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EFFECTS OF ARTERIAL VASODILATORS ON CARDIOVASCULAR HYPERTROPHY AND SYMPATHETIC ACTIVITY IN NORMOTENSIVE WISTAR AND SPONTANEOUSLY HYPERTENSIVE RATS

By

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ABSTRACT

In spontaneously hypertensive rats (SHR), treatment with the arterial vasodilator minoxidil does not prevent or attenuate the progression of cardiovascular hypertrophy despite blood pressure control, and the reason is generally unknown. The purpose of this study, was to examine temporal changes of cardiac and mesenteric arterial structure with respect to changes in volume load and cardiac and arterial sympathetic activity, during chronic treatment of normotensive and SHR with minoxidil alone, or in combination with the diuretic hydrochlorothiazide (HCTZ). The hypothesis to be tested is that an increase in the sympathetic activity and/or cardiac and intravascular volume is involved in causing these structural changes. In normotensive rats, minoxidil induced i) right ventricular hypertrophy (RVH), ii) eccentric left ventricular hypertrophy (LVH), iii) medial hypertrophy of the superior mesenteric artery, iv) intravascular volume expansion, v) increases in cardiac filling pressures, vi) increases in ventricular and arterial norepinephrine turnover rates. In SHR, minoxidil i) decreased blood pressure, ii) potentiated RVH, iii) caused the development of eccentric LVH superimposed on the preexisting hypertrophy, iv) increased the lumen of the superior mesenteric artery, v) prevented further increases in medial hypertrophy of the large and small mesenteric arteries, vi) induced intravascular volume expansion, vii) increased ventricular but decreased arterial norepinephrine turnover rates, and viii) increased elastin content and decreased elastase activity in the large conducting vessels (aorta, superior mesenteric artery). In SHR and normotensive rats, concomitant diuretic treatment prevented intravascular volume expansion, caused concentric LVH rather than eccentric LVH and no longer increased the medial and luminal areas of the superior mesenteric

artery. These results suggest that there are regional differences in the response of the cardiovascular system to minoxidil in SHR and normotensive rats. Some of these differences may relate to differences in regional sympathetic activity, whereas volume load appears to play a modulatory role.

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ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
BP	Blood Pressure
CnBr	Cyanogen Bromide
DOCA	Deoxycorticosterone Acetate
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
HCTZ	Hydrochlorothiazide
HLE	Human Leukocyte Elastase
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVEDP	Left Ventricular End Diastolic Pressure
MAP	Mean Arterial Pressure
MHC	Myosin Heavy Chain
NMC	Nonmyocyte Cells
PDGF	Plasma Derived Growth Factor
PRA	Plasma Renin Activity
r	Radius of Curvature
RAP	Right Atrial Pressure
RAS	Renin-Angiotensin System
RV	Right Ventricle
RVH	Right Ventricular Hypertrophy
SEM	Standard Error of the Mean
SHR	Spontaneously Hypertensive Rat
TGF	Transforming Growth Factor

TPR	Total Peripheral Resistance
2-K, 1-C	Two kidney, one clip
Tw	Wall tension
WT	Wall thickness
WKY	Wistar-Kyoto

PREFACE

The results section of this thesis consists of papers which have been published. Authorship of these papers is shared by my thesis supervisor, Dr. R. M. K. W. Lee, my supervisory committee member, Dr. F. H. H. Leenen, at whose laboratory all the cardiac related experiments were performed, as well as Drs. N. Fields, B. Yuan, and F. W. Keeley who provided valuable technical assistance and scientific input. In the production of each paper, I carried out the experiments, organized and prepared the data for publication, and wrote the first drafts. There was invariably supervisorial revision between the first and final drafts.

1.0 INTRODUCTION

1.1 Cardiac Hypertrophy

1.1.1 Sequalae of Cardiac Hypertrophy

An increase in cardiac mass can be considered an adaptive mechanism serving to compensate for an increased hemodynamic load such as occurs in sustained arterial hypertension. Initially, the increase in cardiac mass serves to reduce wall stress to its normal value. However, as hypertensive cardiovascular disease progresses and cardiac hypertrophy becomes more severe, the following can be observed: i) thickening of the ventricular wall leads to a fall in compliance (Smith *et al.*, 1987), ii) coronary reserve becomes impaired and increases the risk of myocardial ischemia (Wangler *et al.*, 1982; Harrison *et al.*, 1988; Polese *et al.*, 1991), iii) increased frequency of arrhythmias and signs of disturbed repolarization of the myocardium (Messerli *et al.*, 1984), iv) a decrease in left ventricular (LV) pump function that may lead to congestive heart failure (Messerli, 1990, 1996).

Left ventricular hypertrophy (LVH) is a powerful pressure-independent risk factor for cardiovascular morbidity and mortality (Haider *et al.*, 1998). A reduction in LVH improves impaired ventricular filling (Cody *et al.*, 1986), coronary reserve (Canby and Tomanek, 1989), ventricular dysrhythmias (Messerli, 1990), and maintains LV pump function (Schulman *et al.*, 1990).

1.1.2 Stimuli to Cardiac Hypertrophy

Hypertrophy is clearly an adaptive response of the heart to hemodynamic overloads. Hemodynamic and non-hemodynamic factors both play a role in the development of cardiac hypertrophy when they are combined with associated genetic and lifestyle risk

factors (Messerli, 1983). Both volume and pressure load (or preload and afterload) are major hemodynamic determinants of cardiac growth (Grossman, 1980; Messerli, 1996). In several animal models of pressure overload hypertrophy [2K, 1C hypertensive rats (Leenen and De Jong, 1971), rat aortic banding (Mukawa *et al.*, 1997)], a close correlation can be found between blood pressure and degree of cardiac hypertrophy. However, in humans (Devereux *et al.*, 1983; Drayer *et al.*, 1983), or in the spontaneously hypertensive rats (SHR) (Sen *et al.*, 1974), as well as in humans (Devereux *et al.*, 1983; Drayer *et al.*, 1983), the relatively low correlation coefficients between blood pressure and LV mass suggests the importance of other mechanisms. Non-hemodynamic mechanisms such as cardiac sympathetic activity and the renin-angiotensin system have been proposed as playing a major role (Ostman-Smith, 1981; Weber *et al.*, 1995).

1.1.3 Cardiac Remodeling in Hypertension

Cardiac remodeling consists of alterations in size and geometry of the myocardium associated with alterations in wall thickness (Rosado and Lamas, 1997). These changes occur in different conditions such as during growth or after pressure or volume overload. Cardiac muscle normally grows to compensate for the work load imposed on the ventricle. Classically, the most important factor mediating cardiac hypertrophy has been thought to be the systolic force or tension generated by the myocardial fibers (Linzbach, 1960). The relations among LV wall tension, intracavitary pressure and heart size can be expressed by the law of Laplace. Assuming that the ventricle is a thin-walled sphere, wall tension (Tw) is proportional to pressure (P) and the radius of curvature (r): $Tw = Pr/2$. For spheres with a significant wall thickness (WT), this becomes $Tw = Pr/2WT$ or $P = Tw \times 2WT/r$ (Grossman *et al.*, 1975). When a chronically increased pressure load is imposed

on the ventricle, LV wall thickness increases so that the LV peak systolic wall stress remains unchanged or within normal limits (Grossman *et al.*, 1975). Thus, P = a constant (K) X WT/r, so that the ratio of WT/r, or relative wall thickness of the ventricle is directly proportional to the intracavitary pressure.

Pressure-overloaded LV tends to develop concentric hypertrophy defined as an increase in the ratio of wall thickness to radius of the LV cavity (Grossman *et al.*, 1975). These changes occur whether the increased pressure load is due to aortic stenosis, systemic hypertension, or some other lesion. In contrast, volume overloaded LV resulting from high venous return (e.g. intravascular volume expansion or aortic-venous shunt), or cardiac abnormalities such as aortic and mitral regurgitation or atrial septal defect, develop eccentric hypertrophy defined as proportionate increases in LV radius and wall thickness so that relative wall thickness remains within the normal range. In this situation if systolic pressure remains unchanged, wall thickness and the radius of the curvature increases proportionally, causing a "magnification" of the LV.

To examine the role of alterations in systolic and diastolic wall stresses in influencing the pattern and extent of LVH in human beings, Grossman *et al.* (1975), examined systolic and diastolic wall stresses in a series of patients with a normal or chronically pressure-overloaded or chronically volume-overloaded LV. LV systolic pressure was increased only in the pressure-overloaded group, while LV diastolic pressure was increased equally in both the pressure-overloaded and the volume-overloaded group. All patients were well compensated in terms of systolic performance, and LV mass was increased to more than twice of normal in both patient groups indicating significant hypertrophy. LV wall thickness was significantly increased in both groups, but was disproportionately increased in the group with pressure-overload, indicating the presence of concentric hypertrophy. The ratio of LV wall thickness to

internal radius of the LV was normal in patients with LV volume overload, but significantly increased in patients with pressure overload.

These observations led Grossman (1980), to formulate the following hypothesis: When the primary stimulus to hypertrophy is LV pressure overload, the resultant increase in peak wall stress leads to parallel replication of sarcomeres, wall thickening and concentric hypertrophy. The resultant wall thickening is sufficient to return peak systolic stress to normal thus acting as feedback inhibition of the hypertrophic process.

In contrast, when the primary stimulus to hypertrophy is LV volume overload, increased end-diastolic wall stress leads to series replication of sarcomeres, fiber elongation, chamber enlargement and eccentric hypertrophy. Chamber enlargement leads acutely to increased peak systolic wall stress, which in turn causes wall thickening of sufficient magnitude to normalize the systolic stress. Thus, both wall thickening and fiber elongation contribute to the pattern of eccentric hypertrophy.

1.1.3.1 Stretch-Induced Cardiac Hypertrophy

Due to the complexity of the *in vivo* environment, most studies of the effect of mechanical factors on generation of intracellular signals and cell growth have been carried out *in vitro*. Stretching or swelling of the tissue or cell has been identified as the mechanical parameter most closely linked to these events. An increase in protein synthesis by stretch of quiescent papillary muscle has been first observed by Peterson and Lesch (1972). Kira *et al.* (1984), found that an increase in aortic pressure in hearts arrested by potassium or tetrodotoxin accelerates protein synthesis. Increased aortic pressure also decreases the rate of protein degradation (Gordon *et al.*, 1986). An increase in intraventricular pressure from 0 to 25 mm Hg also increases the rate of protein synthesis in tetrodotoxin-arrested hearts (Xenophontos *et al.*, 1986). These findings

support the hypothesis that stretch of the ventricular wall sets in motion a signal transduction pathway that leads to enhanced protein synthesis.

In contrast to quiescent cells, contracting neonatal rat myocytes hypertrophied during 3 days in culture due to accelerated rates of protein and RNA synthesis (McDermott and Morgan, 1989). In adult feline cardiocytes, contracting cells have a larger cardiocyte surface area than nonbeating cells after 2 weeks in culture (Cooper *et al.*, 1986). Linear deformation of adult feline myocytes increases myocyte length by approximately 10 per cent and increases the rate of incorporation of [^{3}H]uridine into nuclear RNA and of [^{3}H]phenylalanine into cytoplasmic protein (Mann *et al.*, 1989). Similarly, stretching rodent neonatal myocytes cultured in a serum-free media by 10-20 percent above resting length causes an increase in protein synthesis without DNA synthesis (Komuro *et al.*, 1990; Sadoshima *et al.*, 1992). These data indicate that stretch is a sufficient stimulus for induction of RNA and protein synthesis in neonatal and adult cardiac myocytes.

Analysis of cell signaling mechanisms shows that stretch activates multiple signal transduction pathways including phospholipases, tyrosine kinases, c-Jun N-terminal protein kinases, p21ras, Raf-1, mitogen-activated protein kinases and their activators and protein kinase C (Komuro *et al.*, 1991; Sadoshima and Izumo, 1993; Komuro and Yazaki, 1993; Yamazaki *et al.*, 1993; Yamazaki *et al.*, 1995; Sadoshima *et al.*, 1996). However, it is unknown which molecules are directly activated by stretch and which molecules are indirectly activated by upstream modulators. Experiments using whole-cell current recording suggest that mechanical stress caused by whole-cell stretch or hypotonic swelling affects a wide variety of ionic channels and currents in the heart, such as the K^+_{ATP} channel (van Wagoner, 1993), a delayed K^+ rectifier channel (Sasaki *et al.*, 1994), a Cl^- channel (Hagiwara *et al.*, 1992), L type Ca^{++} channel (Matsuda *et al.*, 1996), and the NA^+/K^+ pump current (Sasaki *et al.*, 1994). It remains to be determined whether

stretch-activated ion channels can be the initial mechanosensor in stretch-induced cardiac hypertrophy.

Mechanical stimuli also cause a rapid change in gene expression (Komuro *et al.*, 1990; Sadoshima *et al.*, 1992). Linear stretch of cardiac myocytes *in vitro* (an equivalent of volume overload *in vivo*) causes transcriptional activation of immediate-early genes (protooncogenes) (i.e. *c-fos*, *c-jun*, *Egr-1*, and *c-myc*) followed by an induction of the fetal genes - atrial natriuretic factor (ANF), skeletal α-actin, and β-myosin heavy chain (MHC) (Sadoshima *et al.*, 1992). However, cardiac volume overload *in vivo* caused by an abdominal aortocaval fistula, induces a distinct pattern of gene expression involving only the induction of the fetal gene ANF (Calderone *et al.*, 1995). The phenotypic feature of stretched myocytes is very similar to that of pressure overload-induced hypertrophy *in vivo* (Mercadier *et al.*, 1983; Izumo *et al.*, 1988). The fall in myosin ATPase activity in pressure overload (due to aortic banding) rat ventricles, is associated with the transition from adult (V1, α) to fetal (V3, β) MHC (Izumo *et al.*, 1988). Similarly, skeletal α-actin, smooth muscle α-actin, β-tropomyosin, and atrial myosins light chains, present in the fetal ventricle are induced in response to pressure overload (Izumo *et al.*, 1988; Black *et al.*, 1991). Overload of human left atrium, which normally expresses α-MHC, results in the induction of β-MHC proportional to the increase in left atrial transverse diameter (Mercadier *et al.*, 1983).

1.1.3.2 Sympathetic Nervous System

A dissociation between elevated arterial pressure and increased myocardial mass has been demonstrated in hypertensive cardiac hypertrophy in animals (Sen *et al.*, 1974) and humans (Devereux *et al.*, 1983; Drayer *et al.*, 1983). This dissociation suggests the existence of stimuli other than blood pressure that are responsible for the development

and regression of cardiac hypertrophy. Many neural and hormonal stimuli have been implicated in myocardial growth including adrenergic stimuli and peptide growth factors.

Adrenoceptor activation could be a primary effector initiating and maintaining cardiac hypertrophy as a result of increased cardiac sympathetic nerve activity and elevated levels of circulating catecholamines (Ostman-Smith, 1981). In young SHR, an increase in cardiac sympathetic activity as assessed by norepinephrine fractional rate constant, precedes the rise in blood pressure and coincides with the development of cardiac hypertrophy (Adams *et al.*, 1989). Sympathectomy of newborn SHR combined with α_1-adrenoceptor blockade that does not allow re-innervation of the heart, prevents the development of cardiac hypertrophy and hypertension (Korner *et al.*, 1993). Infusion of the α_1/β-agonist norepinephrine into dogs results in significant myocardial hypertrophy, and the changes in ventricular mass are independent of changes in arterial blood pressure, intracardiac pressures, myocardial shortening rate or velocity of shortening, or cardiac work (Gans and Carter, 1970; Laks *et al.*, 1973; King *et al.*, 1987). Stimulation of myocardial β- and α-adrenergic receptors either alone or in combination can result in the development of cardiac hypertrophy, whereas hemodynamic changes play only a minor role (Zeirhut and Zimmer, 1989; Zimmer *et al.*, 1995). Chronic administration of norepinephrine into rats produces concentric LVH, substantially more than can be expected from the modest increase in blood pressure (Newling *et al.*, 1989). Administration of the β-agonist isoproterenol in rats, induces a combination of both concentric and eccentric hypertrophy (Leenen and Harmsen, 1991). This trophic effect of isoproterenol is associated with short lived increases in hemodynamics (Leenen and Harmsen, 1991). In isoproterenol-induced cardiac hypertrophy in rats, the concomitant administration of an angiotensin-converting enzyme (ACE) inhibitor prevents the increase in cardiac tissue angiotensin II and hypertrophy, implicating the cardiac renin-angiotensin system as the mediator of the cardiac trophic response to β-adrenergic

stimulation (Nagamo *et al.*, 1992; Golomb *et al.*, 1994). Isoproterenol-induced cardiac hypertrophy in rats is a dose- and time- dependent event, but so is myocyte necrosis (Stanton *et al.*, 1969). Isoproterenol induces myocyte necrosis, even when administered in low doses and with chronic infusion to avoid transient high catecholamine levels (Knufman *et al.*, 1987). In the presence of myocardial necrosis, the increase in heart weight is an unreliable index of hypertrophy because growth of the remaining myocytes is obscured by loss of necrotic tissue (Knufman *et al.*, 1987). In isoproterenol-induced cardiac hypertrophy in rats, the concomitant administration of an angiotensin-converting enzyme (ACE) inhibitor prevents the increase in cardiac tissue angiotensin II and hypertrophy, implicating the cardiac renin-angiotensin system as the mediator of the cardiac trophic response to isoproterenol (Nagamo *et al.*, 1992; Golomb *et al.*, 1994). These data *in vivo* suggest that stimulation of myocardial α- and β-adrenoceptors may induce cardiac hypertrophy independent of hemodynamic changes.

A direct test of norepinephrine's growth potential has been examined in cardiac myocytes and perfused hearts (Simpson, 1985; Fuller *et al.*, 1990). Many of these studies involve neonatal rat cardiac myocytes in cell culture. In cultured neonatal myocytes, α_1-adrenergic agonists via stimulation of the α_{1A}-adrenergic receptor subtype (Knowlton *et al.*, 1993), induce growth that is characterized by increases in protein synthesis, myocyte surface area, and protein content (Simpson, 1985). α_1-adrenergic stimulation also induces a hypertrophic response in isolated adult myocytes (Ikeda *et al.*, 1991). DNA synthesis is unaffected by α_1-adrenergic stimuli. The phenotypic feature of α_1-adrenergic agonists is very similar to stretched myocyte-induced hypertrophy. The hypertrophic response to α_1-adrenergic stimulation involves activation of protooncogenes (i.e. *c-fos*, *c-jun*, *Egr-1*, and *c-myc*) (Simpson, 1989), and re-expression of genes normally restricted to the fetus, including the embryonic β-MHC, the skeletal isoform of α-actin, ANF, and a fetal L-type calcium channel (Bishopric *et al.*, 1987; Chien *et al.*, 1991; Waspe *et al.*, 1991). The α_1-

signaling pathway appears to involve activation of phospholipase C, diacylglycerol, protein kinase C and intracellular tyrosine kinases (Kariya *et al.*, 1990; Knowlton *et al.*, 1993; Karns *et al.*, 1995). In summary, there is substantial data to indicate that α_1-adrenergic stimuli can mediate cardiac myocyte hypertrophy *in vitro*.

1.1.3.3 The Renin-Angiotensin System

Accumulating evidence suggests that angiotensin II may be a critical factor mediating cardiac hypertrophy *in vivo*. In experimental models of hypertension, most studies show that ACE inhibitors and angiotensin II receptor antagonists suppress both the rise in blood pressure and cardiac hypertrophy. Treatment of rats having aortic coarctation with an ACE inhibitor or angiotensin II type I receptor antagonists (losartan and TCV116) decreases blood pressure and prevents (or causes regression of) LVH by pressure overload (Baker *et al.*, 1990; Bruckschlegel *et al.*, 1995; Kojima *et al.*, 1994). Similarly, cardiac hypertrophy that occurs in rats with aortocaval shunt and volume overload can be prevented with losartan or an ACE inhibitor (quinapril) with high affinity for cardiac tissue ACE (Ruzicka *et al.*, 1993; Ruzicka and Leenen, 1995). In SHR (Pfeffer *et al.*, 1982; Korner and Bobik, 1995; Lundie *et al.*, 1997) or rats with experimentally produced renovascular hypertension (Sen *et al.*, 1981; Leenen and Prowse, 1987), administration of an ACE inhibitor not only prevents the development of hypertension and cardiac hypertrophy but also causes regression of established LVH. Although effects of ACE inhibitors on hypertrophy in these models may be secondary to a decrease in pressure, there is evidence to suggest a direct effect of angiotensin II *in vivo*. Chronic infusion of angiotensin II into rats increases LV mass, a response that occurs even when the pressor activity of the peptide is blocked (Khairallah and Kanabus, 1983). Using a similar model but one in which a subpressor dose of angiotensin II is chronically

(1-2 weeks) infused into rats, Morgan and Baker (1991), found an increase in LV-to-body weight ratios in the animals receiving angiotensin II, even in the absence of blood pressure differences between sham-operated controls and experimental animals.

Treatment of cultured neonatal rat cardiac myocytes with exogenously applied angiotensin II acting through the angiotensin II type I receptor induces the hypertrophic phenotype: i) cellular hypertrophy, ii) the protooncogenes *c-fos*, *c-myc*, *c-jun* and *Egr-1*, and iii) the fetal genes skeletal α-actin, β-MHC and ANF (Sadoshima and Izumo, 1993). These results suggest a trophic action of angiotensin II in the induction of cardiac hypertrophy *in vivo* and *in vitro*.

1.1.3.3.1 The Renin-Angiotensin System and Stretch-Induced Hypertrophy

The role of angiotensin II as a critical mediator of stretch-induced hypertrophy has been shown in the neonatal rat cardiac myocyte system *in vitro*. Mechanical stretch of neonatal rat cardiac myocytes in serum-free culture results in a more than 100-fold increase in angiotensin II concentrations and a recapitulation of the hypertrophic phenotype (Sadoshima *et al.*, 1993). Angiotensin II type I receptor antagonists inhibit the stretch-induced hypertrophic phenotype (Sadoshima *et al.*, 1993).

There is clear evidence for the existence of a cardiac renin-angiotensin system (Lindpainter and Garten, 1991; Baker *et al.*, 1992; Dostal *et al.*, 1992) and its up-regulation chronically in load-induced hypertrophy *in vivo*. mRNA expression of angiotensinogen, renin, ACE, and angiotensin II receptors are all upregulated in cardiac hypertrophy caused by pressure overload and ischemia (Schunkert *et al.*, 1990; Baker *et al.*, 1992; Suzuki *et al.*, 1993; Zhang *et al.*, 1995; Mukawa *et al.*, 1997). At the protein level, there is an upregulation of ACE and angiotensin II receptor binding (Suzuki *et al.*, 1993), and the percent of myocytes containing renin, angiotensin I, and angiotensin II

significantly increase in hypertrophied hearts (Zhang *et al.*, 1995). However, it is not clear whether pressure overload itself rather than angiotensin II is the predominant cause of hypertrophy (Mohabir *et al.*, 1994; Mukawa *et al.*, 1997). In this regard, using the angiotensin II type I knockout mice, aortic constriction induced the expression of the immediate early genes in the heart and an increase in the heart:body weight ratio (Harada *et al.*, 1996). Cardiac volume overload by aortocaval shunt induces initially, an increase in cardiac renin mRNA and cardiac renin activity (Boer *et al.*, 1994), followed by increases in cardiac ACE mRNA and cardiac ACE after 7 days (Iwai *et al.*, 1995), and an increase in angiotensin II receptor density in hypertrophied cardiomyocytes 28 days post aortocaval shunt (Fareh *et al.*, 1996). In cardiac volume overload, cardiac angiotensin II generation appears to depend on cardiac ACE (Ruzicka *et al.*, 1995). Whether an increase in angiotensin II in response to pressure overload is indeed of cardiac origin remains to be determined. The *in vitro* data suggests that the renin-angiotensin system may be involved in stretch-induced hypertrophy through the trophic actions of angiotensin II.

1.1.3.4 Peptide Growth Factors

In neonatal rat cardiocytes in culture, in addition to angiotensin II, other peptides such as type β transforming growth factors induce ($TGF\beta_1$), platelet-derived growth factor, (PDGF), epidermal growth factor (EGF), fibroblast growth factors (a and b FGFs), endothelin and insulin-like growth factor have been shown to induce the hypertrophic phenotype: i) myocyte hypertrophy ii) the protooncogenes *c-fos*, *c-myc*, *Erg-1*, and *c-jun* and iii) provoke a pattern of fetal gene expression including skeletal α-actin, β-MHC and ANF (Parker and Schneider, 1991).

1.2 Arterial Hypertrophy

One of the consequences of hypertension is an increase in vascular resistance and reactivity and as suggested by Folkow (1982), can be explained by structural alterations of the arterial wall. These structural alterations include an increase in wall thickness which results in an increase in the medial wall to lumen ratio and an encroachment of the medial wall into the lumen (Folkow *et al.*, 1970; Folkow, 1982, 1990). Histologic and morphologic studies of arteries from hypertensive humans and animal models of hypertension have documented the presence of medial wall hypertrophy and an increase in the media to lumen ratio in arteries from various vascular beds (Aalkjaer *et al.*, 1987; Lee *et al.*, 1983, 1989; Lee and Triggle, 1986; Ono *et al.*, 1989). Encroachment of the medial wall into the lumen resulting in a permanent narrowing of the lumen diameter has been an inconsistent finding.

1.2.1 Hypertrophy, Hyperplasia or Remodeling

In renal hypertensive rats, as well as in genetic models of chronic hypertension such as the SHR and the Dahl rat, thickening of the media of large elastic arteries (e.g. aorta - arteries with a diameter greater than 500 μm) occurs mainly through smooth muscle cell hypertrophy (increase in cell mass) accompanied by DNA replication (Lee *et al.*, 1983; Lee and Triggle, 1986; Ono *et al.*, 1989). The smooth muscle cells which replicate their DNA do not undergo division, resulting in a high frequency of large polyploid cells in the media (Owens *et al.*, 1981; Owens and Schwartz, 1982; Owens, 1987). Polyploidy is the increase in DNA content within a cell, such that instead of the normal diploid DNA content, some cells may contain twice (tetraploid) or four times (octaploid) the normal amount of DNA in each cell. In contrast, an increase in medial

area in the large elastic arteries of deoxycorticosterone-NaCl (DOCA) hypertensive rats is due to hyperplasia (increase in cell number) of the smooth muscle cells (Lee *et al.*, 1989). In resistance vessels (500 μm in diameter or less) of the SHR, Dahl, DOCA as well as renal hypertensive rats thickening of the media occurs through a hyperplastic (increase in cell number) response (Owens and Schwartz, 1982; Lee *et al.*, 1983, 1989; Lee and Triggle, 1986; Ono *et al.*, 1989).

The processes of hypertrophy and hyperplasia already described clearly involve growth. However, an increased media-to-lumen ratio can also be brought about by rearranging the existing material around a smaller lumen, without a need to invoke a growth response or change in media cross-sectional area. This process has been defined as remodeling (Baumbach and Heistad, 1989). As originally described by Baumbach and Heistad (1989), remodeling defines a developmental process which is distinct from growth, but can also take place in conjunction with the growth process. To quantify the remodeling process, Baumbach and Heistad (1989), have defined a remodeling index, which is the ratio of the calculated change in lumen diameter (normotensive lumen diameter minus calculated remodeled lumen diameter) to the observed difference in lumen diameter (normotensive lumen diameter minus hypertensive lumen diameter). In this regard, Heagerty *et al.* (1993), have reviewed a number of *in vitro* investigations (Aalkjaer *et al.*, 1987; Schiffrin *et al.*, 1993; Korsgaard *et al.*, 1993) of subcutaneous small arteries taken from the gluteal region of essential hypertensive patients and when compared with age- and sex-matched control subjects, showed an increased media-to-lumen ratio. In some cases, there is a tendency towards a small amount of growth, but in all cases the external diameter is less in the vessels from hypertensive individuals, indicating remodeling. Heagerty *et al.* (1993) calculated the remodeling and growth indices of a number of small arteries (less than 300 μm in diameter) from genetically hypertensive rats (Mulvany *et al.*, 1985; Baumbach and Heistad, 1989; Bund *et al.*, 1991;

Deng and Schiffrin, 1992) and reported that, in addition to hyperplastic growth, remodeling also plays an important role. Similar findings have been reported concerning mesenteric small arteries (less than 300 μm in diameter) from transgenic hypertensive rats harboring the mouse Ren-2 gene (Thybo *et al.*, 1992).

The available evidence suggests that the increased media-to-lumen ratio of small arteries (less than 300 μm in diameter) that is seen in essential hypertension and in several animal models of hypertension may be due to remodeling rather than growth. In larger muscular and elastic arteries the evidence for remodeling is lacking. The mechanisms that are responsible for remodeling in hypertension are unknown.

1.2.2 Stimuli to Arterial Hypertrophy

Little is known about the mechanisms responsible for the increase in media thickness in essential hypertension as well as hypertensive animal models. In part, the increase in media thickness appears to be induced by elevated pressure itself and may represent a compensatory mechanism activated by the elevated wall shear stress (reviews - Folkow, 1982; Mulvany and Aalkjaer, 1990; Cowan and Langille, 1996). Lee and Smeda (1985), have suggested that hyperplasia of medial smooth muscle cells is a primary response related to the development of hypertension, whereas hypertrophy of the smooth muscle cells is a secondary adaptive response that can be reversed with antihypertensive treatment. The extent to which regression of hypertrophy is achieved depends on the mode of action of the antihypertensive drug. There is evidence from *in vivo* and *in vitro* data that the sympathetic nervous system and angiotensin II induce trophic effects in the vasculature.

1.2.2.1 Sympathetic Nervous System

The sympathetic nervous system appears to exert a trophic effect on the vasculature to promote growth and remodeling (Bevan, 1975; Bevan and Tsuru, 1979; Hart *et al.*, 1980). In vascular tissues of SHR, a number of studies have demonstrated an increase in sympathetic nerve activity as characterized by an enhanced histofluorescence for norepinephrine, enhanced neuronal uptake of norepinephrine, enhanced number of sympathetic nerves as determined by morphometric analysis, and enhanced levels of norepinephrine (Grobecker *et al.*, 1975; Nakamura, 1977; Cassis *et al.*, 1985; Head, 1989) when compared with WKY controls. This hypernoradrenergic innervation in SHR is accompanied by an increase in nerve growth factor and nerve growth factor mRNA in the mesenteric and caudal arteries (Ueyama *et al.*, 1992; Zettler and Rush, 1993). Deprivation of sympathetic input during periods of growth, alters the structure of individual vessels (Bevan and Tsuru, 1981; Mangiarua *et al.*, 1986; Mangiarua and Lee, 1992). Lee *et al.* (1991), have shown that neonatal sympathectomy of the SHR with a combined treatment of antinerve growth factor and guanethidine not only prevents the hyperplastic response of the smooth muscle cells in the mesenteric arteries but also prevents the development of hypertension. Korner *et al.* (1993), have reported that sympathectomy of newborn SHR combined with α-adrenergic blockade that does not allow re-innervation of vessels prevents the abnormal increases in vascular reactivity, indicative of vascular hypertrophy. With regard to the specific adrenergic receptor involved in smooth muscle cell growth *in vivo*, studies supporting a role for β-adrenergic stimulation in arterial hypertrophy are inconclusive (Leitschuh and Chobanian, 1987; Owens, 1987; Lee *et al.*, 1991b). However, several studies suggest a role for β-adrenergic stimulation in the incidence of polyploidy in smooth muscle cells of large conducting vessels (Leitschuh and Chobanian, 1987; Lee *et al.*, 1992). In DOCA-salt hypertensive

rats, the β-adrenergic antagonist propranolol inhibits the development of hypertrophy and polyploidy in aortic smooth muscle cells (Leitschuh and Chobanian, 1987). Chronic treatment of SHR with nadolol from gestation to 28 weeks of age attenuates the increase in blood pressure, reduces the incidence of polyploidy in the aorta, but does not prevent the development of medial hypertrophy in the mesenteric arteries (Lee *et al.*, 1991b). In contrast, chronic treatment of young SHR with propranolol has no effect on aortic smooth muscle cell hypertrophy or polyploidy, despite an antihypertensive response (Owens, 1987). Studies assessing the role of α-adrenergic receptors in the development of arterial hypertrophy are lacking.

In *in vitro* conditions, catecholamines have been shown to stimulate the growth of smooth muscle cells (Blaes and Boissel, 1983; Yamori *et al.*, 1987; Erlinge *et al.*, 1994; Siwik and Brown, 1996; Xin *et al.*, 1997). Catecholamines have been shown to induce smooth muscle cell hypertrophy (Siwik and Brown, 1996; Xin *et al.*, 1997), smooth muscle cell polyploidy (Yamori *et al.*, 1987), and smooth muscle cell hyperplasia (Blaes and Boissel, 1983; Erlinge *et al.*, 1994) in culture. With regard to the type of adrenergic receptor involved *in vitro*, both α-adrenergic (Blaes and Boissel, 1983) specifically, the $\alpha_{1A/D}$-adrenergic receptor subtype(s) (Siwik and Brown, 1996; Xin *et al.*, 1997) and β_1-adrenergic (Yamori *et al.*, 1987) receptors have been implicated. Although, available evidence suggests that the sympathetic nervous system exerts a trophic effect on the vasculature, direct stimulation of the pathway by either α- or β- adrenergic receptors has not been well established.

1.2.2.2 The Renin-Angiotensin System

The available data regarding a direct role of the renin-angiotensin system in the development of vascular hypertrophy and remodeling in animal models of hypertension is inconclusive. Under *in vivo* conditions, as a consequence of the effect of angiotensin II to increase blood pressure, it is often difficult to distinguish between its direct and indirect effects on vascular hypertrophy associated with hypertension. In this regard, ACE inhibitors (review - Lundie *et al.*, 1997), and angiotensin II type I receptor antagonists (Oddie *et al.*, 1993; Soltis, 1993; Gillies *et al.*, 1997; Gillies *et al.*, 1998) have been shown to inhibit medial hypertrophy and decrease blood pressure. There is evidence that shows angiotensin II acts as a growth factor through nonpressor mechanisms. In rats, infusion of angiotensin II induces the development of medial hypertrophy in the small mesenteric arteries, in the absence of an increase in blood pressure (Griffin *et al.*, 1991; Stassen *et al.*, 1997; Simon *et al.*, 1998). In SHR, the ACE inhibitor captopril, reduces medial hypertrophy of the aorta, in the presence of elevated pressure (Wang and Prewitt, 1990). In another study, Morishita *et al.* (1994), provide evidence for a direct local effect of angiotensin II. Transfection of the ACE gene into intact rat carotid arteries results in an increase local ACE activity that is associated with an increase in DNA synthesis, protein content and wall/lumen ratio. In the same experiment, administration of losartan prevents the increase in local ACE activity and the associated structural alterations. These *in vivo* studies, suggest a direct trophic effect of angiotensin II in the vasculature.

