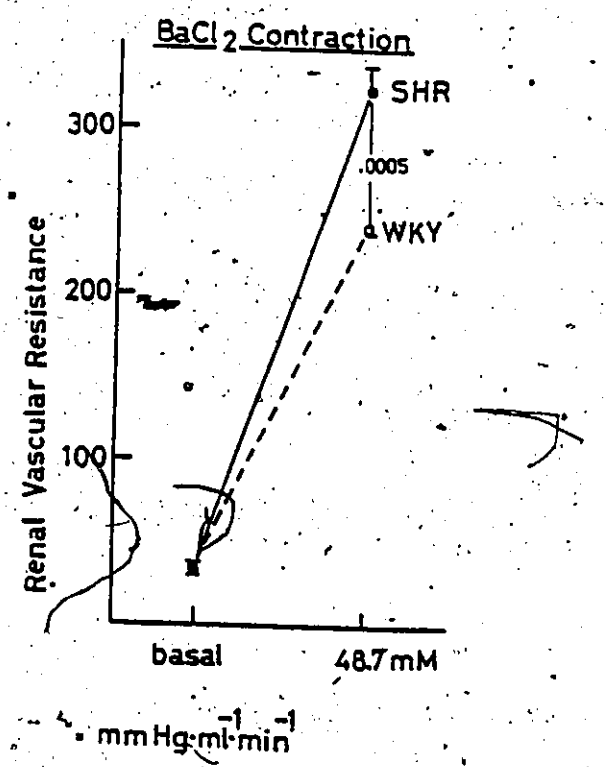

BaCl2 Contraction
Renal Vascular Resistance
300
200
100
SHR
.0005
WKY
basal
48.7mM
mmHg·ml^{-1}·min^{-1}

Fig. 16. Alterations in the sensitivity of the renovasculature to norepinephrine contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The conditions are the same as those outlined in Fig. 10. The response is expressed as a fraction of the maximal renal vascular resistance obtained in response to norepinephrine.

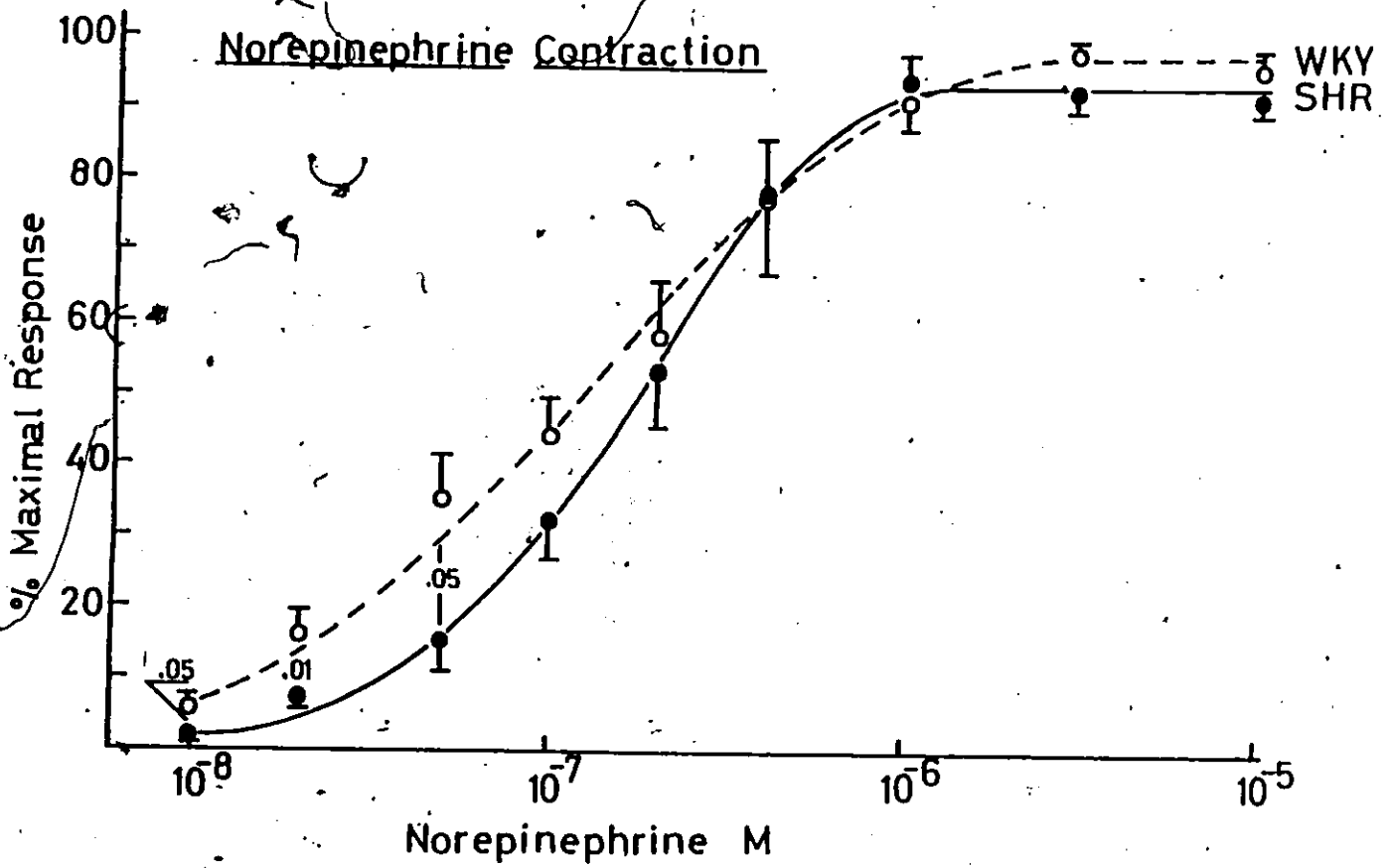

Norepinephrine Contraction
WKY
SHR
% Maximal Response
100
80
60
40
20
.05
.01
.05
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Norepinephrine M

Fig. 17. Alterations in the sensitivity of the renovasculature to $BaCl_2$ contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The conditions are the same as those outlined in Fig. 12. The response is expressed as a fraction of the maximal renal vascular resistance obtained in response to $BaCl_2$.

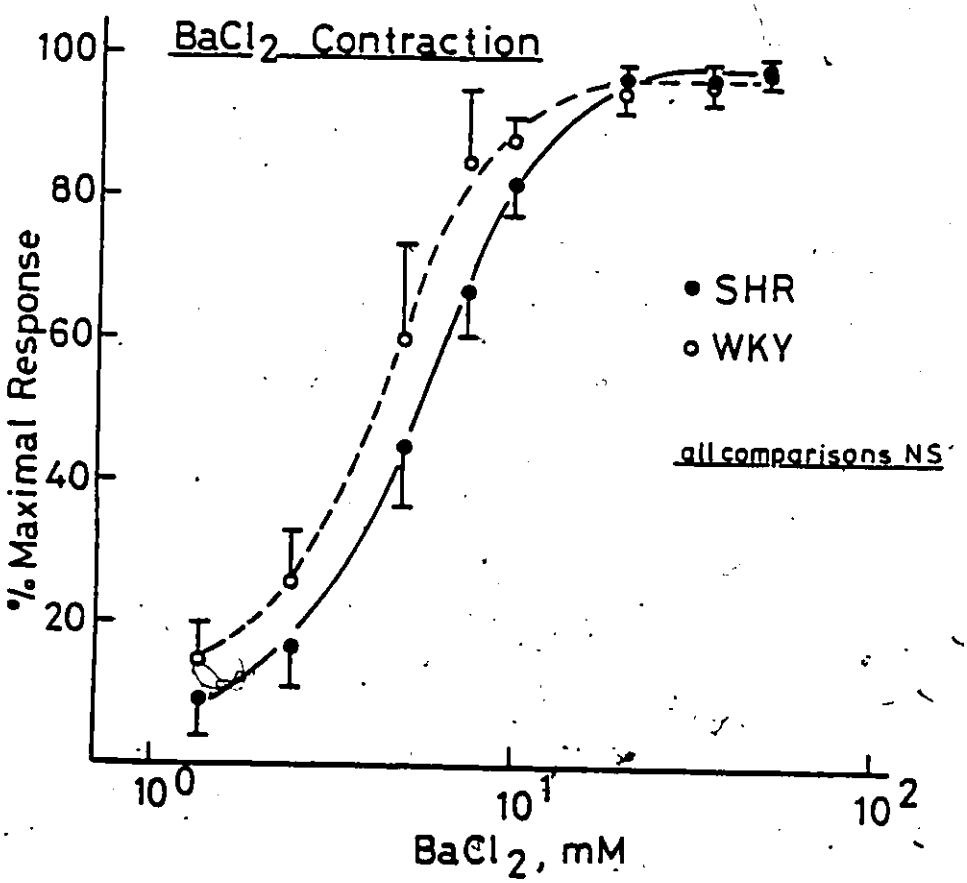

BaCl2 Contraction
100
80
60
40
20
% Maximal Response
• SHR
o WKY
all comparisons NS
10^0
10^1
10^2
BaCl2, mM

Fig. 18. Alterations in the sensitivity of the renovasculature to KCl contraction in SHR with established hypertension and age matched WKY (mean ± SEM).

The conditions are the same as those outlined in Fig. 13. The response is expressed as a fraction of the maximal renal vascular to resistance obtained in response to KCl.

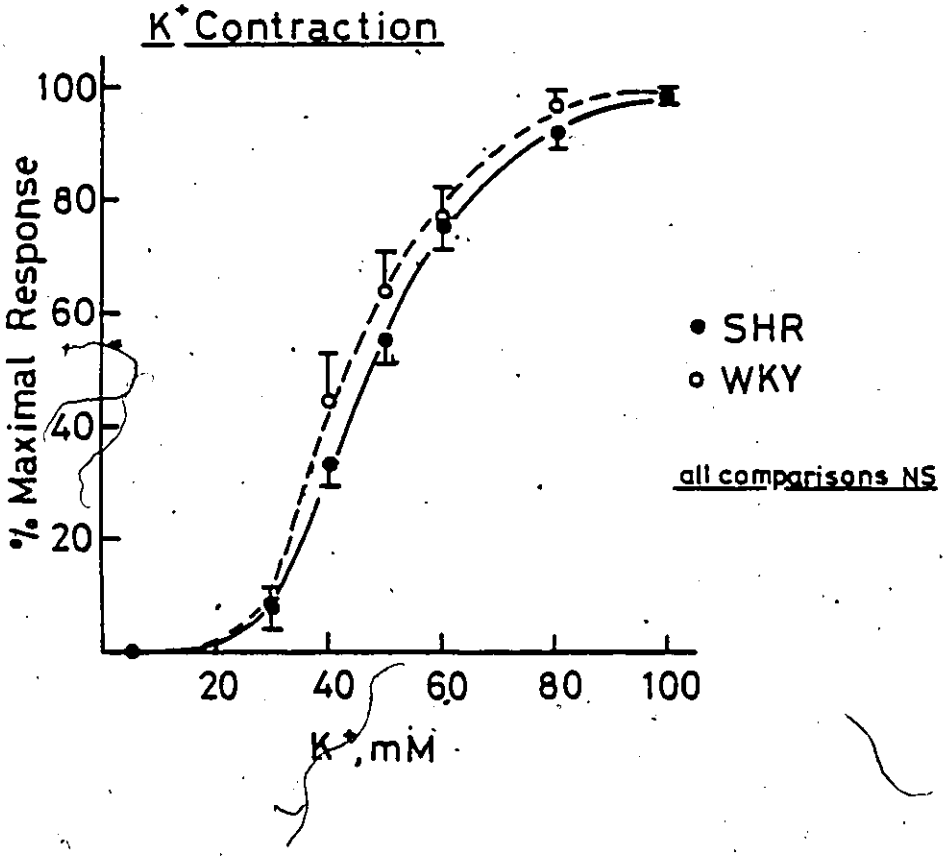

K⁺ Contraction
% Maximal Response
100
80
60
40
20
20
40
60
80
100
K⁺, mM
• SHR
○ WKY
all comparisons NS

Table 13. The norepinephrine, KCl and $BaCl_2$ concentrations producing 20% and 50% of the maximal response in adult SHR and WKY.

	SHR	WKY	P
Perfused Norepinephrine Contraction	n=6	n=7	
20% maximal response (mM x 10^{-8})	6.91±1.28	3.38±.90	0.05
50% maximal response (mM x 10^{-7})	1.77±.34	1.16±.31	NS
KCl Contraction	n=6	n=6	
20% maximal response (mM)	33.2±2.00	33.7±.804	NS
50% maximal response (mM)	47.5±2.18	45.0±2.94	NS
BaCl Contraction	n=4	n=4	
20% maximal response (mM)	2.90±.540	2.05±.790	NS
50% maximal response (mM)	5.75±1.00	4.25±.785	NS

6. Summary of the findings of the dose response studies involving various contractile agents

(i) When renal vascular bed was maximally relaxed with Krebs, the RVR was not significantly different between SHR and WKY.

(ii) Contraction of the renal vasculature with NE or $BaCl_2$ produced a situation where the amplitude of the RVR was higher in SHR than WKY.

(iii) In SHR, beta receptor blockade by propranolol did not potentiate maximal contraction in response to NE.

(iv) K^+ contraction of the renal vasculature produced RVR alterations that were not significantly different when SHR were compared with WKY. However, in SHR the same renal vasculatures produced higher amplitudes of RVR change in response to A_{II} and $BaCl_2$.

(v) The ED_{20} values for contraction in response to NE were higher in SHR than WKY, suggesting a small decrease in the contractile sensitivity of the renal vasculature to this agonist. However, none of the other contractile agents exhibited any alterations in ED_{20} or ED_{50} values when SHR and WKY were compared.

E. Nerve Stimulation Study Involving Established Hypertensive SHR

1. Nerve stimulation frequency vs RVR response

Table 14 outlines the physical characteristics of the SHR and WKY used in the nerve stimulation study. The age of the animals studied was not significantly different. However, within this sample, the

Table 14. Physical characteristics of adult SHR and WKY used in the nerve stimulated contraction study.

	SHR (n=8)	WKY (n=5)	P
Age (wks)	22.7±.456	21.3±.216	NS
Wt (g)	317 ±10.7	347 ±3.94	0.05
BP (mmHg)	188 ±5.14	125 ±3.80	0.005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	5.00±.138	4.33±.219	0.01
Kidney Wt (g)			
Perfused	1.58±.068	1.50±.085	NS
Unperfused	1.63±.076	1.55±.040	NS
	NS	NS	

weight of the SHR was significantly lower than that of the WKY. Consistent with the previous findings, the blood pressure as well as the kidney to body weight ratio was elevated in SHR over WKY. There was no significant difference between the weight of perfused and unperfused kidneys of either SHR or WKY.

Fig. 19 shows the stimulation frequency vs RVR response curves obtained for SHR and WKY. Under conditions where the renal vasculature was perfused with Krebs in the absence of nerve stimulation, the basal RVR was similar between SHR and WKY. When the nerves entering the kidney were stimulated at increasing frequencies using a pulse duration of 2 ms with a 20 mV potential difference between the electrodes, RVR was increased at all frequencies greater than 6 Hz in SHR over WKY.

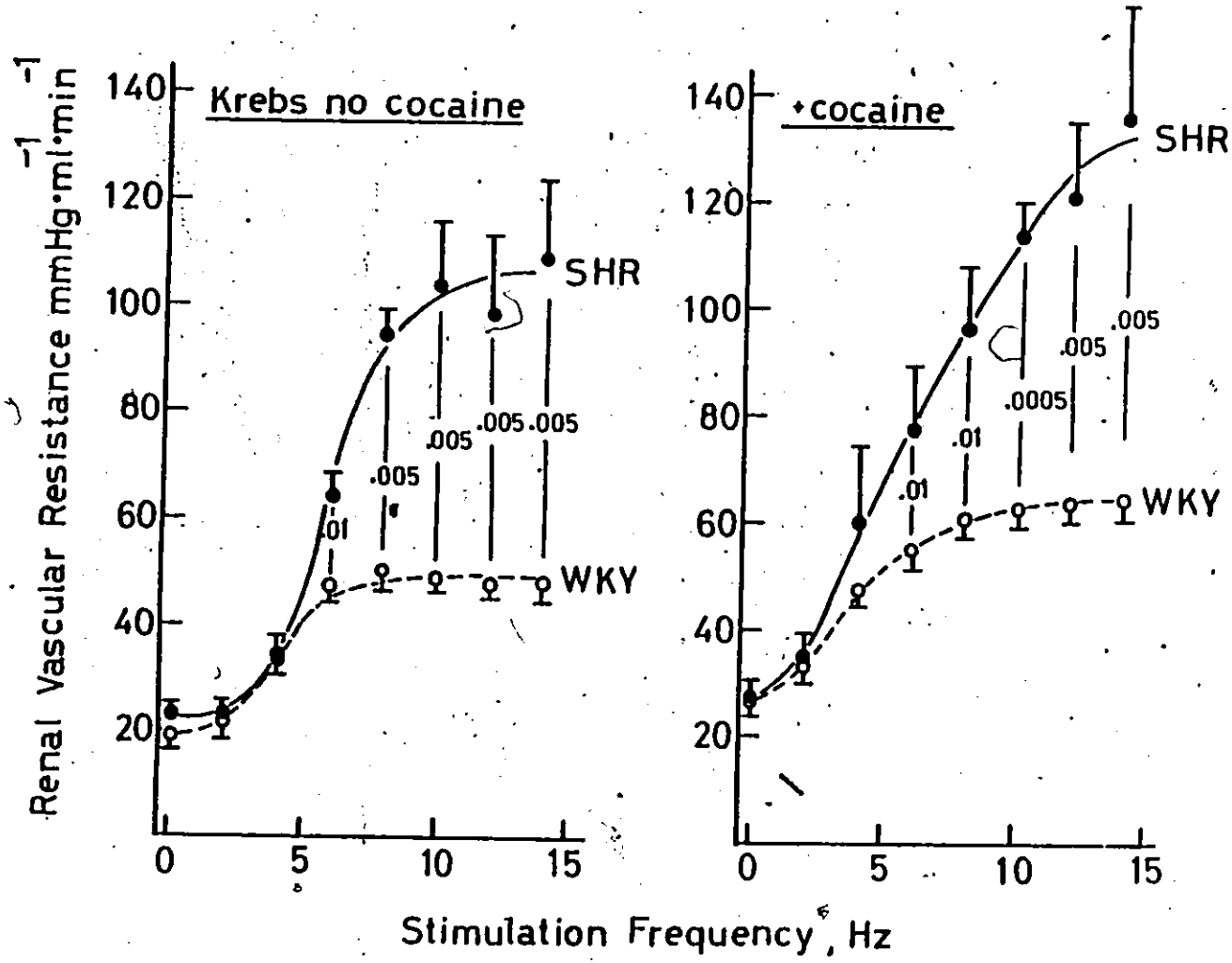
In a subpopulation of these animals (4 SHR and 5 WKY), the neuronal uptake of NE was blocked with 10^{-5} M cocaine. The results are shown in Fig. 20. The typical effect of cocaine was to increase the basal RVR and slightly shift the RVR response curve to the left of the curve obtained in the absence of cocaine. In the presence of cocaine the basal RVR was the same in SHR and WKY, while the amplitude of response was significantly elevated in SHR over WKY at frequencies greater than 6 Hz. Fig. 20 suggests that RVR at maximal levels of stimulation was greater in the presence of cocaine than in its absence (Fig. 19). However, the rats used in the cocaine study had slightly higher RVR at maximal stimulation. To determine whether cocaine potentiated the response in SHR and WKY, the RVR at each frequency in the presence of cocaine was divided by the RVR at the same frequency in the absence of cocaine. Values significantly greater than one, indicate

Fig. 19. (left) Alterations in renal vascular resistance in response to renal nerve stimulation in SHR with established hypertension and age matched WKY (mean ± SEM).

The left kidney of 21 and 22 week old SHR and WKY was perfused with Krebs at a constant flow. Platinum electrodes were placed around the renal artery at an interelectrode distance of 2.5 mm. The nerves entering the kidney were stimulated at varying frequencies using a 2 min train of pulses, each pulse having a 2 msec duration with an interelectrode potential difference of 20 V.

Fig. 20. (right) Alterations in renal vascular resistance in response to renal nerve stimulation in SHR with established hypertension and age matched WKY (mean ± SEM) under conditions where the presynaptic uptake of NE has been blocked.

The conditions of the experiment are identical to those of Fig. 19, except that 10^{-5} M cocaine was added to the Krebs solution to block the neuronal uptake of NE.


Krebs no cocaine
+cocaine
SHR
WKY
.01
.005
.0005
Renal Vascular Resistance mmHg·ml·min
Stimulation Frequency, Hz
0
5
10
15
20
40
60
80
100
120
140

that cocaine increased the amplitude of response. Fig. 21 summarizes the findings. At lower stimulation frequencies and 4 Hz, cocaine significantly potentiated the contractile response in both SHR and WKY. However, at higher stimulation frequencies, cocaine was found to have little effect in altering the RVR. Quantitatively, there was very little difference in the degree of potentiation observed in SHR compared to WKY, although at stimulation frequencies of 10 and 12 Hz the potentiation was higher ($P<0.05$) in WKY than SHR.

2. Alterations in the sensitivity to nerve stimulation

The response of the percentage maximal response for WKY was shifted to the left of that obtained for SHR (Fig. 22) when plotted against stimulation frequency. The proportion of the maximal response was significantly elevated in WKY over SHR at stimulation frequencies of 2, 4 and 6 Hz.

Table 15 shows that the nerve stimulation frequencies producing 20 and 50% maximal responses were reduced in WKY compared to SHR.

3. Receptors involved in the nerve stimulated contractile response

Table 16 contains the physical characteristics of the rats used in these studies. The age and weight of both groups were similar, but blood pressure was elevated in SHR over WKY. Unlike previous studies, the kidney to body weight ratio was not significantly elevated in SHR, while the kidney weights of the perfused and unperfused kidneys were similar in SHR and WKY.

Several receptor antagonists were used to to assess the nerve

Fig. 21. The degree of alteration in nerve mediated responses produced by the blockage of the presynaptic uptake of norepinephrine in the renal vasculature of SHR with established hypertension and age matched WKY (mean ± SEM).

Conditions are the same as those outlined in Fig. 19 and 20. The change in renal vascular resistance is response to each frequency of nerve stimulation in the presence of 10^{-5} M cocaine was divided by the level of response present in the absence of the drug.

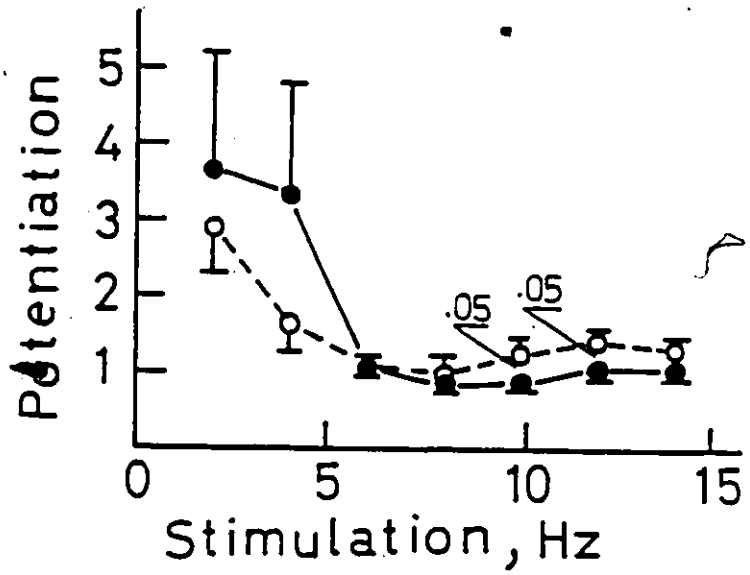

Potentiation
5
4
3
2
1
0
5
10
15
Stimulation, Hz
.05
.05

Fig. 22. Alterations in the sensitivity of the renovascular to nerve mediated contraction in SHR with established hypertension and age matched WKY (mean ± SEM). The conditions of the experiment are identical to those outlined in Fig. 19. The response is expressed as a fraction of the maximal renal vascular resistance obtained in response to nerve stimulation.

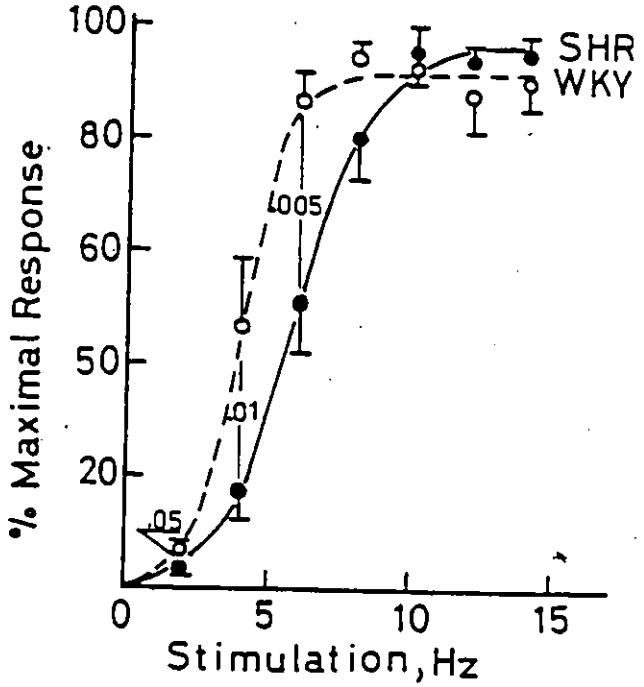

100
80
60
50
20
% Maximal Response
.05
.01
.005
SHR
WKY
0
5
10
15
Stimulation, Hz

Table 15. The stimulation frequency producing 20% and 50% of the maximal response in adult SHR and WKY.

Nerve Stimulated Contraction	SHR (n=5)	WKY (n=5)	P
20% maximal response (Hz)	4.38±.425	2.92±.265	0.01
50% maximal response (Hz)	6.20±.537	3.94±.237	0.005

Table 16. Physical characteristics of adult SHR and WKY used to determine the the receptors involved in the nerve stimulated contractile response.

	SHR (n=4)	WKY (n=4)	P
Age (wks)	23.7±.31	23.1±.085	NS
Wt (g)	318 ±4.4	313 ±4.15	NS
BP (mmHg)	171 ±1.3	123 ±5.95	0.0005
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	4.75±.12	4.56±.114	NS
Kidney Wt (g)			
Perfused	1.51±.57	1.43±.029	NS
Unperfused	1.54±.11	1.42±.044	NS
	NS	NS	

stimulated response. Only propranolol (at 10^{-6} but not 10^{-7} M), a beta antagonist, prazosin (10^{-8} M), an $alpha_1$ receptor antagonist and spiroperidol (10^{-6} M), a dopamine receptor antagonist had any effect in reducing the response. After propranolol (10^{-7} M) + prazosin (10^{-8} M) was introduced into the solution, 10^{-7} and 10^{-6} M yohimbine, an $alpha_2$ receptor antagonist, and phentolamine (10^{-7} M), an $alpha_1$ and $alpha_2$ receptor antagonist had very little effect on the response to nerve stimulation.

Fig. 23 shows the basal perfusion pressure and the response of the renal vasculature to nerve mediated contraction. Here the renal nerves were electrically stimulated with a 2 min, 10 Hz train of pulses with each pulse having a 2 ms duration and a 60 V potential difference. The application of such a stimulus produced a higher response in SHR over WKY animals. The introduction of 10^{-7} M propranolol had very no effect on the response in both SHR and WKY, while 10^{-6} M propranolol (not shown in Fig. 23) either had no further effect, or lowered the response in SHR. This indicated that under the above conditions, beta receptor blockade did not potentiate nerve stimulated contraction. The introduction of 10^{-8} M prazosin, an $alpha_1$ receptor blocker, reduced the response by 85-90%. After prazosin, the responses obtained for SHR and WKY were not significantly different. The small residual response was further decreased by the dopamine receptor antagonist spiroperidol (10^{-6} M). On the basis of these findings, it would appear that nerve stimulated contraction is produced mainly by $alpha_1$ receptors, and to a lesser extent, by dopamine receptors. The proportion of the maximal response attributed to each receptor shown in

Fig. 23. The effect of beta, $alpha_1$ and dopamine receptor antagonists on the renal vascular resistance alterations in response to renal nerve stimulation in SHR with established hypertension and age matched WKY (mean ± SEM).

The left kidney of 23 week old SHR and WKY was perfused with Krebs solution at a constant flow. Subsequently, a 2 min train of nerve stimulation was applied (10 Hz, 2 msec duration, 60 V potential difference). This was repeated in the presence of 10^{-7} M propranolol followed by 10^{-7} M propranolol + 10^{-8} M prazosin, and finally, in the presence of 10^{-7} M propranolol + 10^{-8} M prazosin + 10^{-6} M spiroperidol.

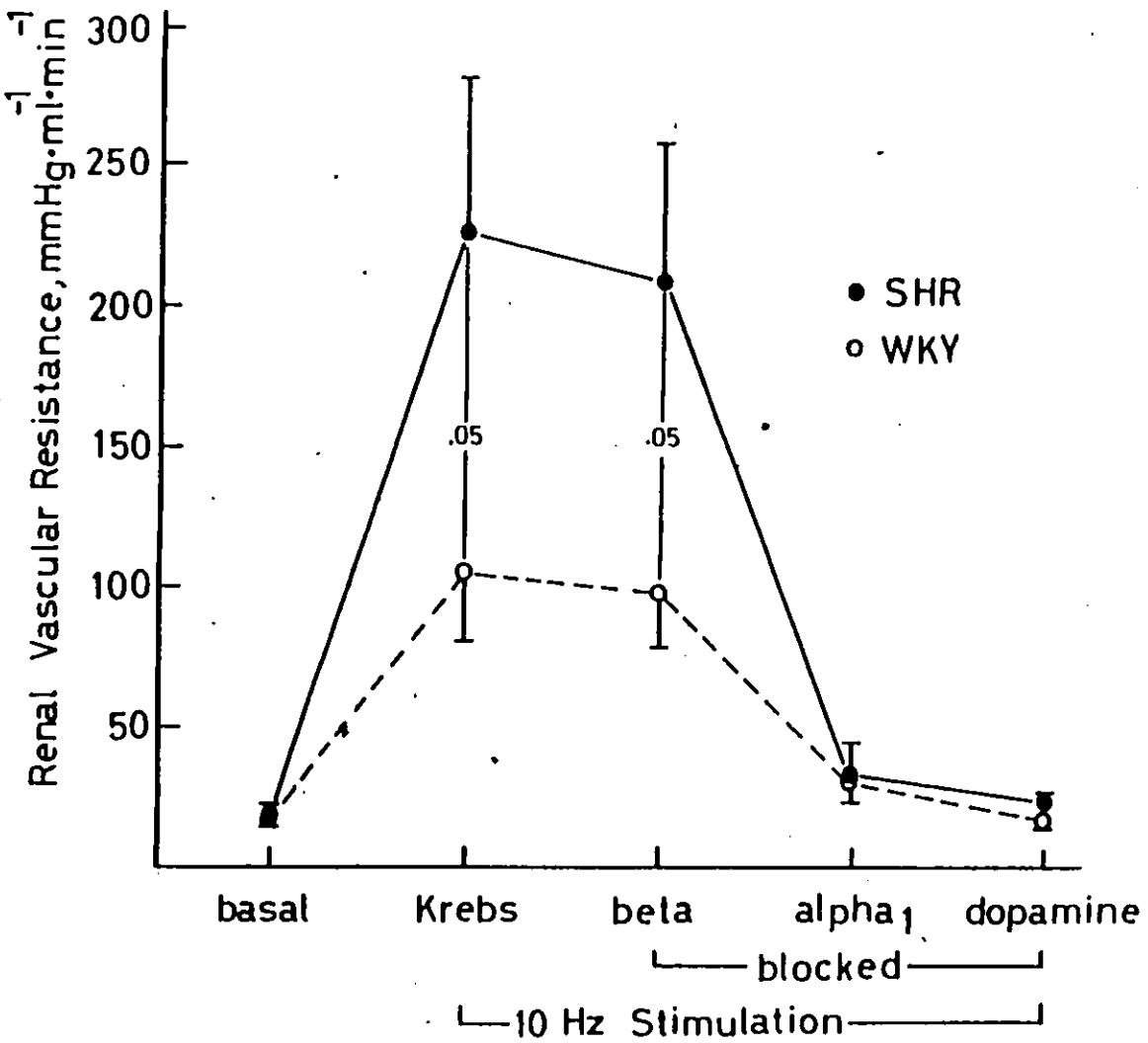

Renal Vascular Resistance, mmHg·ml⁻¹·min⁻¹
300
250
200
150
100
50
.05
.05
• SHR
o WKY
basal
Krebs
beta
alpha 1
dopamine
blocked
10 Hz Stimulation

Table 17. Percent of maximal nerve stimulated response attributed to $alpha_1$ and dopamine receptors in adult SHR and WKY.

