ISOLATED INTRAPULMONARY ARTERIAL RESPONSES TO VASOACTIVE AMINES AND PROSTAGLANDINS

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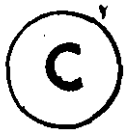

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ABSTRACT

The response of the intrapulmonary artery (IPA) to a variety of endogenous lung amines and prostaglandins (PG) was examined to determine if a differential sensitivity to these vasoactive substances existed between segments taken from two sites on the artery. Longitudinal strips of proximal (PIPA) and distal (DIPA) segments of the left lower lobar intrapulmonary artery were taken from rabbit lungs and isometric tension measured during superfusion abt 37°C with physiological saline. Full or partial dose-response curves were obtained for 5-Hydroxytryptamine (5HT), Histamine (HIS), Norepinephrine (NE), Isoproterenol (IsoP), Arachidonic Acid (AA), PGA_1, PGB_1, PGB_2, PGE_1, PGE_2 and $PGF_{2\alpha}$. In addition to pharmacological studies, length-tension properties of the segments utilized were examined and a qualitative analysis of smooth muscle content and orientation was undertaken. All prostaglandins elicited contractile effects, of varying magnitudes, at high doses. Prostaglandins A_1, E_1, and E_2 produced little or no contractile responses or slight relaxant activity in unstimulated PIPA and DIPA segments at low doses. 5HT contracted both PIPA and DIPA segments in a dose dependent manner, however, proximal segment maximal effects and sensitivity were significantly greater than those of the distal segment. Both PIPA and DIPA segments contracted to HIS and maximal effects were similar in

both segments. Mepyramine (10^{-9}M) antagonized contractile responses to HIS. In the presence of 10^{-7}M mepyramine, HIS produced dose dependent relaxation of precontracted PIPA and DIPA segments. Cimetidine (10^{-5}M) antagonized this relaxation indicating that HIS relaxant effects are mediated by H_2-HIS receptor stimulation in both segments. PIPA segments contracted in response to NE while the DIPA segment responded poorly or not at all suggesting a paucity of alpha adrenoreceptors in distal segments. IsoP produced dose dependent relaxation, that was antagonized by propranolol, of precontracted PIPA and DIPA segments. The dose related contractile response to AA was similar in both PIPA and DIPA segments. These studies indicate that regional differences exist in the response of rabbit IPA to some agonists.

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CHAPTER 1

Review of the Literature

Introduction

Vasoactive substances produced by lung tissue or reaching the lung via the pulmonary artery have been implicated in the local control of pulmonary blood flow and in the response to a number of pathophysiologic conditions including anaphylaxis, pulmonary embolism, hypoxia and pulmonary edema (16,26,29,42,72). Although the exact vasoactive agents involved are unclear, circulating catecholamines, acetylcholine (ACh), angiotensin (Ang), bradykinin (Bk), serotonin (5HT), histamine (HIS) and prostaglandins (PGs) have all been suggested as possible mediators of pulmonary responses (6,26,29,42,72). The nature and site of their actions on pulmonary blood vessels are often altered or obscured by the interaction of the respiratory, vascular and metabolic functions of the lung. Thus complete pharmacological characterization of pulmonary blood vessels would require investigations in the intact lung and studies using isolated pulmonary blood vessels.

Pulmonary vascular resistance is normally low and is greatly influenced not only by vasoactive agents but also by changes in blood flow, pressure in the left atrium, airways and pleural space as well

as by gravity, blood viscosity, blood gases and pH (see Hyman et al, 1978). In addition to a direct effect on pulmonary vascular smooth muscle, vasoactive substances may influence directly or indirectly any number of these factors thus modifying the pulmonary response to these agonists. Changes in pulmonary arterial pressure are minimized by the passive effects of recruitment and vascular distention (42). Furthermore the lungs are uniquely efficient in inactivating a variety of vasoactive agents (29). Thus it has been necessary to develop elaborate techniques to overcome the multitude of active and passive effects of these substances to ascertain, in the intact and isolated lung, their direct action on pulmonary vascular smooth muscle. Owing to the complex nature of intact lung preparations and to time constraints, the studies presented here concern solely isolated strip responses.

Effects of Humoral Agents on Pulmonary Vasoactivity in the Intact and Isolated Lung

Amines

The role of endogenous amines in pulmonary function and the response to immunological and pathological conditions is largely unknown. HIS release has been associated with alveolar hypoxia, pulmonary embolism and anaphylactoid reactions including asthma (see Said, 1974). 5HT has been suggested as a possible humoral mediator of the pulmonary hypoxic and embolic vascular responses (see Said, 1974). The capacity of the lung to remove and inactivate 5HT and norepinephrine (NE) from the circulation has been described (see

Gillis, 1973) although the physiological significance of this function is unknown. When given intravenously, the changes in pulmonary resistance due to vasoactivity of these agents may be obscured by accompanying effects on cardiac output, left atrial pressure, bronchial smooth muscle and airway resistance (6).

The pulmonary effects of biogenic amines have been widely tested. In the intact and isolated lung of a number of species including the cat (70,71), rabbit (34) and dog (40), NE, an alpha adrenoreceptor stimulant, produced pulmonary vasoconstriction whereas isoproterenol (IsoP), a beta adrenoreceptor stimulant, produced vasodilatation (see Somlyo and Somlyo, 1970). Alpha and beta adrenoreceptor blockers, such as phentolamine and propranolol respectively, modified the activity of these catecholamines (see Somlyo and Somlyo, 1970). Pulmonary vasoconstriction, vasodilatation and biphasic responses to HIS have been described in isolated and intact lungs depending on the species while both vasoconstrictor and dilator responses have been reported in the same species (see Chand and Eyre, 1975). Antagonism or potentiation of HIS responses or conversion to an opposite response have all been reported in the presence of HIS H_1 and H_2 receptor antagonists, e.g. mepyramine and cimetidine (or metiamide) respectively (16,67). 5HT actively constricted pulmonary vessels of several mammals including rabbit (34), dog (13,41) and cat (71). 5HT antagonists, structurally related to lysergic acid, inhibit the vasoconstrictor action of 5HT (see Somlyo and Somlyo, 1970).

Other agonists commonly described as eliciting pulmonary vasoconstriction in a number of species include Bk, Ach, and Ang (see Aviado, 1960, and Somlyo and Somlyo, 1970).

Prostaglandins

The PGs are a group of naturally occurring acidic lipids which possess remarkable and diverse pharmacological activity. The biosynthetic pathway for the production of PGs and thromboxanes is outlined in fig. 1-1. The lung is one of the major sites of PG synthesis and inactivation in mammals (42). Enhanced PG synthesis and release has been demonstrated in hypoxia, anaphylaxis, pulmonary embolism and edema (see Said, 1974). A possible role of PGs in the local control of vascular tone and reactivity has been suggested but this has still to be determined (62). With respect to pulmonary function, PGs affect pulmonary arterial pressure, pulmonary blood flow, pleural pressure, bronchomotor tone, lung volume and blood gases (42) tending to obscure their direct effect on pulmonary vascular smooth muscle. Hyman et al (42) have recently published an extensive review of the role of prostaglandins in the lung.

The PGs have been shown to cause marked vasomotor activity in the lung. $PGF_{2\alpha}$ generally has been found to be a potent pulmonary vasoconstrictor in the intact lung, the magnitude of the response varying among species (see Hyman et al, 1978). PGE_1 caused dilation of the pulmonary vasculature of many species (see Hyman et al, 1978). Although PGE_2 was a pulmonary vasoconstrictor in mature intact