In vitro data implicates angiotensin II in vascular smooth muscle cell growth. Angiotensin II induces both hypertrophy, hyperplasia and polyploidy of vascular smooth muscle cells in culture (Geisterfer *et al.*, 1988; Berk *et al.*, 1989; Gibbons *et al.*, 1992). Several signal transduction mechanisms appear to be involved in the growth-promoting

effects of angiotensin II. Angiotensin II may promote growth by stimulating multiple tyrosine kinases, including $pp60^{c\text{-}src}$ kinase, focal adhesion kinase, and Janus kinases (review - Berk and Corson, 1997) which in turn induce the expression of the protooncogenes *c-fos, c-myc, c-jun, and Erg1* (Taubman *et al.*, 1989; Naftilan *et al.*, 1989, 1992). *In vitro* evidence suggests that the growth-promoting effect of angiotensin II is mediated by the angiotensin type I receptor and is blocked selectively by losartan at various levels (Naftilan *et al.*, 1989; Gibbons *et al.*, 1992; Lyall *et al.*, 1992). In rat aortic smooth muscle cells in culture, losartan, but not angiotensin type II receptor antagonists, blocks angiotensin II-induced DNA and protein synthesis, intracellular Ca^{++} mobilization, *c-fos* expression and the phosphoinositide-signaling system (Gibbons *et al.*, 1992; Lyall *et al.*, 1992). Angiotensin II is also known to stimulate the expression of PDGF, bFGF, and $TGF\beta_1$ by vascular smooth muscle (Gibbons *et al.*, 1992; Itoh *et al.*, 1993). The sequential activation of protooncogenes and growth factors may explain, in part, the trophic effect of angiotensin II on vascular smooth muscle. Gibbons *et al.* (1992), have shown that the growth response to angiotensin II is dependent on the interactions of PDGF, bFGF, and $TGF\beta_1$. The data suggests that if $TGF\beta_1$ activation is inhibited or minimized, the principle effect of angiotensin is mitogenesis or hyperplasia resulting from the activation of PDGF and bFGF (Koibuchi *et al.*, 1993). The simultaneous activation of $TGF\beta_1$ results in an inhibition of cell proliferation and leads to hypertrophy. Angiotensin II has also been shown to induce transcription and expression of $\alpha_{1A/D}$-adrenergic receptors in aortic smooth muscle cells and thereby potentiating the effects of catecholamines in the cells (Hu *et al.*, 1995). Thus, from the above discussion it is clear that angiotensin II plays an important role in smooth muscle cell growth *in vitro*.

1.2.2.3 Peptide Growth Factors

Growth-promoting effects on vascular smooth muscle have been described for many other vasoconstrictors, including thromboxane, leukotrienes, vasopressin, substance K and serotonin (review - Hahn *et al.*, 1993). In addition, the endothelium produces a number of factors that regulate smooth muscle cell growth including PDGF, bFGF, insulin-like growth factor I, interleukin I, and endothelin (reviews - Dzau and Gibbons, 1991; Luscher, *et al.*, 1992).

1.3 Cardiovascular Connective Tissue Changes in Hypertension

1.3.1 Ventricular Connective Tissue Changes

The nature of the changes in connective tissue proteins of ventricular tissues ins response to hypertension, is controversial. In all studies, there appears to be an increase in the total amount of ventricular collagen (Buccino *et al.*, 1969; Sen *et al.*, 1976; Sen and Bumpus, 1979; Caspari *et al.*, 1977; Medugorac, 1980; Bonnin *et al.*, 1981; Weber *et al.*, 1988; Yang *et al.*, 1997). Some authors report increases in collagen relative to muscle mass as a consequence of hypertension (Buccino *et al.*, 1969; Medugorac, 1980; Weber *et al.*, 1988). Others describe increases in total amounts but not increases in the proportion by weight of collagen in the ventricle (Caspari *et al.*, 1977; Thiedemann *et al.*, 1983). Still others report that the initial response in cardiac hypertrophy is an increase in cardiac muscle mass, and a decrease in the proportion of collagen in the ventricular wall (Lund *et al.*, 1979; Bonnin *et al.*, 1981; Yang *et al.*, 1997), with the proportion of collagen increasing again only with more prolonged hypertension. Different models of hypertension may also show different responses. A rapid increase in the proportion of

collagen in ventricular tissue has been observed in renal hypertensive rats (Averill *et al.*, 1976) and hypertension induced by coarctation (Lund *et al.*, 1979), as compared to more delayed collagen response in the SHR (Lund *et al.*, 1979). Similarly, Caspari *et al.* (1977), observed an increase in the proportion of collagen in human ventricle only in the presence of a valvular lesion. In contrast, to the increases in LV collagen content in models of pressure overload, LV collagen accumulation decreases in response to chronic cardiac volume overload induced by aortocaval shunt (Ruzicka *et al.*, 1994). Thus, the connective tissue response of ventricular tissue to hypertension may depend on the model, severity and duration of hypertension at the time of measurement.

1.3.2 Arterial Connective Tissue Changes

Biochemical and morphometrical studies on vascular connective tissue have shown that arterial hypertrophy in response to hypertension includes increases in the amounts of collagen and elastin resulting in an increase in the stiffness of the vessel wall (Cox, 1981, 1982a). In general, in DOCA-salt and renal hypertensive rats, an increase in blood pressure results in concomitant increases in total elastin and collagen content in the aorta and mesenteric arteries however, the percentage of elastin and collagen may either decrease or remain unchanged (Wolinsky, 1970, 1971; Rorive *et al.*, 1980; Carlier and Rorive, 1985). In SHR, an increase in collagen and elastin content in the aorta and mesenteric arteries is seen in the prehypertensive and hypertensive phases (Olivetti *et al.*, 1982; Bostrom and Fryklund, 1979; Ehrhalt and Ferrario, 1981). In contrast, others (Berry and Greenwald, 1976; Cox, 1979, 1981, 1982a, 1982b; Brayden *et al.*, 1983; Anversa *et al.*, 1984) report no change or a decrease in elastin and collagen content in the aorta, mesenteric, carotid, and tail arteries of SHR, DOCA-salt and renal hypertensive rats in response to an increase in blood pressure.

As described above, the results on quantitative as well as qualitative changes of elastin and collagen content in the arterial wall in hypertension are not consistent among experiments. Wolinsky (1970b), has emphasized the importance of using changes in the absolute amount of elastin and collagen in the arterial wall for comparative studies, instead of the relative amount, or percentages of a component expressed in terms of total dry weight of the tissue. The rationale is that changes seen in the percentage of a component may only reflect changes in the absolute amounts of other components. However, others (Berry and Greenwald, 1976) have emphasized the importance of connective tissue concentration in the arterial wall, since the tension of the arterial wall depends on the relative amount of each component. The relative distribution of cellular and connective tissue components is probably more important for arterial wall function than the absolute amount of each of the vessel wall components, and all components may change in arterial diseases. Therefore, the relative amount (or concentration) of connective tissue seems to be a reasonable parameter to use in trying to understand arterial pathology. Disagreement among findings may also be due to differences in age, severity of hypertension and hypertensive strain used.

1.3.3 Mechanisms Responsible For Stimulating Connective Tissue Changes

Several studies have demonstrated a stimulatory effect of stretch on collagen-producing cells in culture systems. Studies with elastin are lacking. Stretch induces collagen synthesis by cardiac fibroblasts (Carver *et al.*, 1991; Butt *et al.*, 1995b) and aortic vascular smooth muscle cells in culture (Sumpio *et al.*, 1988). In response to stretch, cardiac fibroblasts (Butt *et al.*, 1995, 1995b) and arterial smooth muscle cells (Li *et al.*, 1997, 1998) release a variety of factors including PDGF, TGFβ, bFGF, and angiotensin II that may act via autocrine-paracrine pathways to induce collagen synthesis.

Increasing evidence suggests a major role of the renin-angiotensin system and especially angiotensin II in the stimulation of collagen synthesis. *In vitro*, angiotensin II via the angiotensin type I receptor induces collagen synthesis in cardiac fibroblast (Villarreal *et al.*, 1993; Brilla *et al.*, 1994) and smooth muscle cell cultures (Scott-Burden *et al.*, 1990; Kato *et al.*, 1991). *In vivo*, several studies show that angiotensin II plays an important trophic role in connective tissue synthesis in the cardiovascular system. In normotensive rats, early treatment with an ACE inhibitor suppresses normal accumulation of ventricular and vascular connective tissue (Keeley *et al.*, 1992). In SHR, chronic ACE inhibition prevents collagen accumulation despite the development of hypertension (Albaladejo *et al.*, 1994). Although these observations with ACE inhibitors imply a role for angiotensin II in such processes, mediation of these effects through other mechanisms, such as increased levels of bradykinin, cannot be ruled out.

1.4. Arterial Vasodilators and Cardiovascular Hypertrophy

1.4.1 Arterial Vasodilators

1.4.1.1 Mechanisms of Action

The antihypertensive agent hydralazine exerts its effect by reducing peripheral resistance. A number of studies (Mclean *et al.*, 1978; Jacobs, 1984; Ebeigbe and Aloamaka, 1985) have reported that hydralazine relaxes vascular smooth muscle; although the precise mechanism is not clearly understood. Hydralazine has been shown to relax contractile responses induced by various agents which depend on receptor and non-receptor activation mechanisms, suggesting that it acts at a point beyond receptor activation in the excitation-contraction sequence. Hydralazine-induced relaxation of

contractile responses maybe due to interference with the influx of Ca^{2+} into the smooth muscle cell or its release from intracellular stores (Mclean *et al.*, 1978; Ebeigbe and Aloamaka, 1985). A direct action of hydralazine on the contractile apparatus of smooth muscle cells has also been proposed (Jacobs, 1984). In this regard, hydralazine has been shown to decrease the phosphorylation of the myosin-P light chains (regulators of the actin-myosin interaction), and inhibit the actin activation of Ca^{2+} dependent myosin ATPase. Further investigations using both intact muscle and subcellular components are required to explain fully the hypotensive actions of hydralazine.

Minoxidil-induced arterial vasodilation is mediated primarily by its active metabolite minoxidil sulfate (Ducharme *et al.*, 1973). The mechanism of action by which minoxidil sulfate relaxes vascular smooth muscle is by opening K^+ channels in smooth muscle cells. Several observations have provided support for this conclusion: minoxidil sulfate-induced vasodilation is blocked by K^+ channel blockers, is attenuated by increases in extracellular $[K^+]$, is associated with stimulation of ^{42}K efflux and is associated with inhibition of agonist-stimulated $^{45}Ca^{2+}$ influx (Meisheri *et al.*, 1988; Newgreen *et al.*, 1990). In addition, electrophysiological measurements show that minoxidil sulfate causes hyperpolarization of smooth muscle cell membranes and enhancement of outward K^+ current (Leblanc *et al.*, 1989). The K_{ATP} channel is the likely target for minoxidil sulfate based on the observation that glyburide, a potent and selective inhibitor of K_{ATP} can selectively block in vitro vascular relaxation by minoxidil sulfate (Newgreen *et al.*, 1990). The molecular mechanism(s) by which minoxidil sulfate activates or opens K^+ channels remains to be elucidated.

In addition to its hypotensive action, minoxidil has also been implicated in hypertrichosis in hypertensive patients (Campese *et al.*, 1979) and in the regrowth of hair

in male pattern baldness (Clissold and Heel, 1987). The mechanism(s) by which minoxidil stimulates hair growth and the target cell(s) involved are unknown.

1.4.1.2 Hemodynamics

Normalization of arterial vasoconstriction by the use of arterial vasodilators appears to be a logical approach to the treatment of hypertension. However, the hemodynamic profile induced by the classical arterial vasodilators - hydralazine and minoxidil - has major drawbacks. The decrease in cardiac outflow impedance caused by the arterial vasodilatation increases the ejection fraction of the LV. Furthermore, the decrease in blood pressure reduces arterial baroreceptor afferent activity causing a decrease in vagal tone and an increase in sympathetic tone (Gilmore *et al.*, 1970; DuCharme *et al.*, 1973; Murphy *et al.*, 1982). The net effect is an increase in heart rate and cardiac contractility as well as vasoconstriction (both directly and through stimulation of renin release; Pettinger *et al.*, 1973; Sinaiko, 1980). The increase in sympathetic tone maybe short-lived due to a rapid resetting of the baroreceptors (Salgado and Krieger, 1978). While the arterial vasoconstriction tends to be opposed by the arterial vasodilator, the vasoconstrictor effects on the venous capacitance vessels remain unopposed, resulting in a decrease in venous compliance, and thus an increase in i) central blood volume, ii) venous return, iii) right atrial pressure, and iv) pulmonary artery pressure. In addition, sodium and water retention (as a result of a lowered renal perfusion pressure), an increase in renal sympathetic drive and activation of the renin-angiotensin-aldosterone system may further increase venous return (Sanz *et al.*, 1990). These hemodynamic effects of an arterial vasodilator may result in a hyperdynamic circulation with several consequences:

i) The increase in cardiac output may minimize the anti-hypertensive effect.

ii) Myocardial oxygen consumption and work may increase due to the increases in myocardial contractility, heart rate and venous return.

iii) Cardiac hypertrophy may develop from this increase in work.

1.4.2 Arterial Vasodilators and Cardiac Hypertrophy

The effects of the classical arterial vasodilators (hydralazine or minoxidil) on cardiac hypertrophy have been first described by Sen and co-workers. In normotensive rats, LVH develops following chronic (6 weeks) treatment with the arterial vasodilator minoxidil (Sen *et al.*, 1977). In SHR, LVH and collagen content increase following 2 weeks of treatment with minoxidil despite a marked reduction in systolic blood pressure (Sen *et al.*, 1977). Treatment of SHR with hydralazine normalizes blood pressure but does not affect LVH, whereas the sympatholytic agent α-methyldopa is less effective in lowering blood pressure but decreases LV weight significantly (Sen *et al.*, 1974). Similarly, hydralazine is more effective in preventing the development of hypertension in young SHR, but shows no salutary effect on LVH or collagen content (Sen *et al.*, 1974). In SHR, some regression in LVH is only noted following long-term treatment (6-9) months with hydralazine (Weiss and Lundgren, 1978). An increase in plasma renin activity (PRA), during the initial weeks of arterial vasodilator treatment, in SHR, suggests that the renin-angiotensin system may play an important permissive role in the cardiac effects of the arterial vasodilators (Sen *et al.*, 1974). However, in a later study, Sen *et al.* (1977), showed that renin stimulation is not sustained with chronic administration of minoxidil; whereas PRA levels return to control levels after several weeks of treatment, cardiac hypertrophy progresses. Moreover, Tarazi *et al.* (1982), suggested that most of the

effects of the renin-angiotensin system are mediated through the sympathetic nervous system. An activated renin-angiotensin system may contribute to the development of cardiac hypertrophy via facilitation of norepinephrine release from sympathetic nerve terminals and stimulation of α_1-receptor-mediated cardiomyocyte growth (Starke, 1977; Simpson, 1985; Bohm *et al.*, 1998). Sen and co-workers (Sen *et al.*, 1977; Tarazi *et al.*, 1982; Sen and Tarazi, 1983), assigned a primary role for increased sympathetic activity in the exacerbation of cardiac hypertrophy produced by minoxidil and hydralazine, based on pharmacological intervention studies showing that the association of i) the β-adrenergic antagonist propranolol in a precise ratio to hydralazine to overcome the reflex cardioadrenergic effect of the arterial vasodilator, leads to moderate blood pressure control without an increase in myocardial catecholamine concentration, and a reduction of myocardial hypertrophy (Sen and Tarazi, 1983), ii) the sympatholytic agent methyldopa with minoxidil reverses cardiac hypertrophy in SHR (Pegram *et al.*, 1982; Sen *et al.*, 1977) and prevents the minoxidil-induced cardiac hypertrophy in normotensive rats (Sen *et al.*, 1977; Pegram *et al.*, 1982).

The above studies (Sen *et al.*, 1977; Tarazi *et al.*, 1982; Sen and Tarazi, 1983) are limited in terms of elucidating the importance of cardiac sympathetic activity, or hemodynamic factors such as volume overload, with respect to their effects on ventricular anatomy, as measurements of intravascular volume or sympathetic activity have not been performed. Furthermore, only LV weight has been assessed without evaluation of changes in LV configuration (concentric versus eccentric hypertrophy) or RV weight, which could provide more information on the role of cardiac volume overload in the observed changes in cardiac anatomy.

Studies by Leenen and co-workers (Fenje and Leenen, 1985; Tsoporis and Leenen, 1986; Leenen and Prowse, 1987) have shown that treatment of 2-K, 1-C hypertensive

rats with either hydralazine or minoxidil initially induces a clear antihypertensive effect but subsequently tolerance develops (within 7 days for hydralazine and 14-21 days for minoxidil). PRA tends to increase during the initial weeks of treatment. General sympathetic tone, as assessed by plasma catecholamines, blood pressure response to hexamethonium and heart rate shows no increases at all at any time of follow up (starting at 2 days of treatment). However, cardiac sympathetic activity remains to be determined. Moderate, but significant increases in plasma volume occur after 7-14 days of treatment. With regard to cardiac anatomy, the two arterial vasodilators induce an increase in LV internal diameter and RV weight (after 14 days of minoxidil, and 21 days of hydralazine), followed 7-14 days later with an increase in LV weight. This pattern of changes is indicative of cardiac volume overload resulting in RVH as well as eccentric LVH, superimposed on the concentric LVH resulting from the pressure overload. Similarly, in normotensive humans, receiving topical minoxidil (6 months) as treatment for male pattern baldness, an increase in LV mass is associated with increases in LV end-diastolic volume and cardiac output indicative of cardiac volume overload (Leenen *et al.*, 1988).

1.4.3 Arterial Vasodilators and Arterial Hypertrophy

Studies on arterial structural alterations in response to the arterial vasodilator minoxidil are lacking. Hydralazine started prenatally and continued postnatally until 21-28 weeks of age, prevents the development of hypertension in SHR but does not prevent the structural alterations of the muscular renal arteries (Smeda and Lee, 1988) or the mesenteric vasculature (Smeda and Lee, 1991). Sano and Tarazi (1987), have shown that 12-week hydralazine treatment of 17-week-old SHR does not induce regression of medial hypertrophy of hindlimb resistance vessels despite normalization blood pressure. Jesperson *et al.* (1985), have reported similar findings in mesenteric resistance vessels of

SHR treated with hydralazine from age of 4 weeks to 6 months. Hydralazine treatment of stroke-prone SHR from the age of 3 months to 6 months results in a decrease in blood pressure, but does not attenuate increases in distensibility and remodeling of cerebral arterioles (Hadju *et al.*, 1991). In SHR, Owens (1987), has shown that hydralazine not only prevents the development of hypertension but also aortic medial hypertrophy. These discrepancies are consistent with the observation that the aorta, a rather elastic artery, is mainly sensitive to blood pressure changes, whereas in peripheral arteries, which are rather muscular, hypertrophy maybe modulated mainly by nonhemodynamic factors, such as the sympathetic nervous system. In studies involving multiple therapeutic regimens, treatment of SHR with hydralazine and guanethidine (Sapru and Wang, 1976), or hydralazine, hydrochlorothiazide plus reserpine (Warshaw *et al.*, 1980; Limas *et al.*, 1983) normalizes blood pressure and results in regression of arterial structure. The above studies suggest that both hemodynamic and nonhemodynamic (i.e. sympathetic hyperactivity) may be important factors in determining the structural response of the vessel to the arterial vasodilator hydralazine.

1.5 Hypothesis

Based on the review outlined above and the results (Fenje and Leenen, 1985; Tsoporis and Leenen, 1986; Leenen and Prowse, 1986) obtained so far, it is hypothesized that:

i) Arterial vasodilator treatment is associated with volume overload/intravascular volume expansion and sympathetic hyperactivity.

ii) Cardiac volume overload/intravascular volume expansion play(s) a major role in the development of RVH, eccentric LVH, and arterial hypertrophy, during chronic

treatment of normotensive Wistar and SHR, with the arterial vasodilators hydralazine or minoxidil.

1.5.1 General Research Plan

In order to test the above hypothesis, the following studies have been designed.

1.5.2.1 Part I -Time Relationships

The time-course of changes in i) blood pressure, ii) sympathetic activity (heart rate, plasma catecholamines and organ (RV, LV, superior and large mesenteric arteries) norepinephrine turnover rates), iii) filling pressures [right atrial (RAP), LV end-diastolic (LVEDP), and LV systolic pressures] - as a measure of cardiac volume overload, iv) plasma and blood volumes - as a measure of intravascular volume expansion, v) LV and RV weights, vi) LV dimensions, vii) morphometric measurements of the mesenteric (superior, large, and small), carotid, and basilar arteries, viii) PRA, and ix) arterial connective tissue content (collagen and elastin), will be assessed during long-term treatment (7, 14, 35, and 70 days) of normotensive rats and SHR with the arterial vasodilators hydralazine and minoxidil.

1.5.2.2 Part II - The Effect of Diuretic Therapy

The importance of intravascular volume expansion in the development of the minoxidil-induced cardiovascular structural alterations will be determined by concurrent treatment with hydrochlorothiazide (HCTZ). The diuretic HCTZ inhibits the action of the Na^+-Cl^- cotransporter, thereby inhibiting reabsorption of sodium and chloride in the distal

tubule, promoting fluid loss, and resulting in a decrease in plasma volume, a decrease in extracellular fluid, and a decrease in cardiac output (Frolich *et al.*, 1960; Shah *et al.*, 1978). In this section, the time course of changes of i) blood pressure, ii) sympathetic activity (heart rate and plasma catecholamines), iii) filling pressures (RAP, LVEDP, and LV systolic pressure), iv) LV and RV weights, v) LV dimensions, vi) morphometric measurements of the mesenteric (superior, large, and small) arteries, vii) PRA, viii) plasma and blood volumes will be assessed during long-term treatment (35 and 70 days) of normotensive rats and SHR with minoxidil or HCTZ alone or in combination (minoxidil plus HCTZ).

1.5.2.3 General Considerations

To assess the general applicability of the hemodynamic and cardiac structural alterations observed during treatment of 2-K, 1-C hypertensive rats with the arterial vasodilators hydralazine and minoxidil, the experiments will be repeated in both normotensive Wistar rats and SHR - the animal model that closely resembles human essential hypertension. The normotensive Wistar rat is free of confounding effects of preexisting cardiovascular pathology and therefore represents a pure model for the study of the interrelationships of possible mechanisms involved in the development of cardiovascular hypertrophy. The normotensive WKY rat is the parent stock of the SHR and does not represent a true normotensive strain.

In 2-K, 1-C hypertensive rats, the two doses of each vasodilator - minoxidil (40 and 120 mg/L drinking water) (Leenen and Prowse 1987), hydralazine (80 and 120 mg/L drinking water) (Tsoporis and Leenen, 1986) - induce qualitatively similar alterations in cardiac morphology. Further experiments will continue with only one dose (120 mg/L drinking water). At this dose (120 mg arterial vasodilator/L drinking water) and taking

into account the water intake and the body weight of the animal, the amount of drug the animal receives per day corresponds to antihypertensive doses given to hypertensive humans (Gottlieb *et al.*, 1972). The dose of HCTZ (250 mg/L drinking water) is based on preliminary experiments in normotensive (unpublished observation) and hypertensive rats (Freis and Ragan, 1976) showing that at this dose of HCTZ is able to prevent the intravascular volume expansion observed with arterial vasodilator alone. The treatment periods have been chosen based on the results obtained in 2-K, 1-C hypertensive rats: at 2 days the maximal antihypertensive effect occurs, at 14 days LV dilation and RVH are observed, and at 35 days a further increase in LV mass occurs. A longer treatment period (i. e. 70 days) may be required to observe arterial structural alterations.

1.5.4 Specific Research Protocols

Chapter 3.1 **Arterial vasodilators, cardiac volume load, and cardiac hypertrophy in normotensive rats**. To assess a possible involvement of cardiac volume overload, in the development of RVH and eccentric LVH, during chronic arterial vasodilator treatment, changes in parameters of cardiac volume load (LVEDP, RAP, plasma and blood volumes) in relation to changes in cardiac anatomy, were evaluated during treatment of normotensive rats, with either hydralazine or minoxidil.

Treatment Protocol

i) 7, 14, 35 and 70 Days Treatment (n per treatment period)

Cardiac Dimensions and Hemodynamics

Wistar rats untreated (n=12)

Wistar rats treated with hydralazine, 120 mg/L (n=12)

Wistar rats treated with minoxidil, 120 mg/L (n=12)

ii) 7, 14, 35 Days of Treatment (n per treatment period)

Cardiac Filling Pressures

Wistar rats untreated (n=10)

Wistar rats treated with hydralazine, 120 mg/L (n=10)

Wistar rats treated with minoxidil, 120 mg/L (n=10)

Chapter 3.2 **Arterial vasodilation and cardiovascular structural changes in normotensive rats.** The objectives of these series of investigations were to i) define the alterations in cardiovascular structure [RV and LV weights, LV dimensions, morphometric measurements of the mesenteric (superior, large, and small), carotid, and basilar arteries], during chronic treatment of normotensive rats with minoxidil and ii) examine possible mechanisms i.e. organ sympathetic activity (LV, RV, superior and large mesenteric norepinephrine turnover rates) and volume load (by the addition of the diuretic HCTZ) that may be responsible for the observed alterations in cardiovascular structure in response to chronic minoxidil treatment.

Treatment Protocol

i) 7 Days of Treatment (n per treatment period)

Cardiac Pressures

Wistar rats untreated (n=10)

Wistar rats treated with HCTZ, 250 mg/L (n=10)

Wistar rats treated with minoxidil, 120 mg/L (n=10)

Wistar rats treated with minoxidil and HCTZ (n=10)

ii) 35 and 70 Days of Treatment (n per treatment period)

Hemodynamics and Cardiovascular Dimensions

Wistar rats untreated (n=16)

Wistar rats treated with minoxidil, 120 mg/L (n=16)

Wistar rats treated with HCTZ, 250mg/L or 500 mg/L (n=16)

Wistar rats treated with minoxidil and HCTZ (n=16)

iii) 35 and 70 Day of Treatment (n per treatment period)

Norepinephrine Turnover Rates

Wistar rats untreated (n=18)

Wistar rats treated with minoxidil, 120 mg/L (n=18)

Chapter 3.3 **Effects of the arterial vasodilator minoxidil on cardiovascular structure and sympathetic activity in spontaneously hypertensive rats**. The aim of this study was to evaluate the effects of long-term treatment with minoxidil alone or in combination with the diuretic HCTZ on i) cardiac design (RV weight, LV weight and dimensions) ii) mesenteric (superior, large and small) arterial structure, iii) regional sympathetic activity [cardiac versus mesenteric (superior and large) norepinephrine turnover rates], in SHR with established hypertension.

Treatment Protocol

i) Pre-Treatment Period

Vascular Dimensions

WKY untreated (n=6)

SHR untreated (n=6)

ii) 35 Day Treatment Period

Cardiovascular Dimensions and Hemodynamics

SHR untreated (n=16)

SHR treated with minoxidil, 120 mg/L (n=16)

SHR treated with HCTZ, 250 mg/L (n=16)

SHR treated with minoxidil, 120 mg/L + HCTZ, 250 mg/L (n=16)

iii) 70 Day Treatment Period

Cardiovascular Dimensions and Hemodynamics

WKY untreated (n=16)

SHR untreated (n=16)

SHR treated with minoxidil, 120 mg/L (n=16)

SHR treated with minoxidil, 120 mg/L + HCTZ 250 mg/L (n=16)

Norepinephrine Turnover Rates

WKY untreated (n=18)

SHR untreated (n=18)

SHR treated with minoxidil, 120 mg/L (n=18)

Chapter 3.4 **Arterial vasodilation and vascular connective tissue changes in spontaneously hypertensive rats**. The purpose of this study was to determine the connective tissue response of the arterial (aorta, carotid, renal, and superior mesenteric) wall in SHR during chronic treatment with minoxidil.

Treatment Protocols

70 Days of Treatment

Connective Tissue Content

SHR untreated (n=6)

SHR minoxidil 120 mg/mL (n=6)

Elastase Activity

SHR untreated (n=6)

SHR minoxidil 120 mg/mL (n=6)

1.6 Clinical Relevance of Proposed Studies

Cardiac hypertrophy due to pressure or volume overload is a compensatory mechanism that normalizes left ventricular wall stress (Grossman, 1980). In the long-term, however, it is associated with depressed contractile performance, and ultimately, LV failure. In addition, it exacerbates the clinical manifestations of coronary artery disease, in particular sudden death (Koyanagi *et al.*, 1982; Anderson *et al.*, 1984). Optimal regression of LVH may result in further reduction of the incidence of hypertension-related cardiovascular disease (Koren *et al.*, 1990; Yurenev *et al.*, 1992; Muiesan *et al.*, 1995; Verdecchia *et al.*, 1998). In this regard, in a preliminary report of 166 initially uncomplicated hypertensive patients followed for 5 years, the risk of a cardiovascular event was greater in subjects where LVH developed or persisted than in those where LV mass remained normal or was reduced (Koren *et al.*, 1990). Data from a

preliminary report by Yurenev *et al.* (1992), on 304 patients with documented LVH followed for 4 years in a multicenter study suggests that failure to reduce LVH was strongly related to mortality. A 10-year follow up of 151 patients with uncomplicated hypertension found a 3.5-fold increase in the risk of a cardiovascular event in the group that developed LVH, compared with the risk in the group with normal initial LV mass and no development of LVH, whereas the risk was almost normalized in the subjects where LVH was reduced (Muiesan *et al.*, 1995). More recently, data from a report by Verdecchia *et al.* (1998), on 430 patients followed for an average of 3 years, the risk of developing a cardiovascular event was four times greater in patients who did not have a reduction in LVH than in those with LVH regression.

Animal studies outlined in this thesis may help to elucidate mechanisms involved in i) the persistence of LVH, ii) development of RVH, and iii) alterations in arterial structure with arterial vasodilators. This may help to define more optimal approaches to drug therapy in humans with hypertension, as well as to a better understanding of mechanisms involved in the development/maintenance of LVH, RVH, and arterial hypertrophy.

2.0 MATERIALS AND METHODS

2.1 Animals

Male Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were obtained from Taconic farms (Germantown, NY, USA) at 16 weeks of age. Male Wistar rats weighing 250 to 260 g were obtained from Charles River Breeding Laboratories, Montreal, Canada. For the myocyte culture experiments, pregnant mothers were obtained from the colonies kept at the Animal Facility, University of California, San Francisco. Rats were housed two to a cage and given food (Purina rat chow, St. Louis, MO, USA; 180 μmol Na^+/g food) and water *ad libitum* and kept on a 12-hour light/dark cycle. Following a 5 day acclimatization period, WKY and SHR were randomized into the appropriate experimental groups (n=6-12/group). Fluid intake and body weight were monitored regularly, and the animals were handled twice weekly.

2.2 Hemodynamics

Different groups of rats were treated for either 7, 14, 35 or 70 days depending on the experimental protocol. Two days prior to the end of each treatment period, surgery was performed under halothane-nitrous oxide and oxygen anesthesia. A PE-50 (Clay Adams, Parsippany, NJ, USA) catheter filled with heparinized saline (100 IU/mL) was inserted into the left carotid artery and exteriorized on the neck for monitoring blood pressure and heart rate for the next 2 days (Toal and Leenen, 1983). Hemodynamic studies were performed in the morning under the same environmental conditions in a quiet study room. Blood pressure and heart rate were recorded in conscious, unrestrained animals after 30 minutes of rest. Following the collection of blood samples (see below), the arterial catheters were reconnected to the pressure transducer and blood pressure was

monitored while the animals recovered for 10 minutes. It is important to note that carotid cannulation itself, by promoting underperfusion of the carotid body, may activate the baroreceptor sympathetic reflex and may perturb the accurate measurement of sympathetic activity. In preliminary experiments however, LV norepinephrine concentrations in cannulated (535±35 ng/g LV weight, n=3) versus uncannulated Wistar normotensive rats (555±42 ng/g LV weight, n=3) were not significantly different suggesting that carotid cannulation alone did not induce sympathetic hyperactivity.

2.3 Blood Sampling - Plasma Catecholamines, Plasma Renin Activity, Plasma Potassium, Plasma and Blood Volumes

On the last day of each treatment period, after recording resting blood pressure, the arterial cannula was removed from the transducer and blood samples were taken for the determination of plasma catecholamines by radioenzymatic assay (Sole and Hussain, 1977), PRA by an antibody-trapping technique (Poulsen and Jorgensen, 1974), plasma potassium by dual-channel integrating flame photometer, and plasma and blood volumes by the radioiodinated human serum albumin (^{131}I-labeled RISA) technique (Toal and Leenen, 1983). Following the collection of blood samples, the arterial catheters were reconnected to the pressure transducer and blood pressure was monitored while the animals recovered for 10 minutes. Then in some experiments, hexamethonium (30 mg/kg) was injected through the carotid catheter and maximal decreases in blood pressure were noted. The decrease in blood pressure after hexamethonium ganglionic blockade is influenced by many factors and may not represent an accurate index of sympathetic activity. For this reason, additional indices of sympathetic activity were measured

including heart rate, plasma catecholamines, LV and RV norepinephrine concentrations and turnover rates (see section 2.6).

2.4 Intracardiac Pressures

For the assessment of intracardiac pressures, the left carotid artery and right jugular vein were cannulated and the catheters (PE-50, Clay Adams) were advanced centrally, the venous catheter being positioned in the right atrium for the determination of RAP and the arterial catheter into the LV for LVEDP and LV systolic pressure measurements. The catheters were filled with heparinized saline (100 U/mL) and exteriorized in the neck. Three hours were given to the rats for recovery. This length of time was found to be sufficient for recovery from anesthesia (Kanda and Flaim, 1984). In addition LV cannulation for 1 or 2 days was associated with increasingly severe aortic insufficiency and mortality. Intracardiac pressures were measured in conscious unrestrained animals after 10, 20, and 30 min of rest. The arterial catheter was attached to a pressure transducer, and LVEDP was recorded at an amplification of 5 mm Hg/cm and at a paper speed of 100 mm/s. The venous catheter was connected to a sensitive low-volume-pressure transducer and RAP was recorded at high amplification (50 mmHg/cm) and at a high paper speed. RAP was determined during both inspiration and expiration. The values of RAP reported were those taken at the end of the expiratory phase.