	SHR (n=4)	WKY (n=4)	P
$Alpha_1$	90.4 ± 2.73	84.9 ± 2.73	NS
Dopamine	6.2 ± 2.3	13.0 ± 2.64	NS

Table 17 is not different between SHR and WKY.

4. Summary of the findings of the nerve stimulation study

(i) Under conditions where the nerves were not stimulated, the vascular resistance was similar between SHR and WKY.

(ii) Nerve stimulation increased the vascular resistance of SHR kidneys to a much greater degree than WKY.

(iii) The blockade of presynaptic NE uptake increased the basal resistance of the kidney (zero nerve stimulation) and shifted the dose response curve slightly to the left (RVR vs stimulation frequency response).

(iv) In the absence of cocaine, the nerve stimulation frequencies producing 20 or 50% maximal responses were less in WKY compared to SHR, thus indicating that the nerve stimulation sensitivity was reduced in SHR over WKY.

(v) Alpha$_1$, and to a much lesser extent, dopamine receptors were involved in mediating the contractile response to nerve stimulation in the renal vasculature. Although the maximal response to nerve stimulation was elevated in SHR over WKY, the proportion of the response attributed to alpha$_1$ and dopamine receptors was not altered.

(vi) The beta receptor did not appear to be involved in nerve stimulated contraction.

F. Pharmacological Studies on Prehypertensive SHR

Table 18 outlines the physical characteristics of the prehypertensive SHR and WKY used in the pharmacological studies. The age, body weight and blood pressure (tail cuff compression method) were not significantly different between SHR and WKY. As in the previous hypertensive groups, the kidney to body weight ratio was elevated SHR. The kidney weight of the perfused left kidney was not significantly altered compared to the kidney weight of the unperfused right kidney in either SHR or WKY.

1. Norepinephrine (NE) concentration vs renal vascular resistance (RVR) response curves

Fig. 24 shows the RVR response to infused NE in the presence of 10^{-6} M cocaine. When the vasculature was relaxed with Krebs the RVR was not different between SHR and WKY. When the renal vasculature was contracted with NE, the response curve obtained for SHR was shifted to the right of the response curve obtained for WKY. Thus, at lower concentrations of NE, WKY exhibited higher amplitude of RVR change than SHR. However, at maximal contraction, the renal vascular resistance in SHR was elevated over WKY.

2. Alterations in the NE contraction sensitivity

As might be suspected from Fig. 24, the contractile sensitivity of the renal vasculature to NE was reduced in SHR when compared to WKY. The proportion of the maximal response produced by each NE dose (Fig. 25) indicate that WKY had a higher fractional response than SHR at 10^{-7}, 2×10^{-7} and 5×10^{-7} M doses of NE. An analysis of the ED_{20} and ED_{50}

Table 18. Physical characteristics of prehypertensive SHR and WKY used in pharmacological studies.

	SHR (n=7)	WKY (n=7)	P
Age (wks)	5.75±.231	5.77±.255	NS
Wt (g)	85.1±8.65	84.5±6.00	NS
BP (mmHg)	114 ±4.31	103 ±5.03	NS
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	7.08±.083	6.64±.151	0.05
Kidney Wt (g)			
Perfused	.60 ±.059	.56 ±.034	NS
Unperfused	.60 ±.068	.50 ±.037	NS
	NS	NS	

Fig. 24. Alterations in renal vascular resistance in response to norepinephrine contraction in prehypertensive SHR and age matched WKY (mean ± SEM).

The left kidney of 5 week old SHR and WKY was perfused with Krebs solution at a constant flow. Responses to cumulative doses of norepinephrine in the presence of 10^{-6} M cocaine were recorded.

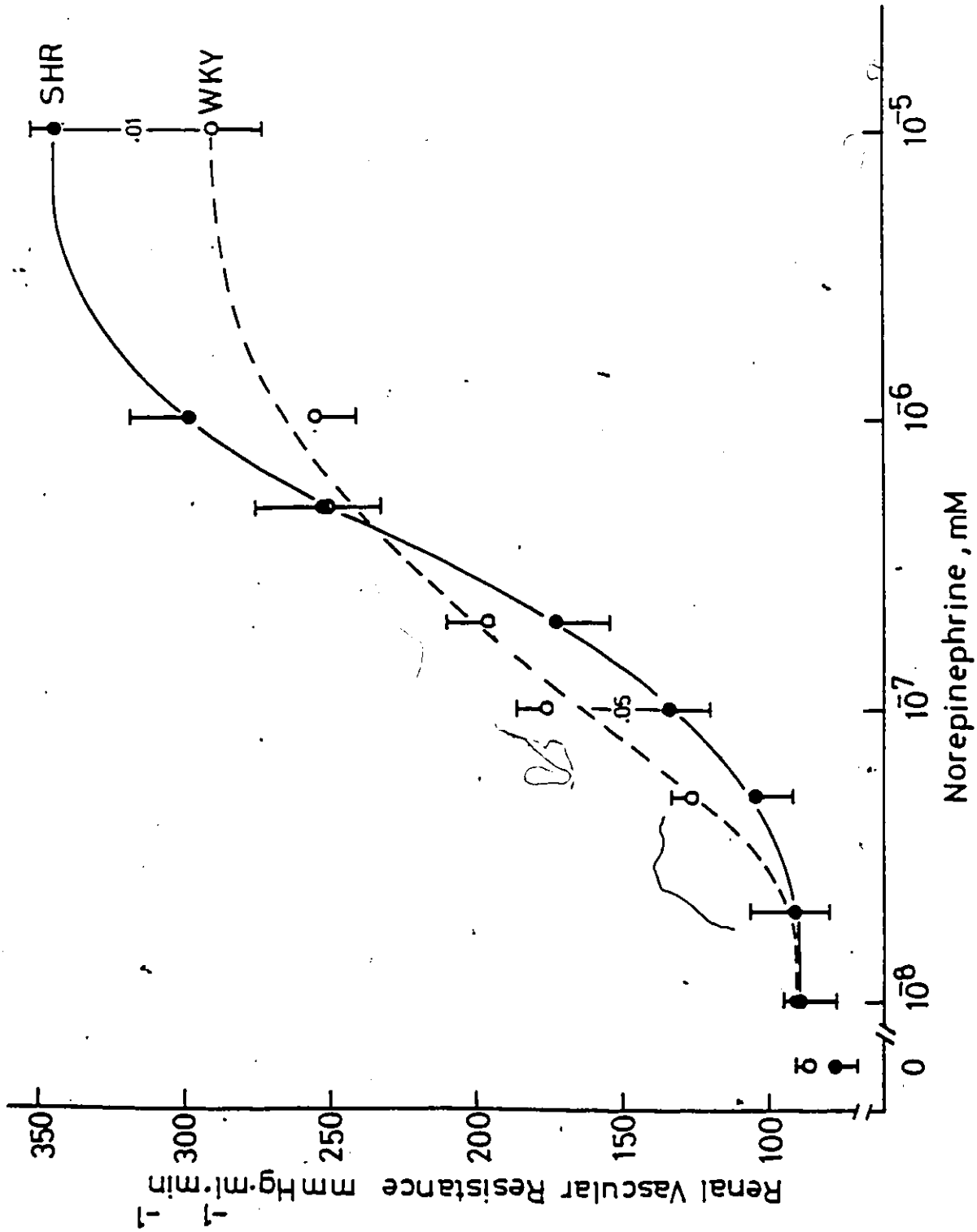

SHR
WKY
.01
.05
Renal Vascular Resistance mmHg.ml.min⁻¹
350
300
250
200
150
100
0
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Norepinephrine, mM

Fig. 25. Alterations in the sensitivity of the renovasculature to norepinephrine contraction in prehypertensive SHR and age matched WKY (mean ± SEM).

The conditions of the experiment are the same as those outlined in Fig. 24. The response is expressed as a fraction of the maximal renal vascular resistance obtained in response to norepinephrine.

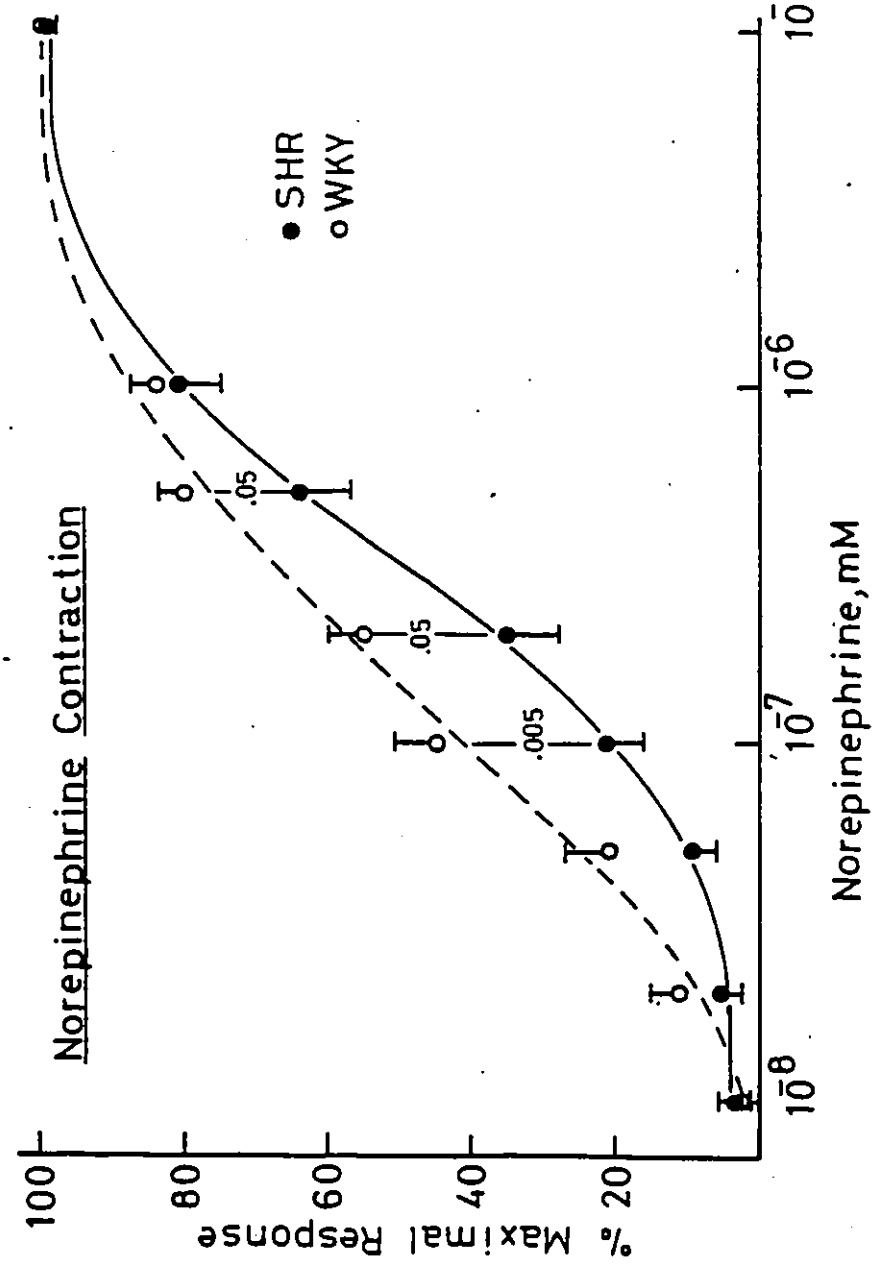

Norepinephrine Contraction
SHR
WKY
.05
.05
.005
% Maximal Response
100
80
60
40
20
10^{-8}
10^{-7}
10^{-6}
10^{-5}
Norepinephrine, mM

Table 19. The norepinephrine concentration producing 20% and 50% of the maximal response in prehypertensive SHR and WKY.

	SHR	WKY	P
Perfused Norepinephrine Contraction	n=6	n=7	
20% maximal response (mM x 10^{-8})	8.78±1.25	5.90±1.20	NS
50% maximal response (mM x 10^{-7})	3.20±.559	1.80±.361	0.05

values for NE contraction (Table 19) showed that ED_{50} values were elevated in SHR over WKY.

G. Nerve Stimulation Study Involving Prehypertensive SHR

1. Stimulation frequency vs RVR response curves

The nerves entering the left kidney were stimulated at frequencies of 2, 4, 6, 8, 10, 14 and 16 Hz using a 2 min train of pulses. Each stimulation pulse had a duration of 2 ms. The potential difference between the electrodes (spaced 2.5 mm apart) was 60 V. The RVR at each frequency of stimulation in the absence of cocaine is outlined in Fig. 26. Under conditions where no stimulus was applied, the RVR was not different between SHR and WKY. The mean RVR response in SHR was higher than WKY at frequencies greater than 4 Hz. However, the alterations were not significant at $P<0.05$.

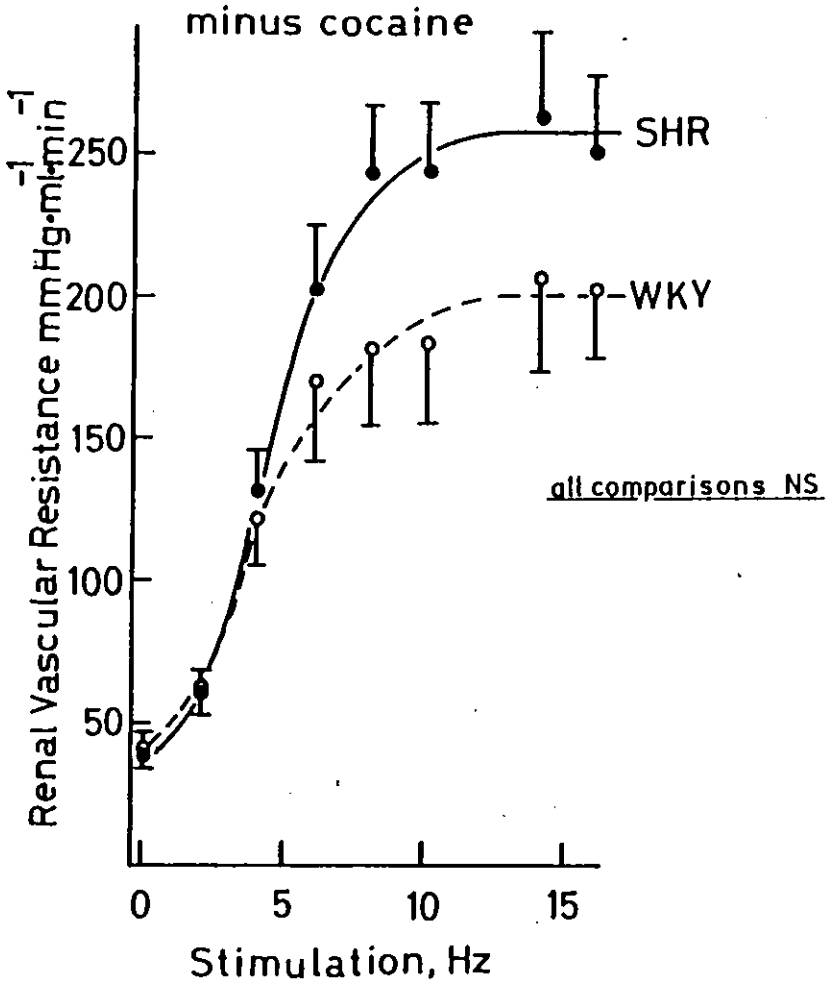
Fig. 27 shows the experiment discussed above repeated under conditions where neuronal uptake of NE was blocked by 10^{-6} M cocaine. As in older SHR with established hypertension, cocaine increased the basal RVR slightly in both SHR and WKY. It was found that at maximal stimulation frequencies in the presence of cocaine the RVR response was slightly reduced in both SHR and WKY compared to the responses previously obtained without cocaine. It is possible that the reduction in the response could have been due to an anaesthetic effect of cocaine on the nerves, or nerve fatigue due to excessive stimulation. However, lowering the cocaine levels and performing the experiment on other rats at one-half maximal levels of nerve stimulation still produced a situation where, in some instances, the degree of response was lower in

Fig. 26. (left) Alterations in renal vascular resistance in response to renal nerve stimulation in prehypertensive SHR and age matched WKY (mean ± SEM).

The left kidney of 5 week old SHR and WKY was perfused with Krebs solution at a constant flow. Platinum electrodes were placed around the renal artery at an interelectrode distance of 2.5 mm. The nerves entering the kidney were stimulated at varying frequencies using a 2 min train of pulses, each pulse having a 2 msec duration with an interelectrode potential difference of 60 V.

Fig. 27. (right) Alterations in renal vascular resistance in response to renal nerve stimulation in prehypertensive SHR and age matched WKY under conditions where the presynaptic uptake of NE was blocked (mean ± SEM).

The conditions of the experiment are identical to those outlined in Fig. 26; 10^{-6} M cocaine was added to the Krebs solution to block the neuronal uptake of NE.


minus cocaine
Renal Vascular Resistance mmHg·ml·min
-1 -1
250
200
150
100
50
SHR
WKY
all comparisons NS
0
5
10
15
Stimulation, Hz

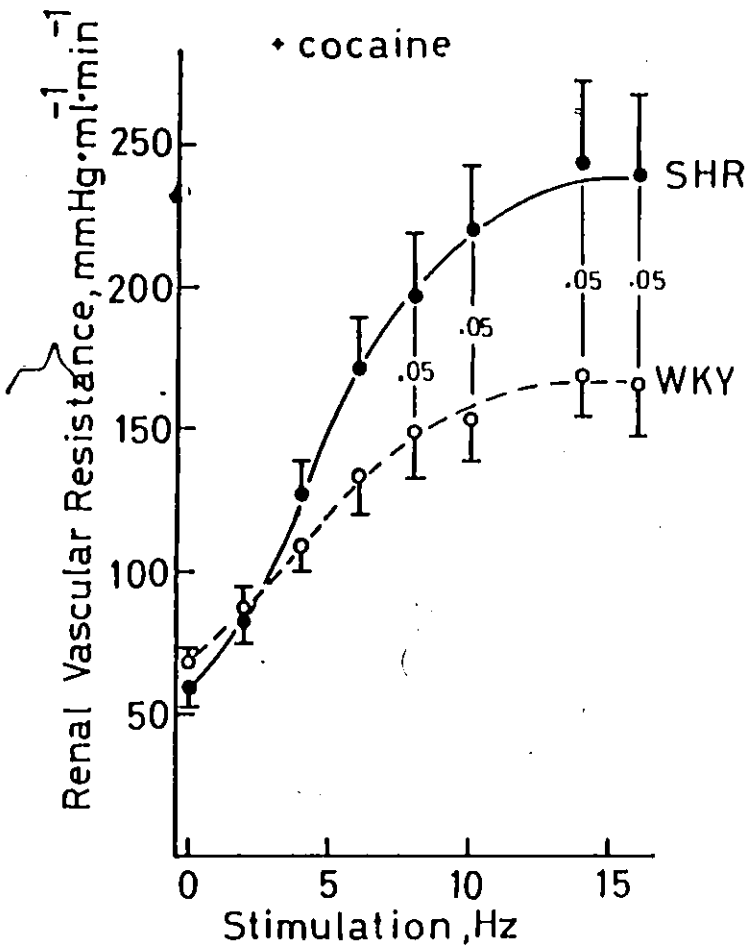

+ cocaine
Renal Vascular Resistance, mmHg·ml·min
-1 -1
250
200
150
100
50
SHR
WKY
.05
.05
.05
.05
0
5
10
15
Stimulation, Hz

Fig. 28. Alterations in renovascular sensitivity in response to nerve mediated contraction in prehypertensive SHR and age matched WKY (mean ± SEM).

The conditions of the experiment are identical to those outlined in Fig. 26. The response is expressed as a fraction of the maximal renal vascular resistance obtained in response to nerve stimulation.

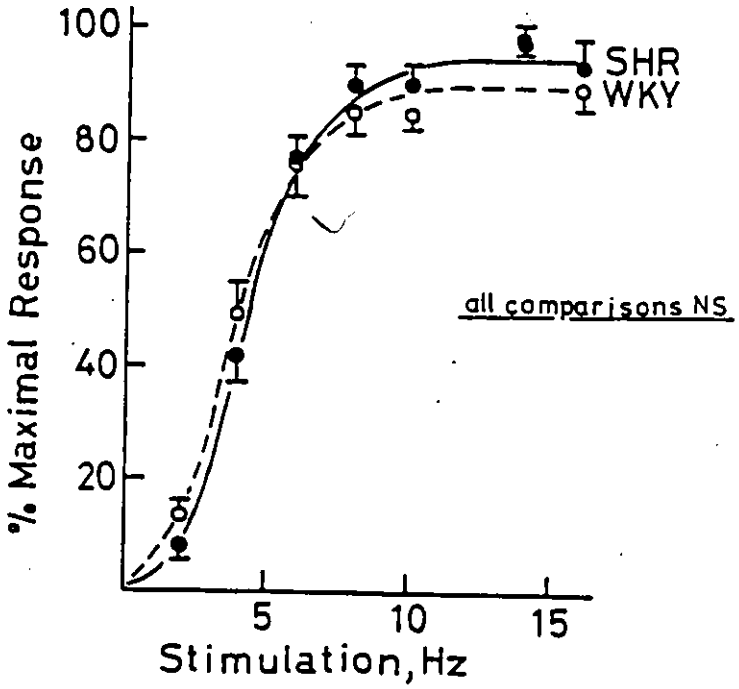

100
80
60
40
20
% Maximal Response
SHR
WKY
all comparisons NS
5
10
15
Stimulation, Hz

Table 20. The stimulation frequencies producing 20%and 50% of the maximal response in prehypertensive SHR and WKY.

Nerve Stimulated Contraction	SHR (n=7)	WKY(n=7)	P
20% maximal response (Hz)	2.94±.217	2.51±.511	NS
50% maximal response (Hz)	4.36±.220	4.13±.300	NS

the presence of cocaine than in its absence. Unlike the frequency response curve obtained in the absence of cocaine, in the presence of cocaine SHR exhibited significantly higher RVR alterations than WKY at nerve stimulation frequencies greater than 8 Hz (Fig. 27).

2. Alterations in the sensitivity to nerve stimulation

The relationship between stimulation frequency and the maximal response was not significantly different in SHR and WKY (Fig. 28). Table 20 also indicates that the nerve stimulation frequencies producing 20 and 50% maximal responses were similar between SHR and WKY.

3. Summary of the pharmacological and electrical studies on prehypertensive SHR

(i) Under conditions where the renal vasculature was relaxed with Krebs solution and not challenged with NE or nerve stimulation the RVR was similar in SHR and WKY.

(ii) NE contraction vs RVR response curves for SHR were shifted to the right compared to WKY. At low doses of NE, WKY responded with greater changes in RVR than did SHR. However, at maximal contraction, the RVR was higher in SHR than WKY.

(iii) The contractile sensitivity to NE was elevated in WKY over SHR.

(iv) Nerve stimulation frequency vs RVR responses showed significantly higher RVR responses in SHR in the presence, but not in the absence of cocaine.

(v) The nerve stimulated contractile sensitivity was unaltered

between SHR and WKY.

H. Hydralazine Treatment Study

1. Blood pressure profile of SHR and WKY treated with hydralazine and non-treated controls

Fig. 29 shows the blood pressure profiles of SHR and WKY treated in utero and postnatally with hydralazine, and untreated control SHR and WKY. Hydralazine reduced the blood pressure of SHR to a level not different than the WKY controls. The blood pressure of the treated SHR was significantly lower than that in the non-treated SHR at ages greater than 6 weeks (Fig. 29). At all ages studied, the blood pressure in the treated SHR and untreated WKY was similar. It should be noted however that hydralazine also reduced the blood pressure in WKY animals at 9, 11, 13, 14 and 21 weeks of age when compared to untreated WKY. Fig. 30 shows a comparison of the blood pressure profiles of treated SHR and WKY. Treated SHR exhibited a higher blood pressure at 13, 15, 17 and 21 weeks of age when compared to treated WKY; however, the absolute differences in blood pressure were small.

2. Morphometric analysis of the structural changes in the renal arteries of in utero and postnatally treated SHR, WKY and untreated SHR and WKY

Table 21 outlines the physical characteristics of the treated and non-treated SHR and WKY used in the morphometric study at the time of sampling. The ages of all the rats used were similar. The hydralazine treated SHR and WKY had lower body weights than their

Fig. 29. Blood pressure versus age profiles of untreated male SHR and WKY, and male SHR and WKY treated *in utero* and postnatally with hydralazine (mean ± SEM).

SHR and WKY females were treated with 100 mg hydralazine per 1 drinking water. Once blood pressure was normalized in SHR, a male rat was introduced to inseminate the female. Hydralazine treatment was continued during pregnancy. Newborn pups were fed 0.0169 mg hydralazine/day/g rat weight by gavage up to weaning (3-4 weeks old). Subsequently, 100 mg hydralazine/l was placed in the drinking water up to 21 weeks of age. The systolic blood pressure was measured using the tail cuff compression method.

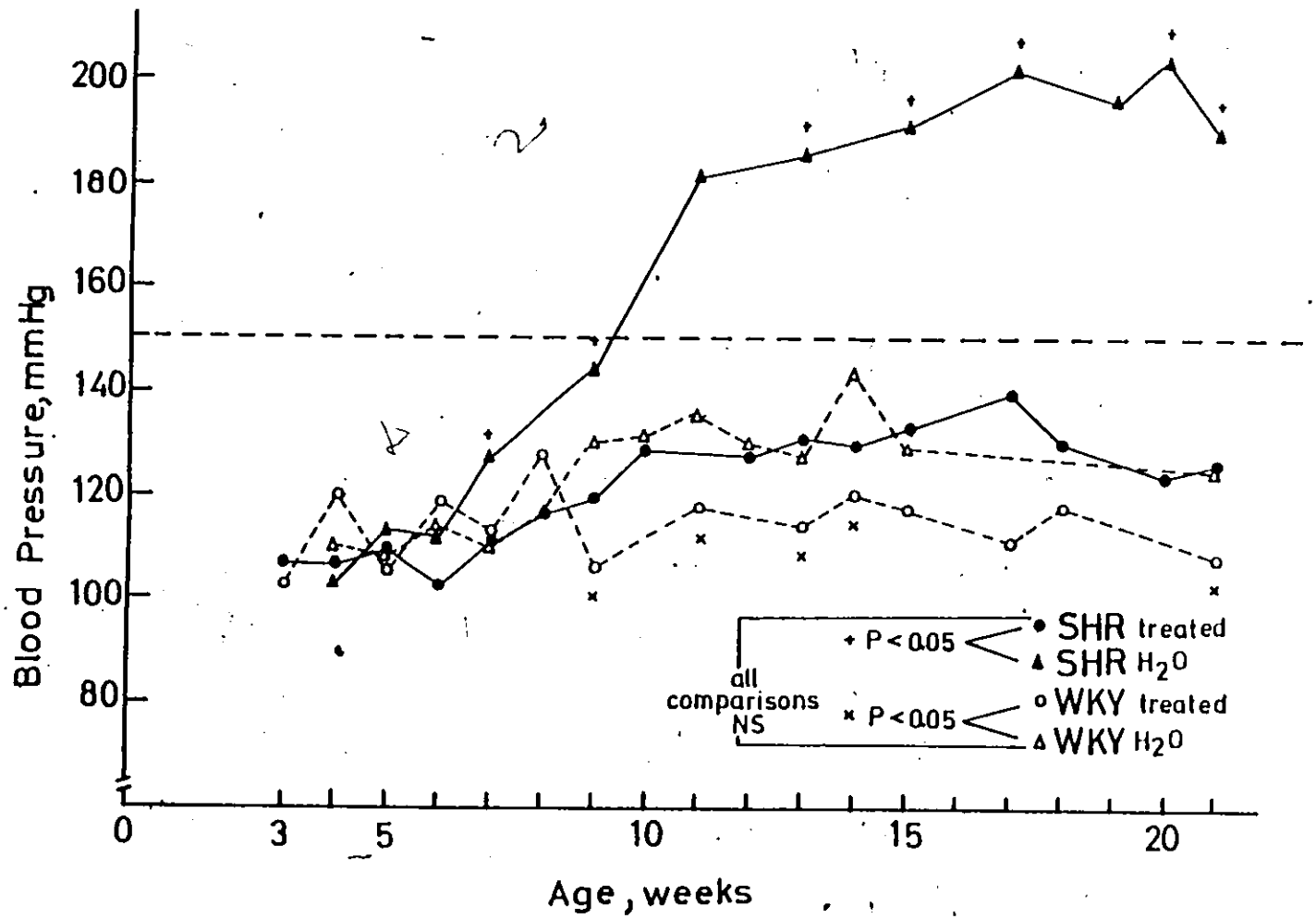

Blood Pressure, mmHg
200
180
160
140
120
100
80
0
3
5
10
15
20
Age, weeks
+ P < 0.05
SHR treated
SHR H2O
all comparisons NS
× P < 0.05
WKY treated
WKY H2O

Fig. 30. Blood pressure versus age profiles of male SHR and WKY (mean ± SEM) treated *in utero* and postnatally with hydralazine.

Conditions are identical to those outlined in Fig. 29.