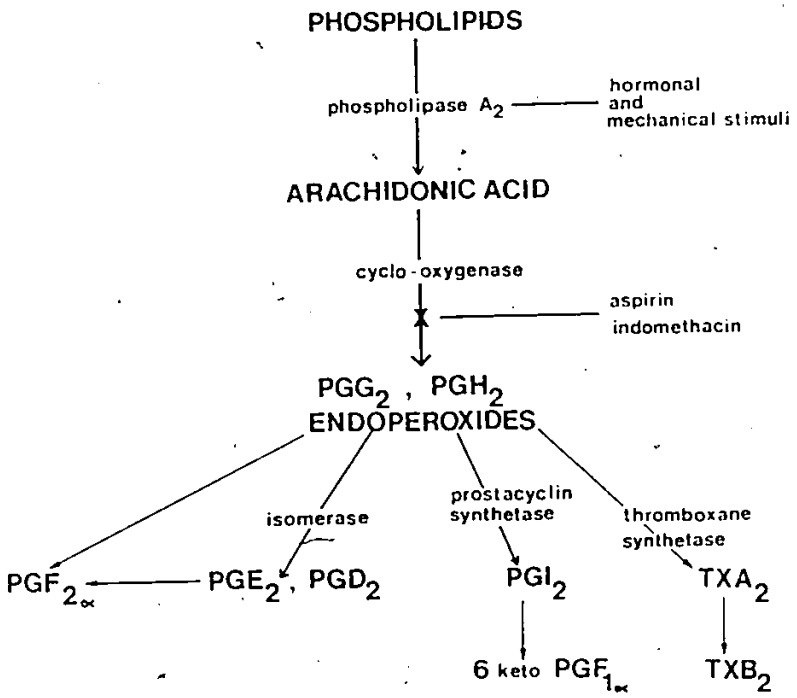


Fig 1-1. Biochemical pathway for production of prostaglandins and thromboxanes. Taken from Kaley, 1978.

animals (see Hyman et al, 1978), a vasodilator response to PGE_2 has been shown in isolated calf and fetal goat lung (57,93). There is little information available about the pulmonary effects of the A and B series of PGs which are thought to be derived from PGs of the E series. In the intact dog, PGA_2 was found to be a moderately active pulmonary vasoconstrictor whereas PGA_1 was a modest pulmonary vasodilator (49). In the isolated rabbit lung PGA_1 produced vasoconstriction (31). PGs of the B series in intact dogs actively constricted pulmonary vessels, PGB_2 being only slightly less active than $PGF_{2\alpha}$ (50).

Arachidonic acid (AA), the precursor of endoperoxides (see fig. 1) produced increased pulmonary vascular resistance when injected into intact spontaneously breathing monkeys, dogs, cats and rabbits (see Hyman et al, 1978). Indomethacin, an inhibitor of the cyclo-oxygenase system that converts AA into PGs and related substances, produced blockade, in the intact dog, of the cardiopulmonary effects of AA whereas increased pulmonary arterial pressure in response to PGE_2 and $PGF_{2\alpha}$ was enhanced by the cyclo-oxygenase inhibitor (52). Recent evidence suggests that the role of other intermediates and products of the PG biosynthetic pathway may be of even greater signficance in pulmonary function and response to pathophysiologic conditions (53). An excellent review of these substances and their activities has been published by Kadowitz et al, 1977.

Owing to the complexity of the pulmonary vascular bed and the ability of neurogenic and hemodynamic factors to influence its

responsiveness it is clearly of value to examine the responses of isolated pulmonary vessels. The use of isolated tissue strips of pulmonary vessels permits examination of the direct or local effects of vasoactive agents on pulmonary vessels. However, while this minimizes extraneous factors, by removing these vessels from their usual physiological environment, the drug actions thus observed may not necessarily be the same as in vivo. Moreover the techniques of isolating these vessels and subsequent physical manipulation entails some risk of modifying their smooth muscle reactivity (26).

Actions of Drugs on Isolated Pulmonary Vessels

The response of the pulmonary artery to a number of vasoactive agents, often under a variety of experimental conditions has been the subject of numerous investigations. In general these studies have utilized a segment of artery, either in its ring form, or, more frequently, spirally cut to a narrow strip, mounted in a tissue bath containing physiological saline solution kept at 37°C. Responses to vasoactive drugs have been measured under conditions of isometric and isotonic recording of tension. Pulmonary blood vessel responses to various agonists have been previously reviewed, in part, by Su and Bevan (1976). Their interesting review of the pharmacology of pulmonary blood vessels included discussion of the mode of action of vasoactive drugs on blood vessels and the factors that influence the effects of drugs with reference to pulmonary blood vessels.

The initial studies examining pulmonary responses most commonly employed extra- or main pulmonary arterial segments. The main pulmonary artery (MPA) is essentially a large conduit artery rich in elastic tissue (88). Its pharmacological characteristics are reportedly very similar to large elastic arteries in the systemic circulation, e.g. the thoracic aorta (10,88). Consequently, it is thought to be of limited hemodynamic significance and thus cannot be considered representative of smaller intrapulmonary arteries. However, numerous studies have employed this vessel resulting in a wealth of pharmacological information. Owing to the large number of these studies, canine, rabbit and cat pulmonary arterial responses to vasoactive amines and prostaglandins have been primarily selected for review, although mention of other species, drug and vessel responses has been made when appropriate.

Extrapulmonary Arterial Responses

The literature pertaining to the responses of isolated pulmonary vessels dates back to, at least, the turn of this century. Franklin (1932), in a paper outlining the actions of E and ACh on canine isolated pulmonary vessels, briefly reviewed the previous literature providing a fascinating account of the early experimental work. Predominantly contractile responses to E are described for pulmonary vessels of many different species including man. Franklin's own experimental work showed constriction of ring segments of canine extrapulmonary arteries and veins to E while ACh relaxed arteries and constricted veins (27).

Bevan (8) reported that electrical stimulation and NE caused contraction of ring segments of rabbit extrapulmonary artery (EPA). HIS, 5HT, and increased extracellular potassium (K^+) have also been shown to contract the pulmonary artery of the rabbbit (11,15,78,86,90). Bevan and Osher (10) examined the relative sensitivity of some blood vessels of the rabbit to sympathomimetic amines and reported that helical strips of extrapulmonary arteries contacted in response to NE, E and a number of other sympathomimetic amines. The sensitivity of the EPA to the amines tested was described as similar to that of the thoracic aorta. Kitamura and coworkers (56) studied prostaglandin responses in the rabbit and reported that $PGF_{2\alpha}$ contracted main pumonary arterial segments while PGE_1 and PGE_2, on their own did not affect muscle tone. However, PGE_1 and PGE_2 did partly relax K^+ and NE induced contractions (56).

Su (85) reported the action of some vasodilator agents on the isolated MPA of the rabbit. Vasoconstrictor agents were used to increase and maintain vascular tone so that relaxant effects could be observed. IsoP produced marked relaxation that was enhanced by cocaine and abolished by dichloroisoprenaline. HIS and E were reported to elicit a slight reduction in elevated tone, in contrast to papaverine and sodium nitrite which greatly reduced the elevated tone (85). The observations of a HIS relaxant response contrasts with previous reports of HIS contractile activity in isolated rabbit pulmonary strips.

Somlyo and Somlyo (77) examined isotonic contractions of helical cut strips of canine MPA. E, NE, phenylephrine (PE), and 5HT

produced significant vasoconstriction. IsoP at low doses failed to demonstrate vasodilator activity in unstimulated strips and did not produce significant vasoconstriction unless applied in extremely high concentrations (77). Isolated spiral strips of cat pulmonary artery and vein contracted in response to HIS, 2-methyl HIS (a relatively specific H_1 HIS receptor agonist), 4-methyl HIS (a relatively specific agonist for H_2 HIS receptors), and 5HT (17). Low doses of IsoP usually produced relaxations of partially contracted pulmonary blood vessels while increased doses induced contractions. PGE_1, PGE_2 and $PGF_{2\alpha}$ all contracted the cat pulmonary artery, however, increased doses of PGE_1 relaxed the artery. Pulmonary veins, partially contracted to $PGF_{2\alpha}$ were relaxed by PGE_1 and PGE_2 (17).

Spirally cut strips of guinea pig MPA have been employed by Okpako in a series of investigations (64,65,66). NE, HIS, E and $PGF_{2\alpha}$ all produced dose related contractions of this preparation (64,64). PGE_2, by itself, produced no effect but when muscle tone was raised by prior addition of HIS to the bath it produced relaxation (64). Also PGE_2 inhibited contractions to HIS and NE, the inhibitory effect persisting at high doses despite washout from the bath. IsoP even in very high doses had no effect causing neither contraction nor relaxation even after alpha adrenoceptor blockade in HIS treated preparations (64,65). Contractions caused by HIS and NE were specifically antagonized by mepyramine and phentolamine respectively (65). $PGF_{2\alpha}$ induced contractile responses were resistant to the actions of phentolamine and mepyramine (64).