2.5 Cardiac Dimensions

At the end of an experiment, the animals were anesthetized with chloroform. The chest cavity was opened and the heart was excised and immediately placed in ice-cold

saline to arrest the heart in diastole and to remove blood. After removal of the atria and great vessels, the ventricles were blotted dry, and the RV was dissected along its septal insertion from the rest of the ventricular mass (Fenje and Leenen, 1985). After obtaining LV and RV weights, a transverse mid-level slice of the LV was obtained by two transverse cuts at one third and two thirds of the length (Idikio *et al.*, 1983). This slice was viewed under a light microscope using a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8 to 10 points around the circular section, and the average was calculated. The internal diameters of the slices were measured from the distal points of the major (anterior-posterior) and minor (septal-lateral) internal diameters.

2.6 Norepinephrine Turnover Rate

Norepinephrine turnover rate was determined by the decline of endogenous norepinephrine in the LV and RV and superior and large mesenteric arteries after its synthesis had been inhibited by metyrosine (α-methyl-DL-p-tyrosine methyl ester hydrochloride) (Sigma, St.Louis, MO) (Brodie *et al.*, 1966; Sole *et al.*, 1975). Metyrosine was administered at a dose of 200 mg/kg subcutaneously in 0.1 mL/100 g body weight, with a second dose of 300 mg/kg body weight given 4.5 hours later. For each experimental protocol 6-8 rats per group were decapitated at 0, 4.5, and 9 hours after the first dose. Following decapitation, the hearts were quickly removed, rinsed in cold saline, blotted dry, and dissected free of atria and great vessels and the RV and LV separated, and placed on dry ice. Subsequently, the superior and large mesenteric arteries were removed, cleaned of contaminating tissue, and also placed on dry ice. The tissues were stored at -85° C. The frozen ventricles were weighed, and 100 mg each of RV and

LV tissue (cut from the apex) was homogenized in 2 mL of iced 0.25 N acetic acid containing glutathione (1.6 mM) and EGTA (0.65 mM) with a polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The portion of mesenteric artery collected was also weighed and homogenized as described above. The homogenates were centrifuged at 5000 g for 20 minutes at 4°C and stored at -85°C until extraction of the catecholamines. The catecholamines in the homogenates were absorbed onto alumina, extracted with acid, and assayed using high performance liquid chromatography with electro-chemical detection (Sole *et al.*, 1975; Shum *et al.*, 1982). The turnover rate constant (k) was calculated from the rate of decline of the logarithm of the tissue norepinephrine concentration (regression coefficient). Half-life and absolute turnover rate were calculated from the following equations: half-life = 0.693/k and absolute turnover rate = [norepinephrine]*k, where [norepinephrine] is the concentration of norepinephrine in each tissue before inhibition of synthesis.

It is possible that metyrosine on its own, by decreasing the ability of the sympathetic nerves to release norepinephrine, may result in a hypodynamic circulation that in turn would enhance sympathetic drive and may complicate the interpretation of the results. To address this issue, Nakamura *et al.* (1971), measured norepinephrine turnover rates in normotensive and hypertensive rats using to different techniques: i) decline of norepinephrine after blockade of synthesis with metyrosine, and ii) decline of specific activity after injection of ^{3}H-norepinephrine. The results of the two methods were in close agreement under the experimental conditions, indicating that the changes in turnover measured resulted not from the pitfalls inherent to one method, but represented real changes of functional significance.

2.7 Arterial Weight

In several experiments, a number of arteries were sampled for determination of vessel weight per millimeter length. The superior mesenteric artery was excised from its origin in the abdominal aorta to a length of 4-5 mm, and a large mesenteric artery (2-3 mm in length) originating from the 7th branch of the superior mesenteric artery was obtained.

2.8 Arterial Morphometry - Light Microscope Measurements

Animals (n=6 per group) were randomized to the appropriate treatments groups according to the experimental protocol. For morphometric analysis, the arterial beds were fixed by perfusion fixation as previously described (mesenteric arterial bed - Lee, 1985). For perfusion of the upper body, immediately following the start of perfusion of the mesenteric arterial bed, a PE-90 catheter was inserted into the abdominal aorta above the mesenteric arteries and advanced into the thoracic aorta distal to the arch. An incision was made in the right jugular vein to allow drainage of perfusate. Perfusion was nonpulsatile from a pressurized reservoir maintained at 100 mm Hg.

The upper body and mesenteric arterial bed were perfused with Krebs solution (pH 7.4, 37°C) for 10-15 min, followed by 20 min perfusion with 2.5% glutaraldehyde in 0.03 M phosphate buffer and 15 min of phosphate-buffer wash. Methylene blue (0.05 %) in phosphate wash buffer was perfused for 2-3 min following buffer wash to aid in the location of small vessels for dissection. Different categories of arteries (see below) were sampled, fixed again in 2.5 % glutaraldehyde for 45 min, washed in 3 changes of wash buffer for 30 min each, post-fixed in 1% osmium tetroxide for 1 hour, en bloc stained with 0.5% aqueous uranyl acetate for 1 h, dehydrated through ethanol series, and

embedded in Spurr's resin. The fixation procedure used has been shown to result in minimal change in smooth muscle cell volume, and the arteries were in a maximally relaxed state (Lee *et al.*, 1983).

Three categories of mesenteric arteries were examined: 1) elastic, conducting arteries (superior mesenteric artery), 2) muscular, reactive arteries (large mesenteric arteries), and 3) arteriolar resistance arteries (small mesenteric artery). From each rat, the number of arteries sampled was 2 for superior mesenteric artery, 4 for large mesenteric artery and 8-10 for small mesenteric artery. After upper body perfusion, 2 samples of the right carotid artery at the level of the neck were taken. Following dissection of the Circle of Willis, 2 samples of the basilar artery were taken from each rat.

Cross-sectional areas of the vessel wall components (e.g., lumen, intima, media) (Lee *et al.*, 1983) were measured at the light microscope level, using a Laboratory Computer Systems Microplanimeter system (Cambridge, MA). One-micron-thick cross-sectional profiles of the arteries from a Zeiss standard 16 microscope were projected onto a digitizing board through a Zeiss drawing tube (San Antonio, TX). A cursor was used to trace the outline of various wall components. Final magnifications used were X135 for carotid and superior mesenteric arteries, X585 for large mesenteric and basilar arteries, and X935 for small mesenteric artery. Correction for eccentricity of the sections due to oblique sectioning angle was applied (Lee *et al.*, 1983). Analysis of variance showed that variations between vessels of each vessel type from each rat were not statistically significant. Therefore, data from each rat for each parameter of measurement were pooled and the means calculated, so that in the analysis, each rat contributed only one value for each parameter.

2.9 Arterial Morphometry - Electron Microscope Measurements

Ultrathin sections (50-70 nm) of the vessels were cut and subsequently photographed in a Philips EM 300 transmission electron microscope using 35-mm film, and the photographs were printed on standard 8x10 inch photographic paper. A multipurpose grid with a P_T=168, and an area of 363.5 cm^2 was used for quantification. Volume density of medial smooth muscle cells and medial intercellular space were determined. Surface density and volume to surface ratios were also measured for smooth muscle cells. Volume to surface ratio and the number of smooth muscle cell layers were used to determine hypertrophy of the cells as previously described (Lee *et al.*, 1983b).

2.10 Connective Tissue Content

The rats were sacrificed by decapitation and using anatomical landmarks, consistent segments of the following tissues were sampled for elastin and collagen content:

i) carotid artery - from the origin at the innominate artery to the bifurcation of the internal and external carotid arteries.

ii) renal artery - from the origin at the aorta to its bifurcation at the hilum of the kidney.

iii) superior mesenteric artery - from the origin at the aorta to its first branch point.

iv) abdominal aorta - from the level of the diaphragm to the iliac bifurcation.

The arterial segments were cleaned of all adhering fat, blood and loose connective tissue, weighed and stored in aluminum foil at - 20° C until biochemical assessment. Determination of insoluble elastin and collagen involved the methodology of Labourene *et al.* (1990), modified from Todorovich-Hunter *et al* (1988). In brief, the freshly dissected segments were digested in 70% formic acid and 5% cyanogen bromide (CNBr)

for 24 hours at 20°C. The residue was washed with boiling water, and the washings were combined with the CNBr extract and saved. The remaining elastin residue was lyophilized and its weight taken as the total elastin content. The combined CNBr extracts were evaporated to dryness, hydrolyzed, redried, and redissolved in water (10 mg tissue/mL). Total hydroxyproline in the extract was determined by a calorimetric assay (Kivirikko *et al.*, 1967), and total collagen was calculated on the assumption that collagen contains approximately 13% hydroxyproline by weight (Keeley *et al.*, 1984).

2.11 Elastase Activity

The animals were sacrificed by decapitation and the whole length of the abdominal aorta and superior mesenteric artery were removed as described above and placed in aluminum foil at - 80°C until biochemical assessment. Elastase activity was assayed by measurement of the degradation of an insoluble [^{3}H]elastin substrate using a method described by Labourene *et al.* (1990), modified from Leake *et al.* (1983). The arteries were washed in 0.9% saline containing 2 mM methylamine, minced, homogenized and sonicated at a concentration of approximately 100 mg/500 ml of saline-methylamine by use of a Polytron (Brinkmann Instruments, Westbury, New York). The tissue was homogenized and sonicated 3 times for 15 seconds at 10,000 rpm with a pulse bandwidth of 4%. The homogenized tissue was then extracted 3 times, at 4°C for 24 hours, with 0.5 M sodium acetate containing 0.2 M EDTA and 0.02% NaN_3 (pH 4.0 at 4°C). The extracts were combined, dialyzed and lyophilized. The sample was reconstituted with 60% saturated ammonium sulfate solution (39 g/100 mL) containing 4 mM methylamine, agitated for 1 hour, and allowed to sit at 4°C for 18 hours. The insoluble material

containing elastase activity was removed and resuspended into 50 mM Tris buffer at a concentration of 0.57 mg original tissue weight/mL.

The radioactive elastin was prepared by $^{3}HNaBH_4$ reduction of calf ligamentum nuchae (933 cpm/mg elastin). Thirty-five microliters of resuspended enzyme (approximately 20 mg of original tissue weight) was added to 20 µl (23 mg) of washed elastin substrate and to buffer. The enzyme substrate mixture was incubated at 37°C for 18 hours. After the incubation, the samples were centrifuged, and 100 µl of supernatant containing the solubilized reaction products was added to 4 mL of scintillation fluid and counted on a beta counter. A standard curve using human leukocyte elastase (HLE) from sputum was constructed with each assay. During the course of the study, it was found that it was necessary to pool vessels to obtain measurable elastase activity.

2.12 Cell Culture

Cell cultures were prepared from one-day-old rats as described previously (Simpson, 1985). In brief, cells were obtained by alternating cycles of trypsinization and mechanical disaggregation. Cells were combined, washed and preplated in the presence of 5% calf serum to reduce the number of contaminating nonmyocardial mesenchymal cells (NMC's). After 30 minutes, the still suspended myocytes were removed from the attached NMCs, counted and diluted to 200,000 viable (trypan blue-negative) cells/mL in culture medium with 5% calf serum. This cell suspension was distributed into 35-mm culture dishes.

The standard culture medium was medium 199 (Hanks's) supplemented with 1.5 mM vitamin B12 and 50 U/mL penicillin. The medium also contained 0.1 mM bromodeoxyuridine to prevent low-level NMC proliferation.

Cell yield was 4-5 million/heart, of which over 90 % were viable. All cultures were kept at 37°C with humidified air with sufficient carbon dioxide (about 1%) to maintain a pH of 7.3.

2.13 Growth Measurements

On culture day 1, after overnight attachment, the cultures were given 1 mL of serum-free medium containing transferrin and insulin (each 10 mg/mL) and treated with either minoxidil (1-100 μM), or the potassium channel activator and active metabolite of minoxidil - minoxidil sulfate (1-100 μM) (Meisheri *et al.*, 1991), or the α_1-adrenergic agonist phenylephrine (20 μM) (Simpson, 1985). After 3 days of treatment, i) cell number was determined by counting the cells using phase contrast microscopy, (Simpson, 1985), and ii) cell size was quantified either by estimation of cell protein by continuous labeling of cell protein with [^{14}]C-phenylalanine (Simpson, 1985) and by direct measurement of cell protein by spectophotometry (Simpson, 1985).

2.14 Drugs

Minoxidil and minoxidil sulfate (Upjohn), hydralazine (Ciba-Geigy), hydrochlorothiazide (Apotex Inc.), hexamethonium bromide and phenylephrine (Sigma) were used.

2.15 Statistics

Results are presented as means ± SEM. Treated/control ratios (cell culture experiments) were tested for deviation from unity by calculation of confidence limits. Statistically significant differences between groups at a given treatment period or between time periods were analyzed by analyses of variance. The least significant difference approach (Duncan Multiple Range Test) was used to locate significant differences; a p level below 0.05 was considered significant.

3.0 **RESULTS**

3.1 ARTERIAL VASODILATORS, CARDIAC VOLUME LOAD, AND CARDIAC HYPERTROPHY IN NORMOTENSIVE RATS.

J. TSOPORIS, B. YUAN, AND F. H. H. LEENEN. AM. J. PHYSIOL. 256:H876-H880, 1989

Arterial vasodilators, cardiac volume load, and cardiac hypertrophy in normotensive rats

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TSOPORIS, JAMES, BAOXUE YUAN, AND FRANS H. H. LEENEN. *Arterial vasodilators, cardiac volume load, and cardiac hypertrophy in normotensive rats.* Am. J. Physiol. 256 (Heart Circ. Physiol. 25): H876–H880, 1989.—To assess a possible involvement of cardiac volume overload in the development of cardiac hypertrophy during chronic arterial vasodilator treatment, changes in indexes of cardiac volume load in relation to changes in cardiac anatomy were evaluated during treatment of normotensive rats with 120 mg/l hydralazine or 120 mg/l minoxidil, with drinking water. Long-term treatment with hydralazine, but not minoxidil, caused small decreases in systolic blood pressure; neither vasodilator affected heart rate with chronic treatment. Arterial vasodilator treatment for 2 wk or more resulted in increases in plasma and blood volumes by 10–20%. Both arterial vasodilators increased right atrial pressure and left ventricular end-diastolic pressure in the initial weeks of treatment. Only the minoxidil group showed a persistent increase in right atrial pressure throughout the treatment period. These hemodynamic changes were associated with increases in left ventricular (LV) internal diameter and right ventricular (RV) weight, and with minoxidil these changes were also associated with increased LV weight. LV wall thickness did not increase. Cardiac volume overload therefore indeed occurs during treatment with arterial vasodilators and may contribute to their effects on cardiac anatomy (i.e., development of RV hypertrophy and, in the case of minoxidil, also, eccentric LV hypertrophy), which are consistent with cardiac volume overload.

hydralazine; minoxidil; left ventricular end-diastolic pressure; right atrial pressure, right ventricular hypertrophy; eccentric left ventricular hypertrophy

TREATMENT of normotensive or hypertensive humans and rats with arterial vasodilators such as hydralazine or minoxidil results in marked hemodynamic changes, which can be defined as a "hyperdynamic circulation." Acutely, these relate to vagal withdrawal and a reflex-mediated increase in sympathetic activity (15, 17, 23). During long-term administration, fluid and sodium retention (5, 9) contribute to persistence of the hyperdynamic circulation. The volume-expanding effects of minoxidil have been well documented in both clinical and experimental studies (2, 4, 5). Regarding hydralazine, volume expansion has also been reported (4, 9, 29), although exchangeable sodium may not increase (18). Long-term arterial vasodilator treatment is associated with the development of right ventricular hypertrophy (RVH) and, in the case of minoxidil, also eccentric left ventricular hypertrophy (LVH) in both hypertensive (14, 19, 30) and normotensive rats (30). Chronic cardiac sympathetic hyperactivity has been implicated in this effect of arterial vasodilators on the heart (20, 25). However, chronic cardiac volume overload also would induce RVH and eccentric LVH (6). Arterial vasodilators may induce cardiac volume overload by causing intravascular volume expansion, as well as by a shift of blood volume to the central compartment (27). The present study was undertaken to evaluate the extent of cardiac volume overload by the assessment of intracardiac pressures during long-term treatment of normotensive rats with the arterial vasodilators hydralazine or minoxidil.

METHODS

Male Wistar rats weighing 250–260 g (Charles River, Montreal, Canada), were housed two to a cage and were given food (Purina rat chow, 180 μmol Na/g food) and water ad libitum and kept on a 12-h light-dark cycle. After a 5-day acclimatization period, animals were randomized into three groups (n = 10–12 per group): untreated and 120 mg/l hydralazine, or 120 mg/l minoxidil with drinking water (1, 14, 29, 30). Fluid intake and body weight were monitored regularly, and the animals were handled twice weekly.

In separate experiments animals were subjected to either 7, 14, or 35 days of treatment. At the end of each treatment period, surgery was performed under halothane-nitrous oxide and oxygen anesthesia. The left carotid artery and right jugular vein were cannulated and the catheters (PE-50, Clay Adams) were advanced centrally, the venous catheter being positioned in the right atrium for the determination of right atrial pressure (RAP) and the arterial catheter into the left ventricle (LV) for LV end-diastolic pressure (LVEDP) and LV systolic pressure measurements. The catheters were filled with heparinized saline (100 U/ml) and exteriorized in the neck. Three hours were given to the rats for recovery. This length of time was found to be sufficient for recovery from anesthesia (12). In addition LV cannulation for 1 or 2 days was associated with increasingly severe aortic insufficiency and mortality, particularly in minoxidil-treated rats. Intracardiac pressures were measured in conscious unrestrained animals after 10, 20, and 30 min of rest by a direct recording technique by using a method previously described (16). The arterial catheter was attached to a pressure transducer, and LVEDP was

recorded at an amplification of 5 mmHg/cm and at a paper speed of 100 mm/s. The venous catheter was connected to a sensitive low-volume-pressure transducer and RAP was recorded at high amplification (50 mmHg/cm) and at high paper speed (100 mm/s). RAP was determined during both inspiration and expiration. The values of RAP shown were those taken at the end of the expiratory phase.

In a separate series of experiments animals were subjected to either 7, 14, 35, or 70 days of treatment. Two days before the end of each treatment period a PE-50 catheter was inserted into a carotid artery. On the last day of each treatment period, plasma and blood volumes were determined by the radioiodinated serum albumin (^{131}I-labeled RISA) technique, as described previously (28). Under chloroform anesthesia the hearts were excised and immediately placed in ice-cold saline to arrest the heart in diastole and to remove blood. LV and RV weights were determined as described by Fenje and Leenen (1). After weighing, a transverse midlevel slice of LV was obtained by two transverse cuts at one-third and two-thirds of the length (10). This slice was viewed under a light microscope with the use of a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8–10 points around the circular section, and the average was calculated. The internal diameters of the slices were measured from the furthest points of the major (anterior-posterior) and minor (septal-lateral) internal diameters.

Results are presented as means ± SE. Statistical significant differences between groups at a given treatment period were analyzed by analysis of variance. The least significant difference approach was used to locate significant differences; $P < 0.05$ was considered significant.

RESULTS

Parameters of Cardiac Volume Load

Left ventricular systolic pressure (Fig. 1). Chronic treatment with hydralazine, but not minoxidil, resulted in small decreases in LV systolic pressure.

LVEDP (Fig. 1). Arterial vasodilator treatment for 7 and 14 days induced a significant ($P < 0.05$) increase in LVEDP. After long-term (5 wk) treatment only a minor [nonsignificant (NS)] increase persisted.

RAP (Fig. 1). Hydralazine induced a significant ($P < 0.05$) increase in RAP only after 7 days of treatment. Minoxidil significantly increased RAP after 7, 14, and 35 days of treatment. Minoxidil induced more marked increases in RAP compared with hydralazine after 7 and 35 days of treatment.

Heart rate (Table 1). Treatment with either hydralazine or minoxidil for 7, 14, or 35 days did not change heart rate.

Plasma and blood volumes (Table 1). A significant increase in plasma volume was seen after 14 days of either treatment, persisting with prolonged treatment, and was similar for the two vasodilators. Blood volume showed a similar time course, but did not reach significance until 35 days of treatment. Minoxidil treatment for 70 days showed a persistent increase in both plasma and blood volume.

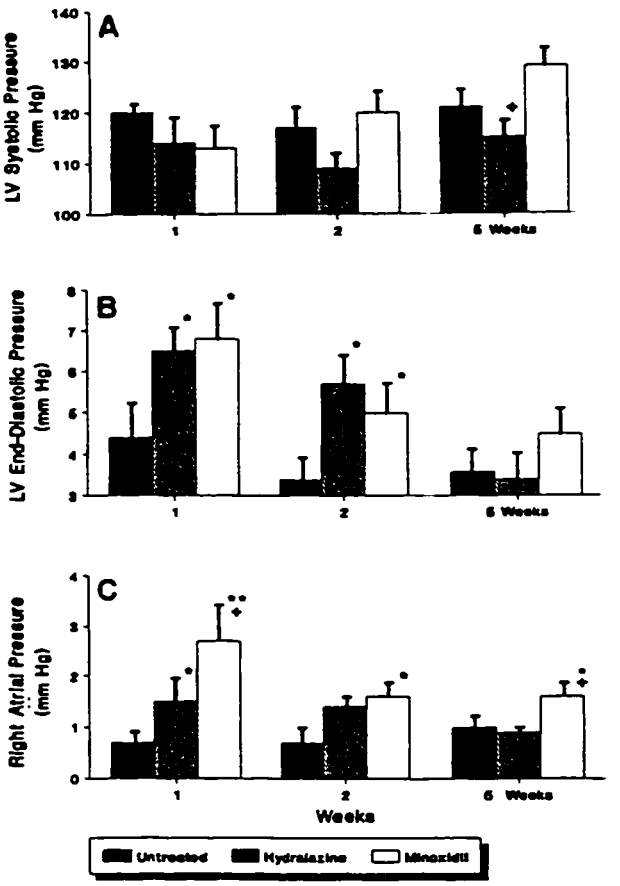

FIG. 1. Left ventricular (LV) systolic pressure (*top*), LV end-diastolic pressure (*middle*), and right atrial pressure (*bottom*) of untreated and minoxidil- (120 mg/l drinking water) or hydralazine-treated (120 mg/l drinking water) normotensive groups. Bars are means ± SE (n = 7–12/group). * $P < 0.05$, ** $P < 0.01$, treated vs. untreated groups; ‡ $P < 0.05$, hydralazine vs. minoxidil.

Cardiac Anatomy

LV and RV weight (Table 2). Seven days of treatment did not change LV or RV weight, normalized for body weight. Two wk of treatment with minoxidil increased both LV and RV weight significantly. Long-term (5 wk) minoxidil treatment resulted in a further ($P < 0.01$) increase. Minoxidil treatment for 70 days resulted in no further increases in LV or RV weight. Hydralazine significantly increased RV weight but only after more prolonged treatment (35 days). LV weight was increased only slightly (not significant) by hydralazine. LV weight was significantly ($P < 0.05$) larger in the minoxidil vs. hydralazine group at both 2 and 5 wk of treatment. RV weights did not differ significantly between the two treatments except for a small ($P = 0.07$) difference at 2 wk.

LV wall thickness and dimensions (Table 2). LV wall thickness showed only minor changes during chronic treatment with either vasodilator. A small ($P < 0.05$)

TABLE 1. *Heart rate, plasma, and blood volumes in untreated and minoxidil- or hydralazine-treated normotensive rats*

Day	Untreated	Hydralazine	Minoxidil
	Heart rate, beats/min		
+7	404±8	392±9	408±14
+14	391±12	398±14	392±8
+35	395±10	390±8	369±11
	Plasma volume, ml/100 g body wt		
+7	3.8±0.1	3.8±0.1	3.9±0.1
+14	3.5±0.1	3.8±0.1*	3.7±0.1*
+35	3.9±0.1	4.5±0.1†	4.5±0.1†
+70	3.5±0.1		4.7±0.1†
	Blood volume, ml/100 g body wt		
+7	7.2±0.3	7.5±0.4	7.4±0.3
+14	6.9±0.1	7.2±0.3	7.0±0.2
+35	7.7±0.2	8.4±0.2†	8.5±0.2†
+70	6.2±0.2		8.7±0.3†

Values are means ± SE (n = 7–12 per group). * $P < 0.05$; † $P < 0.01$ untreated vs. treated groups.

TABLE 2. *Parameters of cardiac anatomy in untreated and minoxidil- or hydralazine-treated normotensive rats*

Day	Untreated	Hydralazine	Minoxidil
	Left ventricular weight, mg/100 g body wt		
+7	180±6	179±5	188±6
+14	167±2	174±3	183±2*‡
+35	175±3	183±4	206±3†‡
+70	180±3		206±4†
	Right ventricular weight, mg/100 g body wt		
+7	47±2	47±2	45±2
+14	42±1	42±1	46±1*
+35	40±1	51±1*	53±1†
+70	42±1		52±2†
	Left ventricular wall thickness, mm		
+7	2.4±0.1	2.5±0.1	2.3±0.1
+14	2.6±0.1	2.5±0.1	2.3±0.1*
+35	2.3±0.1	2.2±0.1	2.4±0.1
+70	2.9±0.1		2.7±0.2
	Left ventricular internal diameter (anterior-posterior axis), mm		
+7	4.2±0.1	4.4±0.1	4.3±0.1
+14	3.7±0.1	4.0±0.1	4.4±0.1*
+35	3.8±0.1	4.6±0.1*	5.3±0.1†§
+70	4.2±0.1		5.9±0.2†

Values are means ± SE (n = 10–12/group). * $P < 0.05$, † $P < 0.01$, untreated vs. treated groups; ‡ $P < 0.05$, § $P < 0.01$, hydralazine vs. minoxidil.

decrease in the minoxidil group was noted at 2 wk.

Treatment with either hydralazine or minoxidil for 7 days did not change LV internal diameters. Minoxidil treatment for 2 wk induced a significant increase in the major (anterior-posterior) LV internal diameter and a more marked ($P < 0.01$) increase after 5 and 10 wk. Hydralazine treatment for 2 wk did not affect the major LV internal diameter, but after 5 wk a significant increase did occur. After 5 wk of treatment, the increase induced by minoxodil was significantly ($P < 0.01$) larger than the one caused by hydralazine. The minor (septal-lateral) LV internal diameter followed a similar pattern, reaching values of 4.2 ± 0.1, 3.8 ± 0.1, and 3.1 ± 0.1 mm at 5 wk for the minoxidil, hydralazine, and untreated groups, respectively.

Body weight and water intake. The untreated and treated groups showed a similar pattern of weight gain and water intake. In the 5-wk experiment, body weights increased from ~250 g at the beginning to 327 ± 6, 334 ± 7, and 338 ± 8 in the untreated, 120 mg/l hydralazine and 120 mg/l minoxidil groups, respectively. At the end of the 5-wk experiment, water intake amounted to 22 ± 2, 24 ± 1, and 23 ± 1 ml·animal^{-1}·day^{-1} in the untreated, 120 mg/l, hydralazine and 120 mg/l minoxidil groups, respectively (resulting in a drug intake of 8.6 ± 1.1 and 8.5 ± 1.0 mg·kg^{-1}·day^{-1}).

DISCUSSION

Long-term administration of either hydralazine or minoxidil to normotensive rats significantly affected both cardiac anatomy and cardiac filling pressures. In confirmation of previous studies (11, 21, 26), minoxidil increased LV weight, whereas hydralazine caused only a small (NS) increase. Both vasodilators significantly increased RV weight and LV internal diameters (later and less by hydralazine) but not LV wall thickness. A similar pattern of changes in cardiac anatomy occurs after chronic treatment of spontaneously hypertensive rats (SHR) or two-kidney, one-clip (2K,1C) hypertensive rats (14, 29, 30). An increase in LV weight associated with dilatation of the LV and little change in LV wall thickness is called eccentric hypertrophy, in contrast to concentric hypertrophy (6), and is generally considered to result from chronic cardiac volume overload (6). Arterial vasodilators may cause cardiac volume overload both by intravascular volume expansion and by a shift of blood from the peripheral to the central blood volume (27).

Renal volume retention may result from a lowered renal perfusion pressure and/or activation of the renin-angiotensin system and renal sympathetic nerves (4). The volume-expanding effects of minoxidil and hydralazine have been well documented in both clinical and experimental studies (4, 5, 14, 30). For example, in hypertensive humans, after 1 wk of minoxidil therapy plasma volume had increased by 783 ml, despite concomitant β-blocker and diuretic therapy and a blood loss of 200 ml for laboratory studies. After 2 mo plasma volume had returned toward normal (5). In acutely saline-loaded SHR and normotensive rats, minoxidil reduced sodium excretion by 70% (4). In 2K,1C hypertensive rats and SHR, long-term treatment with either hydralazine or minoxidil induced significant increases (10–20%) in both plasma and blood volumes (14, 29, 30). However, total exchangeable sodium may not necessarily follow a similar pattern (18). In the present study, in normotensive rats volume expansion was noted after 2 wk of treatment and long-term treatment with either hydralazine and minoxidil resulted in similar increases (15–20%) in both plasma and blood volumes.

To substantiate that cardiac volume overload indeed occurred during long-term treatment with arterial vasodilators, cardiac filling pressures, specifically LVEDP and RAP, were measured as indexes of cardiac volume load. The use of LVEDP and RAP as determinants of

cardiac volume overload has been employed by a number of investigators (3, 16). However, only a few studies (mainly clinical) have assessed the effects of arterial vasodilators on cardiac filling pressures (13, 22, 27). In hypertensive humans, acute therapy with either hydralazine or minoxidil alone or in combination with a β-blocker was associated with a marked rise in pulmonary artery pressure, cardiopulmonary blood volume, and cardiac output (27). Klotman et al. (22) reported no changes in pulmonary artery pressure or right atrial pressure after 5 or 60 days of minoxidil treatment concomitant with hydrochlorothiazide and propranolol therapy in hypertensive humans. In SHR and normotensive rats, hydralazine did not affect LVEDP after 6 mo of treatment (22). No previous studies have assessed the time course of changes in cardiac filling pressures in relation to changes in cardiac anatomy during chronic treatment with arterial vasodilators.

In the present study both arterial vasodilators induced similar increases in LVEDP in the initial weeks of treatment. During more chronic treatment LVEDP returned to control values and remained slightly increased only with minoxidil. In the case of RAP, minoxidil induced significant increases in this parameter throughout the treatment period. Hydralazine significantly increased RAP only in the initial week of treatment. Moreover, minoxidil induced a more marked increase in RAP in the initial week of treatment compared with hydralazine. These findings indicate that cardiac volume overload indeed occurred in the initial weeks of arterial vasodilator treatment; it was more marked in the case of minoxidil and only persisting with minoxidil. When we compare the time course of changes in blood volume and in filling pressures, a clear dissociation is obvious; i.e., filling pressures increase rapidly after start of treatment and only minoxidil causes a persistent increase in RAP, whereas plasma and blood volumes are not yet increased at 1 wk and are not maximal until 5 wk of treatment with either vasodilator. These results indicate that there is no direct correlation between the intravascular volume expansion and cardiac filling pressures. The initial increase in filling pressures is not associated with increases in plasma and blood volumes and may represent a shift of blood from the peripheral to the central compartment (27). Arterial vasodilator treatment may be causing increased sympathetic drive to the venous circulation resulting in venoconstriction and thus increasing venous return. This effect may be particularly prominent during the initial days of therapy to return blood pressure toward normal (30). In addition, localized arterial vasodilation may affect blood flow to low- vs. high-capacitance beds and thus changing effective circulating blood volume and venous return. Hydralazine and minoxidil may have different effects in this regard. It is possible that hydralazine induced arterial vasodilation, leading to high-capacitance beds, thereby maintaining normal venous return and filling pressures, despite intravascular volume expansion during chronic therapy.

Cardiac volume overload therefore indeed occurred during treatment with the two arterial vasodilators and was prominent during the initial weeks of therapy. Alterations in cardiac design were evident after 2 wk of arterial vasodilator treatment and were maximal by 5 wk. Cardiac volume overload therefore preceded the increase in RV weight and (in the case of minoxidil) LV weight, consistent with cardiac volume overload as the possible trigger mechanism. A similar relationship was observed by Ross and co-workers (19, 24). In dogs, creation of an infrarenal aortocaval shunt increased LVEDP from 10 to a maximum of 20 mmHg 2–3 wk later. Left ventricular end-diastolic dimension increased gradually reaching a plateau 5–6 wk after creation of the shunt. At the same time they noted the occurrence of LVH. In the aforementioned studies, as well as our study, the time of maximal increases in filling pressures does not correspond to the time of maximal increases in cardiac weight. A latency period of 2–3 wk in duration is apparent and may relate to a period where the ventricle undergoes structural alterations.

However, the results obtained with hydralazine cannot be explained solely by the above proposed mechanisms: hydralazine did increase LVEDP and this was followed by an increase in LV internal diameters, but LV weight increased only to a minor (NS) degree. It is possible that the small but persistent blood pressure lowering effect of hydralazine sufficiently lowered afterload to offset the trophic effect of increased preload. Alternatively, one cannot exclude that minoxidil induced not only cardiac hypertrophy related to increased preload but also compensatory hypertrophy secondary to focal myocardial necrosis (7, 8).

After long-term arterial vasodilator treatment cardiac filling pressures returned to control values, whereas increases in RV and LV weight (for minoxidil) persisted. It is likely that changes in cardiac anatomy improved cardiac emptying decreasing filling pressures and that only minor increases in filling pressures are sufficient to maintain the changed anatomy. In addition, other mechanisms may play a role in the maintenance of increased cardiac weight. A number of studies (20, 25) have implicated the cardiac sympathetic nerves as the final common pathway in the induction of adaptive cardiac hypertrophy. In a previous study (30) we have shown that cardiac sympathetic hyperactivity indeed accompanies the development of cardiac hypertrophy and persists during long-term arterial vasodilator treatment.

In conclusion, arterial vasodilator treatment of normotensive rats induces cardiac volume overload (as quantified by increases in LVEDP and RAP) in the initial weeks, thereby possibly initiating the development of RVH and, in the case of minoxidil, also eccentric LVH.

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3.1.6 Synopsis

This study shows that arterial vasodilator treatment of normotensive rats induces cardiac volume overload (as quantified by increases in LVEDP and RAP) in the initial weeks of treatment. Only the minoxidil group shows a persistent increase in RAP throughout the treatment period (35 days). Thus, cardiac volume overload occurs during treatment with arterial vasodilators and may contribute to their effects on cardiac anatomy (i.e. development of RVH and, in the case of minoxidil, also, eccentric LVH), which are consistent with cardiac volume overload.