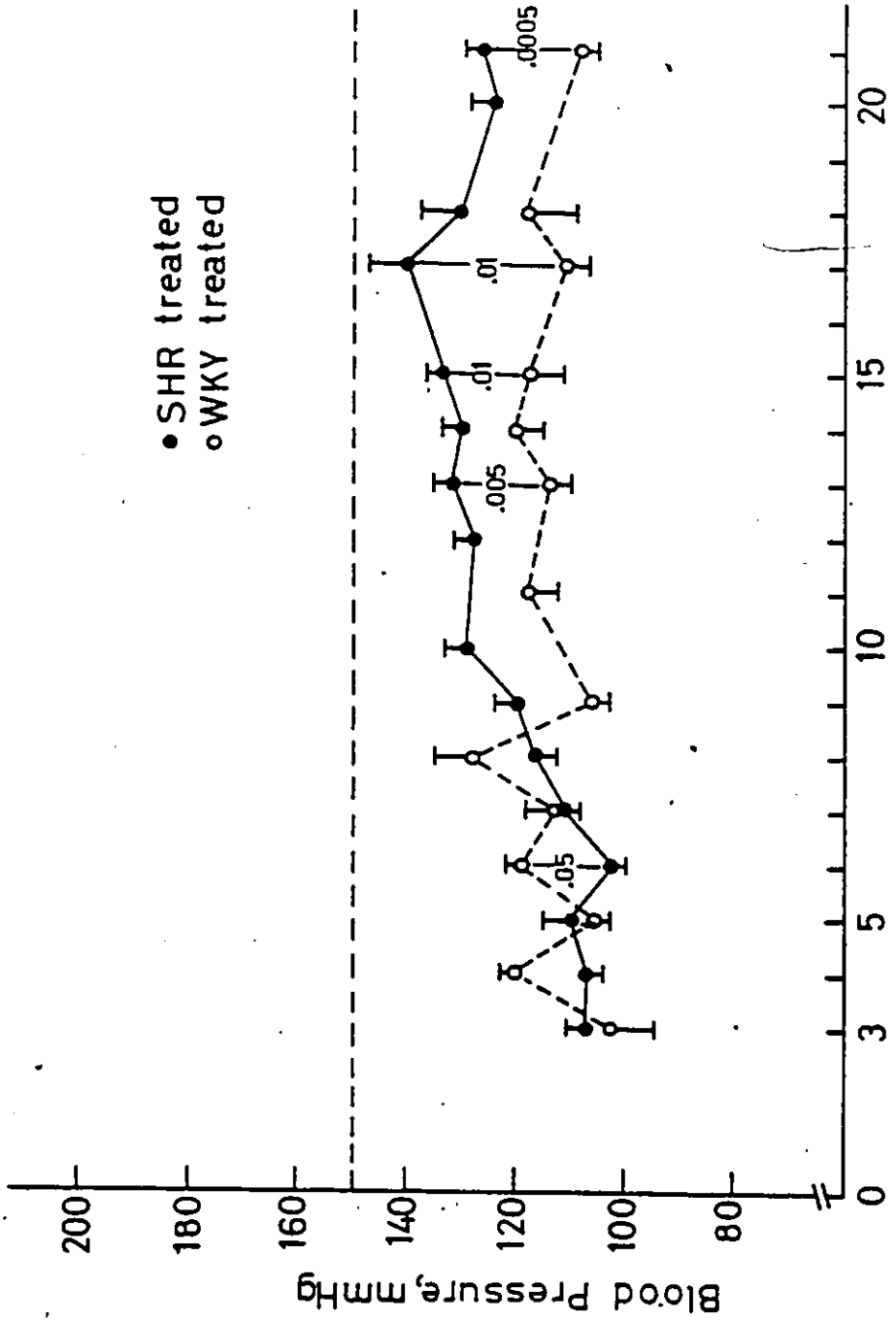

• SHR treated
○ WKY treated
Blood Pressure, mmHg
200
180
160
140
120
100
80
0
3
5
10
15
20
Age, weeks
.05
.005
.01
.01
.0005

Table 21. Physical characteristics of hydralazine and non-treated SHR and WKY at time of sampling for morphometric analysis

		Hydralazine Treated	Non-treated	P
n	SHR	7	6	
	WKY	6	10	
Age (wks)	SHR	21.7±.55	21.3±.15	NS
	WKY	21.3±.23	21.3±.41	NS
	P	NS	NS	
Wt (g)	SHR	304 ±3.9	352 ±16	0.005
	WKY	286 ±4.9	334 ±3.3	0.0005
	P	0.01	NS	
BP (mmHg)	SHR	129 ±3.4	194 ±4.2	0.0005
	WKY	109 ±2.7	121 ±2.2	0.005
	P	0.0005	0.005	
$\frac{\text{Kidney Wt}}{\text{Body Wt}} \times 10^{-3}$	SHR	3.86±.049	4.00±.087	NS
	WKY	3.40±.065	3.53±353	NS
	P	0.005	0.05	
Renal Vascular Resistance ($mmHg.ml.min^{-1}$)	SHR	15.5±.58	25.4±1.97	0.0005
	WKY	15.3±.60	22.7±1.64	0.005
	P	NS	NS	

non-treated counterparts. Within the hydralazine treated group, SHR were heavier than WKY. The blood pressure of treated SHR and WKY was significantly lower than the control SHR and WKY. Within the hydralazine treated group, the mean blood pressure was about 20 mm Hg higher in SHR over WKY, while in the non-treated group there was a 63 mm Hg difference in the mean blood pressure between SHR and WKY. The kidney to body weight ratio was elevated in both treated and non-treated SHR when compared to WKY. Hydralazine treatment did not effect the ratio in either SHR or WKY. The RVR of hydralazine treated SHR and WKY was lower than that of their non-treated SHR and WKY counterparts. However, both hydralazine and non-treated SHR had a similar vascular resistance to treated and non-treated WKY, respectively.

Figures 31 to 35 and Tables 22 and 23 outline the structural alterations present in the renal vasculature of 21 week old control SHR and WKY raised on de-ionized water throughout their life (respectively, SHR_c and WKY_c) and age matched SHR and WKY treated *in utero* and postnatally with hydralazine (respectively, SHR_t and WKY_t). Two statistical tests were used to compare the animal groups; a one way analysis of variance (ANOVA) was used to determine whether a statistically significant difference ($P<0.05$) was present within treated and non-treated animals, tested as a group, and a Student's t test was used to determine which of the test groups were significantly different from the other. To determine the effect of decreased blood pressure on arterial structure, SHR_c with high blood pressure were compared with SHR_t having normalized blood pressure; these groups were also compared with, respectively, WKY_c and WKY_t. In addition, SHR_t were compared to

WKY_c, since both these groups had similar blood pressures vs age profiles (see Fig. 29). To test the effects of hydralazine on arterial structure WKY_t were compared with WKY_c.

(a) Alterations in lumen diameter (LD)

Fig. 31 summarizes the lumen diameter of the renal arteries in the various treatment groups. Within this study, the cortical arteries were divided into arcuate and interlobular arteries using the criteria outlined in the Methods (section H, subsection 3).

(i) The effect of altered blood pressure on LD

The LD of the main renal artery of SHR_c and SHR_t was larger than that present in WKY_c and WKY_t, respectively. In spite of the fact that blood pressure was normalized in SHR_t, the LD of the main renal artery was similar to that of SHR_c with high blood pressure and untreated WKY_c.

The LD of the interlobar arteries of treated SHR_t was larger than that present in either WKY_t or WKY_c, but was not significantly different from control SHR_c. The interlobar arteries of control SHR_c exhibited a larger mean LD than WKY_c, but this was not significant at $P<0.05$. The ANOVA indicated that the LD of arcuate and interlobular arteries was not different between treated and non-treated groups of animals.

(ii) The effect of hydralazine on LD

The LD of the main renal artery was reduced in hydralazine treated WKY_t compared to WKY_c. The LD of the interlobar, arcuate and interlobular arteries was not different between WKY_t and WKY_c.

(b) Alterations in the cross-sectional area (CSA) of intima (endothelium, subendothelial space, IEL)

Fig. 31. Lumen diameters of the main renal, interlobar, arcuate and interlobular arteries of control (SHR_c, WKY_c) and *in utero* and postnatally hydralazine treated (SHR_t, WKY_t) SHR and WKY (mean ± SEM; ANOVA test, MRA, $P<0.01$; Interlobar, $P<0.01$; Arcuate, NS; Interlobular, NS).

Conditions of the experiment are identical to those outlined in Fig. 7.

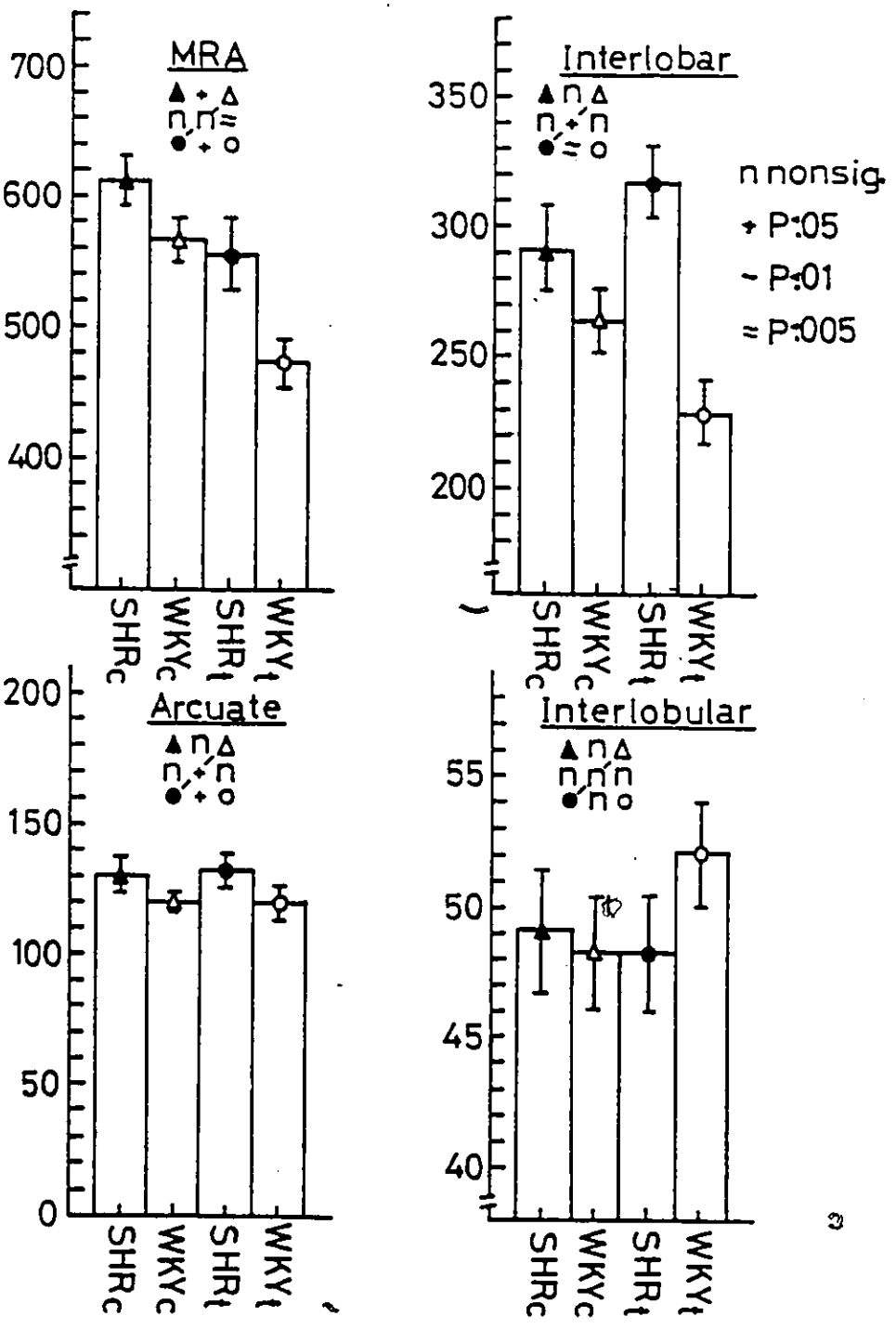

Lumen diameter µm
MRA
Interlobar
Arcuate
Interlobular
n nonsig.
+ P:05
~ P:01
≈ P:005
SHRc
WKYc
SHRt
WKYt

Fig. 32 summarizes the findings in the intima of blood vessels in the various treatment groups.

(i) The effect of altered blood pressure on the intima

When the SHR_c and SHR_t were compared to, respectively, WKY_c and WKY_t, qualitatively similar types of alterations were observed. In the case of SHR_c, renal arteries with a D_I > 95 μm the CSA of intima was greater than WKY_c; arteries with a D_I > 70 μm had greater quantities of intima in SHR_t than WKY_t. The absolute area of intima was increased in the treated groups of animals, particularly in the larger renal arteries. The intima in SHR_t was greater than in SHR_c and WKY_c in all the arterial classes studied.

(ii) The effect if hydralazine on the intima

In arteries with a D_I > 120 μm, WKY_t were found to have greater CSA of intima than WKY_c, hydralazine was associated with increased CSA quantities of intima.

(c) Alterations in the cross-sectional areas of media

Fig. 33 outlines the alterations in the media of various blood vessels in treated and nontreated animals.

(i) The effect of altered blood pressure on the media

The normalization of blood pressure in SHR_t had very little effect on the CSA of arterial media present in SHR. Both SHR_c and SHR_t had increased CSA of media than, respectively, WKY_c and WKY_t in all arterial classes studied. Despite the normalization of blood pressure in SHR_t, when this group was compared to SHR_c, similar CSA quantities of media were present in all the arterial groups, with the exception of those having a D_I between 95 to 120 μm. Furthermore, in all arterial

Fig. 32. The cross-sectional area quantities of intima present in the renal arteries of control (SHR_c, WKY_c) and hydralazine treated (SHR_t, WKY_t) SHR and WKY (mean ± SEM; ANOVA test, all arterial groups were significant at $P<0.05$ or $P<0.01$).

The arteries were perfusion fixed at a constant flow under relaxed conditions. In these measurements, the intima represents the endothelium, subendothelial space and the internal elastic lamina. All cross-sectional area measurements of intima have been compensated for sectioning angle.

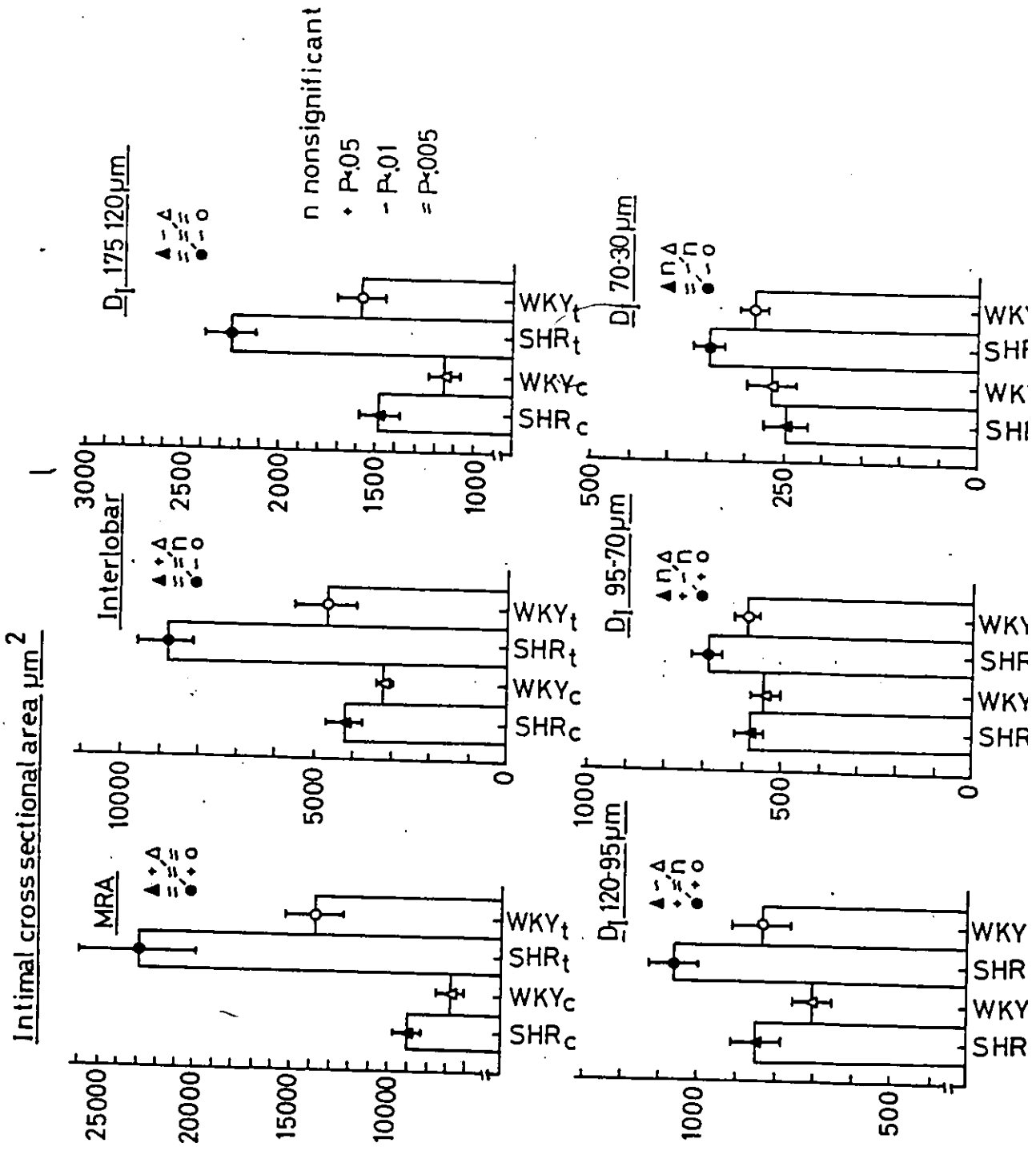
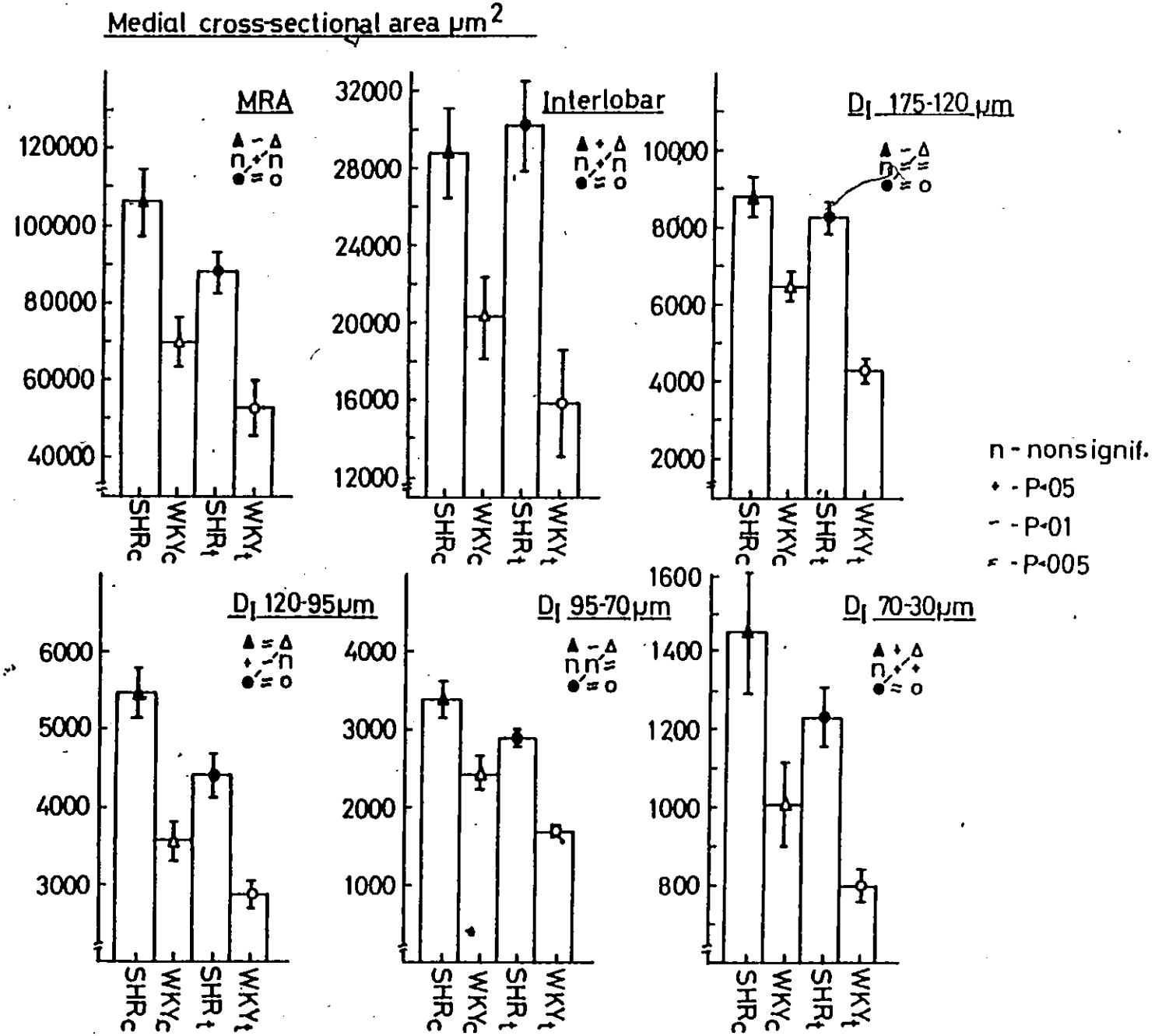

Intimal cross sectional area µm²
MRA
Interlobar
D| 175 120µm
D| 120-95µm
D| 95-70µm
D| 70-30µm
n nonsignificant
+ P<.05
- P<.01
= P<.005
WKYt
SHRt
WKYc
SHRc

Fig. 33. The cross-sectional area quantities of media present in the renal arteries of control (SHR_c, WKY_c) and hydralazine treated (SHR_t, WKY_t) SHR and WKY (mean ± SEM; ANOVA test, all arterial groups were significant, $P<0.01$).

The experimental conditions are identical to those outlined in Fig. 32.


Medial cross-sectional area µm²
MRA
Interlobar
DI 175-120 µm
DI 120-95µm
DI 95-70µm
DI 70-30µm
SHRc
WKYc
SHRt
WKYt
n - nonsignif.
• - P<05
- - P<01
= - P<005

classes, except those having a D_I between 70-95 μm, the media was increased in SHR_t compared to WKY_c. Thus, SHR developed increased CSA quantities of media, irrespective of whether blood pressure was elevated or not.

(ii) The effect of hydralazine on the media

In cortical arteries with a D_I < 175 μm, hydralazine treatment was found to decrease the CSA of media in WKY_t over WKY_c.

(d) Alterations in the numbers of SMC layers present in the media

Fig. 34 outlines the alterations in the numbers of SMC layers present in the media of treated and control animals.

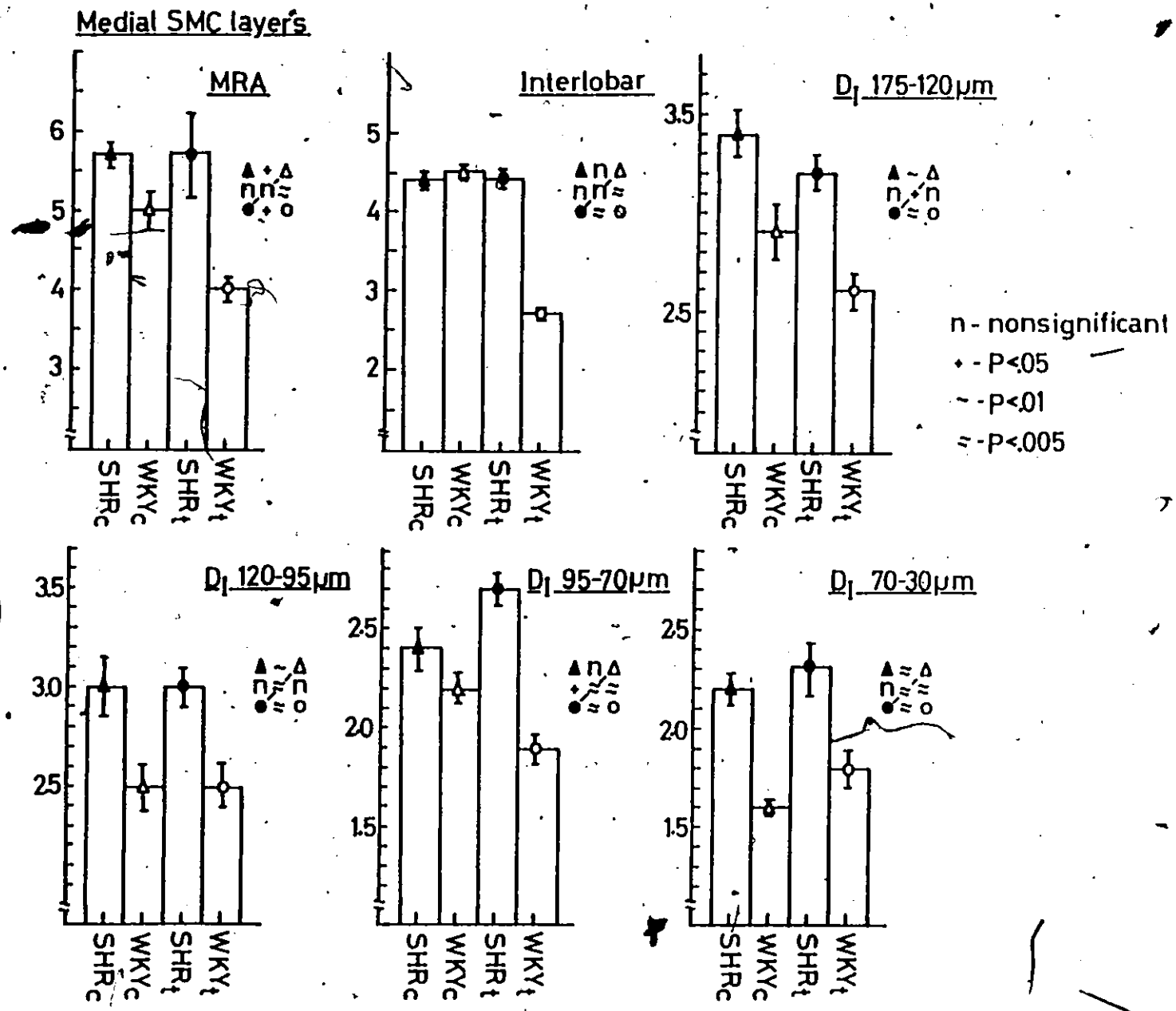
(i) The effect of altered blood pressure on the number of medial SMC layers

In SHR_c, all arterial classes, with the exception of interlobar arteries and arteries with a D_I between 70-95 μm, were found to have a greater number of SMC layers when compared to WKY_c. SHR_t with normalized blood pressure had significantly increased numbers of SMC layers over WKY_t in all arterial classes studied. SHR_t were also found to have greater numbers of SMC layers than WKY_c in all arteries with a D_I < 175 μm. When SHR_t with normalized blood pressure were compared to SHR_c, both groups had similar numbers of SMC layers within the media of all arterial groups with the exception of those having a D_I between 70-95 μm. Thus, increases in medial SMC layers in SHR, was due to SMC hyperplasia, and occurred in spite of the fact that blood pressure was not elevated at any time in these rats.

(ii) The effect of hydralazine on the number of SMC layers in the media

Fig. 34. The numbers of medial smooth muscle cell layers in the renal arteries of control (SHR_c, WKY_c) and hydralazine treated (SHR_t and WKY_t) SHR and WKY (mean ± SEM; ANOVA test, all arterial groups significant, $P<0.01$).

The experimental conditions are identical to those outlined in Fig. 32.


Medial SMC layers
MRA
Interlobar
D1 175-120 µm
D1 120-95 µm
D1 95-70 µm
D1 70-30 µm
SHRc
WKYc
SHRt
WKYt
n - nonsignificant
• - P<.05
~ - P<.01
≈ - P<.005

Treated WKY_t were found to have significantly decreased numbers of SMC layers in the media of interlobar and cortical arteries with a D_I between 70-95 μm when compared to WKY_c. On the other hand, in cortical arteries with a D_I between 30-70 μm, the number of SMC layers was modestly increased in WKY_t over WKY_c.

(e) Alterations in the cross-sectional area of adventitia

Table 22 outlines the alterations in the cross-sectional area of adventitia of the blood vessel wall in the various treatment groups.

(i) The effect of altered blood pressure on the adventitia

The control SHR_c and WKY_c did not exhibit any difference in the cross-sectional area of adventitia in any of the arterial classes studied. However, interlobar and cortical arteries with a D_I between 95-175 μm did exhibit increased amounts of adventitia in SHR_t over WKY_t. SHR_t with normalized blood pressure had main renal and interlobar arteries with a larger cross-sectional area of adventitia than SHR_c and WKY_c, while adventitia in arteries with a D_I < 175 μm was decreased in SHR_t over that present in SHR_c or WKY_c.

(ii) The effect of hydralazine on the adventitia

When WKY_t were compared to WKY_c, the effect of hydralazine depended on vessel size. Larger arteries, such as the main renal and interlobar arteries, had increased adventitia in WKY_t than WKY_c, while smaller arteries with a D_I < 175 μm were found to have less adventitia in WKY_t than WKY_c.

(f) Alterations in the quantities of non-adventitial wall (intima, IEL, media)

Table 23 outlines the alterations that exist in the

Table 22. Cross-sectional area (μm^2) of the adventitia of hydralazine treated and control SHR and WKY.

	Main Renal Artery	Interlobar Artery	175-120 μm	120-95 μm	95-70 μm	70-30 μm
a SHR_c	70335 ± 6271	17533 ± 2185	8667 ± 973	7246 ± 934	5978 ± 577	2426 ± 368
b WKY_c	71862 ± 9686	15301 ± 2167	9993 ± 1528	7739 ± 347	5344 ± 614	2033 ± 207
c SHR_t	131894 ± 5487	33648 ± 2386	6100 ± 685	2421 ± 158	1708 ± 126	812 ± 47
d WKY_t	114180 ± 12799	17533 ± 2186	3435 ± 293	1905 ± 137	1497 ± 62	704 ± 37
a-b-c-d ANOVA	P < .01	P < .01	P < .01	P < .01	P < .01	P < .01
	a NS b .0005 .0005 .025 c NS d	a NS b .0005 .0005 NS c .005 d	a NS b .025 .025 .0005 c .005 d	a NS b .0005 .0005 .0005 c .05 d	a NS b .0005 .0005 .0005 c NS d	a NS b .0005 .0005 .0005 c NS d

Table 23. Cross-sectional area (μm^2) of the media and intima of hydralazine treated and control SHR and WKY.

	Main Renal Artery	Interlobar Artery	175-120 μm	120-95 μm	95-70 μm	70-30 μm
a SHR_e	118130 ± 9191	32950 ± 2570	10313 ± 590	6344 ± 342	3872 ± 216	1696 ± 185
b WKY_c	79966 ± 7144	23600 ± 2133	7683 ± 419	4241 ± 280	2989 ± 232	1272 ± 131
c SHR_t	110985 ± 7429	51913 ± 13761	10490 ± 495	5429 ± 308	3372 ± 317	1556 ± 90
d WKY_t	66591 ± 8562	20238 ± 3614	5878 ± 369	3751 ± 187	2305 ± 104	1093 ± 58
a-b-c-d ANOVA	P< .01	NS	P<.01	P<.01	P<.01	P .01
	a .01 b NS :01 NS c .005 d	a .01 b NS NS NS c .05 d	a .005 b NS .0005 .005 c .0005 d	a .0005 b .05 .01 NS c .0005 d	a .01 b NS .05 .0005 c .0005 d	a .05 b NS .05 NS c .0005 d

non-adventitial portion of the blood vessel wall in the various treatment groups.

(i) The effect of altered blood pressure on the non-adventitial wall

When SHR_t and SHR_c were compared to WKY_t and WKY_c, respectively, and when SHR_t were compared to WKY_c, SHR exhibited increased CSA quantities of non-adventitial wall over WKY. This occurred regardless of whether or not they were treated with hydralazine. When treated SHR_t with normalized blood pressure were compared to SHR_c, there was no significant difference in CSA of wall components in all the arteries with a D_I greater than 120 µm and less than 95 µm. In arteries with a D_I between 95-120 µm, SHR_t exhibited a significantly smaller ($P<0.05$) CSA of wall than SHR_c.

(ii) The effect of hydralazine on the non-adventitial wall

The effect of hydralazine treatment on WKY was to reduce CSA of non-adventitial wall in cortical arteries with a D_I between 120-175 µm, and 70-95 µm. Classes of renal arteries other than those mentioned above were not altered in WKY_t treated with hydralazine.