Despite evidence of a HIS mediated depressor action which was unmasked after mepyramine in guinea pig isolated lungs, Okpako (65) could not demonstrate an inhibitory action of HIS in the isolated artery preparation. In the presence of mepyramine, HIS caused neither relaxation of the arterial segment nor did it inhibit catecholamine induced contractions. While 2-methyl HIS, caused contraction of the isolated artery, 4-methyl HIS caused neither contraction nor relaxation when tested over a wide range of concentrations, and failed to inhibit E induced contractions of preparations bathed in physiological saline containing mepyramine (66).

Thompson, Barer and Shaw, (92) studied the action of HIS on pulmonary vessels of cats, rats and rabbits. HIS contracted helical strips of extrapulmonary artery from all three species, the rabbit and cat strips being more sensitive. Successive doses of HIS caused diminishing effects in all strips studied. In rat pulmonary artery, HIS, when given during contractions produced by various agonists including ACh, BK, E, NE, 5HT and even HIS, was reported to produce a decrease in tension or relaxation. However, specific antagonists were not employed to demonstrate that this effect was mediated by HIS receptors. In contrast, contracted rabbit and cat pulmonary arteries responded to HIS with further increments in tension (92).

Responses of bovine (23), chicken (18), calf (57), rat (69) and sheep (24) main pulmonary arteries to amines and prostaglandins have also been extensively investigated.

Intrapulmonary Arterial Responses

The pulmonary vascular bed is influenced significantly by neural and humoral stimuli. Consequently the responses of isolated intrapulmonary artery (IPA) to vasoactive agents are of considerable interest. Although the pharmacological characteristics of a short segment of blood vessel cannot be considered representative of the response of an entire vascular bed, valuable information concerning the site and nature of the responses to vasoactive substances may be obtained.

Franklin (27) has briefly described earlier work examining isolated intrapulmonary responses to E and ACh. Franklin's own experiments revealed that, in general, canine intrapulmonary vessels gave weak and variable reactions to these agonists. Specifically E produced very small and variable responses to intrapulmonary arteries with contractions predominating while ACh produced no response.

Bohr, Goulet and Taquini (12) examined responses of helically cut intrapulmonary arterial vessels, 0.2 to 0.3 mm in diameter, from rabbits and dogs. The isolated pulmonary vessels contracted in response to ACh, 5HT, KCl and Ang while no response to E or NE was seen unless very high concentrations of these catecholamines were administered. No species differences were evident (12). In a similar study, Lloyd (58), examined helically cut strips of 4th and 5th order intrapulmonary arteries from rabbit and canine lungs. The helical strips were obtained from arterial segments with diameters of 3 mm at

proximal end and 1 mm or less at distal end. Electrical stimulation, 5HT, NE, Ang, ACh and KCl all produced contractile responses. In terms of relative effectiveness, on a molar basis the most active compound was Ang followed in decreasing order by 5HT, NE and ACh. Thus, in contrast to the previous study of Bohr and coworkers (12) the NE response was not found to be deficient. Lloyd reported that while the rabbit strips were perhaps more responsive than the dog, any species differences if present appeared small.

Sundt and Winkelman (91), tested arterial segments from vessels of 0.2 to 0.4 mm diameter from different vascular beds of the rabbit. Their helically cut intrapulmonary arterial strips failed to respond to E, NE and IsoP. In this respect, it can be seen that their observations were similar, in part, to those previously obtained by Bohr and coworkers (12). While 5HT and Ang II consistently produced contractile responses, only about 50% of their lung strips contracted in response to HIS and Bk. However, the thresholds which these workers reported for drug responses were considerably greater than any reported elsewhere.

Responses of isolated helical strips of canine intrapulmonary lobar arteries and veins, 3 - 5 mm. in diameter have been extensively investigated by Joiner and coworkers. NE, HIS, 5HT and $PGF_{2\alpha}$ produced contractile responses in both arteries and veins (44,45,46). These arterial responses to NE and 5HT were similar to those previously reported by Lloyd (12). Mepyramine antagonized HIS responses while

phentolamine was found to inhibit NE and Tyr contractile responses without altering 5HT effects (45,46). Methysergide inhibited 5HT responses but not NE contractions (45). Sodium nitrite and PGE_1, relaxed both arteries and veins (44,46). IsoP at low concentrations relaxed and at higher doses contracted the strips but in the presence of phentolamine, this agonist produced only relaxation suggesting that IsoP stimulated, in part, alpha adrenoreceptors (46).

Joiner and coworkers have conducted a number of studies (47,48,51), demonstrating species variability in the response of intrapulmonary vessels to amines and prostaglandins. In a study of the effects of NE on helical strips of intrapulmonary artery and veins from lungs of dog, sheep, swine and man, all strips were reported to have been contracted in response to a depolarizing solution of K^+ and to NE, although the response of sheep and swine intrapulmonary vein (IPV) to NE were weak (48). In a similar trial, the actions of PGE_1 and $PGF_{2\alpha}$ on intrapulmonary arteries and veins from dog, sheep, swine and human lungs were studied (47). All vascular strips tested had responded well to NE. $PGF_{2\alpha}$ contracted human arterial strips, relaxed slightly sheep arteries and had no effect on dog arteries. PGE_1 relaxed both veins and arteries from dog and sheep. It was noted that by comparison, canine tissues were more responsive to the relaxant effects of nitrite ion than PGE_1. Human arteries usually contracted slightly and human veins usually relaxed slightly to PGE_1. Relaxant effects were examined both in unstimulated vessels

and in vessels contracted submaximally to PE. In either case the magnitude of relaxation was not significantly different. Swine arteries and veins failed to respond to $PGF_{2\alpha}$ or PGE_1 (47).

In an extensive review of the physiological and pharmacological roles of prostaglandins, Kadowitz, Joiner and Hyman (51) have outlined and summarized the responses of isolated intrapulmonary arteries and veins to the prostaglandins and other vasoactive agents. Specific information concerning venous responses may be obtained from their review. The following summarizes, according to species, the arterial responses to prostaglandins and some vasoactive amines as presented in their review (51).

Dog: Prostaglandins A_1, A_2 and E_1 relaxed canine arteries. While the A series of PGs produced maximal relaxation, PGE_1 had minimal relaxant effects. Prostaglandins B_1, B_2, E_2, and $F_{2\alpha}$ had no significant effect while prostaglandins 15-methyl PGE_2 and 15-methyl $PGF_{2\alpha}$ produced minimal contraction.

Swine: Although responding well to NE and HIS, swine arteries were unresponsive to $PGF_{2\alpha}$ and PGE_1 in unstimulated strips.

Sheep: Arteries contracted well to NE, HIS and 5HT. $PGF_{2\alpha}$ produced contractile effects but only of half the magnitude of the amines, while PGE_1 produced relaxation. Prostaglandins A_2 and B_1 had little or no effect on sheep arteries.

Human: Arteries contracted well to NE, ACh, 5HT and HIS. $PGF_{2\alpha}$ was quite active as a stimulatory agent whereas PGE_1 and PGE_2 were less so.

Baboon: $PGF_{2\alpha}$ elicited dose relaxed contractions while PGE_1 either had no effect or induced slight contraction.

Chimpanzee: $PGF_{2\alpha}$ was contractile at high doses while PGE_1 and PGE_2 tended to relax arteries.

Monkey: Arteries contracted well to $PGF_{2\alpha}$ and only slightly to PGE_1.

It can be seen that in primates including man $PGF_{2\alpha}$ consistently contracted intrapulmonary arterial segments. PGE_1 effects, on the other hand, were more variable.

The contractile effects of AA and an epoxymethano analog, chemically similar to PGH_2, have been investigated in helical strips of IPA and IPV isolated from bovine, rabbit and canine lung (32). The analog increased isometric force in a concentration related manner in IPV from the 3 species and in IPA from bovine and rabbit lung. Canine IPA was unresponsive to the analog. AA contracted only rabbit IPA and this response was blocked by indomethacin. In contrast, AA elicited relaxation in canine IPA partially contracted by PE. AA did not affect bovine IPA nor IPV from the three species (32).

In March 1978 the first study describing regional differences in the response of arterial segments from different sites within the rabbit lung was reported by Su and co-workers (89). In arteries larger than 1.4 mm outside diameter the maximal contractile response to NE was comparable to that of KCl (89). The response to NE decreased to 30 - 40% of the K^+ response in arteries 0.6 to 1.4 mm in diameter. In arteries smaller than 0.6 mm both nerve stimulation and application of

NE elicited either very small responses or none at all. The median effective dose, ED_{50}, for NE, (an index of the sensitivity of the adrenergic receptor), did not change with the arterial diameter. All arterial segments constricted in response to 5HT, HIS and KC1 and the maximal effects of these 3 agents were equal (89). The ED_{50} values for 5HT and HIS did not depend on arterial caliber.