3.2 ARTERIAL VASODILATION AND CARDIOVASCULAR STRUCTURAL CHANGES IN NORMOTENSIVE RATS.

J. TSOPORIS, N. FIELDS, R. M. K. W. LEE AND F. H. H. LEENEN. AM. J. PHYSIOL. 260:H1944-H1952, 1991

Arterial vasodilation and cardiovascular structural changes in normotensive rats

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TSOPORIS, JAMES, NICHOLAS FIELDS, ROBERT M. K. W. LEE, AND FRANS H. H. LEENEN. *Arterial vasodilation and cardiovascular structural changes in normotensive rats.* Am. J. Physiol. 260 (Heart Circ. Physiol. 29): H1944–H1952, 1991.— In normotensive rats, the arterial vasodilator minoxidil causes right ventricular hypertrophy (RVH) and eccentric left ventricular hypertrophy (LVH). To assess whether this trophic effect of minoxidil extends to the vasculature and to examine possible mechanisms involved, alterations in cardiac and arterial (superior, large and small mesenteric arteries, carotid and basilar arteries) structure were evaluated in relation to changes in indexes of cardiac volume load and cardiac and arterial sympathetic activity during long-term (35 and 70 days) treatment of normotensive rats with minoxidil alone or in combination with the diuretic hydrochlorothiazide (HCTZ). Minoxidil alone increased LV and RV weights, LV internal diameter, and medial area of the superior mesenteric artery but did not affect any of the other arteries evaluated. When combined with HCTZ, long-term minoxidil caused concentric LVH rather than eccentric LVH and no longer increased the medial area of the superior mesenteric artery. Neither treatment had any persistent effect on blood pressure, heart rate, or plasma catecholamines. However, minoxidil significantly increased cardiac and arterial (superior and large mesenteric artery) norepinephrine turnover rates, cardiac filling pressures, and plasma and blood volumes. When combined with HCTZ, short-term (1 wk) minoxidil still increased cardiac filling pressures. However, intravascular volume expansion during chronic treatment was significantly attenuated. These results suggest that chronic cardiac volume load appears to determine the type of cardiac hypertrophy induced by a nonhemodynamic mechanism (possibly cardiac sympathetic activity) activated by minoxidil. Intravascular volume expansion or increased arterial flow appears to be responsible for medial hypertrophy of the superior mesenteric artery, but absence of a trophic response in other arteries suggests that another, local mechanism contributes.

left ventricular hypertrophy; minoxidil; hydrochlorothiazide

HYPERTENSION RESULTS in left ventricular hypertrophy (LVH) and medial hypertrophy of arteries and resistance vessels. However, normalization of blood pressure (BP) by the various classes of antihypertensive agents causes different degrees of regression of cardiovascular structure toward normal. In the case of arterial vasodilators, in the heart, right ventricular hypertrophy (RVH) and eccentric LVH may actually develop during the first weeks of treatment in both normotensive and hypertensive rats (10, 27). Cardiac volume overload and/or cardiac sympathetic hyperactivity has been implicated as possible causal or contributing mechanisms in the arterial vasodilator-induced alterations in cardiac structure (27, 28). Studies on arterial structural alterations in response to the arterial vasodilator minoxidil are lacking. In most studies involving hydralazine, multiple therapeutic regimens have been employed (e.g., in combination with a sympatholytic and/or a diuretic) making the interpretation of the effects of the individual drugs difficult. Treatment of spontaneously hypertensive rats (SHR) with hydralazine plus guanethidine (19) or hydralazine, hydrochlorothiazide (HCTZ) plus reserpine (12, 16, 30) normalized BP and resulted in regression of arterial structure. In contrast, hydralazine alone, started prenatally and continued postnatally until 21–28 wk of age, prevented the development of hypertension in SHR but did not prevent the structural alterations of the renal arteries despite the absence of hypertension (23). Sano and Tarazi (18) showed that 12-wk hydralazine treatment of 17-wk-old SHR did not induce regression of medial hypertrophy of hindlimb resistance vessels (as inferred from changes in minimal perfusion pressure) despite normalization of BP. Jesperson et al. (6) reported similar findings in mesenteric resistance vessels of SHR treated with hydralazine from the age of 4 wk to 6 mo. On the other hand, in deoxycorticosterone acetate-salt hypertensive rats, hydralazine decreased BP as well as the media-to-lumen ratio of the renal resistance vessels (29) and coronary arteries (1). The above studies suggest that the model of hypertension may be an important factor in determining the structural response of the vessel to antihypertensive agents.

Studies on cardiovascular structural alterations in response to antihypertensive therapies in hypertensive rats are confounded by the interactions of several factors, such as changes in intramural pressure, genetic influences on cardiovascular structure, and the effects of the drugs per se. The latter can be studied using normotensive animals. As yet, there are no studies in normotensive animals outlining the structural arterial alterations induced by chronic arterial vasodilation. Thus the principal objectives of the present investigation are *1*) to define the cardiovascular structural alterations during long-term treatment of adult normotensive rats with the arterial vasodilator minoxidil and *2*) to examine possible mechanisms, i.e., sympathetic activity and volume load, that may be responsible for the observed changes in

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cardiovascular structure in response to long-term minoxidil treatment.

MATERIALS AND METHODS

Male Wistar rats weighing 250–260 g (Charles River Breeding, Montreal, Canada) were housed two per cage and given food (Purina rat chow, 180 μmol Na/g food) and water ad libitum and were kept on a 12-h light and dark cycle. After a 5-day acclimatization period, animals were randomized into the different treatment groups, i.e., monotherapy with minoxidil or HCTZ, or a combination of minoxidil and HCTZ. The latter combination was studied to ascertain whether intravascular volume expansion is involved in the minoxidil-induced cardiovascular structural changes. Three experimental protocols were performed. *1*) Effects of treatment with minoxidil, HCTZ, and the combination of minoxidil and HCTZ for 35 and 70 days on hemodynamics, plasma catecholamines, and cardiac anatomy were examined. Cardiac filling pressures were studied after a 7-day treatment period. *2*) Effects of treatment with minoxidil, HCTZ, and the combination of minoxidil and HCTZ for 35 and 70 days on arterial morphology were studied. *3*) Effect of 35- and 70-day minoxidil treatment on catecholamine turnover in left and right ventricles and superior and large mesenteric arteries was studied. In all experiments, fluid intake and body weight were monitored weekly, and the animals were handled twice weekly.

Experiment I: Assessment of Hemodynamics, Plasma Catecholamines, and Cardiac Anatomy

The animals were randomized into the following groups (n = 8–10/group): untreated, minoxidil (120 mg/l; see Ref. 27), HCTZ (250 mg/l; see Ref. 5), or the combination of minoxidil (120 mg/l) with HCTZ (250 mg/l drinking water). To assess whether this diuretic dose exerted the maximal diuretic action for preventing the minoxidil-induced intravascular volume expansion, in the 35-days experiment a HCTZ dose of 500 mg/l was employed as well.

Two days before the end of each treatment period, under halothane-nitrous oxide and oxygen anesthesia, a PE-50 (Clay Adams, Parsippany, NJ) catheter was inserted into a carotid artery to monitor BP and heart rate for the next 2 days as previously described (27, 28). On the final day of each treatment period, after recording resting BP and heart rate, blood samples were taken for the determination of plasma catecholamines by a radioenzymatic assay (24), plasma renin activity (PRA) by an antibody-trapping technique (17), plasma potassium by dual-channel integrating flame photometer, and plasma and blood volumes by the radioiodinated human serum albumin ^{131}I technique (26). Subsequently, under chloroform anesthesia, the hearts were excised and immediately placed in ice-cold saline to arrest the heart in diastole and to remove blood. LV and RV weights were determined as described previously (27). After weights were obtained, a transverse midlevel slice of left ventricle was obtained by two transverse cuts at one-third and two-thirds of the length (27). This slice was viewed under a light microscope using a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8–10 points around the circular section, and the average was calculated. The internal diameters of the left ventricle were measured from the farthest points of the major (anterior-posterior) and minor (septal-lateral) internal diameters.

In the 70-day treatment period, this was followed by sampling of arteries for vessel weight per millimeter length determination. The superior mesenteric artery was excised from its origin in the abdominal aorta to a length of 4–5 mm, and a large mesenteric artery (2–3 mm in length) originating from the 7th branch of the superior mesenteric artery was obtained.

In a separate experiment, the assessment of intracardiac pressures was undertaken to determine the effect of HCTZ on the minoxidil-induced cardiac volume overload. A treatment period of 7 days was chosen, because cardiac filling pressures have been shown to be maximal at this time (28). Intracardiac pressures were determined as previously described (28). In brief, the left carotid artery and right jugular vein were cannulated and the catheters were advanced centrally; the venous catheter was positioned in the right atrium for the determination of right atrial pressure (RAP), and the arterial catheter was positioned in the left ventricle for LV end-diastolic pressure (LVEDP) and LV systolic pressure measurements. After 3 h of recovery (28), intracardiac pressures were measured in conscious unrestrained animals after 10, 20, and 30 min of rest.

Experiment II: Assessment of Arterial Morphology

Animals (n = 6/group) were randomized to the following groups: untreated, minoxidil (120 mg/l), HCTZ (250 mg/l), and the combination of minoxidil and HCTZ and subjected to either 35 or 70 days of treatment. For morphometric analysis, the arterial beds were fixed by perfusion fixation as previously described (mesenteric arterial bed; see Refs. 7, 8). For perfusion of the upper body, immediately after the start of perfusion of the mesenteric arterial bed, a PE-90 catheter was inserted into the abdominal aorta above the mesenteric arteries and advanced into the thoracic aorta distal to the arch. An incision was made in the right jugular vein to allow drainage of perfusate. Perfusion was nonpulsatile from a pressurized reservoir maintained at 100 mmHg.

The upper body and mesenteric arterial bed were perfused with Krebs solution (pH 7.4, 37°C) for 10–15 min, followed by 20-min perfusion with 2.5% glutaraldehyde (GA) in 0.03 M phosphate buffer and 15 min of phosphate-buffer wash. Methylene blue (0.05%) in phosphate wash buffer was perfused for 2–3 min after buffer wash to aid in the location of small vessels for dissection. Different categories of arteries (see below) were sampled, fixed again in 2.5% GA for 45 min, washed in three changes of wash buffer for 30 min each, postfixed in 1% OsO_4 for 1 h, en bloc stained with 0.5% aqueous uranyl acetate for 1 h, dehydrated through ethanol series, and embedded in Spurr's resin. The fixation procedure used has been shown to result in minimal change in smooth muscle cell volume, and the arteries were in a maximally

relaxed state (8).

Three categories of mesenteric arteries were examined: *1*) elastic conducting arteries (superior mesenteric artery), *2*) muscular reactive arteries (large mesenteric arteries), and *3*) arteriolar resistance arteries (small mesenteric artery). From each rat, the number of arteries sampled was 2 for superior mesenteric artery, 4 for large mesenteric artery, and 8–10 for small mesenteric artery. After upper body perfusion, two samples of the right carotid artery at the level of the neck were taken. From each rat after dissection of the Circle of Willis, two samples of the basilar artery were taken.

Cross-sectional areas of the vessel wall components (e.g., lumen, intima, media; see Ref. 8) were measured at the light microscope level, using a Laboratory Computer Systems (Cambridge, MA) Microplanimeter system. One-micron-thick cross-sectional profiles of the arteries from a Zeiss standard 16 microscope were projected onto a digitizing board through a Zeiss drawing tube (San Antonio, TX). A cursor was used to trace the outline of various wall components. Final magnifications used were ×135 for carotid and superior mesenteric arteries, ×585 for large mesenteric and basilar arteries, and ×935 for small mesenteric artery. Correction for eccentricity of the sections due to oblique sectioning angle was applied (8). Analysis of variance showed that variations between vessels of each vessel type from each rat were not statistically significant. Therefore, data from each rat for each parameter of measurement were pooled and the means calculated, so that in the analysis, each rat contributed only one value for each parameter.

Ultrathin sections (50–70 nm) of the vessels were cut and subsequently photographed in a Philips EM 300 transmission electron microscope using 35-mm film, and the photographs were printed on standard 8 × 10 in. photographic paper. A multipurpose grid with the number of test points (P_T) equal to 168 and an area of 363.5 cm^2 was used for quantification. Volume density of medial smooth muscle cells and medial intercellular space were determined. Surface density and volume-to-surface ratios were also measured for smooth muscle cells. Volume-to-surface ratio and the number of smooth muscle cells were used to determine hypertrophy of the cells as previously described (7).

Experiment III: Assessment of Organ Sympathetic Activity

In this series of experiments, animals were randomized into untreated group or group treated with minoxidil (120 mg/l drinking water) for 35 or 70 days. Norepinephrine (NE) turnover rate was determined by the decline of endogenous NE in the left and right ventricles and superior and large mesenteric arteries after its synthesis had been inhibited by metyrosine (Sigma, St. Louis, MO) as previously described (4, 27). In brief, rats from each group were decapitated either before or 4.5 or 9 h ($n = 6$ at each time period) after metyrosine (300 mg/kg ip) administration, with the rats in the 9-h group receiving a maintenance dose of 300 mg/kg 4.5 h after the first injection. After decapitation, the tissues were removed and stored at −85°C until homogenization. The catecholamines were extracted from the homogenates by adsorption onto alumina and were assayed using high-performance liquid chromatography with electrochemical detection. The turnover rate constant (k) was calculated from the rate of the decline of the logarithm of tissue catecholamine concentration. The absolute turnover rate was calculated from the equation: turnover rate = [NE] × k, where [NE] is the concentration in the tissue before inhibition of synthesis.

Statistical Analysis

Values are expressed as means ± SE. Significant differences between multiple groups were analyzed by analysis of variance. The least significant difference approach was used to locate significant differences. For comparison between two groups the Student's unpaired t test was used. P values at 0.05 or less were considered statistically significant.

RESULTS

Cardiac Anatomy

Minoxidil significantly increased both LV (by 30%) and RV (by 35%) weights after 35 days of treatment, with no further increases after 70 days. HCTZ alone did not affect LV and RV weights. Addition of either dose of HCTZ (250 or 500 mg/l) to minoxidil treatment significantly attenuated the increase in LV weight seen with minoxidil alone (+20% vs. +30%), but significant increases in LV weight persisted. Addition of HCTZ to minoxidil did not affect the increase in RV weight caused by minoxidil (Fig. 1).

Minoxidil increased LV internal diameters after both 35 and 70 days of treatment. HCTZ alone did not change LV dimensions. Addition of HCTZ to the minoxidil treatment significantly attenuated the increase in LV internal diameters to the level of the untreated group. Minoxidil alone did not affect LV wall thickness. Combined with HCTZ, minoxidil tended to increase wall thickness, but these changes were not significant (Fig. 2).

Arterial Morphometry (Light Microscopic Measurements)

Superior mesenteric artery. Thirty-five or 70 days of minoxidil treatment had no significant effect on the luminal area. Minoxidil significantly increased the total vessel wall mass expressed as milligrams per millimeter length after 70 days of treatment (Table 1). This increase in vessel wall area was related solely to an increase in medial area observed after 70 (but not yet after 35) days of treatment (Fig. 3). The corresponding ratio (media/lumen) was similarly increased. Other areas (i.e., adventitial) did not change (data not shown). The number of smooth muscle cell layers was not affected by minoxidil treatment.

HCTZ alone resulted in a significant decrease in the medial area. However, because the luminal area tended to decrease as well, the corresponding ratio (i.e., media/lumen) was not significantly affected by HCTZ. Combin-

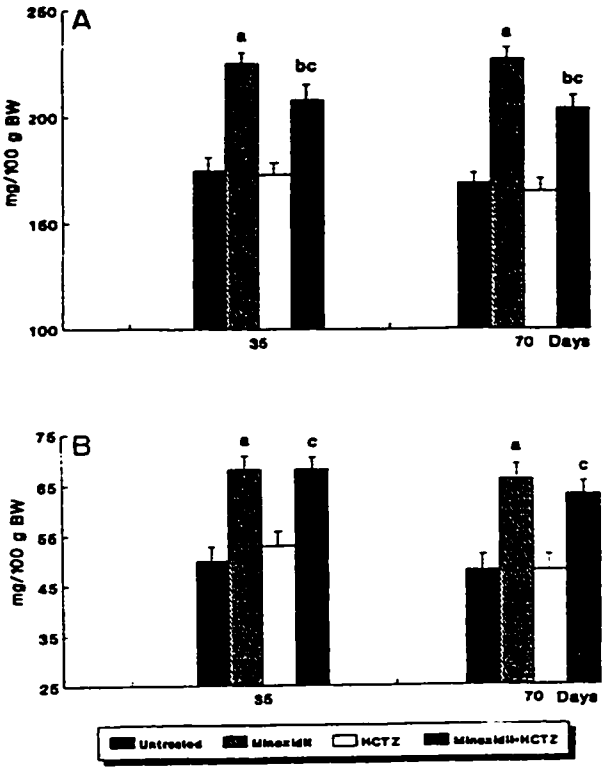

FIG. 1. Effects of minoxidil, HCTZ, or combination of minoxidil and HCTZ on left (*A*) and right (*B*) ventricular weights of normotensive rats after 35 and 70 days of treatment. Bars represent means ± SE (n = 7–10/group). [a,b,c] Significant vs. untreated, minoxidil, and HCTZ, respectively.

ing HCTZ with minoxidil prevented the increases in medial area and corresponding ratio seen with minoxidil alone (Fig. 3).

Large mesenteric artery. Minoxidil significantly increased the luminal area of the large mesenteric artery after 35 and 70 days of treatment (Table 1). Vessel weight and the medial area showed only small, nonsignificant increases. The number of smooth muscle cell layers in the media and the media/lumen ratio were not affected by minoxidil (data not shown).

Carotid, basilar, and small mesenteric artery. Minoxidil did not affect the medial or luminal areas of the carotid, basilar (an example of a cerebral artery), and small mesenteric arteries at 70 days of treatment (Table 1). Other parameters measured were not affected either (data not shown).

Arterial Morphometry (Electron Microscopic Measurements)

Superior mesenteric artery. Minoxidil for 70 days of treatment had no significant effect on the volume densities (vol/unit media vol) of the various components of the media wall (Table 2). However, minoxidil decreased the surface density and increased the volume-to-surface ratio of the smooth muscle cells indicating that the increase in medial area was a result of hypertrophy of smooth muscle cells rather than an increase in intercellular space.

Hemodynamics, Blood Volume, Plasma and Tissue Catecholamines, and PRA

Minoxidil decreases BP and increases heart rate during the initial days of treatment (27), but after chronic treatment no significant effects remain. Chronic treatment with minoxidil and HCTZ did not affect BP and heart rate either (Table 3).

Minoxidil alone induced significant increases in RAP and LVEDP after 7 days of treatment. HCTZ alone had no effect on intracardiac pressures. Minoxidil combined with the diuretic resulted in similar increases in cardiac filling pressures as with minoxidil alone (Table 3).

Plasma and blood volumes were significantly increased after 35 and 70 days of minoxidil treatment. HCTZ alone had no effect on plasma or blood volume. When combined with HCTZ, minoxidil caused only small increases in plasma and blood volume, significantly less than without concomitant diuretic (Table 3).

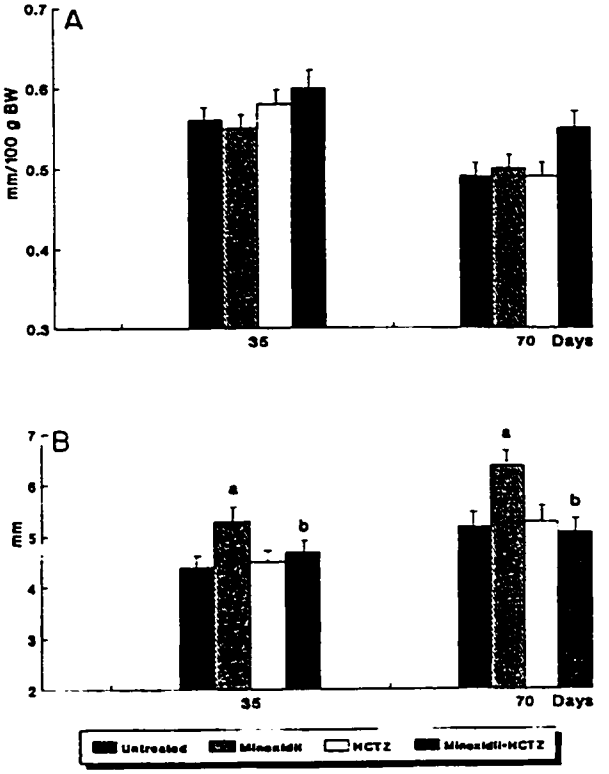

FIG. 2. Effects of minoxidil, HCTZ, and combination of minoxidil and HCTZ on left ventricular (LV) wall thickness (*A*) and LV internal diameter (*B*) of normotensive rats after 35 and 70 days of treatment. Bars represent means ± SE (n = 7–10/group). [a,b] Significant vs. untreated and minoxidil, respectively.

TABLE 1. *Vessel wall dimensions of superior and large mesenteric arteries (35 and 70 days of treatment) and small mesenteric, carotid, and basilar arteries (70 days of treatment) of untreated and minoxidil-treated normotensive rats*

	Untreated	Minoxidil
Luminal area		
Superior mesenteric (10^4 μm^2)		
35 days	72±4	61±5
70 days	55±5	61±5
Large mesenteric (10^3 μm^2)		
35 days	39±4	57±4*
70 days	45±4	58±3*
Small mesenteric (10^3 μm^2)	12±2	12±2
Carotid (10^4 μm^2)	47±4	46±5
Basilar (10^3 μm^2)	54±1	47±1
Vessel wt, mg/mm		
Superior	0.43±0.01	0.50±0.01*
Large mesenteric	0.031±0.003	0.036±0.004
Medial area		
Superior mesenteric (10^4 μm^2)		
35 days	15±1	18±2
70 days	16±2	21±1*
Large mesenteric (10^3 μm^2)		
35 days	10±1	14±2
70 days	14±1	17±3
Small mesenteric (10^3 μm^2)	4.3±0.3	4.3±0.2
Carotid (10^4 μm^2)	12±1	13±1
Basilar (10^3 μm^2)	17±2	12±4
Media/lumen ratio		
Superior mesenteric		
35 days	0.20±0.02	0.29±0.03*
70 days	0.28±0.03	0.40±0.02*
Smooth muscle cell layers in media		
Superior mesenteric		
35 days	6.0±0.3	5.5±0.2
70 days	6.2±0.2	6.5±0.2

Values are means ± SE; n = 6 rats except for smooth muscle cell layer in which n = no. of layers. * Significant vs. untreated.

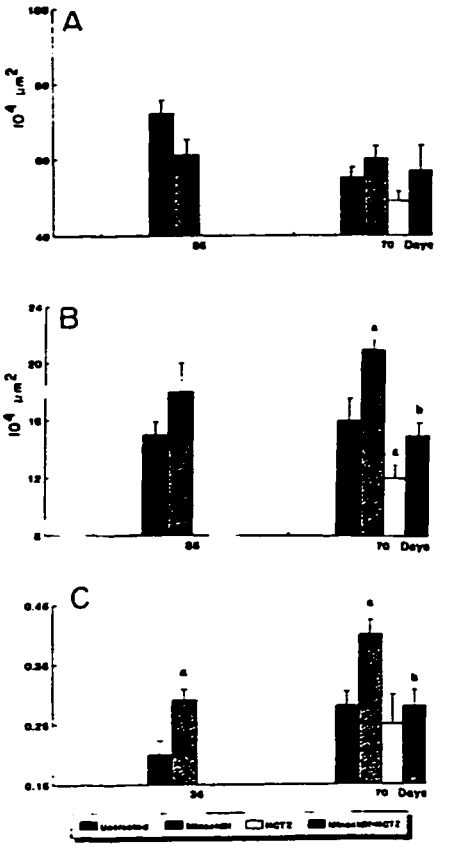

FIG. 3. Effects of minoxidil, HCTZ, and combination of minoxidil and HCTZ on luminal (*A*) and medial (*B*) areas and media/lumen (*C*) ratio of superior mesenteric artery of normotensive rats after 35 and 70 days of treatment. Bars represent means ± SE (n = 6/group) [a,b] Significant vs. untreated and minoxidil, respectively.

Tissue NE concentration (expressed as ng/g tissue) was not affected by either 35 or 70 days of minoxidil treatment. However, per total ventricular weight tissue NE significantly increased in the left ventricle after 70 days of minoxidil treatment (Table 4). Minoxidil treatment for 35 days significantly increased NE turnover rates in the LV and RV as well as in the superior and large mesenteric arteries. After 70 days, significant increases in NE turnover rates persisted in the left and right ventricles and superior mesenteric artery but not in the large mesenteric artery (Figs. 4 and 5, Table 4). In contrast to regional sympathetic activity, plasma catecholamines were not increased by chronic minoxidil treatment (Table 3).

Treatment with either minoxidil or HCTZ alone induced a significant increase in PRA. The combination of minoxidil and HCTZ resulted in a further increase in PRA (Table 3). HCTZ significantly decreased plasma potassium (from 5.4 ±0.1 to 4.0 ± 0.1 mmol/l); this decrease was not influenced by minoxidil.

Body Weight and Water Intake

The untreated and treated groups showed a similar pattern of weight gain, although minoxidil alone tended to increase body weight by 15–20 g. In the 70-day experiment, body weights increased from ~250 g at the beginning to 494 ± 17, 484 ± 20, 508 ± 17, and 476 ± 19 g in the untreated, HCTZ, minoxidil, and the combined HCTZ and minoxidil groups, respectively. At the end of the 70-day experiment water intake amounted to 28 ± 3, 29 ± 2, 27 ± 4, and 30 ± 2 ml in the aforementioned groups, respectively (resulting in a drug intake of 14.9 ±

TABLE 2. *Transmission electron microscopic measurements of media from superior mesenteric arteries of untreated and minoxidil-treated (70 days) normotensive rats*

	Untreated	Minoxidil
Volume density ($\mu m^3/\mu m^3$ media)		
Smooth muscle cells	0.67±0.04	0.75±0.03
Intercellular space	0.033±0.004	0.026±0.003
Elastin	0.12±0.01	0.09±0.01
Collagen	0.19±0.02	0.14±0.02
Surface density ($\mu m^2/\mu m^3$ media)		
Smooth muscle cells	1.3±0.2	0.7±0.1*
Volume/surface ratio ($\mu m^3/\mu m^2$)		
Smooth muscle cells	0.6±0.2	1.2±0.2*

Values are means ± SE; n = 6 rats/group. * Significant vs. untreated.

TABLE 3. *Hemodynamic parameters, plasma and blood volume, plasma catecholamines, and plasma renin activity in untreated, minoxidil, HCTZ, or minoxidil- and HCTZ-treated normotensive rats*

	Day	Untreated	Minoxidil	HCTZ (250 mg/l)	HCTZ (500 mg/l)	Minoxidil + HCTZ (250 mg/l)	Minoxidil + HCTZ (500 mg/l)
Pressure, mmHg							
LV systolic	7	123±4	114±2	116±2		112±3	
LV end-diastolic	7	2.6±0.6	4.9±0.4*	3.1±0.6		4.9±0.6‡	
Right atrial	7	0.2±0.2	1.4±0.2*	0.5±0.2		0.9±0.3‡	
Mean arterial	35	120±5	119±9		111±7	108±6	116±5
	70	114±8	107±9	115±4		114±4	
Heart rate, beats/min	35	429±14	411±15		388±19	396±11	406±7
	70	368±14	375±14	388±12		383±10	
Plasma volume, ml/100 g body wt	35	3.9±0.1	4.5±0.1*		3.6±0.1	4.2±0.1†‡	4.1±0.3
	70	3.3±0.1	4.6±0.1*	3.5±0.1		3.8±0.1†	
Blood volume, ml/100 g body wt	35	7.7±0.2	8.5±0.2*		6.7±0.2	7.2±0.4†	7.2±0.3
	70	6.6±0.3	9.3±0.4*	6.5±0.4		7.4±0.3†‡	
Plasma norepinephrine, pg/ml	70	233±29	241±17	200±13		252±19	
Plasma epinephrine, pg/ml	70	128±10	147±20	149±12		156±20	
Plasma renin activity, ng ANG I·ml^{-1}·h^{-1}	70	10.3±1.4	19.6±2.0*	21.1±2.0*		29.3±3.4†	

Values are means ± SE; n = 7–10 rats. *,†,‡ Significant vs. untreated, minoxidil, and hydrochlorothiazide (HCTZ), respectively.

TABLE 4. *Norepinephrine content and turnover rate in superior and large mesenteric arteries and left and right ventricles of untreated and minoxidil-treated rats*

	35 Days		70 Days	
	Untreated	Minoxidil	Untreated	Minoxidil
Norepinephrine content, ng/g tissue				
Superior mesenteric	5,010±186	4,549±225	4,941±389	4,428±475
Large mesenteric	18,262±977	19,793±1,325	16,650±1,346	15,457±1,237
Left ventricle	536±40	483±34	528±39	530±11
Right ventricle	764±47	645±29	675±45	620±24
Left ventricle*	308±23	364±25	440±33	611±13†
Right ventricle*	125±8	147±7	189±7	208±8
Fractional turnover rate, h^{-1}				
Superior mesenteric	0.08±0.01	0.13±0.02	0.09±0.01	0.12±0.01
Large mesenteric	0.07±0.02	0.08±0.02	0.11±0.01	0.12±0.01
Left ventricle	0.07±0.01	0.12±0.01†	0.08±0.01	0.11±0.01
Right ventricle	0.07±0.01	0.09±0.01	0.08±0.01	0.10±0.01
Absolute turnover rate, ng·g^{-1}·h^{-1}				
Left ventricle	37±3	55±4†	44±3	56±1†
Right ventricle	53±3	60±2†	56±4	63±2

Values are means ± SE; n = 6 rats. * Units are in ng/ventricle. † Significant vs. untreated.

1.8 and 6.4 ± 0.7 mg·kg^{-1}·day^{-1} in the HCTZ and minoxidil groups, respectively; for the group receiving a combination of HCTZ and minoxidil, drug intakes were 15.3 ± 1.5 and 7.3 ± 0.6 mg·kg^{-1}·day^{-1}, respectively).

DISCUSSION

Cardiovascular Structural Alterations

The results of the present investigation show that in normotensive rats, the arterial vasodilator minoxidil not only alters cardiac design but also affects arterial structure. Minoxidil increased the media thickness of the superior mesenteric artery and the lumen of the large mesenteric artery but did not affect the small mesenteric, carotid, and cerebral arteries indicating a lack of parallelism in the arterial response to minoxidil. The increase in media thickness and lumen remained under conditions of maximal arterial relaxation and thus cannot be attributed to artifacts related to differences in contractile state. The increase in the volume-to-surface ratio of the medial smooth muscle cells without a change in the number of cell layers indicates medial hypertrophy rather than hyperplasia.

Studies in normotensive rats addressing the correlation between the effects of antihypertensive therapy on the heart and vasculature are lacking, and most studies in hypertensive rats have selectively examined the effects of arterial vasodilator treatment on either the heart or the vasculature. Most studies (6, 18, 22) show that arterial vasodilators alone cause minimal or no regression of cardiovascular hypertrophy; but when arterial vasodilators are combined with a diuretic (13) a small decrease in cardiac hypertrophy and regression of arterial hypertrophy to normal may occur, and when combined with a diuretic and a sympatholytic agent (16, 30) regression of both cardiac and arterial hypertrophy is possible. These studies implicate both the sympathetic nervous system and volume load as causal or contributory mechanisms in the response of the cardiovascular system to arterial vasodilators.

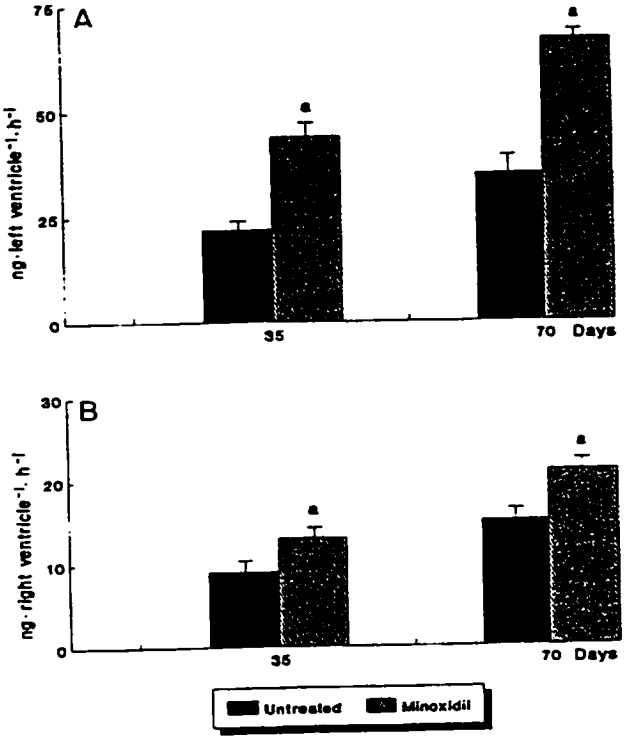

FIG. 4. Absolute turnover rate of norepinephrine in left (*A*) and right (*B*) ventricles of untreated and minoxidil-treated (35 and 70 days) normotensive rats. Bars represent means ± SE (n = 6/group) * Significant vs. untreated.