(g) Alterations in the wall to lumen ratio

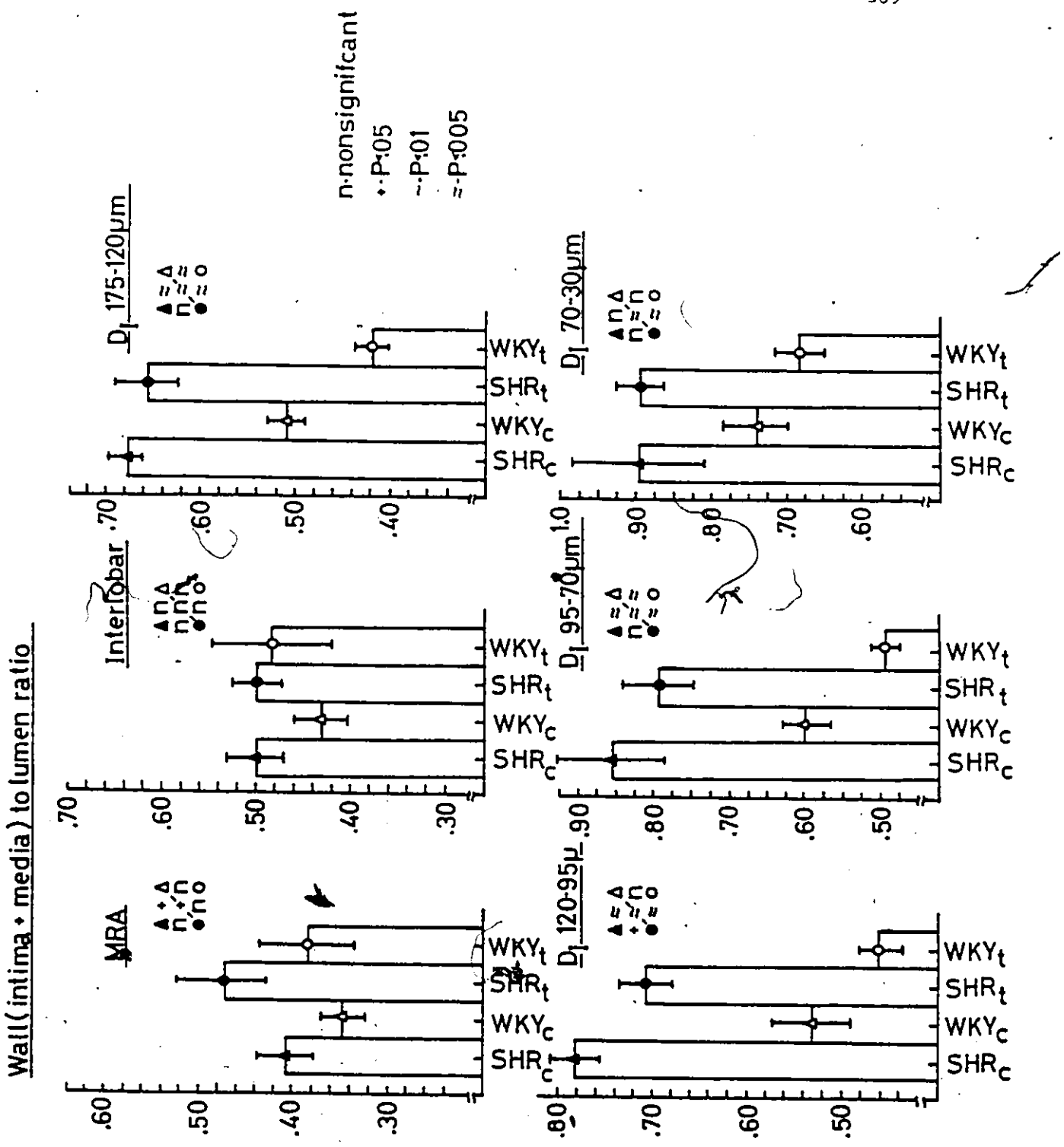
Fig. 35 summarizes the alterations in the wall to lumen ratio in the various treatment groups. The wall to lumen ratio consists of the CSA of the non-adventitial wall divided by the CSA of the lumen.

(i) The effect of blood pressure on the wall to lumen ratio

In arteries with a $D_I < 175$ µm, both SHR_c and SHR_t exhibited a significant increase in the proportion of wall to lumen over, respectively, WKY_c and WKY_t. Within this groups of arteries the ratio

Fig. 35. The wall (intima + media) to lumen ratio of the renal arteries of control (SHR_c, WKY_c) and *in utero* and postnatally hydralazine treated (SHR_t, WKY_t) SHR and WKY (mean ± SEM; ANOVA test, MRA, NS; Interlobar, NS; all other arterial groups significant, $P<0.01$).

The experimental conditions are identical to those outlined in Fig. 32.


Wall(intima+media) to lumen ratio
MRA
Interlobar
D1 175-120µm
D1 95-70µm
D1 70-30µm
D1 120-95µ
n-nonsignificant
+-P<.05
~-P<.01
=-P<.005
WKYt
SHRt
WKYc
SHRc

was also elevated in SHR_t over WKY_c. The ANOVA of the wall to lumen ratio in the main renal and interlobar arteries indicated that no significant alteration in this measurement existed between the test groups. Since in both of the above groups the quantity of the vessel wall was elevated in SHR_c and SHR_t over WKY_c and WKY_t, respectively, the lack of change in the wall to lumen ratio indicates that control and treated SHR had larger main renal and interlobar arteries than WKY_c and WKY_t.

(ii) The effect of hydralazine on the wall to lumen ratio

The wall to lumen ratios of the main renal, interlobar arteries and the cortical arteries with a D_I between 95-120 μm and 30-70 μm were not significantly altered when WKY_t were compared to WKY_c. However, hydralazine treatment of WKY did reduce the wall to lumen ratio in cortical arteries with a D_I between 120-175 μm and 70-95 μm.

3. Hydralazine Withdrawal Experiment

(a) Rats with established hypertension

To study the long term effects of hydralazine on blood pressure, rats with established hypertension were given hydralazine in their drinking water (100 mg/l) for a period of 286 days. Table 24 summarizes the physical characteristics of the SHR prior to the administration of the drug. The average age of the rats was approximately 24 weeks and the blood pressure was 186 ± 5.83 mm Hg. After 5 days of hydralazine treatment the blood pressure dropped to 125 ± 11.44 mm Hg (Fig. 36) and remained within the normotensive range for the 286 day treatment period. Table 25 summarizes the physical characteristics of the SHR after 286

Table 24. Physical characteristics of the SHR in an established phase of hypertension before being treated with hydralazine.

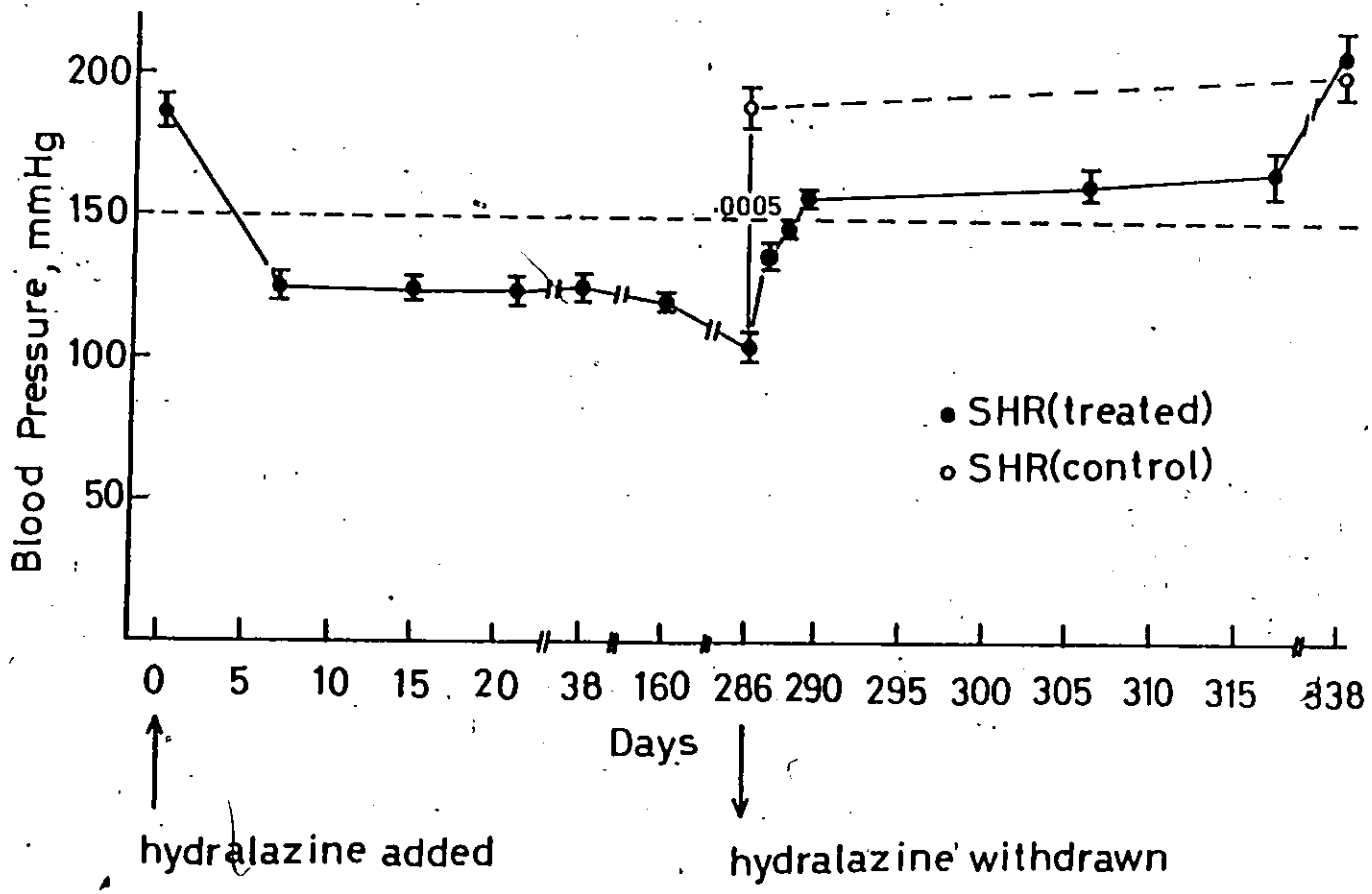
	SHR
Age (wks)	24
Wt (g)	299±8.54
BP (mmHg)	186±5.83

Table 25. Physical characteristics of treated and control SHR during hydralazine withdrawal.

	SHR (treated) n=8	SHR (control) n=9	P
Age (wks)	64.9±.898	66.2±1.23	NS
Wt (g)	356 ±6.58	358 ±11.6	NS
at day 0, hydralazine withdrawn			
BP (mmHg)	105 ±3.77	190±7.61	0.0005
HR (beats/min)	491 ±12.6	458±7.49	0.005
at day 52 after hydralazine withdrawl			
BP (mmHg)	208 ±4.27	203±7.60	NS
HR (beats/min)	465 ±30	487±6.13	NS

Fig. 36. The effect of hydralazine treatment and withdrawal on the blood pressure of SHR in an established phase of hypertension (mean ± SEM).

SHR with established hypertension were treated with 100 mg hydralazine/l drinking water for 286 days, after which time the hydralazine was withdrawn for 56 days. The systolic blood pressure was measured using the tail cuff compression method.


200
150
100
50
Blood Pressure, mmHg
.0005
• SHR(treated)
o SHR(control)
0 5 10 15 20 38 160 286 290 295 300 305 310 315 338
Days
hydralazine added
hydralazine withdrawn

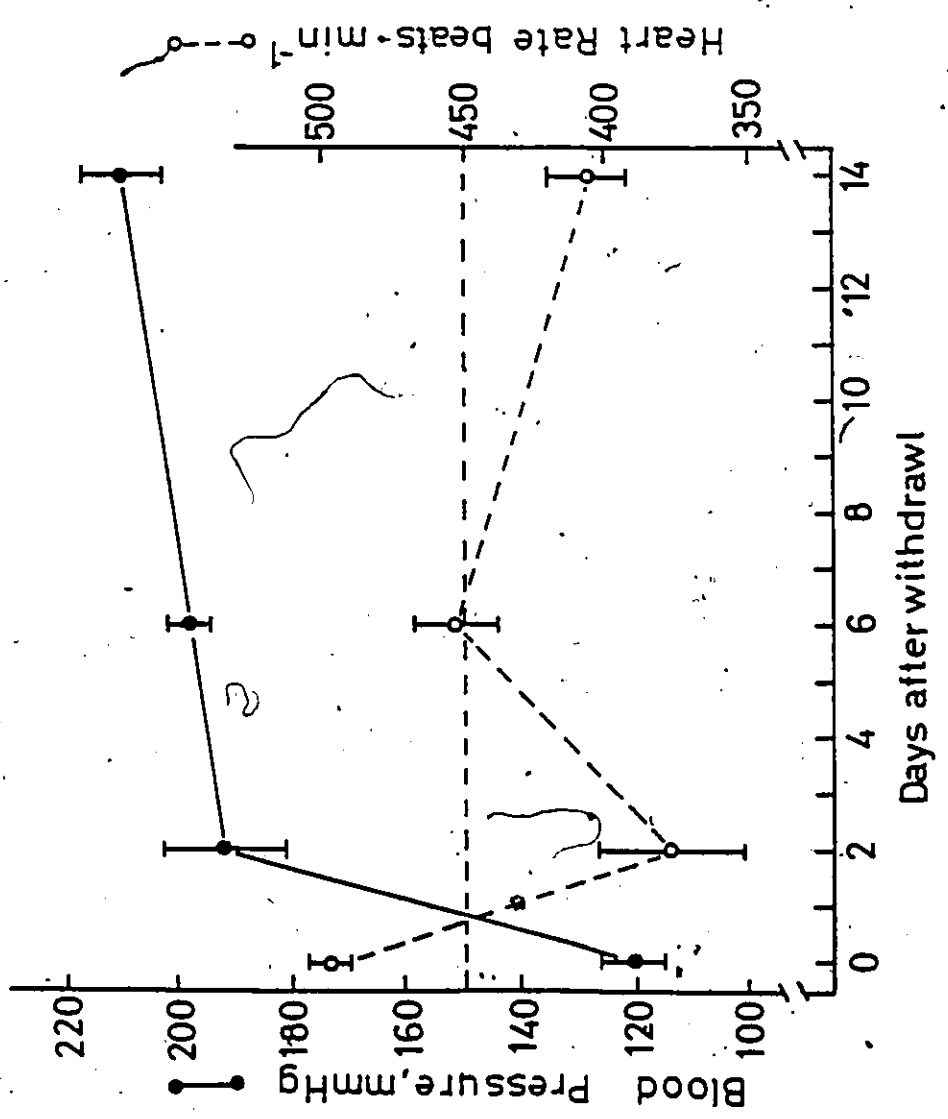
days of hydralazine treatment and age matched SHR controls given de-ionized water for the same length of time. Age and body weight was similar in both groups. However, at the point of hydralazine withdrawal (day 0, Table 25, and day 286, Fig. 36), the blood pressure was significantly decreased and the heart rate increased in the treated SHR group compared to control SHR. When hydralazine was withdrawn from the drinking water, the blood pressure rose quickly over the next four days in the treated rats (Fig. 36). After four days the mean blood pressure of this group (157 ± 3.64 mm Hg) would be considered hypertensive (i.e., a systolic BP greater than 150 mm Hg) but was still less than the control SHR levels. For the next 25 days, the blood pressure did not increase significantly (Fig. 36). However, after 52 days of hydralazine withdrawal, the blood pressure, and as well the heart rate, increased and were similar to the control SHR levels (Table 25 and Fig. 36).

(b) In utero and postnatally treated SHR

Five male SHR treated in utero and postnatally with hydralazine were used in this experiment. The rats were 26 weeks of age and weighed 302 ± 5.27 g at the time of drug withdrawal. The effect of substituting deionized water in place of 100 mg/l hydralazine on blood pressure and heart rate are shown in Fig. 37. Within two days after withdrawal, the blood pressure rose from a mean value of 120 ± 5.29 mm Hg to 192 ± 11.4 mm Hg. Over the next 10 days, the blood pressure rose still further to 210 ± 6.71 mm Hg. Clearly, the in utero and postnatal treatment of SHR with hydralazine did not provide any protection from the mechanisms producing hypertension. The heart rate of the treated SHR was elevated above normal levels during the drug treatment. Withdrawal of the drug

Fig. 37. The effect of hydralazine withdrawal on the blood pressure of SHR treated *in utero* and postnatally with hydralazine up to 26 weeks of age (mean ± SEM).

The systolic blood pressure was measured using the tail cuff compression method.


Heart Rate beats-min⁻¹
500
450
400
350
Blood Pressure, mmHg
220
200
180
160
140
120
100
0
2
4
6
8
10
12
14
Days after withdrawl

resulted in decreased heart rate within two days to values below normal (Fig. 37). Subsequently, the heart rate increased to within normal levels for 26 week old SHR.

4. Summary of Results from the Hydralazine Treatment Study

(i) Hydralazine was found to reduce blood pressure to normal levels in SHR with established hypertension. When administered in utero and postnatally to SHR, hypertension was prevented in the animal for the duration of the experiment (26 weeks).

(ii) The RVR of treated SHR and WKY was not significantly different but was below the resistance values obtained for control SHR and WKY.

(iii) The modifications in lumen diameter between treated and non treated SHR were quite modest. Both treated and non-treated SHR had a larger lumen diameter in the main renal and interlobar arteries than in WKY. Hydralazine treatment of WKY modestly reduced the lumen diameter of both the above classes of arteries. The lumen diameter of the arcuate and interlobular arteries was not different in treated and non-treated animals.

(iv) The cortical arteries with a D_I > 95 μm of both treated and non-treated SHR had increased intima compared to correspondingly treated WKY. Hydralazine treatment of WKY increased the CSA of intima in arteries with a D_I > 120 μm.

(v) The in utero and postnatal normalization of blood pressure had very little effect in reversing the structural changes

within the contractile portion of the vessel wall in SHR. In virtually all the renal vessels studied, the CSA of arterial medial and non-adventitial wall (intima + media), as well as the SMC layers in the media and the wall to lumen ratio, were similar in control and treated SHR. In most arteries, these structural components were increased in both treated and non-treated SHR over both sample groups of WKY.

(vi) Hydralazine treatment of WKY reduced the CSA of media in arteries with a D_I < 175 μm and reduced the number of SMC layers in the main renal, interlobar and cortical arteries with a D_I between 95-70 μm.

(vii) When control SHR_c were compared to WKY_c, no significant alteration in the CSA of adventitia was observed. Hydralazine treated SHR_t did have interlobar and cortical arteries (D_I between 95-175 μm) with larger cross-sectional area of adventitia than WKY_t. The effect of hydralazine treatment on vessel dimensions depended on vessel size. When control SHR and WKY were compared to their hydralazine counterparts, hydralazine treatment was found to increase the adventitia in the main renal and interlobar arteries and decrease the adventitia in cortical arteries with a D_I <175 μm.

(viii) When hydralazine was withdrawn from rats treated for 286 days during established phase of hypertension, blood pressure increased from normal to moderate hypertensive levels 4 days after withdrawal. For the next 25 days, blood pressure

did not change significantly; however after 52 days of hydralazine withdrawal, the blood pressure increased to the level present in control SHR.

(ix) When hydralazine was withdrawn at 21 weeks of age from rats treated <u>in utero</u> and postnatally with the drug, blood pressure rose to established hypertensive levels 2 days after withdrawal.

Discussion

A. Structural alterations in the renal vasculature of SHR with established hypertension

In the present study, SHR with established hypertension had renal vascular resistances that were similar to that of WKY when studied under maximally relaxed conditions. Further, the lumen diameter of the main renal, interlobar, arcuate and interlobular arteries and the preglomerular arterioles of SHR was not altered. There were very modest alterations in the amounts of adventitia, intima or its subcomponents. The most consistent finding was increased arterial media of the prearteriolar renal vessels. This was produced by both an increase in the medial extracellular space, and SMC hypertrophy, hyperplasia or a combination of both alterations. Aside from a small increase in the medial extracellular space, arterioles of SHR exhibited very few alterations when compared to WKY.

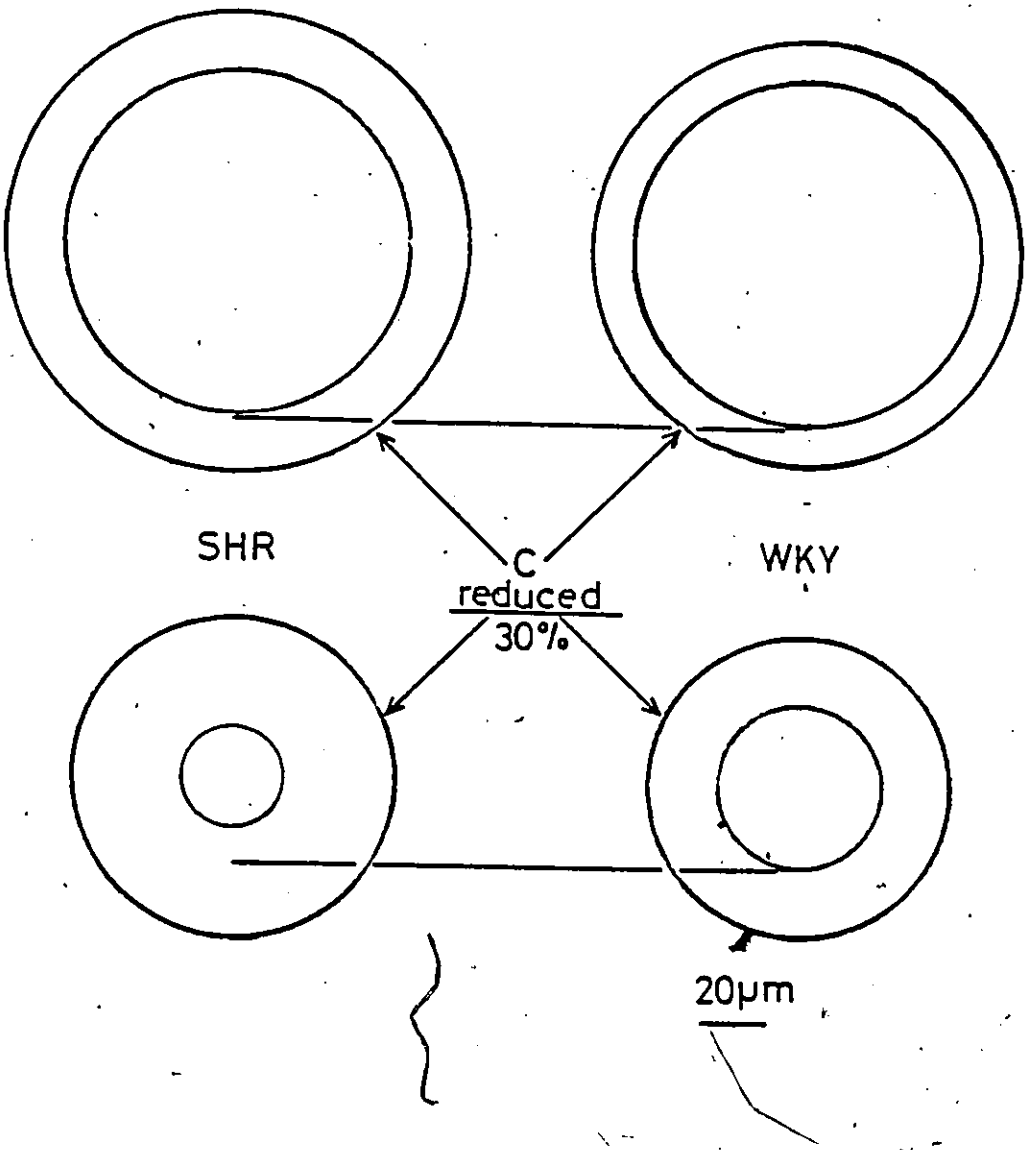
Even though the lumen diameter of the blood vessels was unaltered in SHR with established hypertension, the structural changes observed could be important in maintaining elevated blood pressure. If both WKY and SHR blood vessels were contracted from the adventitia in a manner where 1) each SMC contracts a similar proportion of its length (e.g., 30%) and 2) if during contraction the volume of the media was conserved (which is the case in mesenteric arteries of SHR and WKY (Lee et al., 1983 c)), or was similarly altered in SHR and WKY, the thick

walled hypertensive vessel would occlude its lumen to a greater degree than the thinner walled WKY vessel. Fig. 38 diagramatically illustrates the difference in lumen size under such conditions. Since in vivo the sympathetic nervous system is constantly active (Judy et al., 1976) and maintains vascular smooth muscle cell tone, the increased renal vascular resistance of hypertensive animals would be due to this alone. The presence of increased sympathetic activity and/or blood borne vasoconstrictors in SHR would further increase renal vascular resistance.

The morphology of the renal vasculature of SHR has been studied qualitatively by Mandal et al (1977), semi-quantitatively by Limas et al (1980) and Nordborg and Johansson (1979), and morphometrically by Pang and Scott (1981). Mandal et al (1977) observed that the intrarenal vessels of SHR in an established phase of hypertension appeared to exhibit greater pathological damage and a hypertrophied vascular wall. Limas et al (1980) studied SHR and age matched WKY between the ages of 5 to 48 weeks. After 10 weeks of age when SHR were nearing established hypertension, some intermediate sized intrarenal vessels (30-100 μm, external diameter) exhibited a decreased lumen diameter in SHR over WKY, while in most cases the ratio of the external to internal vessel diameter was significantly elevated in SHR. Such alterations occurred after hypertension development, and it was suggested that a thickening of the vascular wall was a secondary alteration in response to high blood pressure. Nordborg and Johansson (1979), on the other hand, observed an increase in the media to lumen radius ratios of intrarenal vessels with a lumen diameter greater than 100 μm in 15 day old SHR

Fig. 38. A model representing the effect that a thickened vascular wall would have on lumen diameter during arterial contraction.

The upper diagrams represent an intermediate sized renal artery from SHR and WKY with a lumen diameter of 102 μm under relaxed conditions. The wall (intima + IEL + media) to lumen ratio has been drawn to scale to represent mean values present in cortical arteries with a D_I between 120 and 95 μm in 21 week old SHR and WKY (i.e., wall to lumen ratio of 0.738 in SHR, 0.531 in WKY). If the medial cross-sectional area is conserved (as is the case in mesenteric arteries of SHR and WKY, Lee et al., 1983 c) and if the vascular smooth muscle cells are contracted, reducing the external circumferece (C) by 30%, then the lumen of the SHR vessel would be occluded to a much greater degree than the WKY renal vessel. The model implies that even normal levels of sympathetic activity or blood born vasoconstricting agents could act to increase renal vascular resistance in SHR.


SHR
WKY
C
reduced
30%
20µm

with incipient hypertension. The same study, however, failed to observe any alteration in the media to lumen radius of intrarenal vessels from 200 day old SHR with established hypertension. This suggested that the thickened vascular wall observed in young SHR over WKY regressed with age in SHR.

Some of the above findings are inconsistent with the observations of the present study where blood vessel wall thickening was present in virtually all classes of renal arteries prior to blood pressure elevation and during established hypertension in SHR. The differing results could be due to the methods used to study the vasculature. Unlike the present study, Nordborg and Johansson (1979), Limas et al (1980) and Mandal et al (1977) used instillation as opposed to perfusion fixation to prepare the renal vessels for microscopy. With the instillation method collapse and contraction of blood vessels occurs, as demonstrated by the published examples of renal vessels (e.g., Limas et al., 1980). In studies performed by Mandal et al (1977) and Limas et al (1980), no attempt was made to compensate for contraction or sectioning angle in the micrograph. Both of the above ommissions could lead to serious measurement errors. For example, if hypertension is associated with a thickening of the vascular wall and the previously outlined model (Fig. 38) holds true, then blood vessel contraction would occlude the lumen of the hypertensive artery to a greater degree than a normal thin walled blood vessel. Therefore, estimates of the lumen diameter would be less than that of the true lumen and the ratio of media to lumen would depend mainly on the degree of contraction present. Unless a perfect blood vessel cross-section is

obtained (i.e., cut perfectly perpendicular to the longitudinal axis of the vessel) the cross-sectional area of the media and lumen will always be over-estimated.

In studies performed by Nordborg and Johansson (1979), an attempt was made to compensate for blood vessel contraction. Here, a histometric technique was used where the length of the IEL was measured, and the media to lumen radius ratio was determined in a hypothetical state where the IEL of the blood vessel was smooth and circular, and the media of the blood vessel was evenly distributed around the IEL. No attempt was made to compensate for sectioning angle. Such a mathematical compensation is not always adequate. Lee et al (1983 c) have shown that during contraction the IEL of mesenteric arteries not only shortens during contraction, but does so to a greater degree in SHR than WKY vessels. In this study it was observed that when a histometric technique which compensated for sectioning angle was applied to determine the lumen diameter of contracted vessels from SHR, the lumen diamter was underestimated compared to that present in identical relaxed blood vessels. In view of this, the validity of the data presented by Nordborg and Johansson (1979) is questioned.

Pang and Scott (1981) performed a morphometric analysis of the lumen and media of perfusion fixed relaxed main renal arteries from SHR and WKY between the ages of 4 to 18 weeks. Consistent with the observations of the present study, the lumen diameter of the main renal artery, measured in a manner that would compensate for sectioning angle, was similar in SHR and WKY at every age. The analysis of medial components was limited to the proportional cross-sectional area of a

component in relation to the total medial cross-sectional area. Pang and Scott (1981) observed that the proportional composition of the medial components in the main renal artery was similar between SHR and WKY. In the present study, in established hypertensive SHR there was a greater cross-sectional area of SMCs and extracellular space, although the proportion of medial components was similar between SHR and WKY.

Pang and Scott (1981) determined the blood vessel wall thickness of the main renal artery at the vessel's thinnest point. Using this method of measurement, the main renal artery of SHR and WKY was of similar thickness. A consistent finding in the present study was that the main renal artery was not of uniform thickness in either SHR (i.e., see Fig. 2) or WKY. Particularly in SHR, the outer media contains SMCs oriented parallel to the direction of blood flow (longitudinal array). Hence, the average wall thickness may, in fact, be greater in SHR than WKY. In the present study, both pre- and established hypertensive SHR exhibited main renal arteries with increased intima + media, while, within the media increased numbers of SMC layers were present.

The present morphometric study of the renal vascular bed is unique in that 1) blood vessels were perfusion fixed at constant flow rates under maximally relaxed conditions; 2) virtually all the major arterial subcomponents of the intima, media and adventitial layer of the artery were measured and compensated for sectioning angle; and 3) a large representative sample of the renal vasculature was studied ranging from large elastic arteries, such as the main renal artery, to the preglomerular arteriole. In addition, as will be discussed in the next section, the renal vasculature of SHR exhibited contraction dose

response curve characteristics that are consistent with the presence of an unaltered lumen diameter at maximal relaxation and a thickened vascular wall in SHR over WKY.

Morphometric studies performed on the relaxed mesenteric vasculature by Lee et al (1983 a, b) also indicate that SHR in an established phase of high blood pressure development exhibit no alteration in lumen diameter when the superior mesenteric and large and small muscular arteries were measured and compensated for section angle. Wall thickening was present in SHR mesenteric arteries with a lumen diameter of greater than 60 um. This was predominantly produced by SMC hyperplasia, and to a lesser extent by SMC hypertrophy. Consistent with the above results, Mulvany and his colleagues (Mulvany and Halpern, 1977; Mulvany et al., 1979; Warshaw et al., 1979, 1980; Aalkjaer and Mulvany, 1979) observed that both large (LD = 250 μm) and small (LD = 160 μm) mesenteric arteries from SHR, stretched under tension equivalent to transmural pressure of 100 mm Hg, exhibited a thickened vascular wall. Here again, vessel wall thickening was produced primarily by increased numbers of SMCs when compared to WKY. Mulvany's work indicates that in some cases the lumen diameter is decreased in SHR mesenteric arteries (Mulvany et al., 1978; Aalkjaer and Mulvany, 1979), while in other instances it is unaltered (Mulvany and Halpern, 1977; Warshaw et al., 1979, 1980).

B. Structural alterations in the renal vasculature of prehypertensive SHR

Various researchers have suggested that a thickened vascular

wall in SHR is a secondary alteration produced by an elevated blood pressure (Folkow et al., 1971b; Lungren, 1974; Weiss, 1974). Folkow and his colleagues (Folkow et al., 1973) have stated that transient increases in blood pressure produced by stressful situations can cause the vascular wall to thicken and the lumen of the blood vessels to decrease. When such an alteration occurs, blood pressure is permanently elevated. In this regard, SHR are thought to hyper-react to environmental stimuli; hence, it is possible that such action could lead to structural adaptation of the vasculature. Other studies by Folkow and his coworkers (Folkow et al., 1971 b) indicate that vascular adaptation to high blood pressure (as measured by reactivity alterations to NE + $BaCl_2$ at maximal contraction) can be prevented in the hindlimbs of SHR by producing regional hypotension via a clamp placed at the terminal aorta. Based on these results, Folkow (Folkow et al., 1971 b, 1973) hypothesized that, although vascular adaptation is important in permanently elevating blood pressure in SHR, such alterations are not responsible for initiating hypertension.

In view of the above argument, in the present study the renal vasculature of 4 to 5 week old prehypertensive SHR was analyzed morphometrically to determine if structural alterations were present prior to the elevation of blood pressure. However a great deal of argument exists as to whether a prehypertensive stage in fact is present in SHR. Some studies have indicated that SHR are born with hypertension (Gray, 1982). Others indicate a prehypertensive stage that extends from 15 days (Nordborg and Johansson, 1979) to 12 weeks after birth (Moll et al. 1975). Still other researchers have presented data that demonstrate

that at 2 weeks of age SHR exhibit an elevated blood pressure which returns to normal at 4 weeks of age and subsequently increases permanently after 6 weeks of age (Pang and Scott, 1981). The discrepancy in the results is likely produced by differing methods of blood pressure measurement, the degree of inbreeding in an SHR colony, the environmental conditions the colony is kept under and the diet fed to the rats. All these factors can accelerate or delay the onset of hypertension in SHR (Okamoto, 1969). The blood pressure profile of the rats used in the present study was similar to that obtained by Okamoto (1969), who developed the SHR strain. To ensure that the 4 to 5 week old SHR used in the morphometric study were, in fact, prehypertensive, a direct measurement (catheterization of the aorta at the femoral artery junction) of blood pressure was used to obtain the systolic and diastolic blood pressure. Neither this mode of measurement, nor tail cuff compression measurements of the systolic blood pressure of 5 to 6 week old SHR gave any indication that SHR had significantly elevated blood pressure compared to age matched WKY.