Other reports of the use of isolated intrapulmonary arteries, in conjunction with other experimental work, have briefly described responses to various agonists. Houghton and Philips (38) studied isolated human pulmonary arterial and venous tissues employing spirals of muscle cut from vessels dissected after lobectomy or pneumonectomy for carcinoma or bronchiectasis and found that NE, E, HIS and 5HT maximally contracted both arterial and venous segments while IsoP most frequently produced relaxation although contractile effects or no responses were also observed. In their arterial segments, infrequently a biphasic response to IsoP was also obtained consisting of a slight initial relaxation followed by contraction at higher doses. Benumof (5) elicited contractions of helical strips of 4th to 6th order IPA from cat lung in response to 5HT. Thompson and coworker (92), in addition to examining extrapulmonary responses, reported that isolated cat intrapulmonary arteries contracted in response to HIS. Arterial strips, spirally cut from lengths of the major branches of the pulmonary artery within the toad lung were reported to be contracted by E and ACh and relaxed by IsoP (37). A number of studies have utilized

extra- and intrapulmonary arterial strips stimulated under varying degrees of oxygen tension to determine possible mediators of the hypoxic response or to ascertain the effect of hypoxia on vascular smooth muscle responsiveness (19,59,80). Various investigators have examined the response of the rabbit MPA to NE in order to study the relationship between NE induced depolarization and contractions since it now appears that contraction occurs, in part, without measurable change of membrane potential (14,15,33,78,90). These studies are all of interest since they provide additional or confirmatory evidence of isolated pulmonary vascular smooth muscle responsiveness.

Summary of the Literature and Objectives

Thus it can be seen that the ability of pulmonary vascular smooth muscle to respond, both in vivo and in vitro, to many vasoactive agents under a variety of experimental conditions is now well established. Species differences in the responses of pulmonary vessels to vasoactive agents are evident. Furthermore it is apparent that venous responses to an agonist often differ both qualitatively and quantitatively from arterial responses. However, a comparison between studies of isolated strip responses of arteries of different lumenal diameter but of the same species reveals some interesting discrepancies. While Bohr et al (12) and Sundt and Winkelman (91) examined rabbit intrapulmonary vessels .2 to .4 mm in diameter and found the strips poorly or not at all responsive to catecholamines, Lloyd (58) using rabbit intrapulmonary vessels of greater than 1 mm

diameter, found NE an effective agonist. Sundt and Winkelman (91) described contractile responses to HIS and Bk, well known pressor agents, in only 50% of their intrapulmonary strips, although HIS has been shown to consistently contract rabbit extrapulmonary arteries (92). It is recognized that variations and problems in methodology could account for these apparent discrepancies. On the other hand, the limited evidence available suggests there may be signficant differences in responsiveness associated with vessel site, diameter and structure.

Studies in the dog have suggested that pulmonary vascular responsiveness is localized to specific segments for various agonists (13,40,41). In these studies the vascular localization was limited to determining whether lobar pulmonary veins and/or upstream vessels, presumably lobar pulmonary arteries, were actively constricted. The vasoconstrictor response to hypoxia is thought to be localized to arteries of less than 200 μ in diameter, that is, those which lie adjacent to the airspaces (7,55). In light of the differences in potency between isolated lung and isolated extrapulmonary arterial strip responses to $PGF_{2\alpha}$, relative to HIS responses, Okpako (64). proposed that the smaller pulmonary blood vessels might be more sensitive to the action of $PGF_{2\alpha}$.

Variations in responsiveness may be related in part to differences in agonist receptor distribution, differences in neuronal factors or to changes in the histological structure of vessels within the pulmonary vasculature. Okpako's (65) inability to demonstrate a relaxant action of HIS in isolated extrapulmonary arterial strips,

given the isolated lung responses mentioned previously, suggested a difference in the regional distribution of HIS receptors in the pulmonary vascular bed, such that, HIS receptors mediating the depressor effect might be present in higher proportion in arterioles and capillaries. Elastic arteries, transitional arteries, muscular arteries and 'endothelial' arteries or arterioles have been distinguished in the rabbit pulmonary vascular bed (25) and the functional significance of these histological vessel types is imcompletely understood, particularly in the pulmonary circulation. Indeed the term pulmonary arteriole, which is used to describe the terminal branches of the mammalian pulmonary arterial tree, may be misleading since it implies structural and functional similiarities to arterioles of the systemic circulation (74).

The rationale for examining regional differences in responsiveness of an artery is supported by the changes in sensitivity that have been noted in different segments of the same vessel, for example, the aorta, taken from the same animal and studied simultaneously (76). It should be emphasized that there are remarkable differences in the reactivity of vascular smooth muscle taken from vessels of similar lumenal diameter but from different vascular beds. Indeed the heterogeneity in reactivity of vascular smooth muscle is well recognized (see review by Vanhoutte, 1978). Anatomic localization within the pulmonary vascular bed of the responses to various stimuli, including vasoactive agents, should provide insight into the mechanisms

involved in the pulmonary vascular responses in various pathophysiological conditions.

In order to establish whether a differential sensitivity to vasoactive substances existed in the pulmonary vasculature, arterial segments were taken from two sites on the same artery. A proximal intrapulmonary artery was compared with a narrowed distal artery using segments of similar size but representing sites of different lumenal diameter on the same vessel. Responses of the isolated segments to a number of amines and prostaglandins were examined. Specific antagonists were employed in an effort to demonstrate pharmacological blockade and to unmask responses to agonists thought to stimulate more than one type of receptor. To meet these objectives longitudinal intrapulmonary arterial segments from rabbit lung were studied using the superfusion technique of Gaddum (28).

Rabbits were chosen as the experimental animal for study because of the large number of anticipated experiments and the ease of handling this species. The suitability of isolated preparations of pulmonary artery from rabbits for the determination of responses to vasoactive substances has been previously described (12,58,91). Although rabbit pulmonary arterial strips have been widely tested for amine responses, few studies document prostaglandin responses in this species.

The question of the adequacy of the spiral strip technique for preparation of small vessels has been raised (88,89) in light of the

extremely high concentrations of 5HT, HIS, and Ang required to contract small intrapulmonary arteries (0.2 to 0.4 mm in diameter) reported by Sundt and Winkelmann (91). Su and Bevan (88) have suggested that the technical difficulties encountered in handling and cutting spiral strips in vessels of small diameter include the potential for tissue injury which may contribute to a lack of reactivity. To overcome this problem Su et al (89) chose to employ ring segments, in contrast to the present study in which longitudinal segments of artery were used. The ease of preparation of a longitudinal strip in comparison to either helical or ring strips was felt to be highly desirable. A review of the literature has revealed only one report of the use of longitudinal segments. Franklin (27), used a single longitudinal piece of pulmonary artery and found that it contracted in response to adrenaline.

The technique of superfusion in which physiological solutions are passed over tissues suspended in air, is sensitive and particularly suitable for the study of isolated segments of blood vessels. By introducing and withdrawing vasocative agents into and from the superfusing medium drug effects may be elicited and then washed out most effectively and without interruption of flow. Su and Bevan (87) employed the superfusion technique in a study of the release of H^3-NE in spirally cut rabbit main pulmonary arterial strips and reported contractile responses to NE, 5HT and electrical stimulation, thus demonstrating the suitability of this technique. Superfusion has also been used to examine isolated rat pulmonary arterial responses to 5HT and its antagonists (4).

At the time these studies were planned, there were no reported studies on regional differences in isolated pulmonary vessels. As mentioned previously, the recent paper by Su and Bevan (89) confirmed that regional responses for NE did indeed exist. The present studies sought to characterize the responses of intrapulmonary arteries to a number of potential mediators of pulmonary vascular control as well as to determine if there was geographic selectivity of responses.