Volume Factors

In normotensive rats, arterial vasodilators increase cardiac filling pressures in the initial weeks of treatment (present study, see Ref. 28) and cause persistent increases in stroke volume and cardiac output (B. Yuan and F. H. H. Leenen, unpublished observations). Increases in filling pressures precede the volume expansion (28) and are not affected by a diuretic (present study), suggesting that a shift of blood from the peripheral to the central compartment (25) plays a major role. This initial increase in filling pressures may play a role in the initiation of the cardiac trophic response to minoxidil. Chronically, increases in stroke volume and cardiac output persist (likely related now to the intravascular volume expansion), but filling pressures return to normal (28) with development of LV dilation. These persistent increases in stroke volume and cardiac output may thus be prevented and/or blunted by concomitant diuretic therapy. In agreement with previous results in hypertensive rats (13), intravascular volume expansion appears not to be essential for the maintenance of cardiac hypertrophy during arterial vasodilator treatment, because concurrent diuretic treatment prevented most of the increase in blood volume but did not affect the increase in RV weight and only partly inhibited the increase in LV weight. However, concurrent diuretic therapy prevented an increase in left ventricular internal diameter and converted the minoxidil-induced eccentric LVH to concentric LVH. Several explanations are possible for this observation. First, chronic volume expansion and the associated cardiac volume overload may be important with minoxidil alone to maintain filling pressures and diastolic wall stress during chronic treatment, but concomitant diuretic treatment may replace volume overload with another nonhemodynamic mechanism, e.g., (further) increase in sympathetic activity causing concentric hypertrophy (15). Indeed, in hypertensive patients chronic treatment with HCTZ was not associated with regression of LVH but resulted in a decrease in LV internal diameter and an increase in septal wall thickness (31). Alternatively, it is possible that cardiac sympathetic activity is the primary trophic mechanism for minoxidil alone or with concomitant diuretic therapy, whereas volume load determines the pattern of LVH. Increased sympathetic activity can cause both concentric (15) and eccentric (9) hypertrophy, and cardiac volume load possibly plays a modulatory role by determining filling pressures and diastolic wall stress. Studies on the effects of diuretic therapy on cardiac sympathetic activity and central hemodynamics during chronic minoxidil treatment are required to assess these different possibilities. Another trophic mechanism, the renin-angiotensin sys-

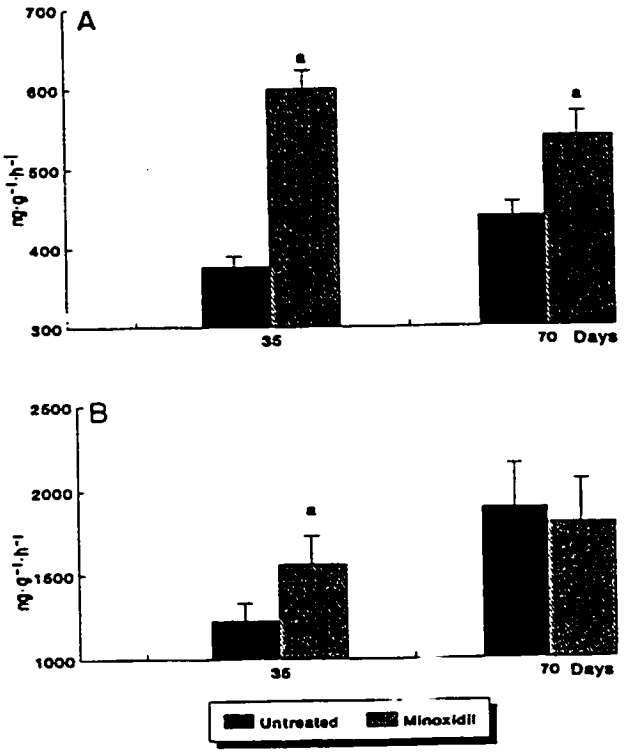

FIG. 5. Absolute turnover rate of norepinephrine in superior (*A*) and large mesenteric (*B*) arteries of untreated and minoxidil-treated (35 and 70 days) normotensive rats. Bars represent mean ± SE (n = 6/group) * Significant vs. untreated.

tem appears at first glance not to play a role, because PRA was similarly increased by minoxidil alone and HCTZ alone, but only minoxidil caused hypertrophy. However, similar plasma renin does not exclude differences in cardiac renin and growth factors relevant for the effectiveness of angiotensin II as a growth promoter.

In contrast to the nonessential role of intravascular volume expansion for the development of minoxidil-induced cardiac hypertrophy, the results suggest that volume expansion may be the trigger mechanism for the development of medial hypertrophy of the superior mesenteric artery, because concurrent diuretic treatment prevented the increase in media thickness of the superior mesenteric artery. The different time course of the development of cardiac hypertrophy (within 3–4 wk) vs. medial hypertrophy of the superior mesenteric artery (within 10 wk) also supports different trophic mechanisms. It is possible that volume expansion or increased cardiac output increases flow through the superior mesenteric artery resulting in vascular growth and that this increased flow was prevented by concomitant diuretic treatment. Mulvany (14) suggested that an increase in flow results in the vascular smooth muscle being stretched thus initiating vascular growth. Supportive evidence that stretch may be initiating vascular growth comes from the studies of Seidel et al. (21) who grafted saphenous veins into a section of saphenous artery, and within 1 wk the lumen diameter of the graft within the artery had increased by nearly 60%, whereas the cross-sectional area had increased by some 70%. Mechanical stretching of fibroblasts also results in mitosis (3). It is tempting to speculate how stretching of the vascular smooth muscle initiates growth. Recent data (32) indicate that hemodynamic shear stress liberates endothelin from endothelial cells, and endothelin has been shown to stimulate protein synthesis in smooth muscle cells (2). It is possible that the trophic effect of minoxidil on the media of the superior mesenteric artery may be mediated by endothelin. However, it remains unclear why medial hypertrophy only occurs in the superior mesenteric artery and not in another elastic artery (carotid artery) or in the muscular arteries (large mesenteric arteries) and arterioles (small mesenteric arteries) assessed. Selective vasodilation by minoxidil or regionally different responses of sympathetic activity (see *Sympathetic Nervous System*) may play a role.

Sympathetic Nervous System

In our previous study (27), as well as in this study, we have shown that the cardiac hypertrophy caused by minoxidil is associated with cardiac sympathetic hyperactivity (as quantified by increases in cardiac norepinephrine turnover rate) without concomitant increases in plasma norepinephrine. Considering that diuretic treatment does not prevent the development and/or persistence of cardiac hypertrophy by arterial vasodilators in normotensive rats (present study), hypertensive rats (13), or hypertensive humans (11), whereas sympatholytics do (22), cardiac sympathetic activity appears to play a primary role in the cardiac trophic responses to arterial vasodilators.

Minoxidil also increased norepinephrine turnover rates in the superior and large mesenteric arteries. Minoxidil increased sympathetic activity to both the superior and large mesenteric arteries after 35 days, but after 70 days sympathetic hyperactivity only persisted in the superior mesenteric artery coinciding with the development of medial hypertrophy in the superior mesenteric artery. Thus the absence of medial hypertrophy in the large mesenteric artery may relate to the absence of persistent sympathetic hyperactivity, suggesting that the arterial hypertrophic response to minoxidil may require both volume expansion and sympathetic hyperactivity. Regarding the possible mechanism causing the arterial sympathetic hyperactivity, it is tempting to speculate that the intravascular volume expansion and higher cardiac output (B. Yuan and F. H. H. Leenen, unpublished observations) increased flow through the mesenteric bed, resulting in increased wall stress and activation of a spinal reflex similar to that observed in the heart (20). If increased flow indeed increased arterial sympathetic activity and the latter played a role in the arterial trophic response to minoxidil, one may expect that concomitant diuretic therapy not only prevented the trophic response but also arterial sympathetic hyperactivity. This experiment still needs to be done.

In conclusion our results show a heterogeneity in the response of the cardiovascular system of normotensive rats to long-term minoxidil treatment i.e., minoxidil-induced RV and eccentric LVH and medial hypertrophy of the superior mesenteric artery but not of other arteries. Concurrent diuretic therapy prevented the medial hypertrophy but converted eccentric LVH to concentric LVH and maintained RVH. These results suggest that in addition to a volume factor other mechanisms, in particular sympathetic hyperactivity, play a role in the minoxidil-induced cardiovascular structural alterations.

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3.2.7 Synopsis

This study shows a heterogeneity in the response of the cardiovascular system of normotensive rats to long-term minoxidil treatment i.e. minoxidil induced RV and eccentric LVH and medial hypertrophy of the superior mesenteric artery but not of other arteries. Concurrent diuretic therapy with HCTZ prevents the medial hypertrophy but converts eccentric LVH to concentric LVH and maintains RVH. These results suggest that chronic cardiac volume load appears to determine the type of cardiac hypertrophy induced by a nonhemodynamic mechanism (possibly cardiac sympathetic hyperactivity) activated by minoxidil. Intravascular volume expansion or increased arterial flow appears to be responsible for medial hypertrophy of the superior mesenteric artery, but absence of a trophic response in other arteries suggests that another local mechanism contributes.

3.3 EFFECTS OF THE ARTERIAL VASODILATOR MINOXIDIL ON CARDIOVASCULAR STRUCTURE AND SYMPATHETIC ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS.

J. TSOPORIS, N. FIELDS, R. M. K. W. LEE AND F. H. H. LEENEN. J. HYPERTENS. 11:1337-1345, 1993

Effects of the arterial vasodilator minoxidil on cardiovascular structure and sympathetic activity in spontaneously hypertensive rats

James Tsoporis*†, Nicholas Fields*, Robert M.K.W. Lee† and Frans H.H. Leenen*

Objective and design: In spontaneously hypertensive rats (SHR) arterial vasodilators do not cause regression and might cause further progression of cardiac hypertrophy. To assess whether these effects extend to the vasculature, and to examine the possible mechanisms involved, cardiac and mesenteric arterial structure was evaluated with respect to changes in cardiac volume load and cardiac and arterial sympathetic activity during long-term (5- and 10-week) treatment of 16-week-old SHR with the arterial vasodilator minoxidil, alone or in combination with the diuretic hydrochlorothiazide.

Results: Despite causing a persistent decrease in blood pressure in SHR, minoxidil further increased left and right ventricular weights and left ventricular internal diameter. In combination with hydrochlorothiazide, minoxidil caused concentric, rather than eccentric, left ventricular hypertrophy. In the mesenteric arterial bed of SHR, minoxidil increased the lumen of the superior mesenteric artery, and prevented further increases in the medial area of the large and small mesenteric arteries. The increase in lumen size of the superior mesenteric artery by minoxidil was abolished when hydrochlorothiazide was added to the treatment. After 10 weeks' treatment with minoxidil, noradrenaline turnover rates were still significantly increased in the left ventricle but were decreased in the mesenteric arteries in the SHR. Minoxidil increased plasma and blood volumes, the increases being largely prevented by concomitant diuretic treatment.

Conclusions: We conclude that there are regional differences in the response of the cardiovascular system to minoxidil in SHR. Some of these differences may be related to differences in regional sympathetic activity, whereas volume load appears to play a modulatory role.

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Keywords: Cardiovascular hypertrophy, minoxidil, diuretic, cardiac sympathetic activity, arterial sympathetic activity, blood volume.

Introduction

Hypertension is associated with left ventricular hypertrophy and medial hypertrophy of conduit and resistance arteries. However, normalization of blood pressure by the various classes of antihypertensive agents causes different degrees of regression of cardiovascular structure towards the normal. Arterial vasodilators, such as minoxidil and hydralazine, are the prototypes of antihypertensive agents which may affect cardiovascular structure adversely. In fact, right ventricular hypertrophy and eccentric left ventricular hypertrophy may develop during the first 5–10 weeks of treatment with

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minoxidil or hydralazine, despite the blood pressure control [1–3]. Similar cardiac structural changes are also induced in normotensive rats [4–6]. Cardiac volume overload and cardiac sympathetic hyperactivity have been implicated as possible causal or contributing mechanisms in these arterial vasodilator-induced alterations in cardiac structure [3,6].

With respect to arterial structural changes, in normotensive Wistar rats minoxidil caused medial hypertrophy of the superior mesenteric artery, but not of other arteries [5]. The effects of minoxidil on hypertension-induced arterial structural changes have not yet been studied, although the effects of hydralazine have been studied, both alone and in combination with a sympatholytic agent. The consistent regression of arterial hypertrophy caused by hydralazine when combined with a sympatholytic agent [7–10], compared with the more variable effects of hydralazine alone [11–14], suggests that, similarly to their effects on the heart, arterial vasodilators also increase arterial sympathetic activity, causing the development or persistence of arterial hypertrophy despite blood pressure control. Such trophic mechanisms may be activated to a different extent depending on the size of the artery (i.e. more active in muscular than in elastic arteries), explaining some of the discrepancies between previous studies [11–14] using hydralazine alone.

In the present study we evaluated the effects of long-term treatment with the arterial vasodilator minoxidil on cardiac design, as well as on the structure of three categories of mesenteric arteries (luminal diameters ranging from 100 to 600 μm) in spontaneously hypertensive rats (SHR) with established hypertension and on regional sympathetic activity (i.e. heart versus mesenteric arteries), and the effects of concomitant administration of a diuretic on minoxidil-induced changes in cardiovascular structure.

Materials and methods

Male Wistar–Kyoto (WKY) rats and SHR, aged 16 weeks, were obtained from Taconic Farms (Germantown, New York, USA). Two rats were housed in each cage, had free access to water and food (Purina rat chow; 180 μmol Na^+/g food; Purina Mills Inc., Richmond, Indiana, USA) and were kept on a 12-h light–dark cycle. Following a 5-day acclimatization period, the rats were randomly assigned to the different treatment groups. Two experimental protocols were performed to investigate the effects of antihypertensive treatment, for 5 or 10 weeks, on haemodynamics, cardiac anatomy and arterial morphology, and the effect of 10 weeks of antihypertensive treatment on catecholamine turnover. WKY rats were used only as untreated normotensive controls. Fluid intake and body weight were monitored regularly and the rats were handled twice a week.

Experiment 1: Assessment of haemodynamics, cardiac anatomy and arterial morphology

SHR were treated with 120 mg/l minoxidil alone or 120 mg/l minoxidil + 250 mg/l of the diuretic hydrochlorothiazide, both in the drinking water [3,15]. The rats were randomly assigned to the following groups: (for the 5-week treatment period) untreated SHR, SHR treated with minoxidil, SHR treated with hydrochlorothiazide and SHR treated with minoxidil + hydrochlorothiazide ($n = 16$ per group); and (for the 10-week period) untreated WKY rats, untreated SHR and SHR treated with minoxidil ($n = 16$ per group).

In 10 rats from each group, 2 days before the end of each treatment period and under halothane–nitrous oxide–oxygen anaesthesia, a PE-50 catheter (Clay Adams, Parsippany, New Jersey, USA) was inserted into a carotid artery to monitor mean arterial pressure and heart rate. On the following day (the final day of the treatment period) resting blood pressure and heart rate were recorded as described previously [3,6], and blood samples were taken for plasma and blood volume determination by the [^{131}I]-human serum albumin technique [16]. Subsequently, under chloroform anaesthesia, the chest cavity was opened, the heart was arrested in diastole by intravenous injection of 1 mol/l KCl, rapidly excised and immediately placed in ice-cold saline to remain in diastole and to remove the blood. Left and right ventricular weights were determined as described previously [3]. After weighing, a transverse midlevel slice of left ventricle was obtained by two transverse cuts at one-third and two-thirds of the length of the ventricle. This slice was viewed under a light microscope using a calibrated (1 mm = 16 units) ocular lens (Macrometer; Olympus, Tokyo, Japan) for measurement of left ventricular wall thickness and internal diameter [3].

The remaining rats (six per group) were used for morphometric analysis of the mesenteric vascular bed. This morphometric analysis was also performed in untreated, 16-week-old SHR and WKY rats (six per group) to assess arterial morphology before initiation of treatment. The mesenteric arterial bed was perfusion-fixed as described previously [17,18]. A flow rate of 1 ml/min per 100 g body weight with perfusion pressures in the range 18–24 mmHg was used to avoid distortion of the luminal size due to high perfusion pressure. The mesenteric arterial bed was perfused with Krebs' solution (pH 7.4, 37°C) for 10–15 min, followed by 20 min perfusion with 2.5% glutaraldehyde in 0.03 mol/l phosphate buffer and a 15-min wash with phosphate buffer. Different categories of arteries (see below) were sampled, fixed again in 2.5% glutaraldehyde for 45 min, washed in three changes of washing buffer for 30 min each,

postfixed in 1% OsO_4 for 1 h, stained (all samples stained together) with 0.5% aqueous uranyl acetate for 1 h, serially dehydrated with ethanol and embedded in Spurr's resin.

Three categories of mesenteric arteries were examined: elastic, conducting vessels (superior mesenteric artery, luminal diameter 500–900 μm; n = 2 per rat); muscular, reactive arteries (large mesenteric arteries, luminal diameter 120–280 μm; n = 4 per rat); and arteriolar resistance arteries (small mesenteric artery, luminal diameter 70–110 μm; n = 8–10 per rat). The average values found for each artery were used for statistical analysis. For consistency, sampling of the arteries was carried out by one person.

Cross-sectional areas of the vessel wall components (i.e. lumen, intima and media) were measured at the light microscope level using an LCS Microplanimeter System (Laboratory Computer Systems, Cambridge, Massachusetts, USA). Cross-sectional profiles (1-μm) of the arteries were projected from a Zeiss standard 16 microscope on to a digitizing board through a Zeiss drawing tube (San Antonio, Texas, USA). A cursor was used to trace the outline of various wall components. Final magnifications used were ×135 for the superior, ×585 for the large and ×935 for the small mesenteric arteries. Correction was made for eccentricity of the sections due to the oblique sectioning angle [18].

To determine whether medial changes reflect a change in smooth muscle cell size or a change in intercellular space, electron microscopic measurements of the large mesenteric artery were made. Ultrathin sections (50- to 70-nm) of the large mesenteric artery were cut and subsequently photographed in a Philips EM 300 transmission electron microscope using 35 mm film, and the photographs were printed on standard 8×10 in. (20.3×25.4 cm) photographic paper. A multipurpose grid, with a total of 168 test points and an area of 363.5 cm^2, was used for quantification. The volume densities of medial smooth muscle cells and medial intercellular space were determined. Surface density and volume : surface ratios were also measured for smooth muscle cells. The volume : surface ratio and the number of smooth muscle cells were used to determine hypertrophy of the cells as described previously [19].

Experiment 2: Assessment of organ sympathetic activity

The rats were randomly assigned to the following groups: untreated WKY rats, untreated SHR and SHR treated with 120 mg/l minoxidil (in their drinking water) for 10 weeks (n = 18 per group). Noradrenaline turnover rate was determined by the decline of endogenous noradrenaline in the left and right ventricles and in the superior and large mesenteric arteries after its synthesis had been inhibited by metyrosine (Sigma Chemical Co., St Louis, Missouri, USA) as described previously [3,4]. In brief, under resting conditions rats from each group were decapitated either before or 4.5 or 9 h after intraperitoneal administration of 300 mg/kg metyrosine (n = 6 at each time point), with the rats in the 9-h group receiving a maintenance dose of 300 mg/kg metyrosine 4.5 h after the first injection. Following decapitation the tissues were removed and stored at −85°C until homogenization. The tissues were homogenized in a solution containing an internal standard (3,4-dihydroxybenzylamine) as well as 1.6 mmol/l glutathione and 0.65 mmol/l ethyleneglyco-bis-(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid. The catecholamines were extracted from the homogenates by absorption on alumina, and were assayed using high-performance liquid chromatography with electrochemical detection. Overall recoveries from homogenization to final analysis were 60–70%. The turnover rate constant was calculated from the rate of decline of the logarithm of tissue catecholamine concentration using linear regression analysis. The absolute turnover rate was calculated as the turnover rate constant multiplied by the concentration of noradrenaline in the tissue before inhibition of noradrenaline synthesis.

Statistical analysis

Values are expressed as means ± SEM. Significant differences between groups were analysed by analysis of variance. The least-significant difference approach was used to locate significant differences. $P \leq 0.05$ was considered statistically significant.

Results

Cardiac anatomy

Minoxidil increased left ventricular weight and internal diameter significantly in the SHR, and left ventricular wall thickness showed a small but significant decrease (Fig. 1). After treatment for 10 weeks (data not shown), the effects of minoxidil on the left ventricle were similar to those after 5 weeks of treatment.

Treatment of SHR with hydrochlorothiazide had no effect on left ventricular weight, internal diameter or wall thickness. When combined with hydrochlorothiazide, minoxidil caused the same increase in left ventricular weight in the SHR as minoxidil alone. However, the increase in left ventricular internal diameter was significantly less than it was without the diuretic, whereas minoxidil combined with hydrochlorothiazide increased left ventricular wall thickness compared with minoxidil alone.

Treatment for 5 weeks with minoxidil increased right ventricular weight significantly, to 96 ± 6 compared

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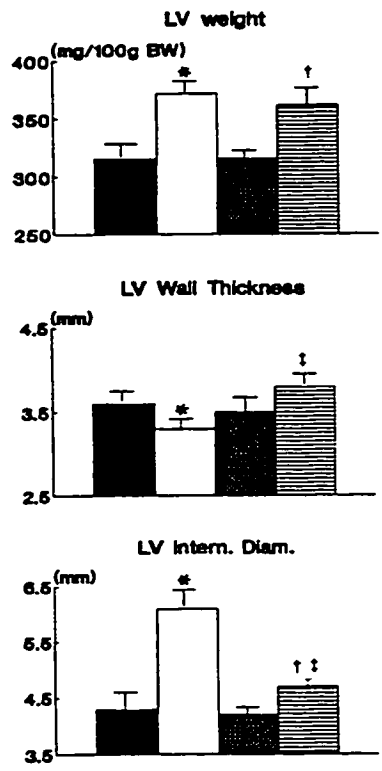

Fig. 1. Left ventricular (LV) weights, wall thickness and internal diameter (Intern. Diam.) of untreated spontaneously hypertensive rats (SHR, ■) and SHR treated for 5 weeks with either minoxidil (□), hydrochlorothiazide (▨) or minoxidil + hydrochlorothiazide (▤). Values are expressed as means ± SEM (n = 7–10 per group). *$P<0.05$, versus untreated SHR; †$P<0.05$, versus hydrochlorothiazide-treated SHR; ‡$P<0.05$, versus minoxidil-treated SHR.

with 66 ± 5 mg/100 g body weight in the untreated SHR. Hydrochlorothiazide did not affect right ventricular weight (77 ± 3 mg/100 g body weight), but prevented the increase caused by minoxidil alone (right ventricular weight upon combination treatment 79 ± 4 mg/100 g body weight).

Arterial morphometry

Light microscopic measurements

The effects of minoxidil on the arteries of SHR varied, depending on the vessel type. In the superior mesenteric artery (Table 1) minoxidil increased the luminal area significantly without changing the medial area, resulting in a significant decrease in the media : lumen ratio. In the large and small mesenteric arteries (Tables 2, 3), treatment with minoxidil had little effect on the lumen, but prevented the further increase in the medial area with age; the medial area after treatment for 10 weeks was maintained at the same level as at the initiation of treatment, resulting in a decreased media : lumen ratio towards the normal.

Treatment with hydrochlorothiazide alone had no effect on the structure of the three mesenteric arteries studied. However, hydrochlorothiazide combined with minoxidil prevented the increase in luminal size in the superior mesenteric arteries (Table 1). In contrast, the combination of hydrochlorothiazide and minoxidil had no effect on the luminal or medial areas of the large and small mesenteric arteries (Tables 2, 3).

The number of smooth muscle cell layers, which was increased in the large and small mesenteric arteries of SHR compared with WKY rats [17], was not affected by any of the treatments (data not shown).

Electron microscopic measurements

The untreated SHR showed a higher volume density of the medial smooth muscle cells from large

Table 1. Vessel wall dimensions of superior mesenteric artery of untreated Wistar–Kyoto (WKY) rats and untreated and treated spontaneously hypertensive rats (SHR).

		SHR treatment group			
	Untreated WKY rats	Untreated	Minoxidil	Hydrochlorothiazide	Combination
Luminal area (×10⁴ μm²)					
Day 0	32 ± 2	33 ± 2			
After 5 weeks		26 ± 3	43 ± 5†	33 ± 2†	30 ± 2‡
After 10 weeks	32 ± 3	33 ± 3	53 ± 4†		
Medial area (×10⁴ μm²)					
Day 0	11 ± 1	18 ± 1*			
After 5 weeks		16 ± 1	20 ± 1	17 ± 2	18 ± 1
After 10 weeks	11 ± 1	18 ± 2*	18 ± 1		
Media : lumen ratio					
Day 0	0.34 ± 0.03	0.52 ± 0.06*			
After 5 weeks		0.61 ± 0.04	0.47 ± 0.01†	0.52 ± 0.04	0.58 ± 0.04
After 10 weeks	0.32 ± 0.02	0.51 ± 0.02*	0.36 ± 0.04†		

Values are expressed as means ± SEM (n = 6 per group). *$P<0.05$, versus WKY rats; †$P<0.05$, versus untreated SHR; ‡$P<0.05$, versus minoxidil-treated SHR.

Table 2. Vessel wall dimensions of the large mesenteric artery of untreated Wistar–Kyoto (WKY) rats and untreated and treated spontaneously hypertensive rats (SHR).

		SHR treatment group			
	Untreated WKY rats	Untreated	Minoxidil	Hydrochlorothiazide	Combination
Luminal area ($\times 10^3\ \mu m^2$)					
Day 0	35±2	35±3			
After 5 weeks		36±3	42±3	33±4	40±5
After 10 weeks	40±4	53±7‡	52±5		
Medial area ($\times 10^3\ \mu m^2$)					
Day 0	9±1	17±1*			
After 5 weeks		20±2	16±1	19±2	17±4
After 10 weeks	11±1	26±3*‡	17±1†		
Media : lumen ratio					
Day 0	0.26±0.01	0.50±0.03*			
After 5 weeks		0.55±0.03	0.38±0.04	0.62±0.01	0.53±0.04
After 10 weeks	0.31±0.04	0.46±0.05*	0.35±0.03†		

Values are expressed as means ± SEM (n = 6 per group). *$P<0.05$, versus WKY rats; †$P<0.05$, versus untreated SHR; ‡$P<0.05$, versus untreated SHR on day 0.

Table 3. Vessel wall dimensions of the small mesenteric artery of untreated Wistar–Kyoto (WKY) rats and untreated and treated spontaneously hypertensive rats (SHR).

		SHR treatment group			
	Untreated WKY rats	Untreated	Minoxidil	Hydrochlorothiazide	Combination
Luminal area ($\times 10^2\ \mu m^2$)					
Day 0	18±1	21±2			
After 5 weeks		20±2	23±3	20±2	21±2
After 10 weeks	19±2	25±3	19±2		
Medial area ($\times 10^2\ \mu m^2$)					
Day 0	5.4±0.1	7.3±0.8			
After 5 weeks		9.1±0.6	7.0±0.3†	8.1±0.7	7.8±0.5
After 10 weeks	5.3±0.5	10.1±1.1*‡	6.9±0.4†		
Media : lumen ratio					
Day 0	0.31±0.03	0.34±0.05			
After 5 weeks		0.39±0.05	0.30±0.04	0.40±0.01	0.37±0.4
After 10 weeks	0.28±0.07	0.41±0.04*	0.35±0.08		

Values are expressed as means ± SEM (n = 6 per group). *$P<0.05$, versus WKY rats; †$P<0.05$, versus untreated SHR; ‡$P<0.05$, versus untreated SHR on day 0.

mesenteric arteries than the SHR treated with minoxidil (Table 4). The resultant increase in the volume : surface ratio of the medial smooth muscle cells suggests that the minoxidil-induced blunting of the increase in medial area reflects an effect on the size of the smooth muscle cells rather than the intercellular space.

Blood pressure

Chronic treatment of SHR with minoxidil resulted in a persistent fall in blood pressure, but not to the level of that in the WKY rats (Table 5). Hydrochlorothiazide alone decreased the blood pressure of the SHR significantly. However, minoxidil combined with hydrochlorothiazide did not cause a more marked antihypertensive response than minoxidil alone (Table 5). The heart rate was not changed by any of the treatments (data not shown).

Tissue catecholamines

In the SHR minoxidil did not affect the noradrenaline content of the right and left ventricles when normalized for heart weight (Table 6). Noradrenaline turnover rates in the left ventricle (increased in SHR compared with WKY rats) were increased further by treatment of the SHR with minoxidil (Fig. 2). Turnover rates in the right ventricle were similar in SHR and WKY rats and were not affected by minoxidil (Fig. 2). In contrast, minoxidil decreased the noradrenaline content of the superior and large mesenteric arteries of the SHR to the levels observed in the WKY rats. Moreover, noradrenaline turnover rates were decreased significantly in both arteries by minoxidil; from normal (versus WKY rats) to low in the superior mesenteric artery and from high (versus WKY rats) to normal in the large mesenteric artery (Fig. 3).

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Table 4. Transmission electron microscopic measurements of the media from large mesenteric arteries of spontaneously hypertensive rats untreated and treated for 10 weeks with minoxidil.

	Untreated	Minoxidil-treated
Volume density ($\mu m^3/\mu m^3$ vessel wall)		
Smooth muscle cells	0.82±0.01	0.75±0.02*
Nucleus	0.057±0.001	0.051±0.002*
Cytoplasm	0.76±0.01	0.70±0.02*
Intercellular space	0.18±0.01	0.25±0.02*
Surface density ($\mu m^2/\mu m^3$ vessel wall)		
Smooth muscle cells	0.21±0.02	0.26±0.02*
Volume:surface ratio ($\mu m^3/\mu m^2$)		
Smooth muscle cells	3.9±0.5	2.7±0.4*

Values are expressed as means±SEM (n=6 per group). *$P<0.05$, versus untreated.

Blood volume

Treatment of the SHR with minoxidil increased plasma and blood volumes significantly, whereas hydrochlorothiazide alone had no effect on plasma and blood volumes in the SHR (Table 5). When combined with minoxidil, hydrochlorothiazide significantly attenuated the increases in plasma and blood volumes seen with minoxidil alone (Table 5).

Body weight and water intake

At the start of treatment the body weight of the WKY rats was significantly higher than that of the SHR. Water intake was significantly lower in the WKY rats than in the SHR (50–60 versus 110–130 ml/kg body weight per day, respectively). In the SHR the untreated and treated groups showed a similar pattern of weight gain and water intake (data not shown).

Discussion

The present study provides three major new findings regarding the effects of the arterial vasodilator minoxidil on cardiovascular structure: the antihypertensive effect of minoxidil in SHR is associated with regional responses of cardiovascular structure;

Table 5. Blood pressure and plasma and blood volume in untreated Wistar–Kyoto (WKY) rats and untreated and treated spontaneously hypertensive rats (SHR).

		SHR treatment group			
	Untreated WKY rats	Untreated	Minoxidil	Hydrochlorothiazide	Combination
Mean arterial pressure (mmHg)					
After 5 weeks		183±4	132±2†	153±5†	133±5§
After 10 weeks	105±6	189±5*	153±7†		
Plasma volume (ml/100 g body weight)					
After 5 weeks		3.5±0.1	4.7±0.3†	3.2±0.1	3.9±0.1‡§
After 10 weeks	3.6±0.1	3.7±0.1	4.9±0.2†		
Blood volume (ml/100 g body weight)					
After 5 weeks		6.6±0.2	8.4±0.4†	5.9±0.3	6.7±0.3‡
After 10 weeks	6.5±0.2	6.7±0.2	10.1±1.0†		

Values are expressed as means±SEM (n=7–10 per group). *$P<0.05$, versus WKY rats; †$P<0.05$, versus untreated SHR; ‡$P<0.05$, versus minoxidil-treated SHR; §$P<0.05$, versus hydrochlorothiazide-treated SHR.

Table 6. Noradrenaline content and fractional turnover rate in the superior and large mesenteric arteries and left and right ventricles in untreated Wistar–Kyoto (WKY) rats, untreated spontaneously hypertensive rats (SHR) and SHR treated with minoxidil for 10 weeks.

		SHR treatment group	
	Untreated WKY rats	Untreated	Minoxidil
Noradrenaline content (ng/g tissue)			
Superior mesenteric artery	4800±167	5786±37*	4862±62†
Large mesenteric artery	17329±478	26106±895*	18728±674†
Left ventricle	524±10	662±26*	612±12
Right ventricle	868±14	921±27	818±24
Noradrenaline content (ng/ventricle)			
Left ventricle	460±19	627±26*	708±27†
Right ventricle	178±6	164±7	209±5†
Fractional turnover rate (/h)			
Superior mesenteric artery	0.08±0.01	0.06±0.01	0.06±0.01
Large mesenteric artery	0.05±0.00	0.07±0.01	0.05±0.01
Left ventricle	0.08±0.01	0.10±0.00	0.12±0.00
Right ventricle	0.04±0.00	0.03±0.01	0.04±0.01

Values are expressed as means±SEM (n=6 per group). *$P<0.05$, versus WKY rats; †$P<0.05$, versus untreated SHR.

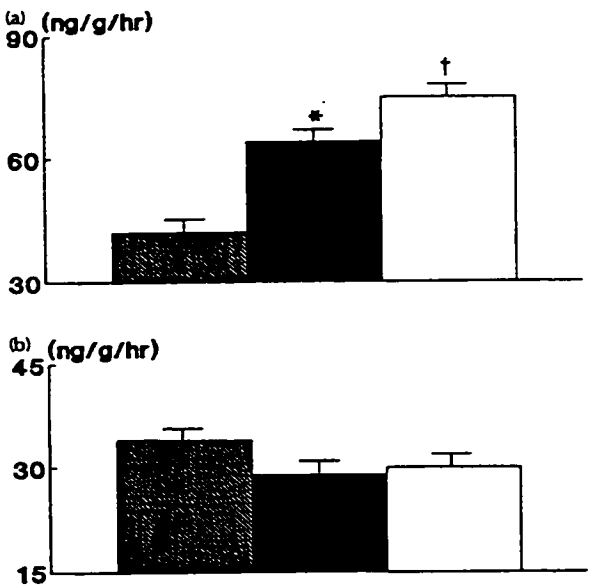

Fig. 2. Absolute turnover rate of noradrenaline in the (a) left and (b) right ventricle of untreated Wistar–Kyoto rats (▨), untreated spontaneously hypertensive rats (SHR, ■) and SHR treated for 10 weeks with minoxidil (□). Values are expressed as means±SEM (n=6 per group). *$P<0.05$, versus Wistar–Kyoto rats; †$P<0.05$, versus untreated SHR.

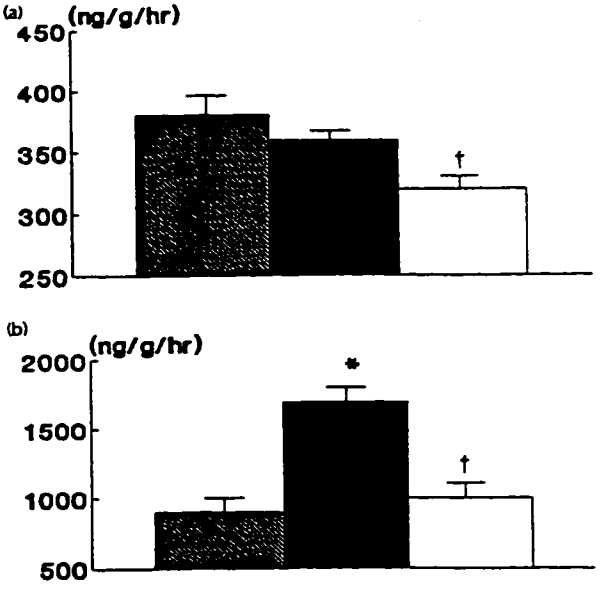

Fig. 3. Absolute turnover rate of noradrenaline in the (a) superior and (b) large mesenteric artery of untreated Wistar–Kyoto rats (▨), untreated spontaneously hypertensive rats (SHR, ■) and SHR treated for 10 weeks with minoxidil (□). Values are expressed as means±SEM (n=6 per group). *$P<0.05$, versus Wistar–Kyoto rats; †$P<0.05$, versus untreated SHR.

minoxidil increases cardiac sympathetic activity but decreases mesenteric arterial sympathetic activity; and concomitant diuretic treatment does not prevent the increase in left ventricular weight, but changes the type of hypertrophy (i.e. concentric instead of eccentric).