Perfusion studies performed on prehypertennsive SHR at a number of flow rates indicated that the renal vascular resistance at maximal dilation is similar to that present in age matched WKY. However, the prehypertensive SHR used in the morphometric study did exhibit a higher renal vascular resistance ($P<0.05$) than WKY when the left kidney was perfused with Krebs, prior to fixation. Morphometric measurements performed on the main renal, interlobar and cortical arteries indicated that the lumen diameter was not altered in any of the above arterial classes when SHR and WKY were compared. This would indicate that the

elevated vascular resistance in this group of SHR resulted from a reduction in the lumen diameter of blood vessels more distal to the points measured (i.e., pre/postglomerular arterioles, capillaries, vasa recta, the veins of the kidney), or from a reduction in the number of blood flow pathways in the SHR kidney. However, since an increase in renal vascular resistance was not observed in the prehypertensive SHR used to study the relation of renal vascular resistance to perfusion flow or in the studies involving the construction of dose response curves, an elevation of renal vascular resistance of the isolated perfused kidney under maximally dilated conditions is not a consistent feature in prehypertensive SHR.

On the other hand, the renal arterial wall of young SHR exhibited many of the same alterations that were present in animals with established hypertension. However, both the magnitude and scope of the alterations was less than that observed in adult SHR. In adult SHR, all the prearteriolar arteries had increased cross-sectional area of the media. In prehypertensive SHR, an increase in the cross-sectional area of the media was limited to the main renal artery and intermediate sized cortical arteries. In adult SHR, most arteries were found to have a 35-50% increase in medial cross-sectional area when compared to WKY. In prehypertensive SHR, cortical arteries with a D_I between 40-60 μm did exhibit a 45% increase in medial cross-sectional area when compared to WKY, while other classes of arteries that were altered in SHR had a 21-26% increase in this arterial component. All classes of renal arteries studied in prehypertensive SHR did exhibit an increase in the wall (intima + media) to lumen area ratio, and almost all the arteries

of SHR had increased numbers of SMC layers within the media. The above findings suggest that medial hypertrophy and SMC hyperplasia occur prior to high blood pressure development in the renal vasculature of SHR.

The above observations are consistent with those of Lee et al (1984, unpublished observations). In this study, it was observed that larger muscular mesenteric arteries (LD approx. 127 μm) sampled from 4 week old prehyptensive SHR also exhibited medial hypertrophy produced by SMC hyperplasia. In this instance, smaller and larger mesenteric arteries were not altered and none of the arterial classes studied exhibited an altered lumen diameter when SHR and WKY were compared. Mulvany and his colleagues (Warshaw et al., 1979; Mulvany and Nyborg, 1980) found that the wall thickness of the second premucosal mesenteric branch was unaltered in 4 week old (Mulvany and Nyborg, 1980) prehypertensive SHR, while at 6 weeks, when blood pressure was slightly but significantly elevated, vessel wall hypertrophy had also occurred (Warshaw et al., 1979). Similar results have been found by Scott and Pang (1983 a) in premucosal mesenteric arteries. An increase in vessel wall thickness was found to coincide with the first evidence of elevated blood pressure.

In studies involving other vascular beds, Karr-Dullien et al (1981) found that tail arteries with an external diameter of 20-60 μm of both newborn and 2 week old SHR exhibited an increased media to lumen ratio over WKY. Gray (1982) also observed that the carotid artery of 12-24 hour old SHR was thickened when compared to age matched WKY. In this latter case, however, blood pressure was already elevated in SHR.

In view of the results of the present study on prehypertensive

SHR and those obtained by other researchers, particularly Lee (1984, unpublished observations), it could at least be possible that structural adaptation of the vascular wall precedes hypertension development and is of primary importance in the establishment of hypertension. This hypothesis is further supported by the results of the hydralazine treatment study discussed in a later section.

C. The site of structural alterations in the vasculature of SHR

It has been hypothesized that hypertension in SHR is associated with a structural adaptation of the vascular wall, in which the lumen diameter of blood vessels is decreased by wall thickening (Folkow et al., 1973). The primary site of this alteration has been suggested to be the so-called "true resistance vessel", the precapillary arteriole. Within the present study, perfusion and morphometric measurements on relaxed renal arteries of pre- and established hypertensive SHR failed to give any indication that lumen diameter is decreased in the relaxed renal arteries of SHR. Furthermore, in established hypertensive SHR, significant alterations in wall thickening, SMC hypertrophy and hyperplasia were less prevalent in smaller blood vessels. Preglomerular arterioles were virtually unaltered in established hypertension. In spite of the widespread belief that arterioles are structurally adapted and help maintain hypertension, there is in fact not a great deal of experimental evidence to support this argument and considerable evidence to the contrary.

In the renal vasculature of SHR, Hsu et al (1982) observed that vascular resistance was elevated in 8 week old conscious SHR. At this

age and in older animals, SHR were found to have preglomerular arterioles with reduced lumen diameters. It was suggested that this alteration was in part responsible for the elevated renal vascular resistance. However, when hydralazine was infused into 12 week old SHR, the elevated renal vascular resistance returned to normal, while the preglomerular arteriole lumen diameter remained unchanged and below normal levels. This would suggest that sites other than the preglomerular arterioles are responsible for the elevated renal vascular resistance and that the presence of SMC tone, as opposed to a permanent fixed decrease in lumen size, is important in maintaining the elevated vascular resistance. Consistent with the latter point, various researchers have observed that the isolated kidneys from SHR with established (Folkow et al., 1971 a; Collis and Vanhoutte, 1977; Fonteles and Jeske, 1980) and incipient hypertension (Collis et al., 1980) do not exhibit a difference in renal vascular resistance compared to WKY, when the renal vasculature is maximally relaxed.

Studies performed on the mesenteric vasculature have similar results. At maximum dilation, the mesenteric vascular resistance is similar between SHR and WKY (Hausler and Finch, 1972; Lee et al., 1983 a). Morphometric studies by Lee and his colleagues on established (Lee et al., 1983 a) and prehypertensive SHR (Lee, 1984, unpublished observations) indicate that in established hypertensive SHR, wall hypertrophy is present in arteries with a lumen diameter greater than 60 um, but not in preterminal and terminal arterioles. In prehypertensive SHR such alterations are limited to even larger classes of arteries.

In vivo microscopy studies by Henrich et al (1978) on the

mesoappendix vasculature of SHR in an early established phase of hypertension also indicate that vessel wall hypertrophy decreases in SHR as the vascular bed is followed towards the capillaries. Under in vivo conditions, the lumen diameter of terminal and preterminal arterioles, and as well the capillaries, was larger in SHR than WKY. In the abdominal vascular bed, Haack et al (1980) found that, despite a 30-40% decrease in the number of arterioles in 5 to 10 week old SHR when compared to WKY, the segmental vascular resistance of the arteriolar portion of the vascular bed was reduced in SHR to half that present in WKY. In vivo microscopy indicated that the terminal and preterminal arterioles had larger lumen diameters which more than compensated for the increase in resistance that could potentially be produced by a decrease in the anatomical numbers of arterioles in SHR. Other studies on cremaster (Bohlen and Lobach, 1978; Chen et al., 1981) and gracilis muscle (Prewitt et al., 1982) vasculature have also failed to demonstrate the presence of vessel wall hypertrophy in the two to three orders of arterioles that preceed the capillaries in SHR. Here again, lumen diameter under in vivo conditions was found to be either unaltered or increased in SHR over WKY. In view of the above results, it appears that structural adaptations in arterioles, such as wall thickening and decreases in lumen diameter, are not the rule.

Prearteriolar structural alterations in the vasculature such as those observed in the present study could still exert an important effect on vascular resistance. The amount of vascular resistance supported by such arteries is significant; for example, in the cremaster muscle of SHR and WKY, approximately 65% of the precapillary pressure

fall occurs in arteries with a lumen diameter greater than 100 μm (Bohlen et al., 1977). Thus, it might be expected that structural and functional alterations in prearteriolar renal vessels could exert an important effect in elevating vascular resistance.

D. Pharmacological and nerve stimulation studies of the renal vasculature of established and prehypertensive SHR

When the renal vascular bed of SHR in an established phase of hypertension was maximally relaxed with Krebs solution, the renal vascular resistance (RVR) was not significantly different between SHR and WKY. However, contraction of the renal vasculature with norepinephrine (NE), $BaCl_2$ or angiotensin (A_{II}) produced a situation where the amplitude of the RVR response was much greater in SHR than WKY (i.e., the renal vasculature of SHR exhibited hyper-reactivity). These findings are consistent with the presence of a renal vasculature that on average, at maximal dilation, contains an unaltered lumen diameter and a thickened vascular wall. Morphometric analysis of the structural alterations of the renal arteries of SHR with established hypertension confirmed this. If such a vascular bed is contracted from the outside of the vessel and the medial mass is conserved, the media of the thicker walled hypertensive vessel would tend to push into the lumen to a greater degree than the thinner walled WKY vessels during equal degrees of SMC shortening. In SHR with established hypertension, it is unlikely that the increased amplitude of RVR response observed during NE contraction is due to an altered efficiency or effectiveness of postsynaptic receptors, since qualitatively, the same response can be elicited by $BaCl_2$ which contracts vascular SMCs via non-receptor

mechanisms, and by A_{II} which vasoconstricts by acting through noradrenergic receptors. Furthermore, ED_{50} values for NE contraction are unchanged, while ED_{20} values are higher in SHR than WKY. This indicates that the kidney vascular smooth muscle cell (SMC) contractile sensitivity to NE is either unaltered or higher in WKY than SHR.

In the case of prehypertensive SHR (as in the established hypertensive group) RVR under relaxed conditions was not different when SHR were compared to WKY. Prehypertensive SHR also exhibited a higher amplitude of RVR response at maximal NE contraction when compared to WKY. The degree of alteration in the renal vascular resistance was less than that observed in adult SHR. At maximal contraction, in response to 10^{-5} M NE, the amplitude of the RVR change in adult SHR was 56% higher than that present in WKY, while in prehypertensive SHR the same dose of NE produced a 32% higher response over that present in WKY. The above results are consistent with the hypothesis that, when compared to WKY, prehypertensive SHR have a renal vascular bed that on average under relaxed conditions, has an unaltered blood vessel lumen diameter and a vascular wall that is thickened, but to a lesser degree than in adult SHR. The presence of such alterations in prehypertensive SHR suggests that blood vessel wall thickening does not develop secondarily to high blood pressure and therefore could be of primary importance in the establishment of hypertension. In both prehypertensive and adult SHR, vascular SMC contractile sensitivity of NE (i.e., the left-right shift of the dose response curve) was either unaltered or higher in WKY than SHR.

In view of the fact that SMC tone is maintained primarily by

periarterial sympathetic nerve activity and not by the circulating levels of NE, the renal vasculature was contracted by stimulating the sympathetic nerves entering the kidney. In SHR with established hypertension, electrical stimulation of the renal nerves was found to increase the renal vascular resistance of SHR kidneys to a greater degree than WKY under normal conditions and under conditions where the neuronal uptake of NE was blocked. This finding is consistent with the fact that the infusion of NE also produced a greater increase in RVR in SHR than WKY, and demonstrates that the reactivity alterations observed in the latter experiments could also be elicited by nerve stimulation.

In experiments involving SHR with established hypertension, maximal frequencies (>10 Hz) of nerve stimulation produced almost twice the RVR in SHR than WKY kidneys. However, under in vivo conditions the renal sympathetic nerve firing activity in SHR is two to three times that of WKY (Judy et al., 1976). Hence, structurally influenced reactivity alterations in response to contraction, in combination with a greater nerve activity, might produce even greater elevations in RVR in SHR over WKY than those observed in the present experiments.

The nerve stimulation studies did not reveal any evidence that the sensitivity to nerve released NE was greater in SHR with established hypertension than in WKY. The stimulation frequencies required to produce 20 and 50% maximal response were higher in SHR than WKY, again suggesting that the NE postsynaptic contractile sensitivity is altered in favour of WKY over SHR.

In prehypertensive SHR maximal levels of nerve stimulation produced higher mean levels of RVR in SHR over WKY; however, these

alterations were not significant at $P<0.05$. When neuronal uptake of NE was blocked by cocaine, SHR exhibited an elevated RVR ($P<0.05$) at stimulation frequencies greater than 8 Hz. The magnitude of the difference in RVR response between SHR and WKY in the presence and absence of cocaine at maximal levels of nerve stimulation was not different. However, one of the effects of cocaine was to decrease the variability in response between individuals within each group. Hence, although in the presence and absence of cocaine the magnitude of RVR response was elevated to a similar degree in SHR over WKY, the smaller degree of variability of measurements in the presence of cocaine permitted statistical significance ($P<0.05$) to be achieved. An argument can be made that if the alterations in RVR are of central importance in producing high blood pressure, the blood pressure of prehypertensive SHR could be maintained normal in spite of the presence of a thickened vascular wall (which would produce a higher vascular reactivity) because (1) the structural changes, although present, are not of sufficient magnitude to dramatically influence vascular resistance in vivo and (2) the elevated renovascular reactivity to NE, in SHR, is counterbalanced in part by a decrease in sensitivity to this agonist. During development, the structural alterations producing an increased reactivity would be augmented and the NE sensitivity of the vascular bed is either normalized (Folkow et al., 1971a) or augmented (Collis and Vanhoutte, 1977) in SHR compared to WKY. This, coupled with the fact that renal nerve firing activity starts to increase in SHR compared to WKY after 5 weeks of age (Judy et al., 1976), may produce a situation where the RVR and blood pressure become elevated in SHR over WKY.

The above results are consistent with some of the studies involving the role of the kidney in the maintenance of high blood pressure in intact SHR. It is known that the renal vascular resistance is increased in SHR over WKY and that this alteration takes place at a very early age. For example, Hsu et al (1982) found that the renal vascular resistance of 8 week old SHR is twice that of WKY. In SHR the site responsible for the altered RVR response appears to be preglomerular, since, although the mean arterial blood pressure is increased in SHR, the blood pressure at the level of the glomerular capillaries is normal (Arendshorst and Beierwaltes, 1979). Consistent with the presence of such an alteration, anaesthetized SHR require a much higher renal arterial pressure to maintain the same glomerular filtration rate as WKY (Arendshorst and Beierwaltes. 1979). In this regard, the SHR kidney in many ways resembles that of the Goldblatt hypertensive rat in that a higher perfusion pressure is required to maintain normal renal blood flow past a restrictive preglomerular vasculature (Byrom, 1969; Coleman et al., 1974).

Although there is a general consensus that RVR is altered in SHR, there is disagreement as to the mechanisms responsible for altering resistance. Studies involving the use of microspheres (Hsu et al., 1982), as well as some semi-quantitative histological studies (Mandal et al., 1977; Limas et al., 1980), indicate that SHR have renal arteries and arterioles with a smaller lumen diameter than WKY. However, perfusion studies of the relaxed renal vasculature of isolated kidneys of old and young SHR (Folkow et al., 1971 a; Collis and Vanhoutte, 1977; Collis et al., 1980; Fonteles and Jeske, 1980), as well as the present

investigation, indicate that the renal vascular resistance is unchanged, suggesting that the mean lumen diameter of relaxed renal arteries is unaltered in SHR over WKY.

Other researchers have studied the possibility that the renal vasculature has an altered reactivity and sensitivity to NE. Consistent with the present investigation, Folkow et al (1971 a) found that SHR kidneys exhibited a higher RVR response than WKY at maximal NE contraction, while NE contractile sensitivity was unchanged. Folkow hypothesized that the reactivity alteration observed could be due to a thickening of the vascular wall secondary to high blood pressure. However, Fonteles and Jeske (1980) failed to find either a sensitivity or reactivity alteration to NE when isolated perfused kidneys of adult SHR and WKY were compared. In the latter study, the lack of such changes could be due to the low blood pressure of SHR used in the investigation (systolic pressure - 130 mm Hg).

Vanhoutte and his coworkers (Collis and Vanhoutte, 1977; Collis et al., 1980; Vanhoutte, 1981) carried out one of the most detailed studies of renal vascular sensitivity and reactivity in SHR. Their findings are, in part, similar to those of the present investigation, namely that in adult SHR, RVR was similar when SHR and WKY were compared at maximally relaxed conditions. NE contraction studies indicated that the SHR renal vasculature was more reactive but, in contrast to the present study, more sensitive to NE contraction. However, in spite of the elevated responsiveness to NE, when the RVR was increased by field stimulating the renal nerves, both SHR and WKY were found to respond in a similar manner (Collis and Vanhoutte, 1977). Other experiments were

performed involving the study of H^3-NE overflow during nerve stimulation and the effect of NE neuronal uptake blockade on RVR responses to nerve stimulation. It was observed that the neuronal uptake of NE was increased and the release of NE was decreased during nerve stimulation (Vanhoutte, 1981). It was hypothesized that in SHR, the increased reponses produced by the presence of hyper-reactivity and sensitivity to NE could be counterbalanced by the presence of decreased levels of NE in the synaptic cleft during nerve stimulation. It was suggested that in adult SHR, RVR is elevated by central increases in sympathetic traffic or by the presence of locally produced or circulating facilitators of the NE release process (Vanhoutte, 1981).

These latter findings of Vanhoutte and his colleagues differ from those of the present study where, in the adult rats, nerve stimulation was found to produce a greater RVR response in SHR than WKY, while the neuronal uptake of NE in SHR was equivalent to that of WKY. Ekas et al (1983 a ,b) have also presented results that are contrary to those of Vanhoutte. In these studies, it was shown that at 2 and 4 Hz stimulation frequencies, the preloaded renal nerves of 20 week old SHR produced twice the H^3-NE overflow present in WKY. Measurements of the accumulation of H^3-NE perfused through the kidney indicated that the uptake of NE is unaltered in the renal vasculature of SHR. Consistent with the present study, Ekas has also demonstrated that SHR exhibit greater increases in RVR than WKY in response to renal nerve stimulation (Ekas et al., 1983 a, b).

Studies involving intact anaesthetized SHR and WKY have not clarified the issue as to whether intrinsic or extrarenal factors are

involved in elevating RVR in SHR. Arendshorst and Beierwaltes (1979) have indicated that RVR is increased in 12 week old SHR. However, when a clamp was placed around the aorta proximal to the renal arteries and the arterial pressure of the renal blood was reduced to that of WKY (from 158 to 114 mm Hg), the renal blood flow and the glomerular filtration rate remained constant, while the renal vascular resistance decreased to normal. It was concluded that a strong influence of an extrarenal vasoconstrictor substance or the presence of intrinsic structural abnormalities did not play a role in elevating RVR in SHR. It was suggested that the elevated RVR in SHR is primarily a consequence of an altered physiological autoregulatory response of afferent arteriolar resistance to an elevated arterial pressure. DiBona and Rios (1978), on the other hand, using techniques similar to those employed by Arendshorst and Beierwaltes (1979) found differing results in volume expanded 15 week old SHR. The RVR of SHR was found to be in excess of two times that of WKY, regardless of whether the mean renal arterial pressure was elevated (177 mm Hg) or reduced to normal (106 mm Hg) by aortic constriction. This latter study suggests that some pressure independent intrinsic renal alteration is responsible for the elevated RVR in SHR.

Very few perfusion studies involving intact vascular beds have been carried out using young SHR. In studies involving 6 week old SHR which were modestly hypertensive with respect to WKY, Vanhoutte and his colleagues (Collis et al., 1980) observed that the RVR of the isolated kidney of SHR was not different from WKY when the vasculature was maximally relaxed. Electrical stimulation of the renal nerves at

frequencies of 6, 8, 10 and 12 Hz resulted in a higher degree of RVR response in SHR as compared to WKY. However, unlike the present study, the elevated amplitude of response was statistically significant both in the presence and absence of cocaine. Furthermore, contrary to the findings of the present study, the reactivity and sensitivity of the renal vasculature to NE contraction were similar when SHR and WKY were compared. It was suggested that the hyper-reactivity of the renal vasculature in response to nerve stimulation in SHR could be due to an increased release of NE from the renal nerves. Other experiments involving the measurement of H^3-NE overflow from the renal nerves did suggest that the release of NE is augmented in young SHR (Vanhoutte, 1981). In the present study, the neuronal release of NE from the renal nerves during nerve stimulation may be augmented in prehypertensive SHR and may contribute in part to the hyper-reactivity observed. However, in view of the fact that the renal vasculature of prehypertensive SHR hyper-reacted to maximal NE contraction when compared to WKY, it appears that postsynaptic alterations in the renal vasculature, such as wall thickening may also play an important role in promoting hyper-reactivity.

Hyper-reactivity in response to NE contraction has also been observed in the isolated hind limb vascular bed of 3 week old SHR (Lais and Brody, 1978). Unlike the renal vasculature of young SHR, the hind limb vasculature also exhibited an increased NE sensitivity. In other experiments, Lais and Brody (1978) observed that the hind limb vasculature of SHR hyper-reacted in response to $BaCl_2$ infusion. Since $BaCl_2$ contracts SMCs via non-receptor mechanisms, the hyper-reactivity

observed in SHR could not be totally explained in terms of alterations in the effectiveness or efficiency of adrenergic receptors in the hind limb vascular bed. In view of this, Lais and Brody (1978) hypothesized that the vascular wall of 3 week old SHR was thickened and produced the hyper-reactivity observed in response to NE contraction. Unfortunately, the blood pressure of the animals studied was not taken, and therefore it is not clear whether the functional changes observed in these experiments preceded hypertension development.

If a thickening of the vascular wall is responsible for the contractile reactivity alterations in the renal vasculature of SHR, it might be expected that all contractile agents, regardless of their mode of contraction would produce an increase in reactivity in SHR over WKY. The findings of the present study are consistent with the above hypothesis, in that the renal arteries of SHR in an established phase of hypertension exhibited an increased reactivity in response to NE, $BaCl_2$ and A_{II}. Similarly, other studies of the renal vasculature (Collis and Vanhoutte, 1977; Bereck et al., 1980) have demonstrated hypercontractile reactivity in response to vasopressin and serotonin in SHR. However, in the present study when the renal vasculature of SHR and WKY was contracted with KCl (in the presence of propranolol and spiroperidol), both groups of rats exhibited similar levels of contractile sensitivity and reactivity, a feature that is inconsistent with the structural hypothesis presented in Fig. 38.

An explanation for the above anomaly could reside in the fact that the mechanisms involved in KCl contraction differ from those of the other contractile agents discussed above. The elevation of

extracellular K^+ depolarizes the VSMC membrane and permits the opening of potential sensitive Ca^{+2} channels, allowing the entry of Ca^{+2} (Droogmans et al., 1977). NE, on the other hand, can under certain circumstances contract VSMCs without altering the membrane potential, i.e., pharmacomechanical coupling (Somlyo and Somlyo, 1968; Droogmans et al., 1977). Unlike NE and A_{II}, which are capable of releasing intracellular stores of Ca^{+2} to elicit contraction, contraction produced by K^+ depolarization requires an extracellular source of Ca^{+2} (Freeman and Daniel, 1973; Van Breeman et al., 1980). Long term NE contraction is also associated with an influx of extracellular Ca^{+2}, however, two to three contractile responses can be elicited in the absence of extracellular Ca^{+2}. Furthermore, there is evidence that the Ca^{+2} channels activated by K^+ depolarization differ from those permitting Ca^{+2} influx during NE contraction. For example, the Ca^{+2} antagonist D600 is more effective in inhibiting $^{45}Ca^{+2}$ influx during K^+, as opposed to NE, contraction, while conversely amirone is more effective in blocking Ca^{+2} entry during NE, as opposed to K^+, contraction (Van Breeman et al., 1980). Van Breeman et al (1980) have suggested that some Ca^{+2} channels are receptor operated, while others are potential sensitive. If this view is correct, NE contraction that is associated with a depolarization of the membrane should open both receptor and potential sensitive Ca^{+2} channels. K^+ depolarization, on the other hand, would affect only the potential sensitive channels.

It seems possible that the mechanisms specific to K^+ induced contraction are less efficient in the VSMCs of SHR than WKY. If this is the case, the presence of hyper-reactivity to contraction produced by a

thickened vascular wall in SHR could be counterbalanced by a decreased contractile responsiveness to K^+. In this regard, there is evidence to suggest that the mechanisms governing the membrane potential are altered in the VSMCs of SHR. Ion flux studies performed by Jones (1973, 1974 a, b), Rb^{86} uptake studies performed by Pamnani et al (1981) and microelectrode measurements of the membrane potential performed by Hermsmeyer (1976 a, b) and Abel and Hermsmeyer (1981) all suggest the presence of a hyperactive Na^+ efflux in the VSMCs of SHR. If in the VSMCs of the renal arteries (unlike the tail arteries) the Na^+/K^+ pump independent portion of the membrane potential remained unaltered between SHR and WKY, a hyperactive Na^+/K^+ pump in SHR could inhibit the degree of depolarization produced by elevating external K^+ levels. Since NE contraction of VSMCs depends in part on mechanisms other than those involving a depolarization of the membrane, an increased Na^+ efflux may not alter NE contraction to the same degree as K^+ contraction, and might, therefore, permit hyper-reactivity in response to NE but not to K^+ to be exhibited in the renal arteries of SHR.

Other studies by Schomig et al (1978) indicate that the hindlimb vasculature of adult SHR does hyper-react to KCl contraction when compared to WKY. However, in these studies no attempt was made to block the effects of NE released from the periarterial nerves during KCl depolarization. The present studies showed that 45% of the maximal KCl response is phentolamine sensitive or can be blocked by 10^{-8} M prazosin + 10^{-6} M spiroperidol. It is essential to have such blocking agents present to ensure that KCl is contracting via SMC depolarization, as opposed to depolarization augmented by an adrenergic response. In view

of the fact the SHR hyper-react to NE, it might be expected that in Schomig et al's (1978) study, SHR would hyper-react to NE released by KCl.

In adult SHR, approximately 85% of the total contractile response to maximal levels of renal nerve stimulation could be inhibited by 10^{-8} M prazosin, a selective $alpha_1$ antagonist. The balance of the response was blocked by 10^{-6} M spiroperidol (a dopamine receptor antagonist), but was not affected by varying concentrations of yohimbine (a selective $alpha_2$ antagonist), phentolamine (an $alpha_1$ and $alpha_2$ antagonist) or methysergide (a 5-HT antagonist). None of these latter agents, when present alone or in combination, further decreased RVR during nerve stimulation after the introduction of 10^{-8} M prazosin. This indicates that in SHR and WKY, nerve mediated contraction of the renal vasculature occurs by activation of $alpha_1$ and dopamine receptors, but not $alpha_2$ or postsynaptic 5-HT receptors. Furthermore, although the amplitude of the maximal nerve stimulated response is higher in SHR over WKY, the proportion of the response attributed to $alpha_1$ and dopamine receptors is similar.

Radioligand binding studies performed by Pettinger et al (1982) on the plasma membrane of whole SHR and WKY kidneys indicates that numerically there are two to three times as many $alpha_2$ receptors as $alpha_1$ receptors in the kidney. Furthermore, both $alpha_1$ and $alpha_2$ receptors are numerically more abundant in SHR than WKY kidneys. In view of the fact that in the present study the proportion of response attributed to $alpha_1$ receptors during nerve stimulation did not differ between SHR and WKY, it would appear that the extra $alpha_1$ receptors

found by Pettinger et al (1982) in the SHR kidneys are (a) present in non-vascular areas, (b) are present on vascular SMCs, but are not coupled to the contractile mechanisms, or (c) are present on vascular SMCs outside synaptic areas where they are not affected by nerve released transmitter.

The $alpha_2$ receptor is present on a variety of VSMCs and its stimulation can elicit a vasoconstrictor response (Timmermans and VanZwieten, 1981). A possible explanation of why renal nerve mediated vasoconstriction does not act through $alpha_2$ receptors could reside in the location of the $alpha_2$ receptor. Various researchers have suggested that the $alpha_1$ receptor is present at neuroeffector junctions, while the $alpha_2$ receptors are present in areas further away from nerve transmitter influence (Timmermans and VanZwieten, 1981; Yamaguchi and Kaplin, 1981). For example, in pithed rats, the pressor effects of exogenous catecholamines are highly susceptible to $alpha_2$ antagonists, while pressor responses due to sympathetic nerve stimulation are more susceptible to $alpha_1$ blockade (Yamaguchi and Kaplin, 1980). Alternatively, it is possible that renal vasculature differs from other vascular beds in that $alpha_2$ receptors are not involved in pressor responses. Consistent with this, Drew and Whiting (1979) have found that in the cat renal vasculature the pressor responses of infused NE can be totally blocked by prazosin, an $alpha_1$ antagonist.

NE acts by stimulation of alpha and beta receptors. In innervated arteries, the beta receptor serves two roles; postsynaptic beta receptors are capable of relaxing VSMCs, while presynaptic $beta_2$ receptors present on sympathetic varicosities can facilitate the release

of NE (Kawasaki et al., 1982 a). Therefore, it is possible that during maximal NE contraction, NE acts to relax the renal arteries postsynaptically, while during nerve stimulation, released NE in addition acts presynaptically to facilitate the further release of NE. The present experiments do not suggest such a hypothesis. When propranolol, a beta receptor antagonist, was introduced into the solution at varying concentrations, no effect on NE or nerve stimulated contraction occurred at lower doses ($< 10^{-7}$ M), while high levels of propranolol ($> 10^{-6}$ M) inhibited contraction. The reduction in contractile response is not unexpected, since at high concentrations this antagonist is capable of blocking alpha, as well as beta receptors (Gulati et al., 1969). These results are consistent with those of Kawasaki et al (1982 a). In these studies, it was observed that, although both isoproterenol (a general beta agonist) and salbutamol (a $beta_2$ selective agonist) were capable of enhancing the release of preloaded H^3-NE from the sympathetic nerves of the mesenteric arteries to a greater degree in SHR than WKY, the presence of propranolol (up to 5×10^{-7}M, a general beta antagonist) or practolol (5×10^{-6} M, a $beta_2$ selective antagonist) did not alter nerve evoked contractile responses in either SHR or WKY.

The lack of involvement of the beta-receptor during NE contraction is also consistent with various other pharmacological studies that indicate that NE is a very weak stimulator of the beta-receptor. When compared to isoproterenol, a full beta agonist, NE has 0.008 times the potency to relax rabbit aortic strips contracted with acetylcholine under conditions where the alpha receptors have been

blocked (Furchgott, 1967). Furthermore, at near maximal levels of NE or KCl contraction, full stimulation of the beta receptor by 10^{-6} or 10^{-5} M isoproterenol produces only a 30% relaxation response (Gadfraind et al., 1978; Kawasaki et al., 1982 a), indicating that maximal beta receptor stimulation is capable of only modest relaxation effects.

E. The effect of hydralazine treatment on the renal vascular structure in SHR and WKY

Wall thickening of the renal vasculature of SHR was mainly due to increased media. This structural change developed to a similar degree in hydralazine treated SHR with normal blood pressure as in control animals with high blood pressure. The cross-sectional area of media, total wall, wall to lumen ratio and the medial SMC layers were similar in the renal vessels of treated and control SHR, but greater than in either treated or control WKY. These results show that wall thickening of the renal vessels of SHR occurs regardless of whether the animal has high blood pressure, and therefore one concludes that high blood pressure is not the primary factor resulting in structural thickening of the renal vessel walls.

Hydralazine reduces blood pressure by vasodilating the systemic arterial vasculature and reducing peripheral resistance (Goldberg et al., 1977; Taylor, 1980). If a thickened vascular wall plays an important role in maintaining high blood pressure in SHR, it is possible that during hydralazine treatment this role may be counteracted by the vasodilating effect of hydralazine. The finding that withdrawal of the drug from the drinking water of SHR treated in utero and postnatally

with hydralazine for 26 weeks did not prevent re-established hypertension within two days, tends to confirm such a mechanism.