CHAPTER 2

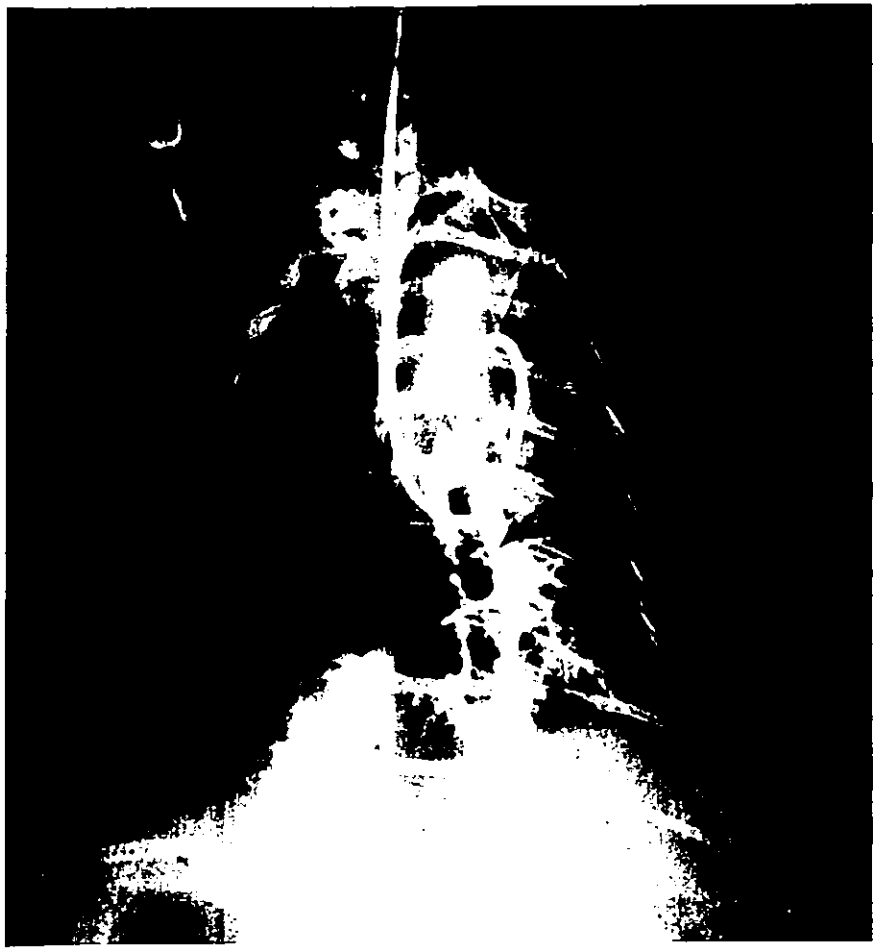
Methods

Experimental Animal and Anaesthesia

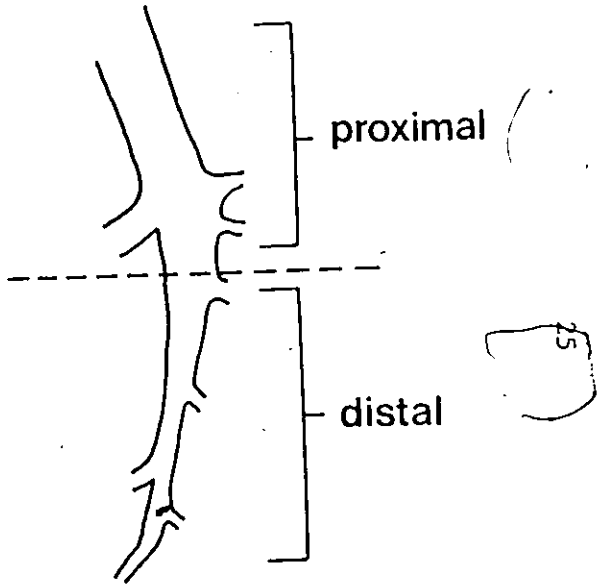
Adult New Zealand White rabbits, of either sex, 2 - 3.5 kg, were sacrificed with an intravenous injection of sodium pentobarbital into the marginal ear vein.

Dissection

Immediately following death the lungs were removed and placed in pre-oxygenated physiological salt solution (PSS) (see below). With the aid of a dissecting microscope (Wild Heerbrugg) the intrapulmonary artery was cut open lengthwise from its point of entry into the left lung and dissected distally as far as possible into the lower lobe. A longitudinal strip, 2 mm or less in width running the length of the artery was then prepared. The strip was removed from the lung by gentle teasing away of parenchyma and cutting off side branches. Any visible parenchymal tissue was removed from the strip, the length and width of which were accurately measured with a calibrated eyepiece micrometer. The strip was then cut to give a proximal and distal segment of equal length, (see fig. 1-2), usually 12-15 mm long. The proximal segment, thus consisted of a strip of the vessel wall of



(a)



(b)

Fig. 1-2. (a) Angiogram of rabbit pulmonary vasculature. The pulmonary artery (PA) and pulmonary vein (PV) of the left lower lobe are marked.
(b) Diagram of intrapulmonary artery showing proximal and distal segment.

about 2 mm in width from that portion of the artery with a mean lumenal diameter of greater than 1 mm. Due to the tapering nature of the artery, the distal segment consisted of the entire vessel wall, the mean lumenal diameter of the artery being less than 1 mm. The width of the distal strip narrowed to between 1.4 and 1.0 mm. At the end of each experiment the wet weight of both tissue segments was obtained.

Superfusion

Fine silk ties were attached to each end of both strips which were then mounted vertically for superfusion and measurement of isometric tension. Each strip was separately superfused, in a parallel circuit (see figs. 1-3 and 1-4), with PSS at a flow rate of about 12 ml/min., maintained by means of a roller pump (Watson Marlow MHRE 20 D). The PSS was continuously drawn from a common reservoir and warmed by heated water jackets (38°C) before superfusing the isolated tissues. The transit time, at a flow rate of 12 ml/min. from the reservoir to the tissues was 90 sec. About 50 - 70 mins. elapsed between onset of anaesthesia and installation of the strips for superfusion.

Physiological Salt Solution (PSS)

The PSS aerated (using a sintered glass filter) with 95% oxygen and 5% carbon dioxide, consisted of (in millimoles): NaCl, 117.6; KCl, 5.36; $CaCl_2$, 2.25; NaH_2PO_4, 1.18; $MgSO_4$: $7H_2O$, 1.16; $NaHCO_3$, 25; and glucose 11.1. The pH, pCO_2 and pO_2 of the PSS

DIAGRAM OF EXPERIMENTAL APPARATUS

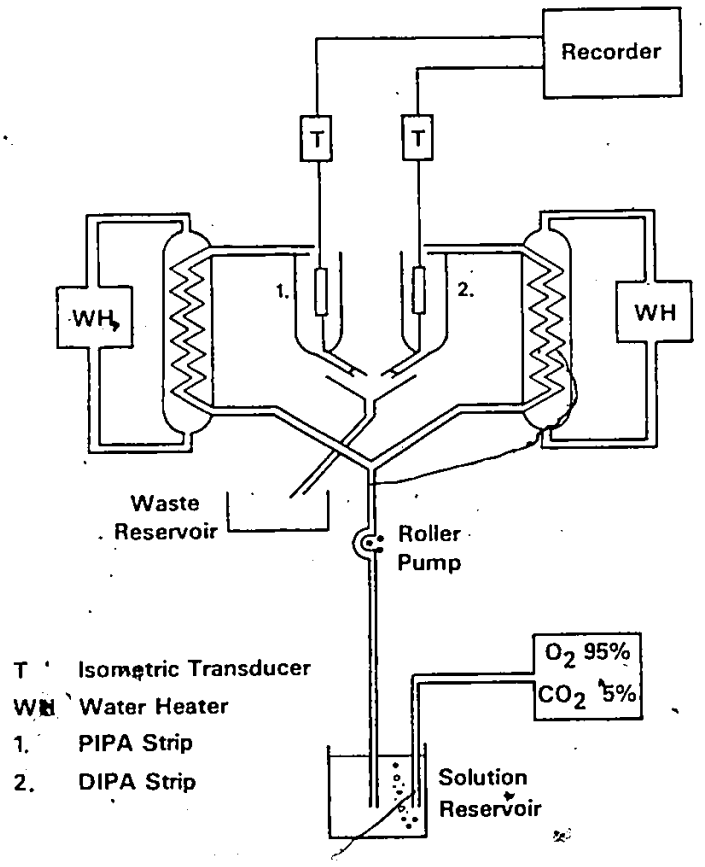


Fig. 1-3.