Cardiovascular structural alterations

As reported previously (for example [3,20]), in the heart of SHR minoxidil further potentiated hypertrophy of the two ventricles. In contrast, in the mesenteric arterial bed of SHR minoxidil increased the luminal area of the superior mesenteric artery and prevented further increases in the medial area of the large and small mesenteric arteries that are associated with more-prolonged hypertension. The latter effects were observed under conditions of maximal arterial relaxation [17], and thus cannot be attributed to artefacts related to differences in contractile state.

As the SHR matured from 16 to 26 weeks of age, further wall thickening was found in the large and small mesenteric arteries but not in the superior mesenteric artery, in which arterial vasodilator treatment induced structural remodelling of the vessel, resulting in a larger lumen and unchanged total medial area, thereby decreasing wall thickness. In contrast, in the large and small mesenteric arteries minoxidil prevented the progression of medial hypertrophy associated with advancing hypertension without affecting the lumen. Most studies to date have compared vascular changes between treated and age-matched control SHR, and a reduction in arterial dimensions in the treated rat was considered to be due to regression of the hypertrophy. The present results show that treatment with minoxidil did not cause regression, but prevented further progression of medial cellular hypertrophy in the large and small mesenteric arteries of SHR. Medial cellular hyperplasia, as indicated by an increase in the number of smooth muscle cell layers in the large and small mesenteric arteries of SHR, was not affected by treatment with minoxidil, which is consistent with findings from previous studies involving vasodilators (for example [11,13]).

Volume factors

The alterations to cardiac design induced by arterial vasodilators implicate cardiac volume overload as a possible causal mechanism. Indeed, increases in filling pressures were found during the initial weeks of arterial vasodilator treatment in normotensive Wistar rats [6]. In contrast, filling pressures in SHR (high compared with in WKY rats) do not increase further, but neither do they decrease as might be expected from the decrease in afterload [21]. Therefore, it is unlikely that cardiac volume overload is the primary mechanism responsible for initiating the cardiac trophic effects of arterial vasodilators in SHR. In a second phase, cardiac volume overload related to intravascular volume expansion might be involved in the cardiac structural alterations, because increas-

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ing the diastolic dimensions while maintaining filling pressure constant increases diastolic wall stress and might thus potentiate or maintain chamber enlargement. However, diuretic therapy concurrent with minoxidil administration prevented the increase in blood volume caused by minoxidil alone, but did not affect the increase in left ventricular weight. The combination therapy did prevent most of the increase in left ventricular diameter induced by minoxidil alone, and converted the minoxidil-induced eccentric left ventricular hypertrophy to concentric left ventricular hypertrophy with increased left ventricular wall thickness. This suggests that cardiac volume load determines the pattern of left ventricular hypertrophy in SHR by determining the filling pressures and diastolic wall stress; for example, high sympathetic activity may lead to eccentric left ventricular hypertrophy in the presence of high filling pressures and to concentric left ventricular hypertrophy in the presence of normal filling pressures. In contrast, the combination therapy prevented the increase in right ventricular weight caused by minoxidil alone, suggesting that volume load is the major determinant in this case.

In the superior mesenteric artery, the present results show that intravascular volume expansion, or increased flow, may be responsible for the increase in luminal area and structural remodelling of the vessel, because the combination therapy prevented the increase in luminal area. The assumed increase in shear stress, generated by volume expansion, might stimulate the release of several factors, including endothelium-derived relaxing factor and prostacyclin, which, in turn, may be responsible for the increase in luminal diameter [22]. Direct effects of minoxidil might also contribute, since minoxidil inhibits both proliferation and protein synthesis in cultured aortic endothelial cells [23]. In contrast, other mechanisms, such as the sympathetic nervous system (see below), appear to dominate in the large and small mesenteric arteries, since no increase in luminal area was induced by minoxidil in these arteries.

Sympathetic nervous system

The persistence or progression of cardiac hypertrophy induced by arterial vasodilators in SHR, despite blood pressure control, is associated with cardiac sympathetic hyperactivity (shown by increases in cardiac noradrenaline turnover). Noradrenaline turnover rates in the left ventricle are increased by minoxidil after 5 [3] as well as 10 weeks of treatment (present study). In contrast, noradrenaline turnover rates in the right ventricle are increased after 5 weeks of treatment, but no longer after 10 weeks of treatment, with minoxidil. Considering that diuretic treatment does not prevent the development or persistence of cardiac hypertrophy induced by arterial vasodilators in normotensive rats [5], or in hypertensive rats (present study), whereas sympatholytic agents such as methyldopa do [20], it appears that sympathetic activity might play a primary role in the cardiac trophic responses to arterial vasodilators. However, hydralazine and minoxidil decrease blood pressure and increase left ventricular sympathetic activity similarly [3], yet only minoxidil increases left ventricular weight and internal diameter. Thus, if left ventricular sympathetic activity is important, other mechanisms, such as the cardiac renin–angiotensin system, might also play a role in the cardiac trophic responses to arterial vasodilators [24].

In the mesenteric arterial bed of SHR, minoxidil decreased sympathetic activity to the superior mesenteric artery significantly and normalized the increased (compared with WKY rats) sympathetic activity to the large mesenteric arteries. However, the structural consequences of vasodilator treatment were different in these two vessel types. In the superior mesenteric artery the combination treatment increased luminal size without affecting the cross-sectional area of the media. The increase in luminal size might be due to the increased blood volume (see above). Lack of effect on the media might be related to the fact that treatment was initiated in adult rats with medial changes in response to fully established hypertension, and that the vasodilators do not cause regression of these established changes. In the large mesenteric artery the minoxidil-induced decreases in blood pressure were associated with the prevention of further medial hypertrophy. To what extent the concomitant decrease in sympathetic hyperactivity participated in the prevention of further arterial medial hypertrophy cannot be assessed from the present data. However, there is evidence to support this concept from Baumbach *et al.* [25], who showed a 44% reduction in cross-sectional area of pial arterioles in 10- to 12-month-old stroke-prone SHR that had undergone unilateral sympathetic denervation at age 1 month. The present experiments clearly establish that the ineffectiveness of arterial vasodilators in causing regression of arterial hypertrophy in SHR is not related to further increases in sympathetic activity to the arteries (as is the case for the heart and for arteries of normotensive rats [5]); arterial sympathetic activity was normalized or even decreased below control levels. Other trophic mechanisms, such as the renin–angiotensin system, have not yet been evaluated in this respect. Sympatholytic agents, while decreasing sympathetic activity, might also affect renin activity, and this might be more relevant.

The present results show opposite effects of minoxidil on cardiac versus arterial sympathetic activity in SHR. Such heterogeneity has not been reported previously for minoxidil or other antihypertensive agents. It is tempting to speculate that minoxidil increased diastolic wall stress in the heart, thereby activating cardio-cardiac reflexes [26], but had op-

posite effects in the arteries. Arterial wall stress may have been decreased sufficiently by the reduction in blood pressure, despite the increase in blood flow. If so, this could explain the persistent increase in arterial sympathetic activity caused by minoxidil in normotensive rats [5] in which the increase in wall stress due to increased blood flow is not offset by a decrease in blood pressure.

In conclusion, the present results show that there are regional differences in cardiovascular structure and sympathetic activity in SHR in response to treatment with arterial vasodilators. The further potentiation of cardiac hypertrophy with minoxidil is probably associated with cardiac sympathetic hyperactivity. Concurrent diuretic therapy converts the eccentric left ventricular hypertrophy caused by minoxidil alone to concentric left ventricular hypertrophy. In the mesenteric bed an increase in the luminal area of the superior mesenteric artery might be related to an increase in blood volume, since it is prevented by concomitant diuretic therapy. The prevention of further medial hypertrophy in the large and small mesenteric arteries by minoxidil might be related to decreases in both blood pressure and sympathetic activity. The present results suggest that regional differences in cardiac and arterial structure in response to arterial vasodilators, despite blood pressure control, might be related, in part, to differences in regional sympathetic activity, and that volume load plays a modulatory role.

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3.3.7 Synopsis

This study shows that minoxidil treatment of SHR causes regional different responses in cardiovascular structure and sympathetic activity. The further potentiation of cardiac hypertrophy with minoxidil is probably associated with cardiac sympathetic hyperactivity. Concurrent diuretic therapy converts the eccentric LVH caused by minoxidil alone to concentric LVH. In the mesenteric bed, an increase in the luminal area of the superior mesenteric artery may be related to an increase in blood volume since it is prevented by concomitant diuretic therapy. The prevention of further medial hypertrophy, in the large and small mesenteric arteries by minoxidil may be related to decreases in both blood pressure and sympathetic activity. These results suggest that regional differences in cardiac and arterial structure in response to minoxidil despite blood pressure control, may be related, in part, to differences in regional sympathetic activity, and that volume load plays a modulatory role.

3.4 ARTERIAL VASODILATION AND VASCULAR CONNECTIVE TISSUE CHANGES IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Arterial Vasodilation and Vascular Connective Tissue Changes in Spontaneously Hypertensive Rats

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Summary: Arterial hypertrophy in response to hypertension includes increases in the connective tissue proteins elastin and collagen. Regression of arterial hypertrophy depends not only on blood pressure normalization but also on the specific antihypertensive treatment. Consequently, each drug class may exert an influence on connective tissue proteins. We evaluated the arterial connective tissue response of 16-week-old spontaneously hypertensive rats (SHRs) to treatment with minoxidil, 120 mg/L, drinking water for 10 weeks. Despite a decrease in blood pressure, minoxidil had no effect on arterial weight or collagen content but increased elastin content in the abdominal aorta, renal, and superior mesenteric arteries. The increase in elastin content in the abdominal aorta and superior mesenteric artery was accompanied by a decrease in tissue elastase activity. Thus the minoxidil-induced increase in arterial elastin content may be related to a direct effect of the drug to decrease elastase activity in these tissues. **Key Words:** Minoxidil—Elastin—Collagen—Spontaneously hypertensive rats (SHRs)—Elastase activity—Arteries.

Cardiovascular hypertrophy in response to hypertension has been shown to include increases in the absolute amounts of elastin and collagen (1–4). Clinical (5) and experimental (6–8) studies have shown that reversal of cardiovascular hypertrophy depends not only on blood pressure normalization but also on the specific antihypertensive treatment. Consequently, the specific effects of each drug class may also exert an influence on connective tissue proteins. With regard to connective tissue changes in the cardiovascular system of spontaneously hypertensive rats (SHRs) in response to arterial vasodilators, studies on arterial tissue are lacking, and studies on cardiac tissue have shown that the persistence of cardiac hypertrophy with hydralazine does not alter connective tissue content or concentration (9), whereas the potentiation of cardiac hypertrophy with minoxidil is accompanied with variable effects on connective tissue proteins. Early studies by Sen et al. (2,6,9) in SHRs showed that minoxidil further increases total collagen content but not concentration in cardiac tissue. Recently we reported a dissociation in the effects of minoxidil on left ventricular versus right ventricular collagen accumulation in SHRs (10). Long-term treatment of SHRs with minoxidil decreases left ventricular collagen content and concentration; however, it increases right ventricular collagen content and concentration, possibly because of minoxidil-induced cardiac volume overload or activation of the renin–angiotensin system or both (10).

The purpose of this study was to determine the connective tissue response of the arterial wall in SHRs during long-term treatment with the arterial vasodilator minoxidil.

MATERIALS AND METHODS

Male SHRs were obtained from Taconic Farms (Germantown, NY, U.S.A.) at age 16 weeks. Rats were housed two per cage and given food (Purina rat chow, PMI, St. Louis, MO, U.S.A., 180 μmol Na/g food) and water ad libitum and kept on a 12-h light-and-dark cycle. After a 5-day acclimatization period, animals were randomized into two groups (n = 18/group): untreated and minoxidil, 120 mg/L drinking water, and subjected to 10 weeks of treatment. Three experimental protocols were performed: (a) the effect of treatment (n = 6/group) on blood pressure, (b) the effect of treatment (n = 6/group) on connective tissue content, and (c) the effect of treatment (n = 6/group) on elastase activity. In all experiments, fluid intake and body weight were monitored weekly, and the animals were handled twice weekly.

In six rats from each group, 2 days before the end of the treatment period and under halothane–nitrous oxide–oxygen anaesthesia, a PE-50 catheter (Clay Adams, Parsippany, NJ,

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U.S.A.) was inserted into a carotid artery to monitor mean arterial pressure. On the following day (final day of the treatment period), resting blood pressure was recorded as described previously (8).

In the second experiment, the rats were killed by decapitation, and by using anatomic landmarks, consistent segments of the following tissues were sampled for elastin and collagen content:

1. Carotid artery, from the origin at the innominate artery to the bifurcation of the internal and external carotid arteries;
2. Renal artery, from the origin at the aorta to its bifurcation at the hilum of the kidney;
3. Superior mesenteric artery, from the origin at the aorta to its first branch point; and
4. Abdominal aorta, from the level of the diaphragm to the iliac bifurcation.

The arterial segments were cleaned of all adhering fat, blood, and loose connective tissue, weighed, and stored in aluminum foil at −20°C until biochemical assessment. Determination of insoluble elastin and collagen involved the method of LaBourene et al. (11) modified from Todorovich-Hunter et al. (12).

In the third experiment, the animals were killed by decapitation, and the whole length of the abdominal aorta and superior mesenteric artery were removed as described and placed in aluminum foil at −80°C until biochemical assessment.

Elastase activity was assayed by measurement of the degradation of an insoluble [^{3}H]elastin substrate with a method described by LaBourene et al. (11) modified from Leake et al. (13). A standard curve with human leukocyte elastase from sputum was constructed with each assay. During the course of the study, we found that it was necessary to pool vessels to obtain measurable elastase activity.

The results are expressed as the mean ± SEM. Statistical differences between two means were determined by Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Long-term (10 weeks) treatment of SHRs with minoxidil resulted in a significant decrease in mean arterial pressure (diastolic blood pressure + 1/3 pulse pressure; 188 ± 6 and 148 ± 8 mm Hg in the SHR untreated and SHR minoxidil groups, respectively) as previously reported (14).

Minoxidil had no significant effect on arterial tissue weights. Minoxidil significantly increased elastin content in the abdominal aorta, superior mesenteric, and renal arteries but had no significant effect on arterial collagen content (Table 1). Minoxidil significantly decreased tissue elastase activity in the abdominal aorta and mesenteric artery (Table 1).

The untreated and treated groups showed a similar pattern of weight gain and food and water intake. In the 10-week experiment, body weights increased from ~250 g at the beginning to 417 ± 6 and 428 ± 6 g in the untreated and minoxidil groups, respectively. At the end of the 10-week experiment, water intake amounted to 32 ± 3 and 30 ± 2 ml/day in the untreated and minoxidil groups, respectively (resulting in a drug intake of 8.6 ± 0.8 mg/kg/day in the minoxidil group).

TABLE 1. *Tissue weight and elastin and collagen concentrations and tissue elastase activity of untreated and 10-week minoxidil-treated SHRs*

Parameter	Untreated	Minoxidil
Tissue		
Abdominal aorta		
Weight (mg/seg)	26.9 ± 2.1	27.2 ± 1.3
Elastin (μg/seg)	6,246 ± 345	74,362 ± 269[a]
Collagen (μg/seg)	2,543 ± 138	2,833 ± 175
Carotid artery		
Weight (mg/seg)	5.4 ± 0.4	5.6 ± 0.4
Elastin (μg/seg)	963 ± 131	1,088 ± 84
Collagen (μg/seg)	780 ± 116	821 ± 57
Renal artery		
Weight (mg/seg)	1.98 ± 0.13	1.98 ± 0.06
Elastin (μg/seg)	102 ± 6	141 ± 10[a]
Collagen (μg/seg)	298 ± 63	300 ± 25
Superior mesenteric artery		
Weight (mg/seg)	3.8 ± 0.3	4.7 ± 0.9
Elastin (μg/seg)	1,653 ± 94	2,254 ± 170[a]
Collagen (μg/seg)	361 ± 33	482 ± 77
Tissue elastase activity (mg HLE equivalence/mg tissue)		
Aorta	1.42 ± 0.08 (3)	0.94 ± 0.18[a] (6)
Mesenteric artery	6.38 ± 0.56 (4)	2.87 ± 0.46[a] (4)

Values expressed as mean ± SEM. It was necessary to pool tissue for detection of elastase activity, so number in parentheses represents number of determinations.
HLE, human leukocyte elastase; SHR, spontaneously hypertensive rat.
[a]Significant vs. untreated group.

DISCUSSION

The results of our study show that in SHRs, long-term minoxidil causes a modest antihypertensive response and different responses in arterial connective tissue protein content. Minoxidil did not alter collagen content but increased elastin content and decreased elastase activity. Although the magnitude of the response varied with individual arteries, the overall effect on connective tissue accumulation appears to be consistent in the elastic arteries investigated. Whether similar changes were present in muscular arteries and resistance vessels remains to be examined. Biochemical analyses of these vessels was difficult because of the limited amount of tissue available.

Previous studies in SHRs have shown that aortic collagen and elastin (3) biosynthesis follow a biphasic pattern. Both collagen and elastin synthesis have been found to exceed Wistar–Kyoto rat control levels in the prehypertensive period (at age of 4 weeks), to decrease in the development of hypertension (to age 14–16 weeks), and to increase again in the period of the established hypertensive state (beyond age 16 weeks). Several studies have shown that this second increase in connective tissue proteins may be prevented, depending on the choice of antihypertensive therapy (6,15).

In our study, a number of explanations are possible for the minoxidil-induced persistence of arterial collagen content, the further increase in arterial elastin content,

and the associated increase in elastase activity. First, a direct drug effect of minoxidil on enzymes that are involved in the synthesis and degradation of connective tissue proteins may be possible. In this regard, minoxidil has been shown directly to influence the activities of the connective tissue enzymes lysine hydroxylase and prolyl hydroxylase (important in collagen biosynthesis) in fibroblast cell lines (16,17). In our study, a direct effect of minoxidil to decrease elastase activity (an enzyme that has been shown selectively to hydrolyze elastin) may explain the further increase in arterial elastin content. The mechanism of action by which minoxidil alters elastase activity remains to be elucidated.

Second, it is possible that the minoxidil-induced alterations in arterial connective tissue proteins, and in particular, the persistence of collagen, may be due to volume loading, sympathetic hyperactivity, or activation of the renin–angiotensin system (or a combination of these)—mechanisms activated by arterial vasodilator administration (13,14). In this regard, in SHRs, studies with hydralazine alone (9) or in combination with a diuretic (18) have shown persistence of arterial collagen content. However, when hydralazine was combined with a sympatholytic agent such as propranolol (9), guanethidine (19), or reserpine (2,15), regression of arterial collagen occurred, suggesting a possible trophic role of the sympathetic nerves on arterial vasodilator connective-tissue alterations. Further studies addressing the effect of minoxidil and sympatholytic agents on arterial connective tissue proteins in SHRs are under way.

In conclusion, our results show that long-term minoxidil, despite a modest antihypertensive response, is associated with the persistence of arterial collagen and a further increase in arterial elastin. The increase in arterial elastin is associated with a decrease in elastase activity. The minoxidil-induced alterations in connective tissue proteins may be due to a direct drug effect on enzymes involved in the biosynthesis and degradation of elastin and collagen, or alternatively may be part of the overall trophic response of the tissue, possibly through an effect on sympathetic nerves.

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3.4.6 Synopsis

The results show that long-term minoxidil, despite a modest antihypertensive response, is associated with the persistence of arterial collagen, and further increase in arterial elastin. The increase in arterial elastin is associated with a decrease in elastase activity. The minoxidil-induced alterations in connective tissue proteins may be due to a direct drug effect on enzymes involved in the biosynthesis and degradation of elastin and collagen or alternatively be part of the overall trophic response of the tissue, possibly through activation of the renin-angiotensin system.

4.0 GENERAL DISCUSSION/FUTURE EXPERIMENTS

4.1. Alterations in Cardiac Anatomy and Potential Mechanisms Involved

In the normotensive Wistar rat, both hydralazine and minoxidil cause modest decreases in blood pressure during the initial days of treatment (Appendix 6.1). However, with continuing treatment tolerance develops and small (hydralazine) to no (minoxidil) decreases in blood pressure remain. During long-term (14-21 days) treatment, both vasodilators induce cardiac hypertrophy. Hydralazine increases RV weight and LV internal diameters without significantly increasing LV weight or LV wall thickness. Minoxidil increases both RV and LV weights, the latter associated with an increase in LV internal diameters but not an increase in LV wall thickness. No further increases in LV internal diameters, RV and LV weights are seen with prolonged (35-70 days) vasodilator treatment.

In SHR, hydralazine and minoxidil cause persistent decreases in blood pressure, although some tolerance develops during long-term treatment (present studies, Appendix 6.1). Despite a persistent antihypertensive effect, hydralazine does not decrease LV weight, whereas minoxidil increases LV weight further as previously reported by Sen and co-workers (Sen *et al.*, 1974; Sen *et al.*, 1977). Both arterial vasodilators increase RV weight further. As in normotensive rats, the minoxidil-induced increase in LV weight is associated with an increase in LV internal diameters and no increase in LV wall thickness. A direct vasodilator trophic response is probably unlikely since neither minoxidil nor its active metabolite minoxidil sulfate (McCall *et al.*, 1983) induce growth of neonatal rat cardiac myocytes in culture (Appendix - Table 1, Figure 1).

The pattern of changes in LV design with arterial vasodilators is similar to that induced by aortocaval shunt (Ruzicka *et al.*, 1993) indicating the development of eccentric LVH, implicating cardiac volume overload as a major contributor to these changes (Grossman, 1980). Morphometric measurements of LV myocytes of

normotensive rats following minoxidil treatment show myocyte elongation, further implicating cardiac volume overload (Moravec *et al.*, 1993). Normotensive [Wistar, Wistar-Kyoto (Appendix - Figures 1, 2)] and hypertensive (SHR, 2-K, 1-C) rats in response to arterial vasodilatation (present studies, Tsoporis and Leenen, 1986; Leenen and Prowse, 1987; Leenen and Tsoporis, 1990), and aortocaval shunt in normotensive rats (Ruzicka *et al.*, 1993) induce similar alterations in cardiac design indicating that these effects are neither strain- nor model-specific and are related to cardiac volume overload.

To substantiate that cardiac volume overload occurs during long-term treatment with arterial vasodilators, cardiac filling pressures, specifically LVEDP and RAP were measured as indices of cardiac volume load in normotensive Wistar rats. Indeed, both vasodilators induced maximal increases in LVEDP and RAP within 7-14 days of treatment before changes in cardiac anatomy occur suggesting that cardiac volume overload precedes the changes in cardiac anatomy, consistent with cardiac volume overload as a possible trigger mechanism. Ross and co-workers (Ross *et al.*, 1971; McCullagh *et al.*, 1972) report a similar relationship. In dogs, creation of an infrarenal aortocaval shunt increases LVEDP from 10-20 mmHg 14-21 days post shunt. LVED dimension increases gradually reaching a plateau 35-42 days after creation of the shunt. At the same time they note the occurrence of LVH. In rats, the creation of an aortocaval shunt results in a marked increase in LVEDP within 7 days and the development of LVH and RVH within 7-28 days (Ruzicka *et al.*, 1993). In response to arterial vasodilation and aortocaval shunt, alterations in cardiac anatomy occur within 21-35 days with no or minimal changes during the long-term (70 days) (present studies, Ross *et al.*, 1971; Ruzicka *et al.*, 1993, 1994). Once the alterations in cardiac mass have been established, LVEDP appears to remain relatively stable (present studies, Ruzicka and Leenen, 1993;

Ruzicka *et al.*, 1993), presumably until heart failure develops, resulting in a further rise in LVEDP (Legault *et al.*, 1990).

In contrast to the increases in filling pressures noted during the initial weeks of arterial vasodilator treatment in normotensive rats, in SHR, neither hydralazine nor minoxidil increase RAP (Yuan and Leenen, 1992). Hydralazine does not affect LVEDP and minoxidil causes only a small, temporary rise in LVEDP (Yuan and Leenen, 1992). The different pattern of cardiac filling may relate to a more marked decrease in afterload induced by arterial vasodilation in SHR versus normotensive rats. Therefore, it is unlikely that cardiac volume overload is the primary mechanism responsible for initiating the cardiac trophic effects of arterial vasodilators in SHR.

In a second phase, cardiac volume overload related to intravascular volume expansion and a shift of blood from the peripheral to the central compartment (Tarazi *et al.*, 1976) may be involved in the cardiac structural alterations induced by arterial vasodilators. In normotensive rats, intravascular volume expansion does not appear to play an essential role in the development of cardiac hypertrophy with arterial vasodilators, since concurrent diuretic treatment prevents most of the increase in blood volume, but does not affect the increase in filling pressures, RV weight and only partly inhibits the increase in LV weight. Similarly, in SHR, concurrent diuretic therapy with minoxidil, prevents the increase in blood volume, but does not affect the increase in LV weight and only partly inhibits the increase in RV weight. In SHR and normotensive rats concurrent diuretic therapy prevents the increase in LV internal diameter and converts the minoxidil-induced eccentric LVH to concentric LVH. Several explanations are possible for this observation. Intravascular volume expansion and the associated cardiac volume overload may be important with minoxidil alone to maintain filling pressures and diastolic wall stress during chronic treatment, but concomitant diuretic therapy may replace volume overload with another nonhemodynamic mechanism e.g. sympathetic

hyperactivity causing concentric hypertrophy (Newling *et al.*, 1989; Zierhut and Zimmer, 1989). Alternatively, it is possible that cardiac sympathetic activity is the primary trophic mechanism for minoxidil alone or with concomitant diuretic therapy, whereas volume load determines the pattern of LVH. Sympathetic hyperactivity can cause both concentric LVH (Newling *et al.*, 1989; Zierhut and Zimmer, 1989) and eccentric LVH (Leenen and Harmsen, 1991), and cardiac volume load may play a modulatory role by determining filling pressures and diastolic wall stress. Studies on the effects of diuretic therapy on cardiac sympathetic activity and central hemodynamics during long-term arterial vasodilator treatment are required to assess these different possibilities.

Cardiac sympathetic activity has been suggested as the primary trophic mechanism for arterial vasodilator-induced cardiac hypertrophy by Sen and co-workers (Sen *et al.*, 1974, 1976, 1977; Sen and Tarazi, 1983). In SHR and normotensive rats, a baroreceptor-reflex-mediated increase in general sympathetic drive occurs on initiation of arterial vasodilator treatment (Appendix 6.1). However, during long-term treatment a resetting of the baroreceptors occurs and general sympathetic tone returns to control levels. The absence of a generalized sympathetic hyperactivity does not exclude a selective increase in sympathetic activity to specific tissues. Indeed, chronic arterial vasodilator treatment of SHR and normotensive rats is associated with cardiac sympathetic hyperactivity, as quantified by increases in ventricular (absolute) norepinephrine turnover rates.

The absolute turnover rate is dependent on both the rate constant k, and the initial tissue concentration. In these studies the arterial vasodilator-induced changes in norepinephrine turnover rates are due primarily to changes in the rate constant of decline of norepinephrine, k, rather than changes in the initial concentrations of the tissues. Changes in k, may reflect a change in the firing rate of the nerves, in the uptake of extraneural degradation of norepinephrine, or a combination of these factors. An increase in the firing rate may lead to an increase in the loss (of norepinephrine) per unit time, and

thus a faster disappearance of norepinephrine from the nerve. Since the changes in norepinephrine turnover rates for arterial vasodilators are not the same in all tissues, it is likely that the changes in turnover rate are the result of changes in the firing rates of the nerves (Fields *et al.*, 1989).

Several mechanisms may be responsible for maintaining cardiac sympathetic hyperactivity in the absence of an increase in general sympathetic activity. A direct cardiostimulatory effect has been suggested for both arterial vasodilators (Greenberg, 1980). Cardiac volume overload in response to arterial vasodilation may contribute to ventricular sympathetic hyperactivity by activating ventricular wall mechanoreceptors and a cardiocardiac reflex (Schwartz *et al.*, 1973). Hyperactivity of the cardiac renin-angiotensin system in response to arterial vasodilation (Ruzicka and Leenen, 1993) may also increase ventricular sympathetic activity via facilitation of norepinephrine release from sympathetic nerve terminals (Starke, 1977). Interestingly, arterial vasodilation specifically upregulates the cardiac α_{1A}-adrenergic receptor (Tsoporis and Simpson, unpublished observation), the subtype that is associated with biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy (Knowlton *et al.*, 1993).

It appears unlikely that there is a direct relationship between ventricular sympathetic activity and the trophic response of the heart to arterial vasodilators, since long-term treatment with either hydralazine or minoxidil increases ventricular sympathetic activity to a similar degree, yet only minoxidil increases LV weight. In this regard, treatment of normotensive rats with the calcium-antagonist nisoldipine induces RV hypertrophy despite a decrease in cardiac sympathetic activity, indicating that an increase in sympathetic activity is not essential for the production of hypertrophy (Fields *et al.*, 1989). Thus, ventricular sympathetic hyperactivity may be involved as a possible contributory mechanism in the cardiac effects of arterial vasodilators.

In addition, to increasing cardiac sympathetic activity, arterial vasodilators also increase plasma renin activity, implicating a role of the renin-angiotensin system. An activated renin-angiotensin system may contribute to development of cardiac hypertrophy via direct cardiac trophic effects of angiotensin II on cardiomyocytes or via facilitation of norepinephrine release from sympathetic nerve terminals and stimulation of α_1-adrenergic receptor mediated growth, via aldosterone mediated volume expansion and via a shift of blood from the peripheral to the central compartment (review - Sadoshima and Izumo, 1997). The possible role of the renin-angiotensin system in minoxidil-induced alterations of cardiac hemodynamics and anatomy has been studied in normotensive rats (Ruzicka and Leenen, 1993). Whether similar mechanisms apply in SHR remains to be tested. In SHR and normotensive rats, PRA increases similarly during the initial days of treatment. In the long-term, PRA returns to control levels in SHR but remains elevated in normotensive rats. In normotensive rats, minoxidil also induces a delayed increase in cardiac renin activity suggesting activation of the cardiac renin-angiotensin system (Ruzicka and Leenen, 1993). An activated renin-angiotensin system through the actions of angiotensin II may contribute to the development of cardiac hypertrophy in response to minoxidil or aortocaval shunt via hemodynamic and/or direct cardiac trophic effects (Ruzicka and Leenen, 1993; Ruzicka *et al.*, 1993, 1994). The angiotensin II receptor antagonist losartan blunts the minoxidil-induced increase in cardiac filling pressures only to a minor extent but prevents LV dilation, RV and LV hypertrophy induced by minoxidil suggesting that the renin-angiotensin system plays a major role in the hypertrophic response of the RV and LV to minoxidil (Ruzicka and Leenen, 1993). In contrast, the ACE inhibitor enalapril normalizes LVEDP and significantly decreases afterload but does not affect the hypertrophic response of the RV and LV to minoxidil (Ruzicka and Leenen, 1993). The low affinity of enalapril for cardiac ACE appears to lead to continuous

angiotensin II generation in the heart and can thus explain the failure of enalapril to attenuate the minoxidil-induced hypertrophic response (Ruzicka *et al.*, 1995). With respect to ACE inhibitors and the minoxidil-induced hypertrophic response, Ruzicka and Leenen (1995), concluded that only prevention of the increase in LVEDP and in plasma and cardiac angiotensin II, attenuates the development of LVH, consistent with the concept that angiotensin II is involved in the development of cardiac hypertrophy by minoxidil by both hemodynamic and trophic effects. In contrast to the development phase, the renin-angiotensin system does not continue as a cardiac trophic stimulus for the maintenance of cardiac hypertrophy induced by minoxidil treatment but indirectly maintains cardiac hypertrophy by contributing to the persistence of filling pressures (Ruzicka *et al.*, 1994).

4.2 Alterations in Arterial Structure and Potential Mechanisms Involved

In the vasculature of Wistar rats, of all the arteries evaluated (carotid, cerebral, and mesenteric), long-term (70 days) treatment with minoxidil increases the medial area of only the superior mesenteric artery (Appendix - Figure 6.4). The increase in medial area of the superior mesenteric artery is associated with an increase in the volume/surface ratio of the medial smooth muscle cells without a change in the number of smooth muscle cell layers indicating smooth muscle cell hypertrophy rather than hyperplasia.

In contrast, to the non essential role of intravascular volume expansion for the development of minoxidil-induced cardiac hypertrophy, volume expansion may be the trigger mechanism for smooth muscle cell hypertrophy of the superior mesenteric artery, since concurrent diuretic therapy prevented the medial hypertrophy of this vessel in normotensive rats. It is possible that minoxidil-induced volume expansion and/or high cardiac output (Ruzicka *et al.*, 1994) increases flow through the superior mesenteric

artery resulting in smooth muscle growth, and concomitant diuretic therapy inhibits smooth muscle cell growth by preventing the increase in flow. An increase in blood flow increases shear stress (Kamiya and Togawa, 1980; Brownlee and Langille, 1991; Malek *et al.*, 1993; Ohno *et al.*, 1995). In response to the increase in shear stress, the endothelium releases numerous growth factors including PDGF A- and B-chains, bFGF, $TGF\beta_1$ and endothelin which in turn may act alone or in combination on smooth muscle cells to induce a trophic response (Malek *et al.*, 1993; Dzau *et al.*, 1994; Resnick and Gimbrone, 1995). It is not clear why medial hypertrophy only occurs in the superior mesenteric artery and not in another elastic artery (carotid) or in the muscular arteries (large mesenteric arteries) or arterioles (small mesenteric arteries). Selective vasodilation by minoxidil or regionally different responses of sympathetic activity may play a role.

Minoxidil increased sympathetic activity (as assessed by norepinephrine turnover rates) to the large and small mesenteric artery after 35 days, but after 70 days sympathetic hyperactivity only persisted in the superior mesenteric artery coinciding with the development of medial hypertrophy in the superior mesenteric artery. The absence of medial hypertrophy in the large mesenteric artery may relate to the absence of persistent sympathetic hyperactivity, suggesting that the arterial hypertrophic response to minoxidil may require both volume expansion and sympathetic hyperactivity. Trophic effects of the sympathetic nervous system on vascular smooth muscle have been described (Bevan and Tsuru, 1979; Blaes and Boissel, 1983; 1984; Lee *et al.*, 1987, 1991; Mangiarua and Lee, 1992; Siwik and Brown, 1996; Xin *et al.*, 1997). With regard to the mechanism causing arterial sympathetic hyperactivity in response to arterial vasodilation, it is tempting to speculate that intravascular volume expansion and a high cardiac output (Ruzicka *et al.*, 1994) increase flow through the mesenteric bed, resulting in an increase in shear stress and activation of a spinal reflex similar to that observed in the heart (Schwartz *et al.*, 1973). If an increase in flow indeed increases arterial sympathetic activity and the latter

plays a role in the arterial trophic response to minoxidil, then concomitant diuretic therapy in addition to preventing the trophic response, should also prevent arterial sympathetic hyperactivity. This experiment still needs to be done.