Other researchers have also studied the relationship of blood pressure and structural alterations in SHR. Mulvany et al (1981 b) used 6-hydroxydopamine to chemically sympathectomize SHR and WKY from birth. At 24 weeks of age, the sympathectomized SHR did exhibit a small but significantly higher blood pressure (12 mm Hg) than similarly treated WKY. Further, Mulvany et al (1981 b) found that the lowering of blood pressure had no effect in reducing the wall thickness of small mesenteric vessels in SHR. In studies performed by Scott and Pang (1983), 2 day old SHR and WKY were injected with capsaicin. Such treatment reduced the mean blood pressure of both 12 week old SHR and WKY by approximately 32 mm Hg compared to untreated SHR and WKY controls. In spite of the fact that the blood pressures of the treated group were lower than their control counterparts, the premucosal jejunal arteries of the former groups were thicker than in the control animals. Furthermore, treated SHR and untreated WKY had similar blood pressures (101 ± 5 vs 91 ± 5 mm Hg, respectively), yet the vascular wall of treated SHR was 55% thicker than the untreated WKY. Scott and Pang (1983b) concluded that factors other than high blood pressure were important in determining vascular wall thickening in SHR, a view which the present study supports. In contrast, the study performed by Mulvany and his colleagues (Mulvany et al., 1981 a), where the antihypertensive agent felodipine was used to reduce the BP of SHR from 6 weeks up to 12 weeks of age, drug treatment reduced the difference in wall thickness observed between SHR and WKY. It was concluded that medial thickening

was a consequence, not a cause, of high blood pressure. However, based on the results which they presented, the latter conclusion can be questioned. The control SHR were normotensive, having at 12 weeks of age a mean blood pressure of only 121 mm Hg, and felodipine treatment produced only a marginal decline in blood pressure of 10 mm Hg. Furthermore, the difference in blood pressure between treated SHR and WKY was 16 mm Hg, while the untreated control group had a difference of only 26 mm Hg. Finally, antihypertensive treatment appeared not to decrease the wall thickness of SHR, but rather to increase the thickness of the WKY vessel walls. In view of 1) the marginal antihypertensive effect of felodipine, 2) the small blood pressure difference between control and treated SHR and WKY, and 3) the lack of regression of structural changes in SHR after treatment, one questions their conclusion that the lowering of blood pressure prevented wall thickening from occurring.

Other researchers have studied the alterations in vascular reactivity that occur in response to antihypertensive treatment. In these experiments an elevation in the amplitude of vascular resistance change from maximal relaxation to maximal contraction in SHR over WKY (i.e., hyper-reactivity) was used as an indicator of a thickened vascular wall in SHR. Folkow et al (1972) immunosympathectomized SHR and WKY at birth using nerve growth factor antiserum. At 32 weeks of age the blood pressure of SHR was reduced from control values of 210 to 139 mm Hg, while similar treatment of WKY lowered their blood pressure from 139 to 113 mm Hg. It was found that the hind limb vasculature of immunosympathectomized SHR with normal blood pressure still exhibited a

higher level of reactivity in response to maximal contraction than either immunosympathectomized or control WKY. However, the reactivity exhibited by denervated SHR was lower than that of the untreated SHR. Thus, it appeared that the severe lowering of blood pressure did partially but not totally attenuate the increased response in SHR thought to be produced by structural adaptations.

Weiss and Lundgren (1978) using similar techniques as those of Folkow et al (1972) studied the effects of antihypertensive drugs metoprolol, hydralazine, propranolol and hydralazine + guanethidine on hind limb vascular reactivity. SHR were treated continuously from weaning to 10 months of age. Each of the drugs studied normalized the blood pressure in SHR. However, the treated SHR exhibited a small but significantly elevated blood pressure over untreated WKY. As in the previous study, it was observed that although hind limb vascular reactivity decreased in SHR subjected to antihypertensive drug treatment, the levels of reactivity were still significantly elevated in the treated SHR over the levels present in WKY, much more so than would be expected based on the differences in blood pressure between these two groups. Both Folkow et al (1972) and Weiss and Lundgren (1978) suggested that SHR resistance vessels might for genetic reasons be more prone to adapt structurally to smaller pressure loads than those of WKY, i.e., a very small elevation in blood pressure may cause a very large structural alteration. In both studies it was suggested that the small elevation in blood pressure observed in treated SHR compared to WKY may be sufficient to produce an exaggerated structural adaptation. However, in Folkow et al's (1972) study, immunosympathectomized SHR had exactly,

the same mean blood pressure as untreated WKY, yet in spite of this, hind limb vascular reactivity was still higher in the former group.

In other studies, Hamilton (1975) found that normalization of BP in SHR from 4 to 16 weeks of age with a combination of reserpine, hydrochlorothiazide and hydralazine eliminated the reactivity alterations to NE and serotonin in the mesenteric vasculature. Unfortunately, the treated SHR were compared to treated Sprague Dawley normotensive control animals and not WKY.

All the above perfusion studies provide indirect evidence for the presence or absence of structural alterations, and none of the groups actually assess structural differences. Researchers such as Hermsmeyer (1976 a, b) have hypothesized that the hyper-reactivity of the SHR vasculature, thought to reflect vascular wall thickening by Folkow et al (1973), is in fact being produced by the presence of a decrease in the non-electrogenic (Na^+ pump independent) portion of the membrane potential. Alternatively, Daniel (1981) has hypothesized that altered Ca^{+2} handling by the VSMCs of SHR could produce similar alterations in vascular contractile reactivity as those observed by Folkow et al (1973). Furthermore, the validity of perfusion experiments depends on the ability of the vasculature to attain maximal contraction. It could be possible that long term drug treatment or the accumulation of the drug itself may cause a functional alteration where maximal pressor reponses are reduced. For example, it has been my experience that a 20 second perfusion of the renal vasculature with 10 mg/l of the vasodilator sodium nitroprusside severely attenuates subsequent NE contractile responses in the kidney (unpublished results). Hydralazine,

as well as other vasodilators, reduces the reponse of arterial smooth muscle to NE, serotonin, epinephrine, angiotensin, $BaCl_2$ and KCl (Taylor, 1980). In the case of hydralazine, autoradiographic studies have shown that the half life of the drug accumulated in the arterial wall is 30 hours (Taylor, 1980). In view of this, a decrease in vascular reactivity, such as that observed in an isolated vascular beds after long term antihypertensive treatment, could be due to the depressor effect of the accumulated drug on pressor responses, as opposed to an alteration in vascular structure.

One of the most consistent alterations observed in SHR and WKY treated with hydralazine is an increase in the intima of arteries with a D_I > 120 μm. In this regard, Spoka et al., (1983) have shown that a significant proportion of hydralazine's ability to relax rabbit aortic strips is exerted through the endothelium and the ability of hydralazine to relax NE contracted tissue is significantly reduced in endothelial-denuded arteries. It was suggested that hydralazine released some constant factor from endothelial cells, which in turn relaxed the arteries. Aerobic pathways also play a critical role in hydralazine relaxation of NE contraction. In carotid arteries, hydralazine relaxation, unlike other forms of vasodilation, cannot take place in the absence of O_2 (Ablad, 1963). In view of the fact that hydralazine relaxation appears to involve endothelial cell metabolism and the synthesis and release of some as yet unknown agent from the endothelium, a side effect of this activity could be endothelial cell hypertrophy and/or hyperplasia. Alternatively, it is possible that the turnover of endothelial cells is increased in the arteries of

hydralazine treated animals. If, during this process, arteries were partially denuded of their endothelium, the regrowth of new endothelium could be associated with the thickening of the subendothelial space. This is similar to the situation that occurs when an artery is mechanically de-endothelialized (Richardson et al., 1980). Increases in endothelial cell size and or number, or a thickening of the subendothelial space could increase the cross-sectional area of the intima in hydralazine treated animals.

In the present study, SHR treated with hydralazine were found to exhibit an elevated heart rate which decreased to normal after hydralazine withdrawal. An elevation in heart rate and cardiac output are often observed in both humans and animals treated with hydralazine (Goldberg,et al., 1977; Taylor, 1980). Theseeffects can be abolished by interrupting the nerves extending from the brain to the heart, indicating that hydralazine does not have a direct effect on the heart (Taylor, 1980). Furthermore, the brain accumulates virtually no hydralazine, suggesting that this drug likely does not act directly on the central nervous system (Taylor, 1980). Hydralazine also has very little effect on the catecholamine content of vascular tissues (Taylor, 1980), indicating that neuronal stores of NE are likely normal. The increase in heart rate observed in hydralazine treated animals could be due to an elevation in sympathetic activity mediated through baroreceptors and caused by the drop in blood pressure (Goldberg et al., 1977; Taylor, 1980). An analogous increase in sympathetic activity has been shown to occur in response to hypotension produced by hemorrhage (Judy et al., 1976). Within the present study, the hypothesis being

tested was that a normalized blood pressure prevents vessel wall thickening in SHR. Hence, an elevated heart rate in the treated animals would not effect the test, since, in spite of this, the blood pressure in treated SHR was normal. The possibility does however exist that the elevated sympathetic nerve activity could exert a trophic influence on the VSMCs. This hypothesis will be examined in the following section.

F. The role of the sympathetic nervous system in producing blood vessel wall thickening in SHR

There is evidence that suggests that the sympathetic nerves can exert a trophic influence on the blood vessel wall. Hart et al (1980) removed one of the two superior cervical ganglia from 8 week old spSHR. This procedure sympathetically denervated the cerebral arteries of one brain hemisphere leaving the other side intact. In 13 month old SHR, the wall to lumen ration of perfusion fixed intraparenchymal brain arteries was elevated on the innervated over the denervated side. Furthermore, the denervated cerebral vessels exhibitied a similar wall to lumen ratio as those present in WKY, in spite of the fact that they were subjected to high blood pressure.

In other experiments, Sadoshima et al (1981) found that the incidence of stroke in spSHR in response to placing 1% saline into the drinking water was dramatically increased if one of the superior cervical ganglia was removed at an early age. Within this study, all the ganglionectomized spSHR (n=28) prematurely died of stroke between the ages of 19 to 23 weeks. Stroke almost always occurred on the side of the brain in which the cerebral blood vessels had been denervated.

Post mortem examination indicated that pial, as well as intraparenchymal blood vessels (ID < 80 μm, compensated for contraction) exhibited a decreased wall to lumen ratio on the denervated over the innervated side of the brain. Both Hart et al (1980) and Sadashima et al (1981) suggested that the sympathetic nerves may exert a trophic influence on the vasculature which can promote vascular wall hypertrophy independent of high blood pressure.

Consistent with the above hypothesis, Bevan (1975) has shown that when rabbit ear arteries are denervated for 2 and 3 weeks, they become atrophied and exhibit a reduced H^3-thymidine uptake into the SMC nuclei when compared to control innervated ear arteries. Studies performed by Cavallero et al (1972, 1973) have shown that the injection of epinephrine results in an increased incorporation of H^3-thymidine into the nuclei of both aorta and pulmonary artery SMCs of rabbits.

The mechanisms through which sympathetic nerves could exert a trophic effect are unknown. However, Bevan (1975) has hypothesized that the beta receptor is involved. Both epinephrine and norepinephrine are capable of acting as beta agonists. There is experimental evidence suggesting that beta receptor stimulation is linked to an increase in SMC cAMP content (Triner et al., 1972). It could be possible that increases in cellular cAMP could stimulate the phosphorylation of histones and initiate DNA synthesis and SMC replication. An overactive sympathetic nervous system, such as that observed in young prehypertensive SHR (Judy et al., 1976), could alter the structure of the vascular bed in a manner conducive to the maintenance of high blood pressure.

Results of the present study do not support the above hypothesis. If the sympathetic nerves did exert a trophic influence on the vasculature, the life long treatment of SHR and WKY with hydralazine and the hyperactivity of the sympathetic nerves associated with such treatment might be expected to produce increased quantities of SMCs in treated over nontreated control SHR and WKY. However, in most cases, the cross-sectional area of media and the numbers of medial SMC layers present in the renal vasculature of treated SHR are similar to that present in untreated control SHR, while in many instances, treated WKY exhibited a reduction in these parameters when compared to control WKY (see Figs. 33 and 34). Other studies by Gray (1982) indicate that the carotid arteries of SHR exhibit a thickened vascular wall over WKY at birth, prior to the development of the sympathetic nervous system. Furthermore, as previously discussed, work by Mulvany et al (1981 b) indicates that neonatal sympathectomy does not prevent the occurrence of vessel wall thickening in the mesenteric arteries of SHR (despite the fact that blood pressure is normalized). The above experiments suggest that pressure and sympathetic nerve independent processes produce blood vessel wall thickening in SHR. In this regard, it is possible that increases in SMC division are genetically programmed in SHR at the level of the VSMC to occur at a certain stage of development.

In the study performed by Mulvany et al (1981 b), as well as the present hydralazine study, the blood pressure of SHR was normalized (by neonatal sympathectomy or hydralazine treatment) while the walls of the blood vessels remained thickened. It could be argued that these studies support the hypothesis that blood vessel wall thickening does not play a

role in elevating blood pressure in SHR since the blood vessel walls remain thickened even though hypertension is absent. This result can be explained in the following manner. If the vascular system of SHR is altered in a manner consistent with the model presented in Fig. 38, wall thickening and the presence of SMC tone (as might be produced by normal sympathetic nerve activity) are required to increase vascular resistance in SHR over WKY. If the mechanisms increasing SMC tone are removed (i.e., by sympathectomy) or counteracted by a vasodilator (i.e., hydralazine) the vasculature will tend to relax, and since under these conditions the blood vessel lumen sizes of SHR and WKY are similar, blood pressure will be normalized. In the case of sympathectomized SHR, unless reinnervation takes place, blood pressure will remain normal. In the case of hydralazine treatment removal of the drug allows the re-establishment of SMC tone and high blood pressure.

The withdrawal of hydralazine at 26 weeks of age from SHR treated in utero and postnatally with the drug quickly re-established hypertension within 2 days. On the other hand, SHR treated with hydralazine for 286 days from an established phase of hypertension (starting from 24 weeks of age) required between 25 to 52 days to reach blood pressure levels present in control SHR. Within this group, blood pressure elevation could be divided into two phases. Within four days the blood pressure quickly rose to moderately hypertensive levels (i.e., 157 mm Hg). Subsequently, the blood pressure did not alter for approximately 25 days. At 52 days after withdrawal, blood pressure was at levels present in SHR controls. The reasons for the different responses observed after hydralazine withdrawal between in utero -

postnatally treated SHR and SHR treated during established hypertension are unknown. One possibility is that long term treatment of SHR during fetal and neonatal development decreases the animal's sensitivity to hydralazine over that present in SHR treated for long periods during the established phase of hypertension. Therefore, drug withdrawal in the former group may allow the re-establishment of hypertension at a faster rate. However, the time differential in the re-establishement of hypertension between the two groups is quite large. After a small abrupt increase in blood pressure very little further change took place for 25 days. Based on the half life of hydralazine within human and animal tissues, this should have been more than sufficient time to eliminate all the hydralazine from SHR. Alternatively, perhaps treatment of SHR in the established phase of hypertension either reverses in part some of the vascular structural changes that contributed to high blood pressure, or in some other manner produces a physical alteration that exerts a short term (25-52days) hypotensive effect. In this regard, Warshaw et al (1980) found that antihypertensive treatment of SHR in the established phase of hypertension had a significant effect in decreasing the SMC content and wall thickness in mesenteric arteries. However, SHR still had arteries with a thicker wall and a greater SMC content than WKY.

E. Summary and Conclusions

Perfusion studies performed on the renal vasculature of SHR with established hypertension indicated that at maximal dilation the renal vascular resistance is similar to that of WKY. Consistent with this finding, the lumen diameter of the main renal, interlobar, arcuate and interlobular arteries, as well as the preglomerular arterioles, of SHR and WKY were similar. Structural alterations were present. A thickened arterial media was observed, particularly in prearteriolar arteries. This alteration was produced by an increase in the medial extracellular space and SMC hyperplasia, hypertrophy or a combination of both the latter alterations. Very little change was observed in the arterial intima, adventitia or its subcomponents.

Qualitatively analogous types of alterations but of a smaller magnitude were found to occur prior to high blood pressure development in SHR. In prehypertensive SHR the renal vascular resistance at maximal relaxation was similar to that present in WKY, while the lumen diameter of the main renal, interlobar and cortical arteries was unaltered between the two groups. In prehypertensive SHR, all the arteries studied exhibited an increased quantity of arterial wall (intima + media) in relation to the lumen and virtually all the arteries exhibited elevated numbers of SMC layers within the media. Since such alterations occurred prior to high blood pressure development in SHR, they could be of primary importance in the initiation of hypertension in this model.

Based on the structural alterations observed it was hypothesized that if the medial cross-sectional area remains constant, contraction of the blood vessel from the adventitial side would produce a situation

where the media of the thicker walled hypertensive vessels would tend to push into the lumen to a greater degree than the thinner walled WKY vessels. Therefore, under relaxed conditions, renal vascular resistance would be expected to be similar between SHR and WKY; however, during contraction, RVR should be elevated to a greater degree in SHR. To test whether the renal vasculature did react in accordance with the proposed model, pharmacological and electrical stimulation experiments were performed on the isolated perfused left kidney of established and prehypertensive SHR and age matched WKY.

When the renal vascular bed of SHR in established and prehypertensive phases of hypertension development was maximally relaxed with Krebs solution, the RVR was similar in both SHR and WKY. Contraction of the renal vasculature of adult SHR with NE, $BaCl_2$, A_{II} or by stimulating the renal nerves (in the presence and absence of cocaine) produced a greater amplitude of RVR response in SHR than WKY (i.e., hyper-reactivity). Prehypertensive SHR also exhibited a higher amplitude of RVR response than WKY at maximal NE contraction. However, the degree of alteration was approximately 43% less than that observed in adult SHR when both groups were compared to WKY. Maximal levels of renal nerve stimulation also produced higher mean levels of RVR in prehypertensive SHR compared to WKY; however, such alterations were only significantly different ($P<0.05$) when the neuronal uptake of NE was blocked by cocaine. The above results are consistent with the hypothesis that SHR have a renal vascular bed that on average has an unaltered lumen diameter and a thickened vascular wall when compared to WKY. However, in the case of prehypertensive SHR, the reactivity

alterations predict that the degree of wall thickening is less than that present in adult SHR in comparison to WKY, a result that is further substantiated by the morphometric findings.

In adult SHR, alpha$_1$, and to a lesser extent, dopamine receptors were responsible for the contractile responses mediated by nerve stimulation. The proportion of the response attributed to the different receptor types was not different between SHR and WKY. The beta receptor did not appear to play an important role in either NE or nerve stimulated contraction.

The in utero and postnatal normalization of blood pressure up to 21 weeks of age with hydralazine has virtually no effect in reversing wall thickening in the SHR renal vasculature. This suggests that such alterations in SHR are not a secondary response to blood pressure. Consistent with the hypothesis that a thickened vascular wall in combination with normal vascular tone is important in maintaining high blood pressure, the withdrawal of hydralazine treatment and presumably vasodilation quickly re-established hypertension in SHR treated in utero and postnatally with hydralazine.

Treatment of established hypertensive rats with hydralazine also reduced blood pressure to normal. Withdrawal of the drug after 286 days of treatment resulted in an abrupt rise in blood pressure for four days to moderate hypertensive levels (157 mm Hg). Subsequently, the re-establishment of full hypertension (> 190 mm Hg) required between 25-52 days. This suggests that long term hydralazine treatment of SHR with established hypertension either produced a short term and partial regression of the mechanisms producing hypertension, or altered some

other unknown factor that produced a hypotensive effect.

Animals treated with hydralazine were found to have a higher heart rate than control animals. This response was found to be reversed by hydralazine withdrawal. An elevation in the heart rate during hydralazine treatment suggests the presence of a hyperactive sympathetic nervous system. Some researchers have indicated that such an alteration could exert a trophic influence of the VSMCs, and thus produce a thickening of the arterial media. The present study does not support this hypothesis. The cross-sectional quantities of the media and medial SMC layers were similar when hydralazine treated SHR were compared to control SHR, while treated WKY exhibited decreased quantities of media and SMC layers over control WKY. This occurred in spite of the fact that the sympathetic nervous system was presumed to be hyperactive during hydralazine treatment.

References

Aalkjaer, G. and Mulvany, M.J. (1979). Morphological and mechanical properties of small mesenteric arteries and veins in spontaneously hypertensive rats. Acta physiol. Scand. 107: 309-317.

Abboud, F.M. (1982). The sympathetic system in hypertension. Hypertension 4 (suppl. 11): II 208-II 225.

Abel, P. and Hermsmeyer, K. (1981). Sympathetic cross-innervation of SHR and genetic controls suggests a trophic influence on vascular muscle membranes. Cir. Res. 49: 1311-1318.

Abe, Y. and Tomita, T. (1968). Cable properties of smooth muscle. J. Physiol. 196: 87-100.

Ablad, B. (1963). A study of the mechanisms of the hemodynamic effects of hydralazine in man. Acta Pharmacol. (Kobenhavn) 20 (suppl. 1): 1-53.

Adelstein, R.S., Conti, M.A. and Hathaway, D.R. (1978). Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3':5' monophosphate dependent

protein kinase. J. Biol. Chem. 253: 8347-8350.

Agarawal, D.K. and Daniel, E.E. (1984). Postsynaptic alpha$_1$ and alpha$_2$ adrenoreceptors in the vascular smooth muscle of spontaneously hypertensive rats (SHR). Fed. Proc. (in press) (Abstract).

Alquist, R.P. (1948). Study of adrenotropic receptors. Am. J. Physiol. 153: 586-600.

Allen, J.C. (1977). Ca+2-binding properties of canine aortic microsomes: lack of effect of cAMP. Blood Vessels 14: 91-104.

Altura, B., Hershy, S. and Zweifach, B. (1965). Effects of a synthetic analogue of vasopressin on vascular smooth muscle. Proc. Soc. Exp. Mol. Med. 119: 258-261.

Altura, B.and Hershy, S. (1967). Pharmacology of neurohypophyseal hormones and their synthetic analogues in the terminal vascular bed. Angiology 18: 428-439.

Altura, B.M. and Altura, B.T. (1977). Vascular smooth muscle and neurohypophyseal hormone. Fed. Proc. 36: 1853-1860.

Amer, M.S., Gomoli, A.W., Perbach, J.L., Ferguson, H.C. and McKinney, G.R. (1974). Aberations of cyclic nucleotide metabolism in

the hearts and vessels of hypertensive rats. Proc. Natl. Acad. Sci. USA 71: 4930–4934.

Amer, M.S. (1979). Cyclic adenosine monophosphate and hypertension in rats. Science 179: 807-809.

Andersson, R. (1973). Role of cyclic AMP and Ca^{+2} in mechanical metabolic events in isometrically contracting vascular smooth muscle. Acta physiol. Scand. 87: 84-95.

Andersson, R., Nilsson, K., Wikeberg, J., Johansson, S., Mohme-Lundholm, E. and Lundholm, L. (1975). Cyclic nucleotides and the contraction of smooth muscle. Adv. Cyclic Nucleotide Res. 5:491-518.

Andresen, M.C. and Brown, A.M. (1980). Baroreceptor function in spontaneously hypertensive rats. Effect of preventing hypertension. Cir. Res. 47: 829-834.

Andresen, M.C., Kuraoko, S. and Brown, A.M. (1980). Baroreceptor function and changes in strain sensitivity in normotensive and spontaneously hypertensive rats. Circ. Res. 47: 821-828.

Antonaccio, M.J., Harris, D., Goldberg, H., High, J.P., and Rubin, B. (1979). The effects of captopril, propranolol and indomethacin on blood pressure and plasma renin activity in

spontaneously hypertensive and normotensive rats. Proc. Soc. Exp. Biol. Med. 162: 429–433.

Arendshorst, W. and Beierwaltes, W.H. (1979). Renal and nephron hemodynamics in spontaneously hypertensive rats. Am. J. Physiol. 236: F246–F251.

Arner, A. and Uvelius, B. (1982). Force-velocity characteristics and active tension in relation to content and orientation of smooth muscle cells in aorta from normotensive and spontaneously hypertensive rats. Circ. Res. 50: 812–821.

Asano, M., Aoki, K. and Matsuda, T. (1982). Reduced beta adreno-receptor interactions of norepinephrine enhanced contraction in the femoral artery from spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 223: 207–214.

Axelrod, J. (1976). Catecholamines and hypertension. Clin. Sci. Mol. Med. 51: 415s–421s.

Bandick, N.R. and Sparks, H.V. (1970). Contractile response of vascular smooth muscle of renal hypertensive rats. Am. J. Physiol. 219(2): 340–344.

Barany, M. (1967). Myosin ATPase and muscle shortening. J. Gen. Physiol. 50(6): 197–218.

Baudouin-Legros, M. and Meyer, P. (1973). Effects of angiotensin, catecholamine and cyclic AMP on calcium storage in aortic microsomes. Br. J. Pharmacol. 47: 377-385.

Baum, T. and Shropshire, A.T. (1973). Reduction of sympathetic outflow by the central administration of L-Dopa, dopamine and norepinephrine. Neuropharmacology 12:49-56.

Beilin, L.J., Bulpitt, C.J., Coles, E.C., Dollery, C.T., Gear, J.S.S., Harper, G., Johnson, B.F., Munrow-Faure, A.D. and Turner, S.C. (1980). Long term antihypertensive drug treatment and blood pressure control in three hypertension clinics. Br. Heart J. 43: 74-79.

Berecek, K., Schwertschlag, U. and Gross, F. (1980). Alterations in renal vascular resistance and reactivity in spontaneous hypertensive rats. Am. J. Physiol. 238: H287-H293.

Bergel, D.H. (1961). Static elastic properties of the arterial wall. J. Physiol. 156: 445-457.

Berry, C.L., Greenwald, S.E. and Rivett, J.F. (1975). Static mechanical properties of the developing and mature rat aorta. Cardiovasc. Res. 9: 669-678.

Berti, F., Bernareggi, V. and Mandelli, V. (1971). Contraction and relaxation of in vitro perfused rat caudal artery: a possible role for cyclic 3', 5'-AMP. Arch. Int. Pharmacodyn. 192: 247-254.

Bevan, J.A., Bevan, R.D. and Duckles, S. (1980). Adrenergic regulation of vascular smooth muscle. In:Handbook of Physiology, Cardiovascular System, Vol.II, American Physiological Society, Bethesda.

Bevan, R.D. (1975). Effect of sympathetic denervation on smooth muscle cell proliferation in the growing rabbit ear artery. Circ. Res. 37: 14-19

Bevan, J.A. (1977). Some functional consequences of variations in adrenergic synaptic cleft width and in nerve density and distribution. Fed. Proc. 36: 2439-2444.

Bhalla, R.C., Webb. R.C. and Brock, T. (1976). cAMP dependent protein kinase activity in blood vessels of spontaneously hypertensive rats. Fed. Proc. 35: 398. (Abstract 1064).

Bhalla, R.C., Sharma, R.V. and Ramanathan, S. (1980). Ontogenetic development of isoproterenol subsensitivity of myocardial adenylate cyclase and B-adrenergic receptors in spontaneously hypertensive rats. Biochim. Biophys. Acta 632: 497-506.

Bhalla, R.C. and Sharma, R.V. (1982). Characteristics of hormone stimulated adenylate cyclase in vascular smooth muscle. Altered activity in spontaneously hypertensive rats. Blood

Vessels 19: 109-116.

Bhargavo, K.P., Raina, N., Misha, N., Shanker, K. and Vrat, S. (1979). Uptake of serotonin by human platelets and its relevance to CNS involvement in hypertension. Life Sci. 25: 195-200.

Birkenhauger, W.H. and De Leeuw, P.W. (1979). Pathophysiological mechanisms in essential hypertension. Pharmacol. Ther. 8:297-319.

Bisset, G.W. and Jones, N. F. (1975). Antidiuretic hormone. In: Recent Advances in Renal Disease, N.F. Jones, Ed., Churchill Livingstone, New York, p 350.

Blaustein, M.P. (1977). Sodium ions, calcium ions, blood pressure regulation: a reassessment and a hypothesis. Am. J. Physiol. 232(3): C165-C173.

Blumenthal, S.J., McConnaughey, and Iams, S.G. (1982). Myocardial adrenergic receptors and adenylate cyclase in the developing spontaneously hypertensive rat. Clin. Exper. Hypertens. - Theory and Practice, A4(6): 883-901.

Bohlen, H.G., Gore, R.W. and Hutchins, P.M. (1977). Comparison of microvascular pressures in normal and spontaneously hypertensive rats. Microvasc. Res. 13: 125-130.

Bohlen, H.G. and Lobach, D. (1978). In vivo study of the microvascular wall characteristics and resting control in young and mature spontaneously hypertensive rats. Blood Vessels 15: 322-330.

Bohlen, H.G. (1979). Arteriolar closure mediated by hyperresponsiveness to norepinephrine in hypertensive rats. Am. J. Physiol. 236: H157-H164.

Boyd, G.W. (1980). The pathophysiology of chronic arterial hypertension: a hypothesis. Clin. Exp. Pharmacol. Physiol. 7: 541-544.

Browning, R.A., Bundman, M., Smith, M. and Myers, J. (1977). Effects of p-chlorophenylalanine (PCPA) and 5,7-dihydroxytryptamine (5,7-DHT) on blood pressure in normotensive and spontaneously hypertensive (SH) rats. Fed. Proc. 36: 1042 (Abstract 4059).

Buckingham, R., Hamilton, T. and Robson, D. (1976). The effects of intracerebroventricular 5,6, dihydroxytryptamine on blood pressure of spontaneously hypertensive rats. Eur. J. Pharmacol. 36: 431-437.

Bulbring, E. (1979). Postjunctional adrenergic mechanisms. Br. Med. Bull. 35: 285-293.

Bunag, R.D., Eferakeya, A.E. and Langdon, D.S. (1975). Enhancement Of hypothalamic pressor responses in spontaneously hypertensive rats. Am. J. Physiol. 228(1): 217-222.

Bunag, R.D. and Eferakeya, A.E. (1976). Immediate hypotensive after-effects of posterior hypothalamic lesions in awake rats with spontaneous, renal or DOCA hypertension. Cardiovasc. Res. 10: 663-671.

Bunag, R.D., Riley, E. and Montello, M. (1976). Sustained pressor responsiveness to prolonged hypothalamic stimulation in awake rats. Am. J. Physiol. 228(1): 217-222.

Bunag, R.D. and Takeda, K. (1979). Sympathetic hyper-responsiveness to hypothalamic stimulation in young hypertensive rats. Am. J. Physiol. 237: R39-R44.

Burstyn, P. (1976). Water and sodium accumulation in rabbits after the administration of prolactin. J. Endocrinol. 68: 15P.

Burton, A.C. (1963). Physical principles of circulatory phenomena: the physical equilibria of heart and blood vessels. In: Handbook of Physiology, Vol. 1, Section 2, Circulation,

Byrom, F.B. (1969). The Hypertensive Vascular Crisis, William

Heineman Medical Books, London.

W.F. Hamilton and P.Dow, Eds., American Physiological Society, p. 85.

Cavellero, C., Di Tondo, U., Mingazzi, P.L., Spagnoli, L.G. and Cavallero, M. (1972). Cell proliferation in the arterial walls of epinephrine-treated rabbits. Experientia 28: 265-266.

Cavallero, C., Di Tondo, U., Mingazzini, P.L., Pesando, P.C. and Spagnoli, L.G. (1973). Cell proliferation in the atherosclerotic lesions of cholesterol fed rabbits. Atherosclerosis 17: 49-62.

Cassidy, P.S., Hoar, P.E. and Kerrick, W. (1979). Irreversible thiophosphorylation and activation of tension in functionally skinned ileum strips by (^{35}S)-ATP S. J. Biol. Chem. 254: 11148-11153.

Chalmers, J.P. (1975). Brain amines and models of experimental hypertension. Circ. Res. 36: 469-480.

Chalmers, J.P. (1978). The nervous system and hypertension. Clin. Sci. Mol. Med. 55: 45s-56s.

Chaturvedi, A.K., Landon, E.J. and Sastry, R. (1978). Influence of

6 (N,N, diethylamino) hexyl 3, 4, 5-trimethoxy benzoate on the responsiveness of aorta to norepinephrine and calcium movements in microsomes from spontaneously hypertensive and normotensive rats. Pharmacology 17:315-322.