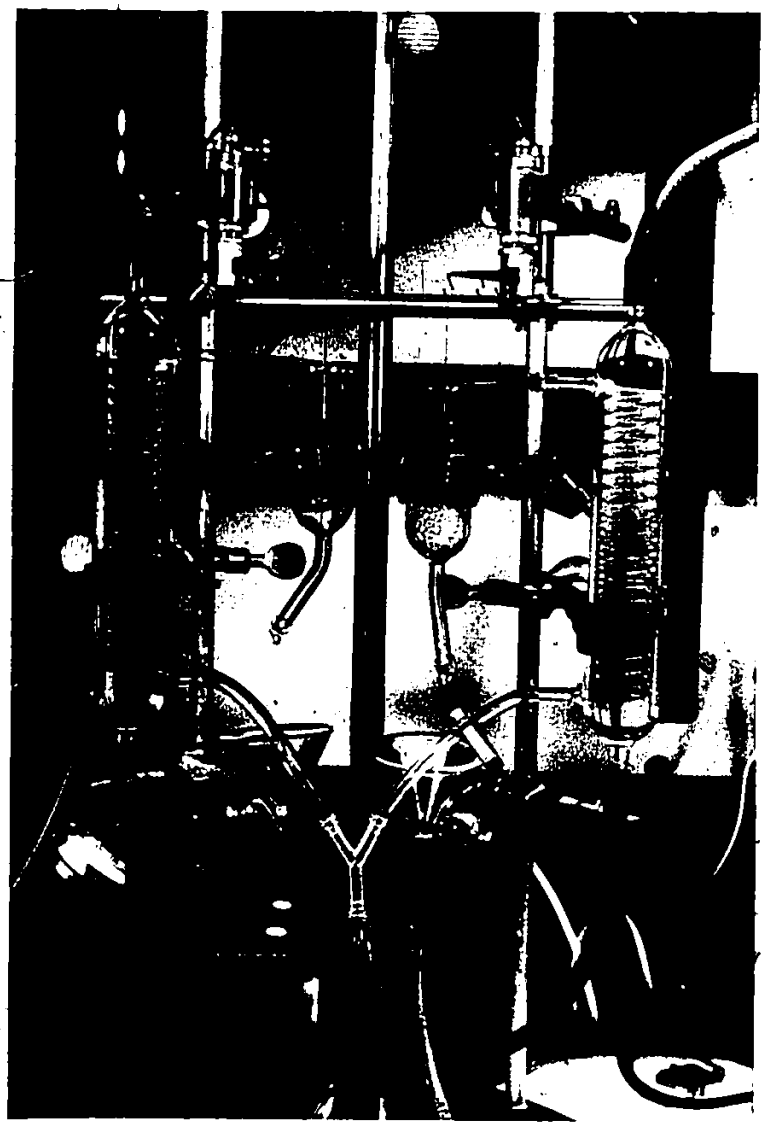


Fig. 1-4. Experimental Apparatus

were determined on 3 separate occasions using the microelectrode method and a pH and blood gas analyser (Instrumentation Laboratory Inc.) following 1 to 2 hours of aeration. The following range of values were obtained: pH, 7.43 - 7.46; P_{O2}, > 600 mmHg and P_{CO2}, 32 - 36 mmHg. Further routine estimations were considered unnecessary. The osmotic pressure of the PSS was determined using an osmometer (Advanced Instruments, model 3 W) and was found to be about 300 mOsm.

Recording

Once mounted, passive (or baseline) tension was applied to the strips by stretching the tissues through manual adjustment of the tissue supporting ligature. Isometric tension was detected by force displacement transducers (Statham gold cell, model UC3) and recorded on a 2 channel Hewlett Packard recorder (model 7702 B) as mg of tension. Balancing and calibration of the recording apparatus was performed at the onset of each experiment. Transducers were mounted on a moveable support permitting accurate adjustment (to 0.5 mm) of the length of the strip.

Experiments

The experimental work may be divided into 3 types: 1. drug response trials; 2. length tension experiments (4 experiments); and 3. fixation of tissues for light and electron miscroscopic analysis (2 experiments). The experimental protocols for each set of experiments were as follows:

1. Drug Response Trials

The drug response trials comprise three groups of experiments.

Group 1. Responses to 5HT, HIS, NE, $PGF_{2\alpha}$ and PGE_2[1] were examined. A baseline tension of between 250 - 350 mg, similar to that employed by previous investigators (12,58,91), was initially placed on both arterial strips. Each agonist was exposed through superfusion for 5 min. with a re-equilibration time of 30 min. The order of the agonists tested was altered with each trial. Additional experiments examining the effects of flow rate and hypoxia, the influence of arterial branches and extrapulmonary arterial responses were also done.

Group 2. Responses to prostagandins A_1, B_1, B_2, and E_2 were examined. A depolarizing K^+ solution was employed as a reference response. The baseline tension was set at about 300 mg. Only two prostaglandins were tested on each strip.

Group 3. Responses to 5HT, HIS, NE, IsoP, $PGF_{2\alpha}$ and AA were examined. Inhibition of responses was studied using specific antagonists. Baseline tension was initially set at 500 mg. Doses of antagonists were chosen based on previous reports of their use with smooth muscle strips and on results from preliminary trials. Owing to high baseline tensions, significant stress relaxation was occasionally seen, particularly in distal strips. Thus it was necessary to increase

[1]5HT - 5 hydroxytryptamine (serotonin), HIS - histamine, NE - norepinephrine, PG - Prostaglandin, AA - arachidonic acid (group 3 experiments).

baseline tension gradually, in a stepwise manner over a period of 10 min. This procedure minimized subsequent stress relaxation. A depolarizing K^+ solution was applied to the tissues following a period of equilibration. If the contractile response to K^+ was less than 75 mg or was biphasic in nature, the baseline tension was reset to 500 mg and K^+ was reapplied after a further period of equalibration. These experiments are summarised for each group. (see table 1).

Following installation in the bath, the isolated tissues were allowed to equilibrate for a minimum of one hour before drug effects were elicited. Drug effects were tested simultaneously on proximal and distal segments. In experiments in groups 2 and 3, a depolarizing potassium solution (123 mM K^+) was applied following the equilibration period in order to establish a reference response. The solution used (123 mM K^+) was identical to PSS except that the NaCl of the PSS was replaced with an equimolar concentration of KCl. (In addition, in all experiments in Group 3, the concentrations of $CaCl_2$ in the depolarizing solution was increased to 5mM from 2.25 mM to ensure maximal activation of contractile units). Dose response curves were obtained by applying drugs, prepared in PSS, to the strips in increasing concentrations. Each drug concentration was prepared separately and applied, in sequence, by replacing the reservoir of Krebs with separate solutions of each drug dose. The time of exposure of the strips to the drugs was carefully controlled. A minimum of 30 minutes perfusion with ordinary PSS was allowed between each drug

Table 1: Drug Response Trials

	baseline tension (mg)	Reference drug	Agonists	Antagonists
Group 1	250-350		5HT, HIS, NE, $PGF_{2\alpha}$, PGE_2	
Group 2	300	K^+ 123 mM	PGA_1, B_1, B_2, E_1 and E_2	
Group 3	500	K^+ 123 mM Ca^{++} 5 mM	5HT	methysergide
			HIS - H_1	mepyramine
			HIS - H_2	cimetidine
			NE	phentolamine
			IsoP	propranolol
			AA $PGF_{2\alpha}$	indomethacin

challenge to enable the agonist to wash out and to permit sufficient time for the tension to return to baseline levels. When antagonists were employed they were added to the PSS superfusing the tissues 30 minutes prior to and during subsequent exposure of the strips to agonists. In some cases relaxant effects of agonists were evaluated in strips that were partially contracted with HIS or $PGF_{2\alpha}$. After the contractile effect reached a steady state the strips were then exposed to increasing doses of relaxant agonist.

Length Tension Experiments

Baseline tension was set at 300 mg and tissues were allowed to equilibrate for 1 hour before a depolarizing K^+ solution (K^+123mM, Ca^{++}5mM in PSS) was applied to establish a reference response. The tissue length in both strips was then reduced to a length at which tension in the strip appeared to be minimal (since further reductions in length did not proportionately reduce tension) and yet permitted adequate superfusion. The length of the strip at this point was measured and designated the initial length. Following an equilibration period of 20 minutes permitting baseline tension to stablize the tissues were stimulated with a contractile agonist for 3 minutes (K^+123mM + Ca^{++}5mM on 3 occasions, HIS 10 µg/ml on 1 occasion). A recovery period of 7 minutes was allowed to enable the agonist to washout and tension to approach baseline levels. The tranducer supports were then adjusted to produce 1 mm increments in tissue lengths. Following a 20 minute equilibration period the tissues were stimulated

again. The above procedures were repeated until the active tension generated by the agonist failed to increase with subsequent exposure, thus giving passive tension and active tension generated for a series of muscle lengths. Data obtained were used to construct active and passive isometric tension - length curves for proximal and distal segments.