In SHR, minoxidil induces structural remodeling of the superior mesenteric artery, resulting in a larger lumen, unchanged total medial area, and a decrease in wall thickness. In the large and small mesenteric arteries, minoxidil prevents the progression of medial hypertrophy associated with advancing hypertension (Lee, 1987b; Korner and Bobik, 1995) (Appendix - Figure 5). The decrease in the volume/surface ratio of the medial smooth muscle cells in the large and small mesenteric arteries indicates the prevention of medial smooth muscle cell hypertrophy rather than hyperplasia.

Intravascular volume expansion in the presence of a decrease in afterload, may be responsible for the increase in luminal area and structural remodeling of the superior mesenteric artery, since concurrent diuretic therapy prevented the increase in the lumen and structural remodeling of this vessel. Luminal enlargement of arteries results from chronically elevated blood flow and shear stress (Kamiya and Togawa, 1980; Zarins *et al.*, 1987; Brownlee and Langille, 1991; Langille 1996; Unthank *et al.*, 1996). Studies by Kamiya and Togawa (1980), and Zarins *et al.* (1987), have demonstrated that luminal enlargement occurs in large arteries when blood flow is increased by an arteriovenous fistula. The assumed increase in shear stress generated by volume expansion/increase in flow may generate endothelium-derived relaxing factors (e.g. nitric oxide) causing remodeling of the vessel (Tesfamarian and Cohen, 1988; Ohno *et al.*, 1993; Dzau and Gibbons, 1991, 1993; Scott-Burden and Vanhoutte, 1994). Studies are needed to clarify the specific stimuli and identify the particular growth factors that mediate the luminal expansion and wall remodeling that occurs in the superior mesenteric artery in response to arterial vasodilation. In contrast, other mechanisms, such as the sympathetic nervous system, appear to dominate in the large and small mesenteric arteries.

In the mesenteric arterial bed, minoxidil significantly decreases sympathetic activity to the superior mesenteric artery and normalizes the increased (compared with WKY rats) sympathetic activity to the large mesenteric arteries. In the superior mesenteric artery, additional overriding influences (hemodynamic versus non-hemodynamic) may in part relate to the absence of regression of medial hypertrophy that may be expected as a result of a decrease in sympathetic activity and blood pressure (see below). In the large mesenteric artery, the minoxidil-induced decrease in blood pressure is associated with the prevention of further medial hypertrophy. To what extent the concomitant decrease in sympathetic hyperactivity participates in the prevention of further arterial hypertrophy cannot be assessed from the present data. However, there is evidence suggesting that the sympathetic nervous system exerts a trophic influence on arterial structure (Bevan and Tsuru, 1979; Blaes and Boissel, 1983; 1984; Lee *et al.*, 1987, 1991; Mangiarua and Lee, 1992; Siwik and Brown, 1996; Xin *et al.*, 1997). In the present study, the inability of arterial vasodilators to cause regression of arterial hypertrophy in SHR is not related to further increases in sympathetic activity to the arteries (as is the case for the heart and for arteries of normotensive rats).

Previous studies suggest that structural alterations (e.g. smooth muscle cell hyperplasia) in the large and small mesenteric arteries of SHR, are already in place during the prehypertensive phase of SHR (Lee *et al.*, 1983, 1983b; Lee and Smeda, 1985) and cannot be abolished by normalizing blood pressure with arterial vasodilators (present studies - Smeda and Lee, 1991). In the superior mesenteric artery of SHR, medial hypertrophy is a secondary alteration that occurs from the presence of hypertension (Lee *et al.*, 1983, 1983b). In this instance, regression of medial hypertrophy of the superior mesenteric artery should be possible with antihypertensive therapy. In the present study, as in the study of Smeda and Lee (1991), arterial vasodilators do not cause regression of medial hypertrophy in the superior mesentery of SHR. Several explanations are possible

for this observation. Folkow and co-workers (Folkow *et al.*, 1972; Weiss and Lundgren, 1978) suggest that the blood vessels of SHR differ from those of WKY rats, that even at normal blood pressures the vasculature of SHR (hyper)responds to produce a thicker wall. Alternatively, trophic mechanisms such as the renin-angiotensin system may play a role. Angiotensin II can stimulate hypertrophic as well as hyperplastic growth of cultured smooth muscle cells (Geisterfer *et al.*, 1988; Stouffer and Owens, 1992). In addition, ACE inhibitors and angiotensin II receptor antagonists seem to be more effective than other drugs in the regression or prevention of arterial structural alterations (Lundie *et al.*, 1997; Gillies et al., 1997, 1998). These observations are consistent with the possibility that ACE inhibitors and angiotensin II receptor antagonists have specific effects on vascular structure independent of their antihypertensive action. In the present study, PRA increases during the initial days of arterial vasodilator treatment in SHR. The short-term increase in the circulating PRA does not exclude a long-term increase in the local vascular (mesenteric artery) renin activity (review - Dzau, 1993), thereby, maintaining arterial hypertrophy through the actions of angiotensin II, despite a decrease in blood pressure. The exact role of the renin-angiotensin system (local and circulating), in the persistence of mesenteric arterial hypertrophy, in response to arterial vasodilators, despite blood pressure control remains to be defined.

In the arterial tree, minoxidil does not alter collagen content, but further increases elastin content in the abdominal aorta, superior mesenteric, and renal arteries, despite blood pressure control. The increase in elastin content of the superior mesenteric artery is not associated with an increase in cross sectional area of the artery. In the absence of an increase in cross sectional area, minoxidil-induced structural remodeling of the artery may result in a size modification of the vessel wall components in particular smooth muscle cells to accommodate the increase in elastin content. The increase in elastin content in the superior mesenteric artery and abdominal aorta is associated with a

decrease in elastase activity (an enzyme that has been shown to selectively hydrolyze elastin). Several explanations are possible. Minoxidil may directly influence enzymes that are involved in the synthesis and degradation of connective tissue proteins. Minoxidil has been shown to directly alter the activities of the connective tissue enzymes lysine hydroxylase and prolyl hydroxylase (important in collagen biosynthesis) in fibroblast cell lines (Hautala *et al.*, 1992; Sharir and Zimmerman, 1993). In the present study, a direct effect of minoxidil to decrease elastase activity may explain the further increase in elastin content. Alternatively, the decrease in elastase activity and increase in elastin content of the large conducting vessels may be due to the minoxidil-induced antihypertensive effect. In stroke-prone SHR, a decrease in blood pressure by reserpine or hydralazine results in an decrease in elastase activity and an increase in elastin content in the aorta (Ito *et al.*, 1986, 1987).

The persistence of connective tissue in response to arterial vasodilation, may be due to volume loading and/or activation of trophic mechanisms (e.g. sympathetic nervous system, renin-angiotensin system). In SHR, hydralazine alone (Albaladejo *et al.*, 1994) or in combination with a diuretic (Limas *et al.*, 1984), does not cause regression of connective tissue content whereas combining an arterial vasodilator with a sympatholytic agent (Ehrhart and Ferrario, 1981) or an ACE inhibitor does, suggesting a trophic effect of sympathetic nerves and angiotensin II. In the present study, a trophic role of sympathetic nerves is unlikely since a decrease in connective tissue content is not seen despite a minoxidil-induced decrease in sympathetic activity to the superior mesenteric artery. Angiotensin II stimulates collagen synthesis in vascular smooth muscle cells (Kato *et al.*, 1991). In addition, chronic ACE inhibition prevents collagen accumulation in SHR, unrelated to a reduction in blood pressure, but associated to a reduction of aortic and not plasma converting enzyme (Albaladejo *et al.*, 1994). Activation of local (arterial)

renin-angiotensin system(s) by minoxidil may play a role in the persistence of connective tissue, despite a decrease in blood pressure.

4.3 Concluding Remarks

In conclusion, our results show that arterial vasodilators induce regional different responses in cardiovascular structure. Chronic minoxidil treatment of SHR and normotensive rats cause RVH and eccentric LVH. The changes in cardiac anatomy are compatible with cardiac volume overload as a major contributor. In normotensive rats, increases in cardiac filling pressures precede the changes in cardiac anatomy. However, chronic cardiac volume overload may not be the sole mechanism, since concurrent diuretic therapy prevents intravascular volume expansion, but converts eccentric LVH to concentric LVH. These results suggest that in addition to volume load other mechanisms, in particular the sympathetic hyperactivity may play a role in the minoxidil-induced alterations in cardiac anatomy.

In normotensive Wistar rats, minoxidil induces the development of medial hypertrophy in the superior mesenteric artery. In SHR, despite an antihypertensive response, minoxidil induces i) structural remodeling of the superior mesenteric artery resulting in a larger lumen, ii) prevents the development of medial smooth muscle cell hypertrophy associated with advancing hypertension in the large and small mesenteric arteries, and iii) increases elastin content in the large conducting vessels. In both SHR and normotensive Wistar rats, concurrent diuretic therapy prevents the structural alterations in the superior mesenteric artery, implicating intravascular volume expansion as a possible mechanism. In SHR, the prevention of further medial hypertrophy in the large and small mesenteric arteries, may be related to decreases in blood pressure and sympathetic activity.

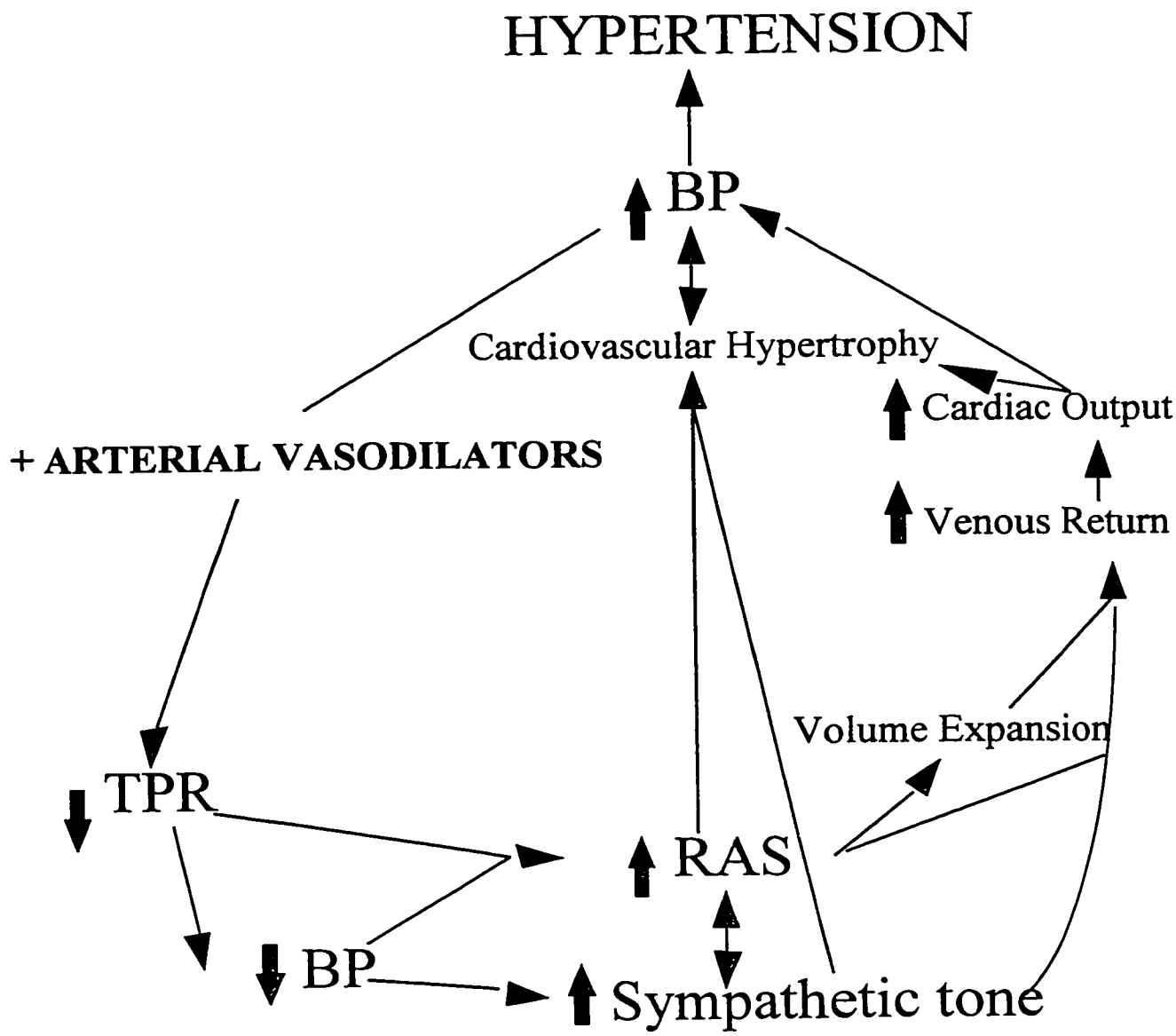
The results suggest that both neurohumoral (sympathetic nervous system) and mechanical (volume loading) factors may be involved as possible causal or contributory mechanisms for the cardiovascular effects of arterial vasodilators as outlined in Figure 5.1. Briefly, in hypertension, an increase in blood pressure is associated with the development of cardiovascular hypertrophy. Treatment with arterial vasodilators reduces blood pressure by decreasing total peripheral resistance (TPR). Arterial vasodilators increase sympathetic activity, and stimulate the renin-angiotensin system (RAS) and via the trophic actions of norepinephrine and angiotensin II respectively, may induce the development of cardiovascular hypertrophy. Arterial vasodilators also induce volume expansion either by activation of the RAS (via the actions aldosterone), or via a shift of blood from the peripheral to the central compartment. Both volume expansion and the increase in sympathetic tone results in an increase venous return, and increase cardiac output. An increase in cardiac output increases blood pressure, and may induce hypertrophy of the heart and blood vessels.

4.4 Future Experiments

The results obtained from these experiments suggest that arterial vasodilator treatment of normotensive Wistar and SHR is associated with sympathetic hyperactivity. However, the importance of the sympathetic nervous system for the development of cardiovascular structural changes during treatment with arterial vasodilators remains to be determined. For this, future experiments should focus on eliminating the peripheral sympathetic nervous system by administration of guanethidine with or without removal of the adrenal medullae, or more specifically by removal of the adrenal medullae alone, or by α_1- or β- adrenergic blockade. The demonstration by Leenen and co-workers

(Ruzicka and Leenen, 1993; Ruzicka *et al.*, 1994) that the angiotensin II receptor blocker losartan prevents the remodeling of the heart by minoxidil in normotensive rats, indicates the importance of the renin-angiotensin system in minoxidil-induced cardiac hypertrophy. Further experiments employing the concomitant administration of ACE inhibitors or angiotensin II receptor blockers are required to assess the importance of the renin-angiotensin system in the minoxidil-induced cardiovascular structural alterations in hypertensive rats. The results suggest that intravascular volume expansion or increased arterial flow appear to be responsible for arterial smooth muscle cell hypertrophy in response to minoxidil treatment of normotensive and SHRs. Further experiments are required to define the actual trigger mechanism i.e. growth factors (e.g. PDGF, TGFβ, endothelin, angiotensin II, EFG, aFGF, bFGF) and possible signaling pathways that may be involved in translating the physical stress of intravascular volume expansion/increased flow into biochemical stimulation and smooth muscle cell hypertrophy. It is expected that these experiments will provide a more clear insight into mechanisms responsible for the cardiovascular structural alterations during treatment with arterial vasodilators.

Figure 5.1 Schematic representation of arterial vasodilator-induced cardiovascular hypertrophy. BP = Blood pressure; TPR = Total peripheral resistance.


HYPERTENSION
BP
Cardiovascular Hypertrophy
Cardiac Output
+ ARTERIAL VASODILATORS
Venous Return
Volume Expansion
TPR
RAS
BP
Sympathetic tone

5.0 **REFERENCES**

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6.0 APPENDIX

6.1 EFFECTS OF ARTERIAL VASODILATORS ON CARDIAC HYPERTROPHY AND SYMPATHETIC ACTIVITY IN RATS.

J. TSOPORIS, AND F. H. H. LEENEN. HYPERTENSION 11:376-386, 1988

Effects of Arterial Vasodilators on Cardiac Hypertrophy and Sympathetic Activity in Rats

JAMES TSOPORIS AND FRANS H. H. LEENEN

SUMMARY In spontaneously hypertensive rats (SHR), the progression (or absence of regression) of cardiac hypertrophy despite adequate blood pressure (BP) control by arterial vasodilators has been attributed to increased cardiac sympathetic activity. We evaluated changes in indices of general and cardiac sympathetic tone in relation to changes in cardiac anatomy during treatment of normotensive rats and SHR with hydralazine, 120 mg/L, or minoxidil, 120 mg/L of drinking water. In SHR, both vasodilators reduced BP rapidly and consistently. Significant increases in heart rate and plasma norepinephrine were observed only in the initial 2 days of arterial vasodilator treatment. After 5 weeks of treatment, marked increases in left and right ventricular sympathetic activity (as assessed by norepinephrine turnover rates) were present, but no increase was seen in heart rate and plasma norepinephrine. Intravascular volume expansion was observed on Day 14 of minoxidil and Day 35 of hydralazine treatment. Prolonged treatment with minoxidil induced significant increases in left ventricular internal diameter, as well as in left and right ventricular weights, but not in the wall thickness of the left ventricle. Treatment with hydralazine did not affect left ventricular weight and caused a small increase in the weight of the right ventricle. In normotensive rats, both vasodilators initially decreased BP, but tolerance developed within 1 to 2 weeks of treatment. Plasma norepinephrine and heart rate showed increases only at Day 1 of either treatment, whereas cardiac sympathetic hyperactivity persisted at 2 and 5 weeks of treatment. Changes in cardiac anatomy were qualitatively similar to those observed in SHR. We conclude that, during treatment of normotensive rats and SHR with arterial vasodilators, cardiac sympathetic hyperactivity persists and may be involved in the cardiac effects of arterial vasodilators. However, other mechanisms, such as chronic cardiac volume overload, may also play an important role, particularly with minoxidil. (Hypertension 11: 376–386, 1988)

KEY WORDS • hydralazine • minoxidil • cardiac hypertrophy • cardiac sympathetic activity • blood volume

IN spontaneously hypertensive rats (SHR), but also in other hypertensive models (e.g., two-kidney, one clip [2K1C]) as well as in normotensive rats, long-term treatment with arterial vasodilators such as hydralazine and minoxidil is associated with progression (or absence of regression) of cardiac hypertrophy.[1–6] Chronic sympathetic hyperactivity has been implicated in this effect on the heart.[7,8] It has been assumed that cardiac sympathetic hyperactivity during long-term treatment of SHR with arterial vasodilators persists based on 1) the measurement of myocardial catecholamine content showing a 20% increase in myocardial catecholamines in SHR treated with hydralazine for 6 weeks[8] and 2) pharmacological intervention studies showing that the association of the sympatholytic agent methyldopa with minoxidil reversed cardiac hypertrophy in SHR[3,5] and prevented the minoxidil-induced cardiac hypertrophy in normotensive rats.[5] However, this represents rather indirect and circumstantial evidence for sympathetic hyperactivity.

Whereas an acute reflex-mediated increase in sympathetic activity following the administration of an arterial vasodilator has been well documented,[9–11] one might expect resetting of the baroreceptors[12] during long-term treatment and therefore a return of sympathetic tone toward baseline. However, except for heart rate, no data in SHR are available in this regard. In SHR the (presumably baroreceptor reflex–mediated)

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increases in heart rate and cardiac index disappeared within 2 hours after a single administration of hydralazine despite a persistent antihypertensive effect.[13] Following long-term treatment of SHR with hydralazine for 3 to 24 weeks, increases in heart rate (by 5–10%) were reported in some studies[3, 14, 15] but not in other studies.[8, 16] Similar discrepancies are evident in normotensive rats. Kohlmann et al.[17] reported that, after 2 weeks of hydralazine treatment, a small blood pressure (BP)–lowering effect persisted and plasma catecholamines, heart rate, and the turnover rate of norepinephrine in the heart were all increased. However, other studies[3, 14, 18] failed to find an increase in heart rate despite a small reduction in BP following long-term hydralazine administration. The reason for these inconsistent findings is not obvious. However, the validity of heart rate as an index of cardiac or general sympathetic activity is limited. To the best of our knowledge, no studies have assessed cardiac or even general sympathetic activity more directly during long-term treatment of SHR with either hydralazine or minoxidil. To provide a clearer insight into the long-term effects of arterial vasodilators in normotensive rats and SHR on cardiac sympathetic activity and cardiac hypertrophy, we evaluated the time course of changes in general sympathetic activity (plasma catecholamines and BP response to hexamethonium) versus cardiac sympathetic activity (heart rate and left [LV] and right ventricular [RV] norepinephrine turnover rate) in relationship to changes in intravascular volume and cardiac anatomy during long-term treatment of normotensive rats and SHR with the arterial vasodilators hydralazine and minoxidil.

Materials and Methods

Male Wistar-Kyoto rats (WKY) and SHR were obtained from Taconic Farms (Germantown, NY, USA) at 16 weeks of age. Male Wistar rats weighing 250 to 260 g were obtained from Charles River Breeding Laboratories, Montreal, Canada. Rats were housed two to a cage and given food (Purina rat chow, St. Louis, MO, USA; 180 μmol Na/g food) and water ad libitum and kept on a 12-hour light/dark cycle. Following a 5-day acclimatization period, WKY and SHR were randomized into four groups ($n = 8–10$/group): untreated normotensive (WKY), untreated hypertensive, hypertensive treated with hydralazine, 120 mg/L, and hypertensive treated with minoxidil, 120 mg/L of drinking water.[2, 4–6] In separate experiments, normotensive Wistar rats were randomized into three groups ($n = 10–12$/group): untreated, hydralazine-treated, or minoxidil-treated. Fluid intake and body weight were monitored regularly, and the animals were handled twice weekly.

Different groups of rats were treated for either 2, 14, or 35 days. Two days before the end of the 14- and 35-day treatments, a PE-50 (Clay Adams, Parsippany, NJ, USA) catheter was inserted into a carotid artery for monitoring of BP and heart rate for the next 2 days.[19] In the 2-day experiment, a carotid artery was cannulated 48 hours before the beginning of drug treatment and measurements were done on Days 0, 1, and 2 of treatment. Hemodynamic studies were performed in the morning under the same environmental conditions in a quiet study room. BP and heart rate were recorded in conscious, unrestrained animals after 30 minutes of rest. On the last day of each treatment period, after recording resting BP, blood samples were taken in all groups from the carotid catheter for determination of plasma catecholamines in duplicate by radioenzymatic assay[20] and in SHR alone for plasma renin activity (PRA) by an antibody-trapping technique.[21] For this, whole arterial blood (400 μl) was allowed to flow directly into chilled microcentrifuge tubes containing 0.26 M EGTA and 0.2 M glutathione and an additional 400 μl was collected into 0.0026 M EDTA (for PRA). Subsequently, plasma and blood volume were determined by the radioiodinated human serum albumin–iodine-131 technique requiring 300 μl of blood, as described previously.[22] Following the collection of blood samples, the arterial catheters were reconnected to the pressure transducer and BP was monitored while the animals recovered for 10 minutes. Then, hexamethonium (30 mg/kg) was injected through the carotid catheter and maximal decreases in BP were noted.

At the end of an experiment, the animals were anesthetized with chloroform. The hearts were excised and immediately placed in ice-cold saline to arrest the heart in diastole and to remove blood. LV and RV weights were determined as described by Fenje and Leenen.[1] After the weighing procedure, a transverse mid-level slice of LV was obtained by two transverse cuts at one third and two thirds of the length.[23] This slice was viewed under a light microscope using a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8 to 10 points around the circular section, and the average was calculated. The internal diameters of the slices were measured from the farthest points of the major (anterior–posterior) and minor (septal–lateral) internal diameters. In the 5-week experiments, dry LV and RV weights were determined after drying the ventricles for 24 hours in an oven at 37°C.

Norepinephrine turnover rates were estimated after 1, 14, or 35 days of treatment in normotensive Wistar rats and after 35 days of treatment in SHR. Turnover rate was determined by the decline of endogenous norepinephrine in the left and right ventricles after its synthesis had been inhibited by metyrosine (α-methyl-DL-*p*-tyrosine methyl ester hydrochloride). Metyrosine was dissolved in distilled water and administered subcutaneously at 200 mg/kg body weight, with a second dose of 100 mg/kg body weight given 4.5 hours later. For the experiments in normotensive rats, six rats per group (untreated; hydralazine-treated, 120 mg/L; and minoxidil-treated, 120 mg/L) per treatment period (1, 14, and 35 days) were decapitated 0, 4.5, and 9 hours after the first dose. Rats were allocated in groups of nine (one for each of the three treatments at the three time points), providing six disappearance curves per treatment group for statistical analysis. In the SHR experiment, six rats per group (untreated WKY, un-

treated SHR, and SHR treated for 5 weeks with either hydralazine, 120 mg/L, or minoxidil, 120 mg/L) were decapitated 0, 4.5, and 9 hours after the first dose. Rats were allocated in groups of 12 (one for each of the four groups at the three time points), providing six disappearance curves per group for statistical analysis. The hearts were quickly removed, rinsed in cold saline, blotted dry, and dissected free of atria and great vessels. Left and right ventricles were separated, frozen in dry ice, stored at −70°C, and processed for assay within 24 hours. The frozen ventricles were weighed, and 100 mg each of RV and LV tissue (cut from the apex) was homogenized in 2 ml of iced 0.2 N acetic acid with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 5000 *g* for 20 minutes at 4°C. The extraction of norepinephrine from the supernates and subsequent analysis by high performance liquid chromatography with electrical chemical detection were performed as described by Shum et al.[24] and Sole et al.[25] The turnover rate constant (k) was calculated from the rate of decline of the logarithm of the tissue norepinephrine concentration (regression coefficient). Half-life and turnover rate were calculated from the following equations: half-life = $0.693/k$ and turnover rate = [norepinephrine] · k, where [norepinephrine] is the concentration of norepinephrine in each tissue before inhibition of synthesis.

Results are presented as means ± SEM. Statistically significant differences between groups at a given treatment period were analyzed by analyses of variance. The least significant difference approach was used to locate significant differences; a p level below 0.05 was considered significant.

Results

Blood Pressure

Treatment of normotensive rats induced a clear drop in BP during the initial days of treatment (Table 1). However, tolerance developed within 1 to 2 weeks of treatment, and a small decrease in BP persisted only for hydralazine.

Treatment of SHR with either hydralazine or minoxidil resulted in a significant fall in mean arterial pressure (MAP = diastolic BP + 1/3 pulse pressure) from 170–180 (pretreatment level) to 110–120 mm Hg in the initial days of treatment. Prolonged treatment (14 or 35 days) kept MAP at or slightly above the level seen in untreated WKY.

General Sympathetic Activity

Plasma Catecholamines

Both vasodilators significantly increased plasma norepinephrine in normotensive rats on Day 1 of treatment (Figure 1). However, after 2, 14, and 35 days of either treatment, norepinephrine levels were similar to control values. Plasma norepinephrine concentration of untreated SHR was significantly higher than that in WKY. Treatment of SHR for 1 or 2 days resulted in a significant increase in plasma norepinephrine (more marked in the hydralazine group) compared with untreated SHR. After 14 and 35 days of treatment, however, norepinephrine levels were not significantly different between treated SHR and untreated SHR.

WKY and untreated SHR showed similar values for plasma epinephrine of around 100 pg/mL. Plasma epinephrine concentration was not affected by treatment in SHR and normotensive rats (data not shown).

BP Response to Hexamethonium

In normotensive rats, both vasodilators significantly potentiated the BP-lowering response to hexamethonium after 1 day of treatment but not after long term treatment (Figure 2). Ganglionic blockade caused significantly larger decreases in BP of untreated SHR as compared with untreated WKY. Treatment of SHR with hydralazine or minoxidil significantly reduced the BP response to hexamethonium. Differences in percent changes were less marked, and the inhibitory effect of treatment on the response to hexamethonium

TABLE 1. *BP and Heart Rate in Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil*

Variable	Normotensive Wistar rats			Untreated WKY	SHR		
	Untreated	Hydralazine	Minoxidil		Untreated	Hydralazine	Minoxidil
MAP (mm Hg)							
Day 0	106 ± 4	113 ± 4	111 ± 4	109 ± 6	175 ± 6	177 ± 9	174 ± 4
Day 1	102 ± 5	86 ± 3*	85 ± 2*	110 ± 5	172 ± 6	127 ± 11*	114 ± 9*
Day 2	113 ± 3	89 ± 3*	98 ± 5*	106 ± 6	167 ± 4	117 ± 5*	111 ± 5*
Day 14	109 ± 4	104 ± 5	109 ± 5	98 ± 4	169 ± 5	130 ± 9*	126 ± 7*
Day 35	120 ± 5	106 ± 4†‡	119 ± 9	112 ± 4	180 ± 5	126 ± 6†	122 ± 9*
Heart rate (beats/min)							
Day 0	404 ± 14	413 ± 11	424 ± 11	398 ± 17	414 ± 13	370 ± 22	392 ± 15
Day 1	390 ± 18	471 ± 9*	470 ± 13*	398 ± 19	415 ± 12	444 ± 16†	445 ± 19†
Day 2	428 ± 23	419 ± 16	444 ± 14	363 ± 11	384 ± 17	442 ± 17†	458 ± 19†
Day 14	390 ± 11	378 ± 12	387 ± 10	337 ± 14	349 ± 13	330 ± 11	354 ± 10
Day 35	429 ± 14	398 ± 16	411 ± 15	343 ± 13	385 ± 19	365 ± 18	339 ± 19†

Values represent means ± SEM (n = 8–10/group for WKY and SHR; n = 10–12/group for normotensive Wistar rats).
*$p < 0.01$, †$p < 0.05$ compared with untreated SHR or untreated normotensive Wistar rats.
‡$p < 0.05$, compared with minoxidil.

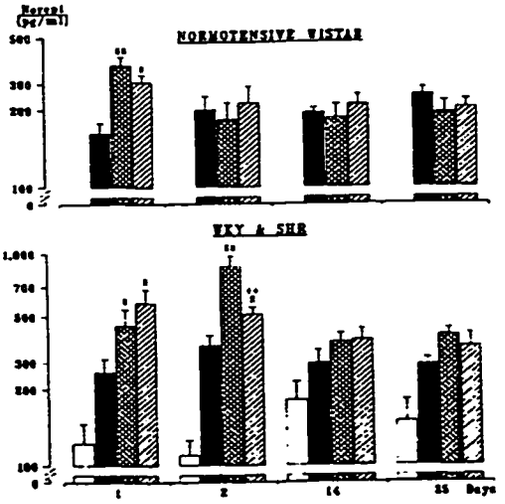

FIGURE 1. *Plasma norepinephrine in untreated normotensive Wistar rats (■), WKY (□), and SHR (■), as well as normotensive Wistar rats treated with either hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■) and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). Single ($p<0.05$) and double asterisks ($p<0.01$) indicate significant difference compared with untreated rats. Double dagger indicates significant difference ($p<0.01$) compared with hydralazine.*

was only consistently significant for minoxidil (data not shown).

Cardiac Sympathetic Activity

Heart Rate

Both vasodilators significantly increased heart rate in normotensive rats on Day 1 of treatment, but this increase disappeared after 2 days and heart rate subsequently remained at control levels (see Table 1). Heart rate in untreated SHR tended to be higher than that in untreated WKY (p = NS). In the initial days of treatment, hydralazine and minoxidil induced similar increases in heart rate. Subsequently, heart rate returned to the level seen in untreated SHR and remained at or below control levels with prolonged treatment.

Norepinephrine Turnover Rate

Correlation coefficients (range, 0.92–0.99) for the linear regressions of norepinephrine versus time were all significant ($p<0.001$). Both vasodilators significantly increased the three parameters of LV sympathetic activity in normotensive rats after 1, 14, and 35 days of treatment. In the right ventricle, minoxidil and hydralazine increased fractional turnover rate on Day 1 and absolute turnover rate and half-life of norepinephrine on Days 1 and 35 of treatment (Figure 3, Table 2).

A significant increase in the fractional turnover rate (k) of endogenous norepinephrine, absolute turnover rate, and decrease in half-life were observed in the left but not in the right ventricle of untreated SHR as compared with untreated WKY. Treatment of SHR with either hydralazine or minoxidil for 5 weeks significantly increased cardiac sympathetic activity in both ventricles in a similar manner, as assessed by fractional turnover rate, absolute turnover rate, and half-life of norepinephrine (Figure 4; see Table 2).

Plasma and Blood Volumes

In normotensive rats, both vasodilators significantly increased plasma volume after 2 and 5 weeks of treatment and blood volume after 5 weeks (Table 3). Untreated WKY and SHR showed small, nonsignificant differences in plasma and blood volume (see Table 3). After 14 days of treatment, an increase in plasma volume was observed only with minoxidil. Both vasodilators increased plasma volume after 35 days of treatment, but this increase was more pronounced in the minoxidil group. A significant increase in blood volume was seen only after 35 days of treatment with minoxidil.

PRA

Untreated WKY and SHR showed small, nonsignificant differences in PRA (Table 4). Both hydralazine and minoxidil caused moderate (50–100%) increases in PRA during the initial 2 days of treatment. However, during long-term treatment for either 2 or 5 weeks, PRA in treated and untreated SHR was very similar.