Chen, I.I.H., Prewitt, R.L. and Dowell, R.F. (1981). Microvascular rarefaction in spontaneously hypertensive rat cremaster muscle. Am. J. Physiol. 241: H300-H310.

Cheng, J.B. and Shibata, S. (1980). Pressor response to 5 hydroxytryptamine, norepinephrine and KCl in perfused hindlimb preparation from spontaneous hypertensive rat. J. Pharmacol. Exp. Ther. 214: 488-495.

Coquil, J.F. and Hamet, P. (1980). Activity of cyclic AMP dependent protein kinase in heart and aorta of spontaneously hypertensive rat. Proc. Soc. Exp. Biol. Med. 164: 569-575.

Collis, M.G. and Vanhoutte, P.M. (1977). Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats. Circ. Res. 43: 293-300.

Collis, M.G., DeMey, C. and Vanhoutte, P.M. (1980). Renal vascular reactivity in young spontaneously hypertensive rat. Hypertension 1: 45-52.

Collins, G.A. and Sutter, M.C. (1975). Quantitative aspects of cyclic AMP and relaxation in the rabbit anterior mesenteric portal vein. Can. J. Physiol. Pharmacol. 53: 989-997.

Coleman, T.G., Cowley, A.W. and Guyton, A.C. (1974). Experimental hypertension and long term control of arterial pressure. In: Cardiovascular Physiology, MTP International Review of Science Physiology Series One, Vol. 1, A.C. Guyton and C.E. Jones, Eds., University Park Press, Baltimore. 259pp.

Coupar, I.M. and Mclennan, P.L. (1978). The influence of prostaglandins on noradrenaline-induced vasoconstriction in isolated perfused mesenteric blood vessels of the rat. Br. J. Pharmacol. 62: 51-59.

Cowley, A.W., Monas, E. and Guyton, A.C. (1974). Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circ. Res. 34: 505-514.

Cowley, A.W. (1980). The concept of autoregulation of total blood flow and its role in hypertension. In: Topics in Hypertension, J.H. Laragh, Ed., York Medical Books, USA.

Cox, R.H. (1979). Comparison of arterial wall mechanics in normo-

tensive and spontaneously hypertensive rats. Am. J. Physiol. 236: H159-H167.

Cox, R.H. (1981). Basis for the altered arterial wall mechanisms in spontaneously hypertensive rat. Hypertension 3: 485-495.

Crofton, J.T., Share, L., Shade, R.E., Allen, C. and Tarnowski, D. (1978). Vasopressin in the rat with spontaneous hypertension. Am. J. Physiol. 235: H361-H366.

Dadkar, N.K., Aroskar, V.A. and Dohadwalla, A.N. (1980). Peripheral vascular smooth muscle relaxation in normotensive and hypertensive rats. J. Pharm. Pharmacol. 32: 74-76.

Dahl, L.K., Knudsen, K.D. and Iwai, J. (1969). Humoral transmission of hypertension; evidence from parabiosis. Circ. Res. 24 (suppl. 1): I21-I32.

Daniel, E.E., Crankshaw, D. and Kwan, C.Y. (1979). Intracellular sources of calcium for activation of smooth muscle. In: Trends in Autonomic Pharmacology, Vol. 1, S. Kalsner, Ed., Urban and Schwarzenburg, Baltimore.

Daniel, E.E. (1981). Role of altered vascular smooth muscle function in hypertension. In: Vasodilation, P.M. Vanhoutte and I. Leusen, Eds., Raven Press, New York.

Dietz, R., Schomig, A., Haebarg, H., Mann, J.F.E., Rascher, W., Luth, J.B., Grunherz, N. and Gross, F. (1978). Studies on the pathogenesis of spontaneous hypertension of rats. Hypertension 43 (suppl. 1): 98-106.

De Quattro, V., Campese, V., Luivey, A., Yen, G. and Kypridakis, G. (1976). Low response of serum dopamine B hydroxylase to stimuli in primary hypertension. Biochem. Med. 15: 1-9.

DeMey, C., Zannekeyn, L. and Vanhoutte, P.M. (1978). Comparison of tachyphylaxis to 5 hydroxy-tryptamine and angiotensin II in the isolated perfused rat kidney of spontaneously hypertensive rat. Arch. Int. Pharmacodyn. 236(2): 298-299.

Deth, R. and van Breeman, C. (1977). Agonist induced release of intracellular Ca^{+2} in the rabbit aorta. J. Membr. Biol. 30: 363-380.

DiBona, G.F. and Rios, L.L. (1978). Mechanisms of exaggerated diuresis in spontaneously hypertensive rats. Am. J. Physiol. 235: F409-F416.

Doba, N. and Reis, D.J. (1974). Role of central and peripheral adrenergic mechanisms in neurogenic hypertension produced by brainstem lesions in the rat. Circ. Res. 34: 293-301.

Dobrowska, R., Sherry, J., Aromatorio, D. and Hartshorne, D. (1978). Modulator protein as a component of the myosin light chain kinase from chicken gizzard. Biochem. 17: 253-258.

Donnelly, T.E. (1978). Lack of altered cyclic nucleotide phosphodiesterase activity in the aorta and heart of the spontaneously hypertensive rat. Biochim. Biophys. Acta 542: 245-252.

Drew, G.M. and Whiting, S.B. (1979). Evidence for two distinct types of post-synaptic alpha adrenoreceptor in vascular smooth muscle in vivo. Br. J. Pharmacol. 67: 207-215.

Droogmans, G., Raeymaekers, L. and Casteels, R. (1977). Electro- and pharmacomechanical coupling in smooth muscle cells of the rabbit ear artery. J. Gen. Physiol. 70: 129-148.

Dutta, S.N., Guha, D. and Pradhan, S.N. (1975). Cardiovascular effects of central microinjections of apomorphine in cats. Arch. Int. Pharmacodyn. 215: 259-265.

Ebashi, S., Mikawa, T., Hirata, M., Toyo-oka, T. and Nonomura, Y. (1977). Regulatory proteins of smooth muscle. In: Excitation-Contraction Coupling in Smooth Muscle, R. Casteel, T. Godfraind and J.C. Ruegg, Eds., Elsevier/North Holland Biomedical Press.

Ebihara, A. (1972). Effect of spontaneous and renal hypertension on a parabiotic partner in rats. In: Spontaneous Hypertension K. Okamoto, Ed., Springer-Verlag, New York.

Ehrhart, L.A. and Ferrario, C.M. (1981). Collagen metabolism and reversal of aortic medial hypertrophy in spontaneous hypertensive rats treated with methyldopa. Hypertension 3: 479-484.

Ekas, R.D. and Lokhandwala, M.F. (1981). Sympathetic nerve function and vascular reactivity in spontaneously hypertensive rats. Am. J. Physiol. 241: R379-R384.

Ekas, R.D., Steenberg, M.L. and Lokhandwala, M.F. (1983a). Increased norepinephrine release during sympathetic nerve stimulation and its inhibition by adenosine in the isolated perfused kidney of spontaneously hypertensive rats. Clin. Exper. Hypertens. - Theory and Practice A5(1):41-48.

Ekas, R.D., Steenberg, M.L., Woods, M.S. and Lokhandwala, M.F. (1983b). Presynaptic alpha and beta adrenoreceptor stimulation and norepinephrine release in the spontaneously hypertensive rat. Hypertension 5(2): 198-204.

Ferrone, R.A. and Antonaccio, M.J. (1979). Prevention of the

development of spontaneous hypertension in rats by captopril (SQ 14,225). Eur. J. Pharmacol. 60: 131-137.

Ferrone, R.A., Walsh, G.M., Tsuchiya, M. and Frohlich, E.D. (1979). Comparison of hemodynamics in conscious spontaneous and renal hypertensive rats. Am. J. Physiol. 236: H403-H408.

Ferrone, R.A., Heron, G.L. and Antonaccio, M.J. (1980). Comparison of the acute and chronic hemodynamic effects of captopril and guanethidine in spontaneously hypertensive rats. Clin. Exp. Hypertens. 2 (2): 242-247.

Field, F.P., Janis, R.A. and Triggle, D.J. (1972). Aortic reactivity with genetic and experimental renal hypertension. Can. J. Physiol. Pharmacol. 50: 1072-1079.

Filczewski, M. and Bogucka, E. (1979). Reactivity of normotensive and spontaneously hypertensive rats (SHR) to some antihypertensive agents after acute and chronic treatment. Pol. J. Pharmacol. Pharm. 31: 127-137.

Finch, L., Haeusler, G., Kuhn, H. and Thoenen, H. (1972). The recovery of vascular adrenergic function in the rat after chemical sympathectomy with 6-hydroxydopamine. Br. J. Pharmac. 44: 357P-358P.

Fink, G.D. and Brody, M.J. (1979). Renal vascular resistance and reactivity in spontaneously hypertensive rat. Am. J. Physiol. 237(2): F128-F132.

Fiodart, J.M., Rorive, G.L., Nusgens, B.V. and Lapiere, G.M. (1978). The relationship between blood pressure and aortic collagen metabolism in renal hypertensive rats. Clin. Sci. Mol. Med. 55: 275-295.

Fitzpatrick, D.F. and Szentwanyi, A. (1977). Stimulation of calcium uptake into aortic microsomes by cAMP and cyclic AMP dependent protein kinase. Nauyn-SchmiedebergsArch. Pharmacol. 298: 255-257.

Folkow, B., Hallback, M., Lundgren, Y. and Weiss, L. (1969). Structural based increase in flow resistance in spontaneously hypertensive rats. Acta. physiol. scand. 330 (suppl.): 94.

Folkow, B., Hallback, M., Lundgren, Y. and Weiss, L. (1970a). Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats. Acta. physiol. scand. 80: 93-106.

Folkow, B., Hallback, M., Lundgren, Y. and Weiss, L. (1970b). Structurally based increase of flow resistance in spontaneously

hypertensive rats. Acta. physiol. scand. 79: 373-378.

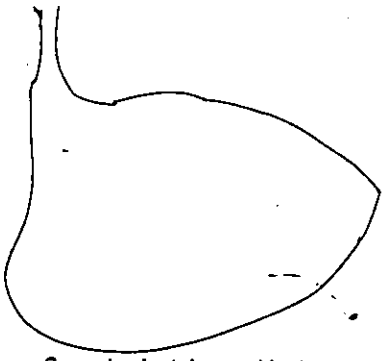
Folkow, B., Hallback, M., Lundgren, Y. and Weiss, L. (1971a). Renal vascular resistance in spontaneously hypertensive rats. Acta physiol. scand. 83: 96-105.

Folkow, B., Gurevich, M., Hallback, M., Lundgren, Y. and Weiss, L. (1971b). The hemodynamic consequences of regional hypotension in spontaneously hypertensive and normotensive rats. Acta physiol. scand. 83: 532-541.

Folkow, B., Hallback, M., Lundgren, Y. and Weiss, L. (1972). The effects of "Immunosympathectomy" on blood pressure and vascular "reactivity" in normal and spontaneously hypertensive rats. Acta physiol. scand. 84: 512-524.

Folkow, B., Hallback, M., Lundgren, Y., Sivertsson, R. and Weiss, L. (1973). The importance of adaptive changes in vascular design for the establishment of primary hypertension studied in man and spontaneously hypertensive rats. Circ. Res. (suppl. 1) 32: I12-I16.

Folkow, B., Gothberg, G., Lundin, S. and Ricksten, S.E. (1977). Structural resetting of the renal vascular bed in spontaneously hypertensive rats (SHR). Acta physiol. scand. 100: 217.



Folkow, B. (1979). Constriction-distension relationships of resistance vessels in normo- and hypertension. Clin. Sci. 57: 235-255.

Folkow, B. (1982). Physiological aspects of primary hypertension. Physiol. Rev. 62: 348-504.

Fonteles, M. and Jeske, A. (1980). Vasoactivity and vascular escape in isolated perfused kidneys from normotensive versus spontaneously hypertensive rats. Gen. Pharmacol. 11:293-296.

Forman, B.H. and Mulrow, P.J. (1974). Effect of propranolol on blood pressure and plasma renin activity in the spontaneously hypertensive rat. Circ. Res. 35: 215-221.

Fourman, J. and Moffat, D.B. (1971). The vascular architecture of the rat kidney. In: The Blood Vessels of the Kidney, J. Fourman and D.B. Moffat, Eds., Blackwell Scientific, Oxford, p. 26.

Frearson, N., Focant, B. and Perry, S.V. (1976). Phosphorylation of a light chain component of myosin from smooth muscle. FEBS Letters 63: 27-32.

Freeman, D.J. and Daniel, E.E. (1973). Ca^{+2} movement in vascular smooth muscle and its detection using lanthanum as a tool.

Can. J. Physiol. Pharmacol. 51: 900-913.

Friedman, S.M. and Friedman, C.L. (1976). Cell permeability, sodium transport and the hypertensive process in the rat. Circ. Res. 37: 433-446.

Fries, E.D., Notargiacomo, A. and Burris, J.F. (1981). Comparative antihypertensive study of diuretic, reserpine and hydralazine in the spontaneously hypertensive rat. Proc. Soc. Exp. Biol. Med. 166: 364-368.

Furchgott, R.F. (1967). The pharmacological differentiation of adrenergic receptors. Ann. N.Y. Acad. Sci. 139: 553-570.

Furchgott, R.F. (1972). The classification of adrenoreceptors. An evaluation from the standpoint of receptor theory. In: Catecholamines, H. Blaschko and E. Muscholl, Eds., Springer-Verlag, New York.

Gadfraind, T. and Dieu, D. (1978). Influence of ageing on isoprenaline relaxation of aortae from normal and hypertensive rats. Arch. Int. Pharmacodyn. 236: 300-302.

Gifford, R.W. (1976). Peripheral adrenergic blockers: guanethidine, reserpine and bethanidine. In: The Spectrum of Antihypertensive Drug Therapy, G. Onesti and D.T. Lowenthal, Eds.,

Biomedical Information Corporation, New York.

Goldberg, L.I., Rick, J.H. and Oparil, S. (1977). Management and treatment of hypertension. In: Hypertension, J. Genest, E. Koiw and O. Kuchel, Eds., McGraw-Hill, New York. p. 990.

Goldberg, M.T. and Triggle, C.R. (1978). Elevated vascular reactivity in timolol treated spontaneously hypertensive rats. Can. J. Pharmacol. 56: 1072-1075.

Goldberg, N.D., Haddox, M.K., Nicol, S.E., Glass, D.B., Sanford, C.H., Kuehl, F.A. and Estensen, R. (1975). Biologic regulation through opposing influences of cGMP and cAMP: The Yin Yang hypothesis. Adv. Cyclic Nucleotide Res. 5:307-338.

Golenhofen, K. (1975). Structural characteristics, mechanisms of contraction, innervation and proliferation of smooth muscle cells. Adv. Exp. Med. Biol. 57: 46-52.

Gray, S.D. (1982). Anatomical and physiological aspects of cardiovascular funtion in Wistar-Kyoto and spontaneously hypertensive rats at birth. Clin. Sci. 63: 383s-385s.

Greenberg, S., Palmer, E.C. and Wilborn, W.M. (1978). Pressure-independent hypertrophy of veins and pulmonary arteries of spontaneously hypertensive rats. Characterization of function,

structural and biochemical changes. Clin. Sci. Mol. Med. 55: 31s-36s.

Greenberg, S. (1980). Venous function in hypertension. In: Trends in Pharmacological Sciences, Elsevier/North Holland Biomedical Press, New York.

Greenberg, S. (1981). Effect of chronic administration of clonidine propranolol and alpha methyldopa on extensibility and biochemical properties of the veins in renal and spontaneous hypertension. J. Pharmacol. Exp. Ther. 218: 779-790.

Greenwald, S.E. and Berry, C.L. (1978). Static mechanical properties and chemical composition of the aorta of spontaneously hypertensive rats. A comparison with the effects of induced hypertension. Cardiovasc. Res. 12: 364-372.

Grobecker, H., Roizen, M., Weise, V., Saavedra, J.M. and Kopin, I.J. (1975). Sympathoadrenalmedullary activity in young, spontaneously hypertensive rats. Nature 258: 267.

Gupta, R.C., Bhalla, R.C. and Sharma, R.V. (1982). Altered distribution and properties of cAMP dependent protein kinase isozymes in spontaneously hypertensive rat aorta. Biochem. Pharmacol. 31: 1837-1841.

Gulati, O.D., Gokdale, S.D., Parikh, H.M., Vdwadia, B.P. and Krishnamurty, V.S.R. (1969). Evidence for a sympathetic alpha receptor blocking action of beta receptor blocking agents. J. Pharmacol. Exp. Ther. 16: 35-43.

Guyton, A.C., Hall, J.E., Lhmeier, T.E., Jackson, T.E. and Manning, R.D. Jr. (1981). The Ninth J.A.F. Stevenson Memorial Lecture: The many role of the kidney in arterial pressure control and hypertension. Can. J. Physiol. Pharmacol. 59: 513-519.

Haack, D.W., Schaffer, J.J. and Simpson, J.G. (1980). Comparisons of cutaneous microvessels from spontaneously hypertensive, normotensive Wistar-Kyoto and normal Wistar rats (4085). Proc. Soc. Exp. Biol. Med. 164: 453-458.

Haeusler, G. and Haefely, W. (1970). Pre- and postjunctional supersensitivity of the mesenteric artery preparation from normotensive and hypertensive rats. Naunyn-Schmiedebergs Arch. Pharmacol. 266:18-33.

Haeusler, G. and Finch, L. (1972a). Vascular resistance and reactivity to various vasoconstrictor agents in hypertensive rats. In: Spontaneous Hypertension, Its Pathogenesis and Complications, K. Okamoto, Ed., Springer-Verlag, New York.

Haeusler, G. and Finch, L. (1972b). Vascular reactivity to 5 hydroxytryptamine and hypertension in the rat. Naunyn-Schmiedebergs Arch. Pharmacol. 272:101-116.

Hallback, M., Lundgren, Y. and Weiss, L. (1971). Reactivity to norepinephrine of aortic strips and portal veins from spontaneously hypertensive and normotensive rats. Acta physiol. scand. 81: 176-181.

Hallback, M. and Folkow, B. (1974). Cardiovascular response to acute mental stress in spontaneously hypertensive rats. Acta physiol. scand. 90: 684-698.

Hamilton, T.C. (1975). Influence of antihypertensive drug treatment on vascular reactivity in spontaneously hypertensive rats. Br. J. Pharmacol. 54: 429-436.

Hansen, T.R., Abrams, G.D. and Bohr, D.F. (1974). Role of pressure in structural and functional changes in arteries of hypertensive rats. Circ. Res. 34/35 (suppl. 1): I101-I107.

Harder, D.R., Contey, S.J., Willems, W.J. and Stekiel, W.J. (1981). Norepinephrine effect on in situ venous membrane potential in spontaneously hypertensive rats. Am. J. Physiol. 240: H837-H842.

Hart, M.N., Heistad, D.D. and Brody, M.J. (1980). Effect of chronic hypertension and sympathetic denervation on the wall to lumen ratio of cerebral vessels. Hypertension 2: 419-423.

Hartshorne, D.J. (1980). Biochemical basis for contraction of vascular smooth muscle. Chest 78: 140-149.

Hartshorne, D.J. and Gorecka, A. (1980). The biochemistry of the contractile proteins of smooth muscle. In: Handbook of Physiology. Section 2. The Cardiovascular System, Vol.II, Vascular Smooth Muscle, A.P. Somlyo and H.V. Sparks, Eds., American Physiological Society, Bethesda.

Haudenschild, C.C., Prescott, M.F. and Chobanian, A.V. (1980). Effects of hypertension and its reversal on aortic intima lesions of the rat. Hypertension 2: 33-44.

Hayashi, T. and Nakamura, K. (1981). Cerebral neuronal activity in spontaneously hypertensive rats as demonstrated by the ^{14}C-deoxyglucose method. Naunyn-Schmiedebergs Arch. Pharmacol. 316: 331-339.

Heistad, D.D. and Marcus, M.L. (1978). Evidence that neural mechanisms do not have important effects on the cerebral blood flow. Circ. Res. 42: 295-296.

Henrich, H., Hertel, R. and Assman, R. (1978). Structural differences in the mesentery microcirculation between normotensive and spontaneously hypertensive rats. Pflugers Arch. 375: 153-159.

Hermsmeyer, K. (1976a). Electrogenesis of increased norepinephrine sensitivity of arterial vascular muscle in hypertension. Circ. Res. 38: 362-374.

Hermsmeyer, K. (1976b). Cellular basis for increased sensitivity of vascular smooth muscle in spontaneously hypertensive rats. Circ. Res. 38 (suppl. 2): II53-II57.

Hermsmeyer, K., Ingram, D., Ingram, M.J., Abel, P.W. and Trapani, A. (1980). K^+ content of caudal artery in SHR/KNR indicates a lower activity coefficient in SHR. Fed. Proc. 39: 813 (Abstract 2877).

Hermsmeyer, K. (1981). Membrane potential mechanisms in experimental hypertension. In: New Trends in Arterial Hypertension, INSERM, Symposium No. 17, M. Worcel et al., Eds., Elsevier/ North Holland, Biochemical Press.

Hertel, R., Henrich, H. and Assmann, R. (1978). Intravital measurements of arteriolar pressure and tangential wall stress in normotensive and spontaneously hypertensive rats (established

hypertension). Experientia 34: 865-867.

Hirata, M., Mikawa, T,. Nonomura, Y. and Ebashi, S. (1977). Ca^{+2} regulation in vascular smooth muscle. J. Biochem. 82: 1793-96.

Hirata, M., Mikawa, T., Nonomura, Y. and Ebashi, S. (1980). Ca^{+2} regulation in vascular smooth muscle. ii Ca^{+2} binding of aorta Leiotonin. J. Biochem. 87: 367-368.

Holloway, E.T. and Bohr, D.F. (1973). Reactivity of vascualr smooth muscle in hypertensive rats. Circ. Res. 33: 678-685.

Horrobin, D.F., Burstyn, P.G., Lloyd, I.J., Durkin, N., Lipton, A. and Muiruri, K.L. (1971). Actions of prolactin in human renal function. Lancet 2: 352-354.

Horrobin, D.F., Manku, M.S. and Burstyn, P.G. (1973). Effects of intravenous prolactin infusion on arterial blood pressure in rabbits. Cardiovasc. Res. 7: 585-587.

Horrobin, D.F., Mtabaji, J.P. and Manku. M.S. (1976). Cortisol in physiological concentrations acts within minutes to modify effects of prolactin and growth hormone on prostaglandin secretion. Med. Hypothesis 2: 219-226.

Hsu, C.H., Slavicek, J.H. and Kurtz, T.W. (1982). Segmental renal

vascular resistance in the spontaneously hypertensive rat. Am. J. Physiol. 242: H961-H966.

Hume, W.R. (1980). Proline and thymidine uptake in rabbit ear artery segments in vitro increased in chronic tangential load. Hypertension 2: 738-743.

Hutchins, P.M. and Darnell, A.E. (1974). Observations of a decreased number of arterioles in spontaneously hypertensive rats. Circ. Res. 34/35 (suppl. 1): I161-I165.

Ichijima, K. (1969). Morphological studies on the peripheral small arteries of spontaneously hypertensive rats. Jap. Circ. J. 33: 785-813.

Ikeda, H., Shino, A. and Nagaoka, A. (1979). Effects of chemical sympathectomy on hypertension and stroke in stroke prone spontaneously hypertensive rats. Eur. J. Pharmacol. 53: 173-179.

Irinoda, K., Matsuyama, S. and Takahashi, S. (1972). A comparison of the fundus finding between essential hypertension in human beings and spontaneously hypertensive rats. In: Spontaneous Hypertension, K. Okamoto, Ed., Springer-Verlag, New York.

Iriuchijima, J. (1983). Regional blood flow in conscious spontan-

eously hypertensive rats. Jap. J. Physiol. 33: 41-50.

Iwatsuki, K., Cardinale, G.J., Spector, S. and Udenfreind, S. (1977). Hypertension: Increases of collagen biosynthesis in arteries but not in veins. Science 198: 403-404.

Jenkins, D.M. and Perry, L.A. (1978). Plasma prolactin in pregnancy induced hypertension. Br. J. Obstet. Gynaecol. 85: 754-757.

Johnson, E.M. and Macia, R.A. (1979). Unique resistance to guanethidine induced chemical sympathectomy of spontaneously hypertensive rats. A resistance overcome by treatment with antibody to Nerve Growth Factor. Circ. Res. 45: 243-249.

Jones, A.W. (1973). Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats. Influences of aldosterone, norepinephrine and angiotensin. Circ. Res. 33: 563-572.

Jones, A.W. (1974a). Altered ion transport in large and small arteries from spontaneously hypertensive rats and influence of Ca^{+2}. Circ. Res. 34 (suppl. 1): I 117-I 222.

Jones, A.W. (1974b). Reactivity of ion fluxes in rat aorta during hypertension and circulatory control. Fed. Proc. 33: 133-137.

Jones, L.M. and Michell, R.H. (1978). Stimulus-response coupling

at alpha adrenergic receptors. Biochem. Soc. Trans. 6: 673-688.

Judy, W.V., Watanabe, A.M., Henry, D.P., Besch, H.R., Murphy, W.R. and Hockel, G.M. (1976). Sympathetic nerve activity: Role in regulation of blood pressure in the spontaneously hypertensive rat. Circ. Res. 38 (suppl. 2): II 22- II 29.

Judy, W.V., Watanabe, A.M., Henry, D.P., Besch, H.R. and Aprison, B. (1978). Effect of L-dopa on sympathetic nerve activity and pressure in the spontaneously hypertensive rat. Circ. Res. 43: 24-28.

Justevich, J.C., Robinson, D.S. and Whitehorn, D. (1978). Effect of hypothalamic stimulation in spontaneously hypertensive and Wistar Kyoto rats. Eur. J. Pharmacol. 51: 429-439.

Kamikawa, Y., Cline, W.H. and Su, C. (1980). Diminished purinergic modulation of vascular adrenergic neurotransmission in spontaneously hypertensive rats. Eur. J. Pharmacol. 66: 347-353.

Kannan, M.S. and Daniel, E.E. (1978). Formation of gap junctions by treatment in vitro with potassium conductance blockers. J. Cell Biol. 48: 338-348.

Karr-Dullien, V., Bloomquist, E.I., Beringer, T. and El-Bermani, A.I.

(1981). Arterial morphometry in neonatal and infant spontaneously hypertensive rats. Blood Vessels 18: 253-262.

Kattenberg, D.M. (1981). Cyclic AMP and arterial smooth muscle calcium regulation. Ph.D. Thesis, McMaster University, Hamilton, Ontario, Canada.

Kawabe, K., Watanabe, T.X., Shiono, K. and Sokabe, H. (1978). Influence on blood pressure or renal isografts between spontaneously hypertensive and normotensive rats utilizing the F_1 hybrids. Jap. Heart J. 19: 886.

Kawasaki, H., Cline, W.H. and Su, C. (1982a). Enhanced presynaptic beta adrenoreceptor mediated modulation of vascular adrenergic neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 223: 721-728.

Kawasaki, H., Cline, W.H. and Su, C. (1982b). Enhanced angiotensin-mediated facilitation of adrenergic neurotransmission in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 221: 112-116.

Keatinge, W.R. (1964). Mechanism of adrenergic stimulation of mammalian arteries and its failure at low temperatures. J. Physiol. 174: 184-205.

Keatinge, W.R. (1980). Mechanisms of response to vasoconstrictor hormones. In: Local Mechanisms Controlling Blood Vessels, W.R. Keatinge and M.C. Hareman, Eds., Academic Press, London.

Kimani, J.K. (1981). Structural evidence for insertion of collagen fibres to smooth muscle cells in the carotid arterial system of the giraffe (Giraffa camelopardalis). Cell Tissue Res. 214: 219-224.

Klenerova, V., Albrecht, I. and Hynie, S. (1975). The activity of adenylate cyclase and phosphodiesterase in hearts and aortas of spontaneously hypertensive rats. Pharmacol. Res. Commun. 7: 453-462.

Kline, R.L., Stuart, P.J. and Merccer, P.F. (1980). Effect of renal denervation on arterial pressure and renal norepinephrine concentration in Wistar Kyoto and spontaneously hypertensive rats. Can. J. Physiol. 58: 1384-1388.

Kramer, G.L. and Hardman, J. (1980). Cyclic nucleotides and blood vessel contraction. In: Handbook of Physiology, section 2, The Cardiovascular System, D.F. Bohr, A.P. Somlyo and H.V. Sparks, Eds., American Physiological Society, Bethesda, Md. USA.

Kroegher, E.A. and Stephens, N.L. (1975). Effects of tetraethylammonium on tonic airway smooth muscle initiation of phasic electrical activity. Am. J. Physiol. 228: 633-636.

Kubo, T. and Hashimoto, M. (1978). Effects of intraventricular and intraspinal 6-hydroxydopamine on blood pressure of spontaneously hypertensive rats. Arch. Int. Pharmacodyn. 232: 166-176.

Kuriyama, H. and Suzuki, H. (1978). Electrical property and chemical sensitivity of vascular smooth muscles in normotensive and spontaneously hypertensive rats. J. Physiol. 285: 409-424.

Kwan, C.Y., Garfield, R. and Daniel, E.E. (1979). An improved procedure for the isolation of plasma membranes from rat mesenteric arteries. J. Mol. Cell. Cardiol. 11: 639-659.

Kwan, C.Y., Belbeck, L. and Daniel, E.E. (1980a). Abnormal biochemistry of the vascular smooth muscle plasma membrane as an important factor in the initiation and maintenance of hypertension in rats. Blood Vessels 16: 259-268.

Kwan, C.Y., Belbeck, L. and Daniel, E.E. (1980b). Characteristics of the arterial plasma membrane in renovascular hypertension in rats. Blood Vessels 17: 131-140.

Kwan, C.Y., Belbeck, L. and Daniel, E.E. (1980c). Abnormal biochemistry of vascular smooth muscle plasma membrane isolated from hypertensive rats. Mol. Pharmacol. 17: 137-140.

Kwan, C.Y. and Daniel, E.E. (1981a). Calcium transport by plasma membrane vesicles isolated from vascular smooth muscle of normal and hypertensive rats. In: Vasodilation, P.M. Vanhoutte and I. Leusen, Eds., Raven Press, New York.

Kwan, C.Y. and Daniel, E.E. (1981b). Biochemical abnormalities of venous plasma membrane fraction isolated from spontaneously hypertensive rats. Eur. J. Pharmacol. 75: 321-324.

Kwan, C.Y. and Daniel, E.E. (1982). Arterial muscle membrane abnormalities of hydralazine treated spontaneously hypertensive rats. Eur. J. Pharmacol. 82: 187-190.

Laird, J.F. (1977). Renal denervation delays blood pressure increase in spontaneously hypertensive rats. Experientia 33: 339-340.

Lais, L.T., Shaffer, R.A. and Brody, M.J. (1974). Neurogenic and humoral factors controlling vascular resistance in spontaneously hypertensive rats. Circ. Res. 35: 764-774.

Lais, L.T. and Brody, M.J. (1975). Mechanisms of vascular hyper-

responsiveness in the spontaneously hypertensive rat. Circ. Res. 36/37 (suppl. 1): I 216-I 222.

Lais, L.T. and Brody, M.J. (1978). Vasoconstrictor hyper-responsiveness: an early pathogenic mechanism in the spontaneously hypertensive rat. Eur. J. Pharmacol. 47: 177-189.

Lee, R.M.K.W., Garfield, R.E., Forrest, J.B. and Daniel, E.E. (1980). Dimensional changes of cultured smooth muscle cells due to the preparatory process for transmission electron microscopy. J. Microsc. 120:85-91.

Lee, R.M.K.W., McKenzie, R., Kobayashi, K., Garfield, R.E., Forrest, J.B. and Daniel, E.E. (1981). Effect of glutaraldehyde fixative osmolarities on smooth muscle cell volume, osmotic reactivity of the cells after fixation. J. Microsc. 125: 77-88.

Lee, R.M.K.W., Garfield, R.E., Forrest, J.B. and Daniel, E.E (1983a). Morphometric study of structural changes in the mesenteric blood vessels of spontaneously hypertensive rats. Blood Vessel 20: 57-71.