Fixation of tissues

Proximal and distal segments, prepared as above, were mounted in the baths under 300 mg passive tension and a contractile response to 5HT (1 μg/ml) was obtained following an hour of equilibration. Following a further period of equilibration the tissues were again contracted and then fixed by superfusing 2.5% glutaraldehyde (4°C, pH 7.4, 350 mOsm) over the tissues for 15 minutes. Tissue samples were then processed for light and electron microscopic examination, by routine procedures using Epon embedded specimens. A qualitative analysis of smooth muscle content and orientation in both strips was undertaken.

Data and Statistics

Contractile responses are expressed as (a) change in active tension developed (mg), (b) as tension output (g/cm^2), (c) as a percent of maximal response to a given agonist or, (d) as percentage reference response. The tension output (g/cm^2) was calculated by dividing the force (g) generated by the cross sectional area (CSA, cm^2) of the strip. The latter was estimated by utilizing the mass

(g), length (cm) and density of the strip (g/cm^3), according to the formula CSA = mass/(density x length). A value of 1.05 g/cm^3 was assumed for the density of the muscle strips (36). Relaxant effects are expressed as percent relaxation calculated as either percentage decrease in induced tone or percent of maximal relaxation. Data are expressed as the arithmetic mean $\pm$ 1 S.D. The concentration of agonist that produced 50% of its maximal response (ED_{50}) was determined in the following manner. A regression equation was obtained for each experiment by linear regression analysis, using the least squares method, of the data derived from those points which could be seen lying on the linear portion of the mean response (% maximum tension) - dose curve. Students t test was used to compare means and probability values of $P < 0.05$ were accepted as denoting significance. Analysis of data was done with Texas Instrument SR-51 11 and TI - Programmable 57 calculators.

Drugs

The vasoactive agents used in this study were:

	Source
L - arterenol bitartrate	Sigma
5 - hydroxytryptamine creatine sulfate complex	Sigma
histamine dihydrochloride	Sigma
isoproterenol hydrochloride	Sigma
arachidonic acid	Sigma

Prostaglandins A_1	
B_1, B_2	gifts of the Upjohn Co.
E_1, E_2	Kalamazoo, MI.
$F_{2\alpha}$	

The antagonists used were:

methysergide hydrogen maleinate	a gift of Sandoz
cimetidine hydrochloride	Smith, Kline and French
mepyramine maleate	a gift of Poulenc Ltd.
phentolamine mesylate	CIBA
propranolol hydrochloride	a gift of ICI
indomethacin	Sigma

Preparation of Drugs

Concentrated stock solutions of all amines were prepared daily in PSS and refrigerated until used. All crystalline prostaglandins were prepared as concentrated solutions (10 mg/ml) in absolute ethanol. PGB_2, an oil, was prepared in an identical manner. Stock solutions of prostaglandins and arachidonic acid in benzene were stored in a freezer. In the case of the amines and prostaglandins, aliquots of solution and vehicles were appropriately diluted in PSS and stored on ice and either used immediately or refrigerated. Arachidonic acid, in benzene, was prepared daily, protected from light, in 10% absolute ethanol and 90% Na_2CO_3 (100 mg/ml) as a 3 mg/ml stock solution. For repetitive trials, it was necessary to prepare concentrated solutions (10 mg/ml) of IsoP for each exposure. Catecholamines were

prepared in PSS containing either 50 mg/l ascorbic acid (Group 1 experiments) or 0.03 mM ethylenediamine tetracetic acid disodium calcium salt (Na_2Ca EDTA, Sigma) (Group 2 and 3 experiments). Phenoxybenzamine was prepared in absolute ethanol and a small quanitity of 1N HCl. Indomethacin was prepared daily as the sodium salt using 100 mg/ml Na_2CO_3. All drug concentrations expressed are final concentrations of the superfused fluid (ng/ml or molar). Molar concentrations of drug solutions were calculated from the anhydrous molecular weights which were derived either from the manufacturer's data or from the Merck Index (61).

CHAPTER 3

Results

Group 1-Amine Dose Response Trials

The responses of proximal and distal segments of rabbit intrapulmonary artery to 5HT, HIS and NE were examined in a series of six experiments. The dose ranges employed were selected on the basis of a number of preliminary experiments. Baseline tensions, initially set at between 250 and 350 mg, were seen to decline gradually during the equilibration period to a lesser value that was generally well maintained. Following drug response trials, small changes of about 10 mg in baseline tension, relative to initial values, were common.

The effects of 5HT, HIS and NE, as a percent of maximum tension developed, on proximal segments are illustrated in fig. 2-1, while distal segment responses to 5HT and HIS are shown in fig. 2-2. Specific characteristics of individual drug responses are outlined in the following paragraphs. In general, it can be seen that both 5HT and HIS elicited dose related contractile activity in both PIPA and DIPA segments. A difference in sensitivity to these amines is evident, the threshold concentration for the 5HT response being much lower than for HIS. The proximal response to NE was characterized by dose related contractile activity to a maximum followed by a decline in active tension at the highest dose. Sensitivity of the PIPA segment to NE

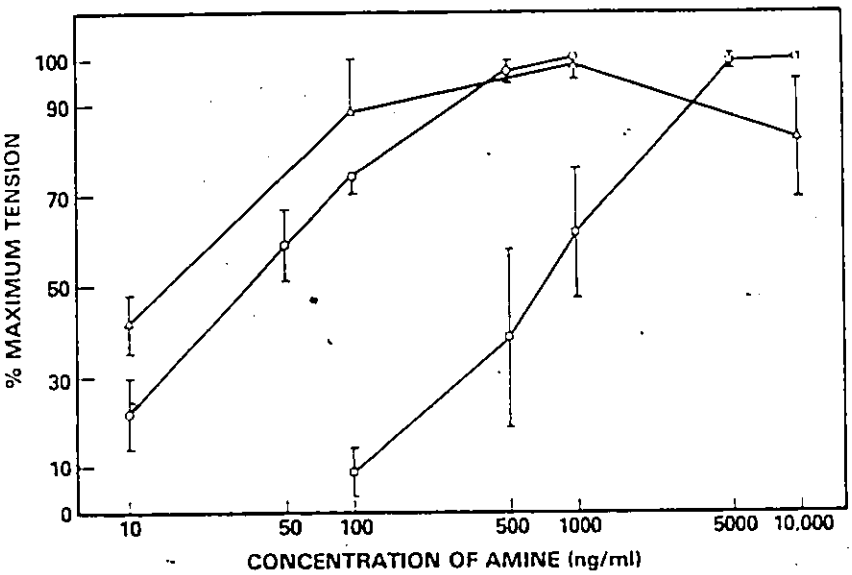


Fig 2-1. Response of proximal rabbit intrapulmonary arterial segment to 5HT (○), HIS (□) and NE (△). Each point represents a mean ± 1 S.D of 5 (HIS responses) of 6 experiments (5HT and NE responses).