Cardiac Anatomy

Right and Left Ventricular Weights

Minoxidil increased LV and RV weights in normotensive rats after 2 weeks of treatment, and this re-

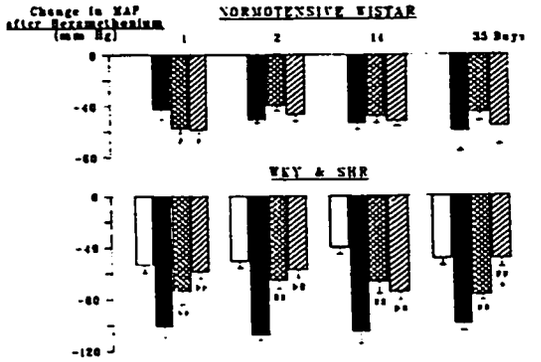

FIGURE 2. *Blood pressure response to hexamethonium in untreated normotensive Wistar rats (■), WKY (□), and SHR (■), as well as normotensive Wistar rats treated with either hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■), and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). Single ($p<0.05$) and double asterisks ($p<0.01$) indicate significant difference compared with untreated rats. Dagger indicates significant difference ($p<0.05$) compared with hydralazine.*

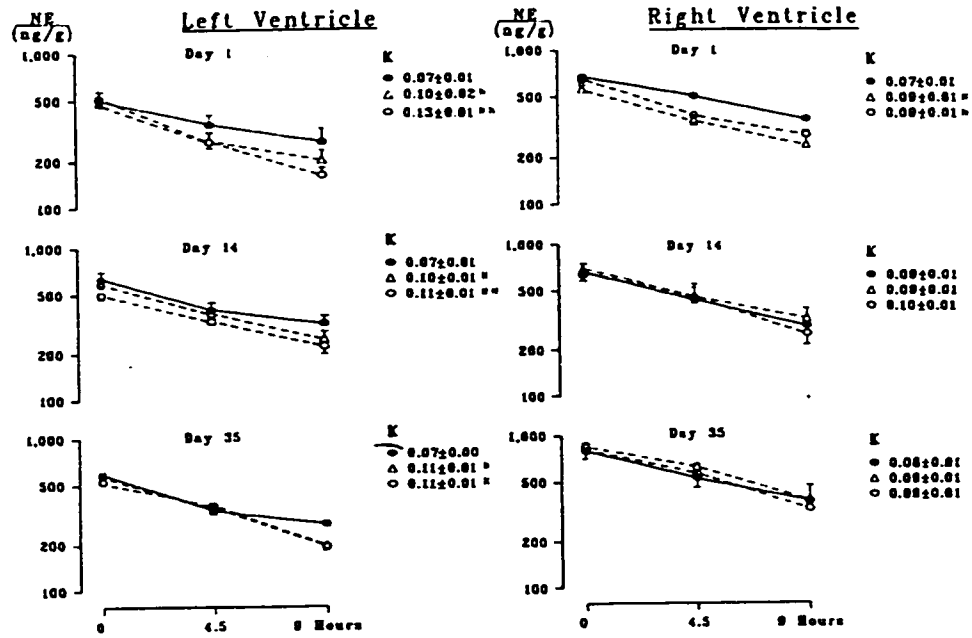

FIGURE 3. *Norepinephrine (NE) turnover rates in the left and right ventricles of normotensive Wistar rats, untreated (●) or treated with either hydralazine, 120 mg/L (△), or minoxidil, 120 mg/L (○), for 1, 14, or 35 days. K = slope = fractional turnover rate of norepinephrine. Points represent means ± SEM (n = 6/group). Single (p<0.05) and double asterisks (p<0.01) indicate significant difference compared with untreated Wistar rats.*

TABLE 2. *Half-life and Turnover Rate of Norepinephrine in the Left and Right Ventricles of Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil*

Variable	Left ventricle		Right ventricle	
	Half-life (hr)	Turnover rate (ng/g/hr)	Half-life (hr)	Turnover rate (ng/g/hr)
Normotensive Wistar rats				
Day 1				
Control	10.6 ± 1.5	35 ± 8	10.7 ± 1.1	45 ± 6
Hydralazine	8.3 ± 0.6*	49 ± 9	7.5 ± 0.5*	53 ± 3
Minoxidil	5.7 ± 0.6†	66 ± 8*	7.4 ± 0.9*	66 ± 10*
Day 14				
Control	9.5 ± 0.7	47 ± 7	8.3 ± 0.8	58 ± 6
Hydralazine	7.0 ± 0.7*	59 ± 6	6.9 ± 0.5	63 ± 4
Minoxidil	7.3 ± 0.5*	48 ± 3	7.9 ± 0.5	57 ± 4
Day 35				
Control	9.4 ± 0.5	44 ± 3	10.1 ± 1.8	60 ± 11
Hydralazine	6.2 ± 0.4*	66 ± 11*	7.5 ± 0.4*	80 ± 6*
Minoxidil	6.6 ± 0.6*	59 ± 8*	7.6 ± 0.5*	75 ± 9
Untreated WKY, Day 35	7.9 ± 0.8	60 ± 8	7.6 ± 0.3	70 ± 5
SHR, Day 35				
Untreated	5.0 ± 0.2	84 ± 6	6.8 ± 0.5	90 ± 6
Hydralazine	4.2 ± 0.2	104 ± 5*	4.9 ± 0.2*	124 ± 8*
Minoxidil	4.0 ± 0.4*	108 ± 13*	4.8 ± 0.3*	112 ± 8*

Values represent means ± SEM (n = 18/group).
*$p<0.05$, †$p<0.01$, compared with untreated SHR or untreated normotensive Wistar rats.

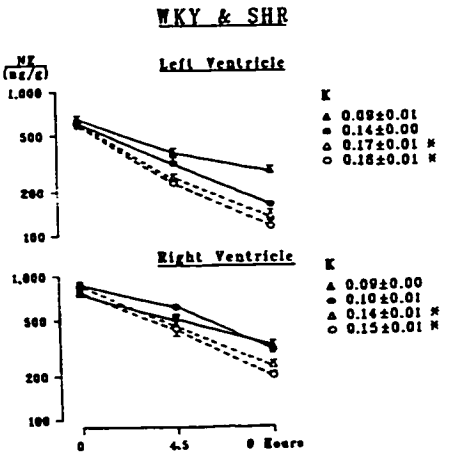

FIGURE 4. *Norepinephrine (NE) turnover rates in the left and right ventricles of untreated WKY (▲) and SHR (●) and SHR treated with either hydralazine, 120 mg/L (△), or minoxidil, 120 mg/L (○) for 35 days. K = slope = fractional turnover rate of norepinephrine. Points represent means ± SEM (n = 6/group). Asterisk indicates significant difference (p<0.05) compared with untreated SHR.*

sponse was more marked ($p<0.01$) after 5 weeks. Hydralazine increased RV weight, but only after 5 weeks of treatment, and did not affect LV weight. LV weight was greater ($p<0.05$) in the minoxidil-treated than in the hydralazine-treated group at both 2 and 5 weeks of treatment.

LV weight of untreated SHR showed an increase of about 50% over that of untreated WKY. Minoxidil treatment caused a significant increase in LV weight after 14 days, and this response was more marked ($p<0.01$) after 35 days of treatment. Treatment with hydralazine had no effect on LV weight (Figure 5). RV weight was significantly increased in untreated SHR compared with WKY. Treatment with either hydralazine or minoxidil induced similar increases in RV weight after 14 days. After 35 days, treatment with minoxidil resulted in a more marked ($p<0.05$) increase and was greater than that observed with hydralazine treatment ($p<0.05$; Figure 6).

Dry LV and RV weights of the various groups were determined in the 5-week experiments and showed a similar pattern to that seen with wet weights (see Table 4). Dry/wet ratios for LV and RV weights showed no significant differences between treated and untreated groups.

Left Ventricular Wall Thickness and Dimensions

The increase in LV wall thickness in untreated SHR as compared with untreated WKY was significant at each treatment period. Long-term treatment with either hydralazine or minoxidil had no effect on LV wall thickness of SHR or of normotensive Wistar rats (see Table 4).

LV internal diameters (the major or anterior–posterior diameter; Figure 7) were significantly increased in untreated SHR as compared to untreated WKY. Minoxidil-induced increases in LV internal diameters were seen after 14 days and were more pronounced ($p<0.05$) after 35 days. Treatment with hydralazine was associated with small, nonsignificant increases. The minor (septal–lateral) LV internal diameter followed a similar pattern, reaching values of 2.7 ± 0.1, 3.3 ± 0.1, 3.4 ± 0.1, and 4.0 ± 0.2 mm at 5 weeks for untreated WKY, untreated SHR, hydralazine-treated SHR and minoxidil-treated SHR, respectively.

In normotensive rats, the two vasodilators caused a similar pattern of changes as in SHR. The increases in LV internal diameters caused by minoxidil were significantly larger ($p<0.01$) than the ones caused by hydralazine.

Body Weight and Water Intake

In normotensive Wistar rats, body weight increased to 327 ± 6, 334 ± 7, and 338 ± 8 g at the end of the 5-week experiment for the untreated, hydralazine-treated, and minoxidil-treated groups, respectively. At the end of this experiment, water intake amounted to 7 ± 1 ml/100 g/day in all three groups, resulting in a drug intake of 8.6 ± 1.1 mg/kg/day for hydralazine and 8.5 ± 1.0 mg/kg/day for minoxidil.

Body weight was significantly higher and water in-

TABLE 3. *Plasma and Blood Volumes in Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil*

	Normotensive Wistar rats			Untreated	SHR		
Variable	Untreated	Hydralazine	Minoxidil	WKY	Untreated	Hydralazine	Minoxidil
Plasma volume (ml/100 g body weight)							
Day 2	3.8±0.1	4.0±0.1	4.0±0.1	3.7±0.1	3.9±0.1	3.9±0.1	3.9±0.1
Day 14	3.5±0.1	3.8±0.1*	3.7±0.1*	3.6±0.1	3.7±0.1	3.7±0.1	4.1±0.1*
Day 35	3.9±0.1	4.5±0.1†	4.5±0.1†	3.8±0.1	3.8±0.1	4.1±0.1*	4.5±0.1*,‡
Blood volume (ml/100 g body weight)							
Day 2	7.2±0.2	7.7±0.1	7.6±0.2	6.5±0.2	6.5±0.1	6.9±0.1	6.9±0.1
Day 14	6.9±0.1	7.2±0.3	7.0±0.2	6.7±0.2	7.1±0.3	6.9±0.2	6.9±0.2
Day 35	7.7±0.2	8.4±0.2†	8.5±0.2†	7.2±0.2	7.5±0.3	7.9±0.2	8.8±0.3*,‡

Values represent means ± SEM (n = 8–10/group for WKY and SHR; n = 10–12/group for normotensive Wistar rats).
*$p<0.05$, †$p<0.01$, compared with untreated SHR or untreated normotensive Wistar rats.
‡$p<0.05$, compared with hydralazine.

TABLE 4. *PRA and Parameters of Cardiac Anatomy in Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil*

Variable	Untreated WKY	SHR			Normotensive Wistar rats		
		Untreated	Hydralazine	Minoxidil	Untreated	Hydralazine	Minoxidil
PRA (ng Ang I/ml/hr)							
Day 1	3.9±1.0	5.2±1.6	10.4±1.6*	7.1±2.0	—	—	—
Day 2	4.8±1.0	6.1±1.7	10.4±2.6*	11.4±2.1†	—	—	—
Day 14	4.1±1.0	4.9±0.6	5.3±0.9	5.5±0.8	—	—	—
Day 35	3.1±0.8	2.7±0.4	2.1±0.2	3.2±0.5	—	—	—
Dry LV weight (mg/100 g body weight)§	53±1	76±2	75±2	91±2†	44±1	44±1	50±1†‡
Dry RV weight (mg/100 g body weight)§	12±0.4	15±0.7	16±0.6	21±1.3†	9.3±0.4	10.7±0.3*	11.7±0.4†
LV wall thickness (mm)§	2.6±0.05	3.2±0.1	3.0±0.1	3.2±0.1	2.3±0.07	2.2±0.03	2.4±0.07

Values represent means ± SEM (n = 8–10/group for WKY and SHR; n = 10–12/group for normotensive Wistar rats). Ang I = angiotensin I; LV = left ventricular; RV = right ventricular.
*$p<0.05$, †$p<0.01$, compared with untreated SHR or untreated normotensive Wistar rats.
‡$p<0.01$, compared with hydralazine.
§Day 35.

take significantly less in untreated WKY as compared with untreated SHR. The untreated and treated hypertensive groups showed a similar pattern of weight gain and water intake. At the end of the 5-week experiment, body weight amounted to 432 ± 8, 328 ± 4, 334 ± 4, and 335 ± 6 g, and water intake, 11 ± 1, 16 ± 1, 13 ± 1, and 13 ± 1 ml/100 g body weight/day in the untreated WKY, untreated SHR, and SHR treated with either hydralazine, 120 mg/L, or minoxidil, 120 mg/L. Drug intakes amounted to 16.1 ± 1 and 16.1 ± 0.6 mg/kg/day for hydralazine and minoxidil, respectively.

Discussion

Studies by Sen et al.[4,5] established that BP control alone is not sufficient to bring about regression of cardiac hypertrophy in SHR. In agreement with these studies, our results show that long-term treatment of SHR with hydralazine or minoxidil normalizes BP but that hydralazine treatment results in the persistence of LV hypertrophy (LVH) whereas minoxidil treatment leads to a 20% increase in LVH. More prolonged treatment of SHR with hydralazine (3–9 months) does result in a small decrease in cardiac hypertrophy (about

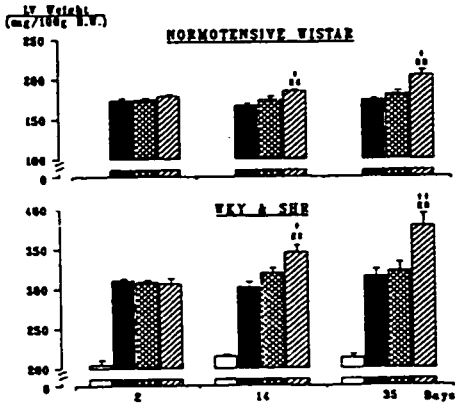

FIGURE 5. *Left ventricular (LV) weight of untreated normotensive Wistar rats (■), WKY (□), and SHR (■), as well as normotensive Wistar rats treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■), and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). BW = body weight. Single ($p<0.05$) and double asterisks ($p<0.01$) indicate significant difference compared with untreated rats. Single ($p<0.05$) and double daggers ($p<0.01$) indicate significant difference compared with hydralazine.*

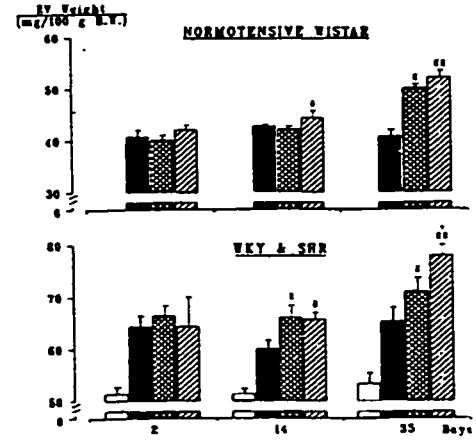

FIGURE 6. *Right ventricular (RV) weight of untreated normotensive Wistar rats (■), WKY (□), and SHR (■), as well as normotensive Wistar rats treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■), and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). BW = body weight. Single ($p<0.05$) and double asterisks ($p<0.01$) indicate significant difference compared with untreated rats. Single ($p<0.05$) and double daggers ($p<0.01$) indicate significant difference compared with hydralazine.*

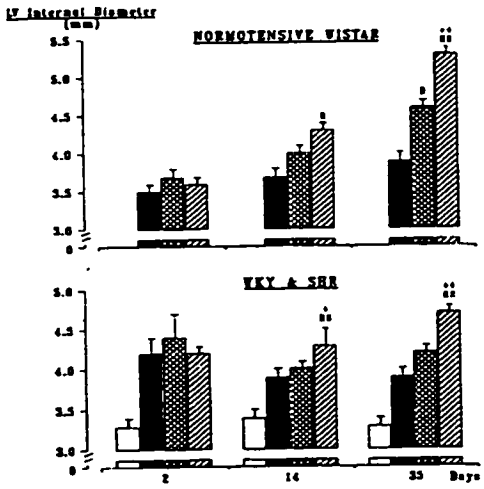

FIGURE 7. *Left ventricular (LV) internal diameter (major axis) of untreated normotensive Wistar rats (■), WKY (□), and SHR (■), as well as normotensive Wistar rats treated with either hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■) and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). Single (p<0.05) and double asterisks (p<0.01) indicate significant difference compared with untreated rats. Single (p<0.05) and double daggers (p<0.01) indicate significant difference compared with hydralazine.*

10%), but not a return to normal levels despite prolonged normotension.[15, 26, 27] Our results in normotensive rats also confirm previous reports showing that minoxidil treatment, but not hydralazine treatment,[3, 14] causes a significant increase in LV weight.[5] None of the previous studies, however, assessed changes in cardiac anatomy. Measurements of cardiac mass alone will not discriminate between the different types of cardiac adaptation that occur in response to a pressure load or a volume load. The former initiates concentric hypertrophy with increased wall thickness, whereas the latter leads to eccentric hypertrophy with an increase in luminal size and only a small increase in wall thickness.[28] When the ventricular architecture of SHR and WKY is compared, SHR show an increase in LV and RV weights and increased ventricular dimensions,[29] suggesting that both pressure and volume overload participate in the development of cardiac hypertrophy in SHR. Our results clearly show that the increase in LV weight during long-term minoxidil treatment is associated with a significant increase in RV weight as well as LV internal diameters but not in LV wall thickness. In the case of hydralazine, LV weight and anatomy remained unchanged and only a small increase in RV weight was observed. Therefore, long-term minoxidil treatment of SHR initiated the development of eccentric LV hypertrophy superimposed on the preexisting hypertrophy. This response is not specific for SHR. In normotensive rats, long-term minoxidil treatment also resulted in the development of RV hypertrophy (RVH) and eccentric LVH, whereas hydralazine treatment also caused RVH but only a minor increase in LV internal diameter and no increase in LV weight. In 2K1C hypertensive rats, treatment with either hydralazine or minoxidil (5–8 weeks) decreased BP only temporarily but resulted in the potentiation of RVH and the development of eccentric LVH superimposed on the preexisting LVH.[2, 6] However, the heart of SHR appears more responsive to minoxidil, having more marked increases in LV and RV weights and LV dimensions despite normal BP than are seen in 2K1C hypertensive rats with persistence of hypertension. Increases in dry weight (see Table 4) and absence of changes in dry/wet ratios indicate that the increases in ventricular weight represent hypertrophy and not increases in myocardial water content (edema). However, morphometric measurements will be needed to confirm the actual type of hypertrophy.

Chronic cardiac volume overload could be responsible for these changes in cardiac anatomy.[28] Minoxidil is known to induce salt and water retention in response to a lowered renal perfusion pressure, or activation of the renin-angiotensin system and renal sympathetic nerves, or both.[30] The sodium-retaining effect of hydralazine is less obvious.[16] Our results in SHR show increases in both plasma and blood volumes of about 5 to 8% and 15 to 20% following long-term (5 weeks) hydralazine and minoxidil treatment, respectively, and of about 10% in the normotensive rats. It is possible (but not very likely) that these small increases in blood volume together with a shift of blood to the central blood volume[31] cause sufficient cardiac volume overload to explain the persistence or progression of cardiac hypertrophy despite BP control in SHR and the development of cardiac hypertrophy in normotensive rats during treatment with the two arterial vasodilators. Measurement of cardiac filling pressure and cardiac output will be needed to substantiate the extent of cardiac volume overload occurring during long-term treatment with arterial vasodilators. Only one study,[3] showing a 20% increase in cardiac output after 3 weeks of hydralazine treatment in SHR, has been reported. In addition, minoxidil administration has been associated with a rise in pulmonary arterial pressure in hypertensive patients.[32] Pulmonary hypertension would result in the development of RVH but might also cause an unexpected increase in LV weight.[33]

As an alternative to volume overload, previous studies have implicated the cardiac sympathetic nerves in the persistence or progression of cardiac hypertrophy caused by arterial vasodilators.[7, 8] This hypothesis was based on measurement of ventricular norepinephrine content and pharmacological intervention studies. Both methyldopa and hydralazine lowered BP significantly in SHR, but ventricular weight was reduced only in rats treated with methyldopa.[3, 4] Hydralazine treatment of SHR was associated with a 20% increase in ventricular norepinephrine content.[8] In the same study, regression of cardiac hypertrophy occurred when hydralazine was combined with propranolol in a

dose that reduced ventricular norepinephrine. However, ventricular norepinephrine content is an inaccurate assessment of cardiac sympathetic activity. Moreover, it is not known what type of hypertrophy (concentric vs eccentric) would be induced by chronic cardiac sympathetic hyperactivity.

In the present study, we obtained more direct measures of general and cardiac sympathetic activity. Sympathetic hyperactivity has been extensively documented in SHR versus WKY,[34] and in the present study we observed increases in plasma norepinephrine, BP response to hexamethonium, and LV norepinephrine turnover rate and a trend toward increased heart rate. Treatment of SHR with either arterial vasodilator for 1 and 2 days increased general sympathetic activity, as reflected by increases in heart rate and plasma norepinephrine concentration. This activation of the sympathetic nervous system likely occurred secondary to the decrease in arterial pressure. Of interest, this sympathetic hyperactivity was associated with a decreased response to hexamethonium in SHR. Similar results were obtained in normotensive rats, except that the sympathetic hyperactivity was associated with the anticipated increased response to ganglionic blockade with hexamethonium. These results indicate that the vasodilators inhibited the pressor responses to increased sympathetic tone in SHR but not in normotensive rats. A similar — presumably reflex-mediated — increase in sympathetic drive (manifesting itself as increases in heart rate and plasma norepinephrine) associated with short-term arterial vasodilator treatment has been reported in normotensive and hypertensive humans,[9, 11] normotensive rats,[3] SHR,[3, 14] and 2K1C hypertensive rats.[2]

During long-term treatment one may expect resetting of the arterial baroreceptors[12] and a return of sympathetic tone toward baseline. Indeed, in our studies, during long-term treatment (2 and 5 weeks) of both SHR and normotensive rats, heart rate returned to control values, as did plasma norepinephrine. Struyker-Boudier et al.[13] reported that the increase in heart rate after hydralazine treatment of SHR was brief in comparison with the prolonged decrease seen in BP. Other studies[8, 16] also reported no increases in heart rate during long-term treatment of SHR with hydralazine. Others[3, 14, 15] have reported small (5–10%) increases in heart rate following long-term (3–25 weeks) hydralazine treatment of SHR. Similar discrepant results have been reported for normotensive rats (as noted in the introductory section). Persistent sympathetic hyperactivity may partly relate to the mode of drug administration: through the drinking water or twice daily through a gastric tube.[17] The latter may cause more intermittent BP effects and, possibly, less resetting of baroreceptors. Taking into account our results in SHR, normotensive rats, and 2K1C hypertensive rats[2, 6] as well as the balance of other studies, we believe that a baroreceptor reflex–mediated increase in general sympathetic drive occurs on initiation of arterial vasodilator treatment; however, during long-term treatment a resetting of the baroreceptors occurs and general sympathetic tone returns to control levels. However, absence of generalized sympathetic hyperactivity does not exclude selective, persistent increased activity to specific organs. Indeed, LV and RV norepinephrine turnover rates (both absolute and fractional) in SHR were significantly increased after 5 weeks of treatment with either minoxidil or hydralazine. In normotensive rats, increases were found both on Day 1 — when evidence of generalized sympathetic hyperactivity was present — and selectively on Days 14 and 35. To our knowledge, no previous studies have assessed the effects of arterial vasodilators on ventricular sympathetic activity in hypertensive rats. In normotensive rats, treatment with hydralazine for 2 weeks[17] was associated with a significant increase in ventricular norepinephrine turnover rate. Of interest, despite significant increases in norepinephrine turnover rates, initial ventricular norepinephrine content did not differ, either between untreated WKY and SHR or in treated versus untreated SHR or normotensive rats.[17] As outlined previously,[35] changes in the turnover rate of norepinephrine are clearly a better indicator of sympathetic tone than changes in the ventricular content of the amine, which may remain constant, increase, or decline in the presence of an increased turnover rate.

The use of norepinephrine turnover rate as a direct measurement of cardiac sympathetic activity has been employed by a number of investigators.[17, 25, 36] In the absence of an increase in general sympathetic activity, the question arises as to the mechanism responsible for maintaining ventricular sympathetic hyperactivity during treatment of SHR or normotensive rats with arterial vasodilators. A direct cardiostimulatory effect has been suggested for both arterial vasodilators.[37, 38] In addition, it is tempting to speculate that the observed intravascular volume expansion did cause cardiac volume overload and distention of the ventricles and thus activate ventricular wall mechanoreceptors and a cardiocardiac reflex,[39] maintaining ventricular sympathetic hyperactivity. Persistent hyperactivity of the renin-angiotensin system also could contribute to increased norepinephrine release[40] and cardiac hypertrophy.[41] However, PRA increased only during the initial days of treatment and then normalized, thus reflecting the time course of generalized sympathetic activity.

Our results show that increased ventricular sympathetic activity accompanies the development and persistence or progression of cardiac hypertrophy in normotensive rats and SHR treated with either hydralazine of minoxidil. As such, these results would appear compatible with the previously stated hypothesis.[7, 8] However, assessment of the density and responsiveness of cardiac adrenergic receptors will be needed to exclude down-regulation (as a primary or secondary event) resulting in normal effective sympathetic activity. Moreover, both vasodilators increased ventricular sympathetic activity to a similar degree, and both normalized BP rather similarly in SHR; yet in SHR, hydralazine treatment maintained LVH at pretreatment levels and caused only a small increase in RV weight, whereas minoxidil caused marked increases in RV weight, LV

weight, and LV internal diameters. Similarly, in normotensive rats, long-term treatment with either arterial vasodilator increased LV norepinephrine turnover to a similar degree, yet only minoxidil increased LV weight. Therefore, it appears unlikely that there is a direct relationship between ventricular sympathetic activity and the trophic response of the heart to treatment with arterial vasodilators. Our results indicate that, if ventricular sympathetic activity is involved in cardiac hypertrophy, other mechanisms (e.g., extent of LV and RV volume overload) are needed for the full expression of the trophic response or other mechanisms (e.g., down-regulation of receptors) can prevent this effect.

The present results showed that long-term arterial vasodilator treatment of SHR with established hypertension produces a sustained decrease in BP, in agreement with previous studies with hydralazine[4, 14–16] and minoxidil.[5] In contrast, in normotensive rats[3, 17] and 2K1C hypertensive rats,[1, 2, 6] tolerance develops to the BP-lowering effect of arterial vasodilators. Several mechanisms can be evoked to explain the different BP response of SHR as compared with the other models. It is tempting to speculate that the vascular smooth muscle of SHR is different and is not able to develop tolerance to the relaxant effect of the two arterial vasodilators. In support of this concept of different responses, long-term treatment with either vasodilator decreases the hypotensive response to ganglionic blockade only in SHR, suggesting a decreased responsiveness of vascular smooth muscle to pressor mechanisms.

In conclusion, the present study clearly shows that despite adequate BP control, long-term arterial vasodilator treatment of SHR at the doses used results in the persistence of cardiac hypertrophy with hydralazine and, in the case of minoxidil, potentiation of RVH and development of eccentric LVH. Normotensive rats exhibit similar cardiac effects. In both animal models, generalized sympathetic hyperactivity only occurs during the initial period but a selective increase in ventricular sympathetic activity persists during long-term treatment. Both ventricular sympathetic hyperactivity and volume overload may be involved as possible causal or contributory mechanisms for the cardiac effects of arterial vasodilators.

Acknowledgments

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Table 6.1 The effects of minoxidil (1-100 μM), minoxidil sulfate (1-100 μM) and phenylephrine (20 μM) on myocyte protein in rat cultured neonatal myocytes.

	Cell Protein (treated/control-fold increase)		
Days of Treatment/ Treatment	1	2	3
Minoxidil (μM)			
1	1.07±0.05	1.04±0.06	1.03±0.04
10	1.02±0.03	1.01±0.03	1.06±0.05
100	1.01±0.04	1.03±0.04	1.02±0.02
Minoxidil Sulphate (μM)			
1	1.06±0.08	1.02±0.07	1.05±0.04
10	1.05±0.05	1.08±0.05	1.02±0.06
100	1.03±0.01	1.02±0.04	1.06±0.05
Phenylephrine (μM)			
20	1.52±0.05*	1.87±0.06*	2.10±0.13*

Cells were labeled with [^{14}C]phenylalanine in the presence of the agents shown. Cell protein was determined 3 days after additions. Each value represents the mean treated:control ratio ± SEM. n=6. *significant versus vehicle-treated control cells - where the cell protein content is assigned 1.00±0.00.

Figure 6.1. Fluorescence micrographs showing the effects of minoxidil sulfate (10 μM) and phenylephrine (20 μM) on myocyte size.

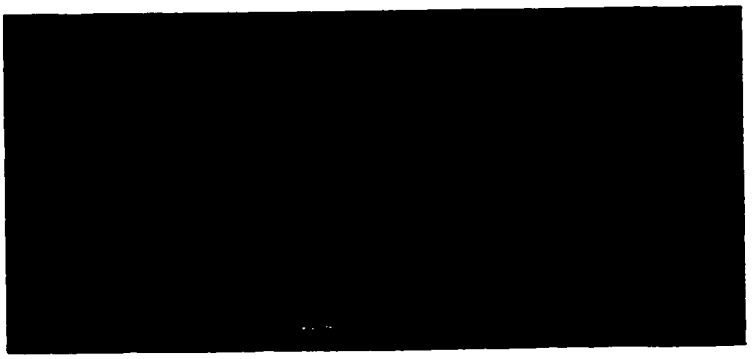
Panel A control myocytes treated with vehicle.

Panel B myocytes treated with 10 μM minoxidil sulfate.

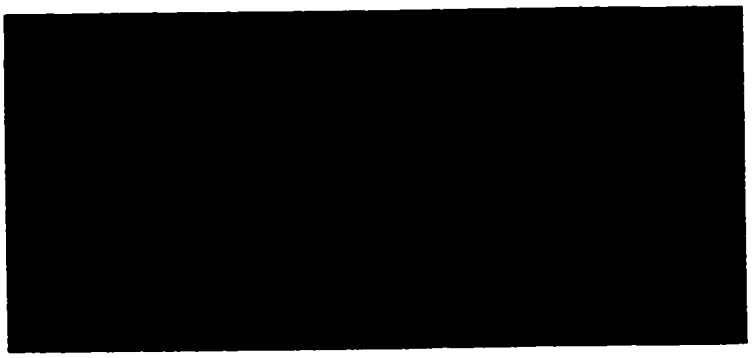
Panel C myocytes treated with 20 μM phenylephrine.

The magnification is 64X.

A.



B.



C.

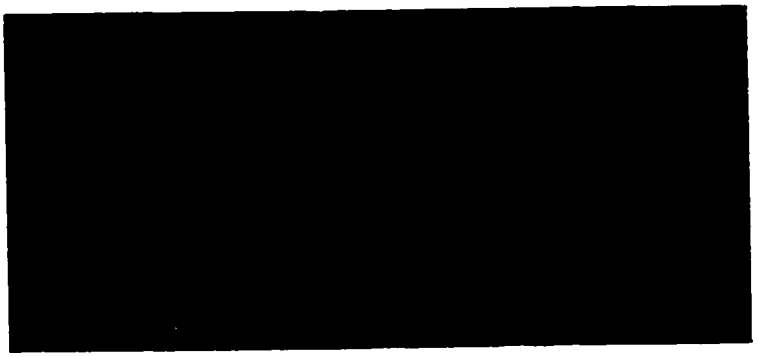
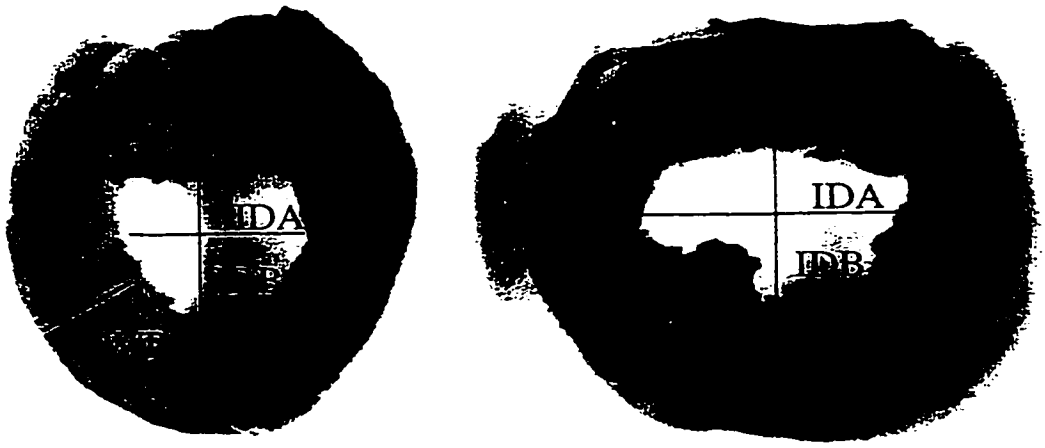


Figure 6.2 Comparison of ventricles of untreated (i) versus minoxidil treated (35 days) (ii) WKY rats. The solid line separates the left ventricle (lv) from the right ventricle (rv).

A. B.

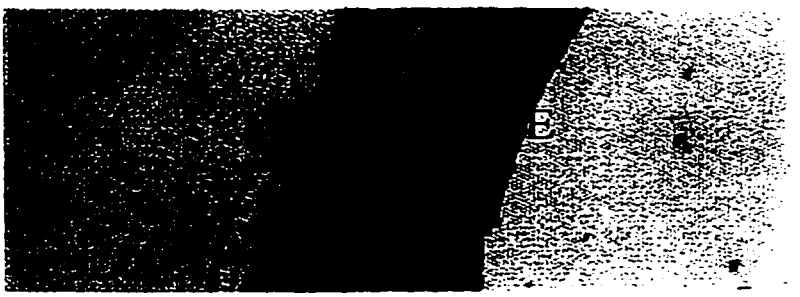
Figure 6.3 Comparison of a mid-level sagittal slice of the left ventricle of untreated (a) versus minoxidil-treated (35 days) (b) WKY rat. Highlighted are LV wall thickness (wt) and LV internal diameters, anterior-posterior axis (ida) and septal-lateral axis (idb).



A. B.

Figure 6.4. Light micrographs of cross-sections of superior mesenteric arteries from untreated (A) and minoxidil-treated (70 days) (B) Wistar rats. Note the minoxidil-induced increase in the thickness of the media. E = Endothelium; S = Smooth muscle cells; A = Adventitia; L = Elastic lamina; EL = External elastic lamina.

A.



B.

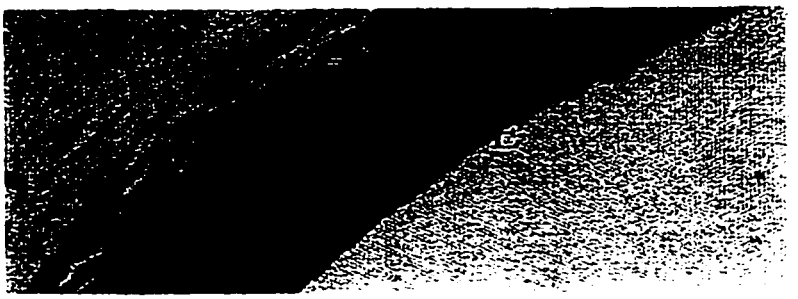


Figure 6.5 Light micrographs of cross-sections of large mesenteric arteries from untreated (Day 0) (A), minoxidil-treated (70 days) (B) and untreated (Day 70) (C) SHR. Note that minoxidil prevented the increase in medial thickness with age. E = Endothelium; M = Media, A = Adventitia.

A.

B.

C.

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