Lee, R.M.K.W., Forrest, J.B., Garfield, R.E. and Daniel, E.E. (1983b). Ultrastructural changes in mesenteric arteries from spontaneously hypertensive rats. Blood Vessels 20: 72-91.

Lee, R.M.K.W., Forrest, J.B., Garfield, R.E. and Daniel, E.E. (1983c).

Comparison of blood vessel wall dimensions in normotensive and hypertensive rats by histometric and morphometric methods. Blood Vessels 20: 245-254.

Lee, R.M.K.W., Smeda, J.S., McKenzie, R., Forrest, J.B., Garfield, R.E. and Daniel, E.E. (1983d): The effect of neonatal treatments with guanethidine or hydralazine on the blood pressure of spontaneously hypertensive rats. Fed. Proc. 42: 343 (Abstract 300).

Lefevre-Borg, F., Roach, A.G., Gomeni, R. and Cavero, I. (1979). Mechanism of antihypertensive activity of orally administered prazosin in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 1: 13-42.

Lefkowitz, R.J. and Hoffman, B.B. (1980). Adrenergic receptors. Adv. Cyclic Nucleot. Res. 12: 37-47.

Lehman, W., Kendrick-Jones, J. and Szenti-Gyorgi, A.G. (1972). Myosin-linked regulatory systems: Comparative studies. Cold Spring Harbor Symp. Quant. Biol. 37: 319-330.

Liedholm, H., Wahlin-Boll, E., Hanson, A., Ingmarsson, I. and Melander, A. (1982). Transplacental passage and breast milk concentrations of hydralazine. Eur. J. Clin. Pharmacol. 21: 417-419.

Limas, C. and Limas, C.J. (1978). Reduced number of beta adrenergic receptors in the myocardium of spontaneously hypertensive rats. Biochem. Biophys. Res. Commun. 83: 710-714.

Limas, C. and Limas, C.J. (1979). Decreased numbers of beta adrenergic receptors in hypertensive vessels. Biochim. Biophys. Acta 582: 533-536.

Limas, C., Westrum, B. and Limas, C.J. (1980). The evolution of vascular changes in the spontaneously hypertensive rat. Am. J. Pathol. 98: 357-384.

Limas, C., Westrum, B., Limas, C.J. (1983). Effect of antihypertensive therapy on the vascular changes of spontaneous hypertensive rats. Am. J. Pathol., 111: 380-393.

Lund, D.D., Twietmeyer, T.A., Schmid. P.G. and Tomanek, R.J. (1979). Independent changes in cardiac muscle fibers and connective tissue in rats with spontaneous hypertension, aortic constriction and hypoxia. Cardiovasc. Res. 13: 39-44.

Lundgren, Y. (1974). Adaptive changes of cardiovascular design in spontaneous and renal hypertension. Acta physiol. scand. 408 (suppl.): 1-62.

Lundgren, Y. and Weiss, L. (1979). Cardiovascular design after reversal of long standing renal hypertension in rats. Clin. Sci. 57: 19s-21s.

Mandal, A.K., Bell, R.D., Norquist, J.A. and Lindeman, R.D. (1977). Anatomical pathology and pathogenesis of the lesions of the small arteries and arterioles of the kidney in essential hypertension. Pathol. Ann. 12:331-371.

Manku, M.S., Nassar, B.A. and Horrobin, D.F. (1973). Effects of prolactin on the response of rat aortic and arteriolar smooth muscle preparations to noradrenalin and angiotensin. Lancet 2; 991-994.

McGiff, J.C. and Quilley, C.P. (1981). The rat with spontaneous genetic hypertension is not a suitable model of essential hypertension. Circ. Res. 48: 455-463.

McGrath, M.A. and Shepherd, F.T. (1978). Histamine and 5-hydroxy-tryptamine inhibition of transmitter release mediated by H_2 and 5-hydroxytryptamine receptors. Fed. Proc. 37: 195-198.

McGregor, D.D. and Smirk, F.H. (1968). Vascular response in mesenteric arteries from genetic and renal hypertensive rats. Am. J. Physiol. 214: 1429-1433.

McMurtry, J.P., Kazama, N. and Wexler, B.C. (1979). Effects of bromocryptine on hormone and blood pressure levels in the spontaneously hypertensive rat. Proc. Soc. Exp. Biol. Med. 161: 186-188.

Mekata, F. (1971). Electrophysiological studies of the smooth muscle cell membrane of the rabbit common carotid artery. J. Gen. Physiol. 57: 738-751.

Mekata, F. and Keatinge, W.R. (1975). Electrical behaviour of inner and outer smooth muscle of the sheep carotid artery. Nature 258: 534-535.

Mikawa, T. (1979). Freezing of the calcium regulated structures of gizzard thin filaments by glutaraldehyde. J. Biochem. 85: 879-881.

Mikawa, T, Nonomuro, Y., Hirata, M., Ebashi, S, and Kakiuchi, S. (1978). Involvement of an acidic protein in regulation of smooth muscle contraction by tropomyosin-leiotonin system. J. Biochem. 84: 1633-1636.

Mohring, J., Kintz, J. and Schoun, J. (1978). Role of vasopressin in blood pressure control of spontaneously hypertensive rats. Clin. Sci. Mol. Med. 55: 247s-250s.

Moll, D., Dale, S.L. and Melby, J.C. (1975). Adrenal steroidogenesis in the spontaneously hypertensive rat. Endocrinology 96: 416-420.

Moore, L., Hurwitz, L., Davenport, G.R. and Landon, E.J. (1975). Energy dependent calcium uptake activity of microsomes from the aorta of normal and hypertensive rats. Biochim. Biophys. Acta 413: 432-443.

Mtabaji, J., Manku, M.S. and Horrobin, D. (1976). Vascular action of furosemide and bumetonide on the rat superior mesenteric vascular bed, interactions with prolactin and prostaglandins. Can. J. Physiol. Pharmacol. 54: 357-366.

Mulvany, M.J. and Halpern, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ. Res. 41: 19-26.

Mulvany, M. J., Hansen, P.K. and Aalkjaer, C. (1978). Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media and an increased number of smooth muscle cell layers. Circ. Res. 43: 854-864.

Mulvany, M.J. and Nyborg, N. (1980). An increased calcium sensitivity

of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. Br. J. Pharmacol. 71: 585-596.

Mulvany, M.J., Korsgaard, N. and Nyborg, N. (1981a). Evidence that the increased calcium sensitivity of resistance vessels in spontaneously hypertensive rats is an intrinsic defect of their vascular smooth muscle. Clin. Exp. Hyper. 3(4): 749-61.

Mulvany, M.J., Korsgaard, N., Nyborg, N. and Nilsson, H. (1981b). Chemical denervation does not affect the medial hypertrophy and increased calcium sensitivity of mesenteric resistance vessels from spontaneously hypertensive rats. Clin. Sci. 61: 61s-63s.

Mulvany, M.J., Nilsson, H., Nyborg, N. and Mikkelsen, E. (1982). Are isolated femoral resistance vessels or tail arteries good models for the hindquarter vasculature of spontaneously hypertensive rats. Acta physiol. scand. 116: 275-283.

Mulvany, M.J. (1983). Do resistance abnormalities contribute to the elevated blood pressure of spontaneously hypertensive rats. Blood Vessels 20: 1-22.

Murthy, V.W., Gilbert, J.C., Goldberg, L.I. and Kuo, J.F. (1976). Dopamine-sensitive adenylate cyclase in canine renal artery. J. Pharm. Pharmacol. 28: 567-571.

Nagaoka, A., Toyoda, S. and Iwaksuka, H. (1978). Increased renal vascular reactivity to norepinephrine in stroke-prone spontaneously hypertensive rats (SHR). Life Sci. 23: 1159-66.

Nagatsu, T., Ikuta, K., Numata, Y., Kata, T. and Sano, M. (1976). Vascular and brain dopamine beta hydroxylase activity in young spontaneously hypertensive rats. Science 191: 290-291.

Nagatsu, T., Kato, T., Hashimoto, Y., Numata, Y., Yamori, Y. and Okamoto, K. (1978). Dopamine beta hydroxylase activity in stroke-prone spontaneously hypertensive rats. Experientia 34: 305-306.

Nagoaka, A. and Lovenberg, W. (1977). Regional changes in the activities of aminergic biosynthetic enzymes in the brains of hypertensive rats. Eur. J. Pharmacol. 43: 297-306.

Nakamura, K. and Nakamura, K. (1977). Enhanced sympathetic activity in young spontaneously hypertensive rats is not the trigger mechanism for genetic hypertension. Naunyn-Schmiedebergs Arch. Pharmacol. 299: 143-148.

Nakamura, N. and Nakamura, K. (1978a). Selective activation of noradrenergic neurons in the brainstem and spinal cords of young spontaneously hypertensive rats. Experientia 34: 1042-1043.

Nakamura, N. and Nakamura, K. (1978b). Role of brainstem and spinal noradrenergic and adrenergic neurons in the development and maintenance of hypertension in spontaneously hypertensive rats. Naunyn-Schmiedebergs Arch. Pharmacol. 305: 127-133.

Nishiyama, K., Nishiyama, A. and Frolich, E.D. (1976). Regional blood flow in normotensive and spontaneously hypertensive rats. Am. J. Physiol. 230: 691-698.

Nordborg, C. and Johansson, B.B. (1979). The ratio between the thickness of media and internal radius in cerebral, mesenteric and renal vessels in spontaneously hypertensive rats. Clin. Sci. 57 (suppl.): 27s-29s.

Nordborg, C. and Johansson, B.B. (1980). Morphometric study on cerebral vessels in spontaneously hypertensive rats. Stroke 11: 266-270.

Oakes, B.W. (1980). An ultrastructural study of the effect of B-aminopropionitrile fumarate (BAPN) on elastin formation by rat aortic smooth muscle cells in culture. Micron 11: 463-64.

Ogawa, M. and Ozaki, M. (1978). Inhibitory effect of B-aminopropionitrile on the development of hypertension in spontaneously hypertensive rats. Jap. J. Pharmacol. 28: 785-788.

Okamoto, K. and Aori, K. (1963) Development of a strain of spontaneously hypertensive rats. Jap.Circ.J. 27:282-293.

Okamoto, K. (1969). Spontaneous hypertension in rats. Int. Rev. Exp. Pathol. 7:227-270.

Okamoto, K., Yamori, Y., Ooshima, A., Park, C., Haebara, H., Matsumoto, M., Tanaka, T., Okuda, T., Hazama, F. and Kyogoku, M. (1972). Establishment of an inbred strain of spontaneously hypertensive rat and genetic factors involved in hypertension. In: Spontaneous Hypertension, K. Okamoto, Ed., Springer-Verlag, New York. pp. 1-8.

Okamoto, K., Yamori, Y. and Nagaoka, A. (1974). The establishment of the stroke prone hypertensive rat. Circ. Res. 34 (suppl.): I 143-I 153.

Ooshima, A., Fuller, G.C., Cardinale, G.J., Spector, S. and Udenfriend, S. (1974). Increased collagen synthesis in blood vessels of hypertensive rats and its reversal by antihypertensive agents. Proc. Natl. Acad. Sci., USA 71:3019-3023.

Oparil, S. and Cutilletta, A.F. (1980). Hemodynamic response to vascular expansion following immunosympathectomy in spontaneously hypertensive rats. Hypertension 2: 304-310.

Owens, G.K., Rabinovitch, P.S. and Schwartz, S.M. (1981). Smooth

muscle cell hypertrophy versus hyperplasia in hypertension. Proc. Natl. Acad. Sci. USA 78: 7759-7763.

Page, E. and Oparil, S. (1978). Effect of peripheral sympathectomy on left ventricular ultrastructure in young spontaneously hypertensive rats. J. Mol. Cell. Cardiol. 10: 310-305.

Palermo, A., Constantini, C., Mara, G. and Libretti, A. (1981). Role of the sympathetic nervous system in spontaneous hypertension: changes in central adrenoreceptors and plasma catecholamine levels. Clin. Sci. 61: 195s-198s.

Pamnani, M.B., Clough, D.L., Huot, S.J. and Haddy, F.J. (1981). Sodium-potassium pump activity in experimental hypertension. In: Vasodilation, P.M. Vanhoutte and I. Leusen, Eds., Raven Press, New York.

Pang, C.Y. and Sutter, M.C. (1980). Hydralazine prevents contractile responses in the aorta but not portal vein strips in hypertensive rats. Blood Vessels 17: 293-301.

Pang, S.C. and Scott, T.M. (1981). Stereological analysis of the tunica media of the aorta and renal artery during the development of hypertension in the spontaneously hypertensive rat. J. Anat. 133: 513-526.

Pegram, B.L., Bevan, R.D. and Bevan, J.D. (1976). The facial vein of the rabbit: neurogenic vasodilation mediated by beta adrenergic receptors. Circ. Res. 39: 854-860.

Pettinger, W.A., Sanchez, A., Saavedra, J., Haywood, J.R., Gandler, T. Rodes, T. (1982). Altered renal alpha$_2$ adrenergic receptor regulation in genetically hypertensive rats. Hypertension 4 (suppl. II):II 188-II 192.

Prewitt, R.L., Chen, I.I.H. and Dowell, R. (1982). Development of microvascular rarefaction in the spontaneously hypertensive rat. Am. J. Physiol. 243: H243-H251.

Prino, R., Dolfini, E., Mennini, T., Palermo, A. and Libretti, A. (1981). Reduced serotonin uptake by spontaneously hypertensive rat platelets. Life Sci. 29: 2375-2379.

Przuntek, H. and Philippu, A. (1973). Reduced pressor responses to stimulation of the locus coerulus after lesion of the posterior hypothalamus. Naunyn-Schmiedebergs Arch. Pharmacol. 276: 119-122.

Ramanathan, S. and Shibata, S. (1974). Cyclic AMP in blood vessels of spontaneously hypertensive rats. Blood Vessels 11: 312-318.

Rapoport, R.M. and Bevan, J.A. (1983). Effect of contraction on the subsequent responsiveness and maximum contractility of the rabbit ear artery and saphenous vein in vitro. Blood Vessels 20: 44-55.

Richardson, M., Innatowycz, I. and Moore, S. (1980). Glycosaminoglycan in the rabbit aortic wall following balloon catheter de-endothelialization. Lab. Invest. 43: 509-516.

Richer, C., Doussae, M.P. and Guidicelli, J.F. (1982). MK 421 and the prevention of genetic hypertension development in young spontaneously hypertensive rats. Eur. J. Pharmacol. 79: 23-9.

Richer, C., Doussau, M.P. and Guidicelli, J.F. (1983). Effects of captopril and enolapril on regional vascular resistance and reactivity in spontaneously hypertensive rats. Hypertension 5: 312-320.

Robberecht, P., Winand, J., Chatelain, P., Polochek, P., Camus, J.C., DeNeef, P. and Christophe, J. (1981). Comparison of beta adrenergic receptors and the adenylate cyclase system with muscarinic receptors and guanylate cydlase activities in the heart of spontaneously hypertensive rats. Biochem. Pharmacol. 30: 385-387.

Rodbell, M. (1980). The role of hormone receptors and GTP regulatory proteins in membrane transduction. Nature 284: 17-22.

Saavedra, J.M., Grobecker, H. and Axelrod, J. (1978). Changes in central catecholaminergic neurons in the spontaneously (genetic) hypertensive rat. Circ. Res. 42: 529-534.

Sadoshima, S., Busija, D., Brody, M. and Heistad, D. (1981). Sympathetic nerves protect against stroke in stroke prone hypertensive rats. Hypertension (suppl. 1):I 124-I 127.

Saito, H. (1981). Clonidine withdrawal hypertension in spontaneously hypertensive rats. Trends Pharmacol. Sci. 2(7): 176-7.

Saito, A. and Lee, T.J.F. (1982). Adrenergic innervation of the brain arteries in spontaneously hypertensive rats. Fed. Proc. 41: 1649 (Abstract 8064).

Sands, H., Sinclair, D. and Mascali, J. (1976). Cyclic AMP and protein kinase in spontaneously hypertensive rat aorta and tissue cultured aortic smooth muscle cells. Blood Vessels 13: 361-373.

Schaectelin, G., Regoli, D. and Gross, F. (1963). Bioassay of renin like pressor material by isovolemic cross circulation.

Am. J. Physiol. 205: 303-306.

Schmitz, J.M., Graham, R.M., Sagalosky, A. and Pettinger, W.A. (1981). Renal alpha$_1$ and alpha$_2$ adrenergic receptors: Biochemical and pharmacologial correlations. J. Pharmacol. Exp. Ther. 219: 400-406.

Schomig, A., Dietz, R., Rascher, W., Luth, J.B., Mann, J., Schmidt, M. and Weber, J. (1978). Sympathetic vascular tone in spontaneous hypertension of rats. Klin. Wschr. 56 (suppl. 1): 131-138.

Scott, T.M. and Pang, S.C. (1983a). The correlation between the development of sympathetic innervation and the development of medial hypertrophy in jeujunal arteries in normotensive and spontaneously hypertensive rats. J. Auton. Nerv. Syst. 8: 25-32.

Scott, T.M. and Pang, S.C. (1983b). Changes in jeujunal arteries in spontaneously hypertensive and normotensive rats following neonatal treatment with capsaicin. Acta Stereol. 2(1): 127-33.

Scriabine, A. (1980). Pharmacology of Antihypertensive Drugs, A. Scriabine, Ed., Raven Press, New York.

Sen, S., Smeby, R.R. and Bumpus, F.M. (1972). Renin in rats with

spontaneous hypertension. Circ. Res. 31: 876-880.

Sen, S., Tarazi, R.C., Khairallah, P.A. and Bumpus, F.M. (1974). Cardiac hypertrophy in spontaneously hypertensive rats. Circ. Res. 35: 775-781.

Sen, S. and Bumpus, F.M. (1979). Collagen synthesis in development and reversal of cardiac hypertrophy in spontaneously hypertensive rats. Am. J. Cardiol. 44: 954-958.

Shepherd, J.T., Lorenz, R.R., Tyce, G.M. and Vanhoutte, P.M. (1978). Acetylcholine-inhibition of transmitter release from adrenergic nerve terminals mediated by muscarinic receptors. Fed. Proc. 37: 191-194.

Sheridan, P.J., Kozar, L.G. and Benson, S.C. (1979). Increased lysyl oxidase activity in aortas of hypertensive rats and the effect of B-aminopropionitrile. Exp. Mol. Pathol. 30: 315-324.

Shibata, S., Kurahashi, K. and Kuchii, M. (1973). A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive and normotensive rats. Blood Vessels 17: 246-256.

Shiono, K. and Sokabe, H. (1976). Renin angiotensin system in spontaneously hypertensive rats. Am. J. Physiol. 231: 1295-1299.

Sinaiko, A.R., Cooper, M.J. and Mirkin, B.L. (1980). Effect of neonatal sympathectomy with 6-hydroxydopamine on the reactivity of the renin-angiotensin system in spontaneously hypertensive rats. Clin. Sci. 59: 123-129.

Smith, M.L., Browning, R.A. and Myers, J.H. (1979). The in vivo rate of serotonin synthesis in brain and spinal cord of young SHR. Eur. J. Pharmacol. 53: 301-305.

Sobieszek, A. and Bremel, R.D. (1975). Preparations and properties of vertebrate smooth muscle myofibriles and actinomyosin. Eur.J. Biochem. 55: 49-60.

Sobieszek, A. and Small, J.V. (1976). Myosin linked calcium regulation in vertebrate smooth muscle. J. Mol. Biol. 101: 75-92.

Somlyo, A.P. and Somlyo, A.V. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. J. Pharmacol. Exp. Ther. 159: 129-145.

Somlyo, A. (1975). Structural characteristics, mechanisms of contraction, innervation and proliferation of smooth muscle cells. Adv. Exp. Med. Biol. 57: 34-46.

Somlyo, A.P., Somlyo, A.V. and Shuman, H. (1979). Electron probe

analysis of vascular smooth muscle. J. Cell Biol. 81: 316-335.

Sowers, J.R., Resch, G., Tempel, G., Herzog, J. and Colantino, M. (1979). Hyperprolactinemia in spontaneously hypertensive rats. Acta Endocrinol. 90: 1-7

Spector, S., Fleisch, J.H., Maling, H.M. and Brodie, B.B. (1969). Vascular smooth muscle reactivity in normotensive and hypertensive rats. Science 166: 1300-1301.

Spector, S., Ooshima, A., Iwaksuki, K., Fuller, G., Cardinale, G. and Uienfriend, S. (1978). Increased collagen biosynthesis by hypertension and reversal by antihypertensive drugs. Blood Vessels 15: 176-182.

Spokas, E.G., Folco, G., Guilley, J., Chandler, P. and McGiff, J.C. (1983). Endothelial mechanisms in the vascular action of hydralazine. Hypertension 5 (suppl.1):I 107-I 111.

Steer, M. (1977). Adrenergic receptors. Clin.Endo.Metab. 6(3):577-598.

Stills, W.J.S. (1979). The effect of chronic hypertension on the aortic intima of the rat. Exp. Mol. Pathol. 31: 1-9.

Stumpe, K.O., Kolloch, R. and Higuchi, M. (1977). Hyperprolactinemia and antihypertensive effects of bromocryptine in essential hypertension. Lancet 1: 211-214.

Sutter, M.C. and Ljung, B. (1977). Contractility, muscle mass and agonist sensitivity of isolated portal veins from normo- and hypertensive rats. Acta physiol. scand. 99: 484-495.

Swamy, V.C. and Triggle, D.J. (1980a). The reactivity of iliac vascular strips from spontaneously hypertensive and normotensive rats. Blood Vessels 17: 246-256.

Swamy, V.C. and Triggle, D.J. (1980b). The response of carotid vascular strips from spontaneously hypertensive and normotensive rats. Can. J. Physiol. Pharmacol. 58: 53-59.

Takeda, K. and Bunag, R.D. (1978). Sympathetic hyper-reactivity during hypothalamic stimulation in spontaneously hypertensive rats. J. Clin. Invest. 62: 642-648.

Takeda, K., Nakagawa, Y., Hashimoto, T., Sakurai, H. and Imai, S. (1979). Effects of several beta blocking agents on the development of hypertension in spontaneously hypertensive rats. Jap. J. Pharmacol. 29: 171-178.

Taylor, D.G. (1980). Hydralazine. In: Pharmacology of Antihypertensive Drugs, A. Scarabine, Ed., Raven Press, New York. pp. 407-414.

Timmermans, P.B. and Van Zweiten, P.A. (1981). The postsynaptic alpha$_2$-adrenoreceptor. J. Auton. Pharmacol. 1: 171-183.

Thoren, P. and Ricksten, S.E. (1979). Recording of renal and splanchnic sympathetic nervous activity in normotensive and spontaneously hypertensive rats. Clin. Sci. 57 (suppl. 5): 197s-199s.

Tobia, A.J., Walsh, G.M., Tadepalli, A.S. and Lee, J.Y. (1974). Unaltered distribution of cardiac output in the conscious young spontaneously hypertensive rat: Evidence for uniform elevation of regional vascular resistance. Blood Vessels 11: 287-294.

Tobian, L. (1974). How sodium and the kidney relate to the hypertensive arteriole. Fed. Proc. 33: 138-142.

Tobian, L., Johnson, M.A., Lange, J. and Magraw, S. (1975). Effect of varying perfusion pressure on the output of sodium and renin and the vascular resistance in kidneys of rats with post salt hypertension and Kyoto spontaneous hypertension. Circ. Res. 36/37 (suppl. 1):I 162-I 170.

Touw, K.B., Haywood, J.R., Shaffer, R.A. and Brody, M.J. (1980). Contribution of the sympathetic nervous system to vascular

resistance in conscious young and adult spontaneously hypertensive rats. Hypertension 2(4): 408-418.

Tomanek, R.J,. Davis, J.W. and Anderson, S.C. (1979). The effects of alpha-methyldopa on cardiac hypertrophy in spontaneously hypertensive rats: ultrastructural, stereological and morphometric measurements. Cardiovasc. Res. 13: 173-182.

Triner, L., Vulliemoz, Y., Verosky, M., Habif, D.V. and Nahas, G.G. (1972). Adenyl cyclase-phosphodiesterase system in arterial smooth muscle. Life Sci. 11: 817-824.

Triner, L., Vulliemoz, Y,. Verosky, M. and Manger, W.M. (1975). Cyclic adenosine monophosphate and vascular reactivity in spontaneously hypertensive rats. Biochem. Pharmacol. 24: 743-745.

Van Breemen, C., Aaronson, P., Loutzenhiser, R. and Meisheri, K. (1980). Ca^{+2} movement in smooth muscle. Chest 78: 157-165.

Van Loh, D. and Bohr, D.F. (1973). Membrane potentials of smooth muscle cells of isolated resistance vessels. Proc. Soc. Exp. Biol. Med. 144: 513-516.

Vanhoutte, P.M. (1978). Adrenergic neuroeffector interaction in the blood vessel wall. Fed. Proc. 37: 181-186.

Vanhoutte, P.M. (1981). Release and disposition of norepinephrine in the blood vessel wall of spontaneously hypertensive rat. In: New Trends in Arterial Hypertension, INSERM, Symposium No. 17, M. Worcel et al., Eds., Elsevier/North Holland Biomedical Press, New York.

Vanhoutte, P.M. and Rimele, T.J. (1982/83). Role of the endothelium in the control of vascular smooth muscle function. J. Physiol. (Paris) 78: 681-686.

Vavra, I., Tom, H. and Greselin, E. (1973). Chronic propranolol treatment in young spontaneously hypertensive and normotensive rats. Can. J. Physiol. Pharmacol. 51: 727-732.

Verhoeghe, R.H., Lorenz, R.R., McGrath, M.A., Shepherd, J.T. and Vanhoutte, P.M. (1978). Metabolic modulation of transmitter release-adenosine, adenine nucleotides, potassium, hyperosmolarity and hydrogen ion. Fed. Proc. 37: 208-211.

Versteeg, D.H.G., Palkovits, M., Van der Gugten, J., Wijnen, H.L.J.M., Smeets, G.W.M. and De Jong, W. (1976). Catecholamine content of individual brain regions of spontaneously hypertensive rats. Brain Res. 112: 429-434.

Warshaw, D.M., Mulvany, M.J. and Halpern, W. (1979). Mechanical and morphological properties of arterial resistance vessels in young and old spontaneously hypertensive rats. Circ. Res. 45: 250-259.

Warshaw, D.M., Root, D.T. and Halpern, W. (1980). Effects of anti-hypertensive drug therapy on the morphology and mechanics of resistance arteries from spontaneously hypertensive rats. Blood Vessels 17: 257-270.

Webb, R.C. and Bhalla, R.C. (1976). Ca^{+2} sequestration by sub-cellular fractions isolated from vascular smooth muscle. Effect of cyclic nucleotides and protaglandins. J. Mol. Cell. Cardiol. 8: 145-157.

Webb, R.C. and Bohr, D.F. (1978). Potassium induced relaxation as an indicator of Na^{+}-K^{+} ATPase activity in vascular smooth muscle. Blood Vessels 15: 198-207.

Webb, R.C. and Bohr, D.F. (1979). Potassium relaxation of vascular smooth muscle from spontaneously hypertensive rats. Blood Vessels 16: 71-79.

Webb, R.C. and Bohr, D.F. (1980). Vascular reactivity in hyper-tension: Altered effect of ouabain. Experientia 36: 220-22.

Webb, R.C. and Vanhoutte, P.M. (1981a). Cocaine and contractile responses of vascular smooth muscle from spontaneously hypertensive rats. Arch. Int. Pharmacodyn. Ther. 253: 241-256.

Webb, R.C., Vanhoutte, P.M. and Bohr, D.F. (1981b). Adrenergic neurotransmission in vascular smooth muscle from spontaneously hypertensive rats. Hypertension 3: 93-103.

Wei, J.W., Janis, R.A. and Daniel, E.E. (1976a). Studies on subcellular fractions from mesenteric arteries of spontaneously hypertensive rats. Alterations in both Ca^{+2} uptake and enzyme activities. Blood Vessels 13: 293-308.

Wei, J.W., Janis, R.A. and Daniel, E.E. (1976b). Calcium accumulation and enzymatic activities of subcellular fractions from aortas and ventricles of genetically hypertensive rats. Circ. Res. 39: 133-140.

Weibel, E. and Bolender, R.P. (1973). Stereological techniques for electron microscopic morphometry. In: Principles and Techniques of Electron Microscopy, Vol. 3, M.A. Hyatt, Ed., Van Nostrand Reinhold, New York. p. 239.

Weiner, N. (1980). Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: The Pharmacological Basis of

Therapeutics, A.G. Gilman, L.S. Goodman and A. Gilman, Eds., MacMillan Pub. Co., New York.

Weinshilboum, R.M. and Axelrod, J. (1971). Serum dopamine B hyroxylase: decrease after chemical sympathectomy. Science 173: 931-933.

Weinshilboum, R.M., Kvetnansky, R., Axelrod, J. and Kopin, I. (1971). Elevation of dopamine B hydroxylase with forced immobilization. Nature (New Biology) 230: 287-288.

Weiss, L. (1974). Aspects of the relation between functional and structural cardiovascular factors in primary hypertension. Acta physiol.scand. 409(suppl.): 1-58.

Weiss, L. and Lundgren, Y. (1978). Chronic antihypertensive drug treatment in young spontaneously hypertensive rats: effects on arterial blood pressure, cardiovascular reactivity and vascular design. Cardiovasc. Res. 12: 744-751.

Weiss, L., Webb, R.C. and Smith, C.B. (1983). Comparison of arterial smooth muscle and neural alpha$_2$ adrenoreceptors from spontaneously hypertensive and Wistar Kyoto rats. Circ. (part II) 68: III-59 (abstract 233).

Wells, J.N. and Hardman, J.G. (1977). Cyclic nucleotide phosphodi-

esterases. Adv. Cyclic. Nucleotide Res. 8:119-143.

Westfall, T.C. (1977). Focal regulation of adrenergic neurotransmission. Physiol. Rev. 57: 660-728.

Whall, C.W., Myers, M.M. and Halpern, W. (1980). Norepinephrine sensitivity, tension development and neuronal uptake in resistance arteries from spontaneously hypertensive and normotensive rats. Blood Vessels 17: 1-15.

Whall, C.W., Havlik, R.J., Halpern, W. and Bohr, D.F. (1983). Potassium depolarization of adrenergic varicosities in resistance arteries from SHR and WKY rats. Blood Vessels 20: 23-33.

Wijnen, H., Palkovits, M., DeJong, W. and Versteeg, D. (1978). Elevated adrenal content in nuclei of medulla oblongata and hypothalamus during the development of spontaneous hypertension. Brain Res. 157: 191-195.

Wijnen, H., Spierenburg, H.A., De Kloet, R., DeJong, W. and Versteeg, D. (1980). Decrease in noradrenergic activity in hypothalamic nuclei during the development of spontaneous hypertension. Brain Res. 184: 153-162.

Yamaguchi, I. and Koplin, I.J. (1980). Differential inhibition of

alpha-1 and alpha-2 adrenoreceptor mediated pressor responses in pithed rats. J. Pharmacol. Exp. Ther. 214: 275-281.

Yamori, Y., Lovenberg, W. and Sjoedsma, A. (1970). Norepinephrine metabolism in brainstem of spontaneously hypertensive rats. Science 170: 544-546.

Yamori, Y. (1976). Neural and non-neural mechanisims in spontaneous hypertension. Clin. Sci. Mol. Med. 51: 431s-434s.

Yamori, Y. and Ohta, K. (1979). Chemical analysis of vascular collagen in stroke prone spontaneously hypertensive rats. Jap. Circ. J. 43: 963-969.

Yomaida, I., Murao, M., Togashi, H., Shimamura, K., Koike, Y., Monama, Y. and Saito, H. (1979). Effects of long term administration and withdrawal of clonidine on activity of sympathetic efferent nerve unit in spontaneously hypertensive rats. Neurosci. Lett. 15: 249-251.

Zandberg, P., Palkovits, M. and DeJong, W. (1978). Effect of various lesions in the nucleus tractus solitarii of the rat on blood pressure, heart rate and reflex response. Clin. Exp. Hypertens. 1(3): 355-379.

Zimmerman, B.G. (1978). Action of angiotensin on adrenergic nerve

endings. Fed. Proc. 37: 199-202.