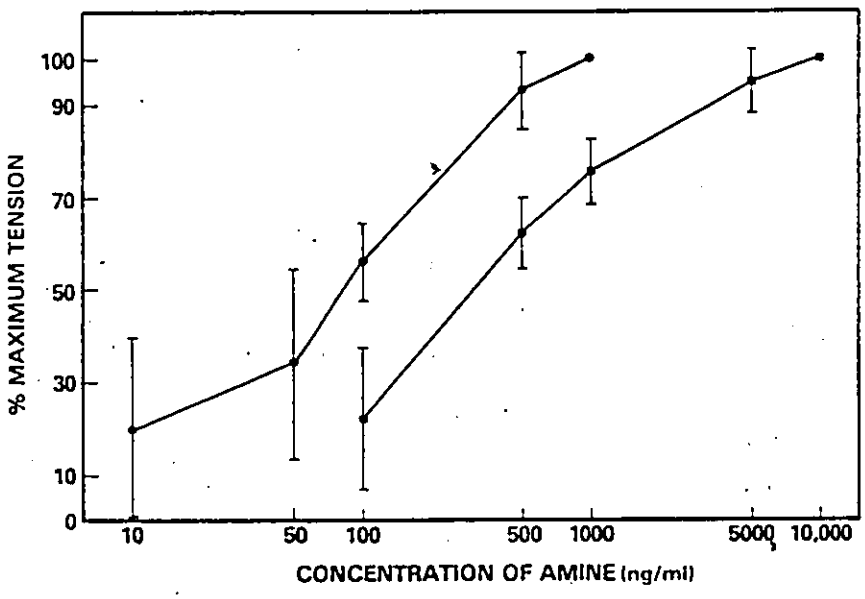


Fig. 2-2. Response of distal rabbit intrapulmonary arterial segment to 5HT (●) and HIS (■). Each point represents a mean ± 1 S.D. of 5 experiments.

more closely resembled that to 5HT. Distal responses to NE are not illustrated in fig. 2-2, because of great variability in response, but are detailed below under NE responses. The order of application of the three amines did not appear to influence tissue responsiveness. Since analysis of the experimental data in terms of mg of active tension produced considerable variability in drug responses, attempts were made to reduce this variability by normalizing responses in terms of tissue mass i.e. g/cm^2. This did not alter the inherent variability of these responses and thus have not been reported here. However, normalizing responses relative to a reference response to high K+ was employed in Group 2 and 3 experiments.

5HT

The dose dependent contractile responses induced by 5HT in proximal segments were rapid and well maintained at high doses. The active tension elicited by 1 µg/ml of 5HT was 110 $\pm$ 46.6 (mean $\pm$ 1 S.D., n = 6). Distal segments responded to 5HT with graded contractile activity in three experiments. However, very poor responses were obtained in three other experiments. On one occasion a maximal effect of only 5 mg of active tension was seen and because of an unusual response to HIS, (see below), data from this DIPA segment were not included in the determination of mean responses. The mean response to 1 µg/ml 5HT was 45.0 $\pm$ 34.6 mg (n = 5). Replacement of the superfusate with PSS free of 5HT resulted in a gradual relaxation of both strips to the baseline level of tension.

HIS

Proximal and distal segment responses to HIS were attained within two minutes but were not as well maintained as 5HT responses at high doses. The maximum contraction produced by HIS in PIRA segments was 95.8 ± 57.0 mg, a response comparable to the 5HT induced maximal response but elicited at a much greater dose of 10 µg/ml. In one experiment the maximum response to HIS was very poor (maximum of 15 mg). The distal response to HIS was characterized by contractile responses in four experiments and an unusual response, henceforth designated as "biphasic", in the other two experiments of this group. Examples of both responses are illustrated in fig. 2-3. The biphasic response was characterized by a decline in tension relative to the baseline or previous response (relaxant phase), followed by an increase in tension which did not necessarily exceed the baseline tension (contractile phase). Following the biphasic response, washout of the agonist was accompanied by a return to the initial baseline tension. In one of the experiments characterized by biphasic responses even at the highest dose of HIS the tension did not return to the baseline while 5HT and NE responses were extremely poor. Data from this distal segment were not used because of the unusual relaxant nature of the response. However, in all other experiments tension increased above baseline and the mean response to 10 µg/ml of HIS was 117.0 ± 78.2 mg (n = 5).

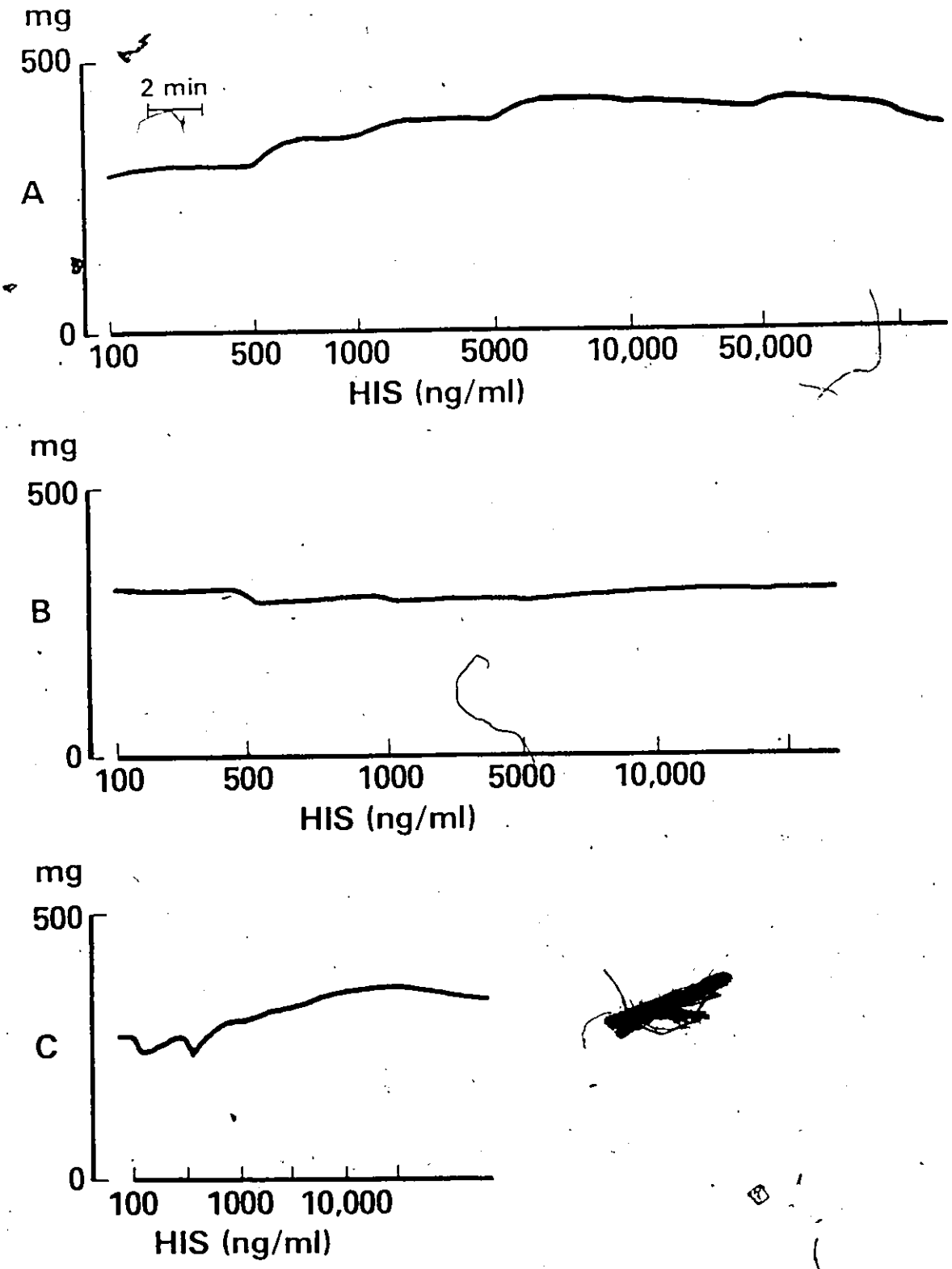


Fig. 2-3. Effects of HIS on distal segment of rabbit intrapulmonary artery. A - contractile response, B and C - biphasic responses.

NE

NE contracted proximal segments although it was observed that the highest dose produced a decline in active tension. The maximal response, produced by 1 μg/ml of NE, was 56.7 ± 8.8 mg (n = 6). With washing of the tissue this decline in tension was reversed with an increase in tension reaching a maximum in 2 - 10 minutes comparable to the drug elicited maximal response. Subsequently tension declined gradually to baseline levels.

Distal segments responded poorly to NE. Small and highly variable contractile and relaxant effects were observed. The mean maximal contractile response, obtained at 100 ng/ml, was 9.2 ± 7.4 mg (n = 5). Further increases in doses of NE produced a decline in mean tension to below the baseline. Similar to the PIPA segment, with washing of the tissue, tension increased to a maximum equal to or greater than the drug elicited maximal response.

Comparison of Amines

For the purpose of comparing the magnitudes of amine contractile effects, responses have been expressed as a percent of the mean maximum response to HIS for proximal segments, (fig. 2-4), and distal segments (fig. 2-5). In addition, proximal segment responses have been expressed in fig. 2.6, as a percent of the mean maximal response to 5HT. Analysis of proximal amine responses in terms of the HIS response was complicated by a single very poor response to HIS, despite reasonable responses to 5HT and NE, obtained in one experiment

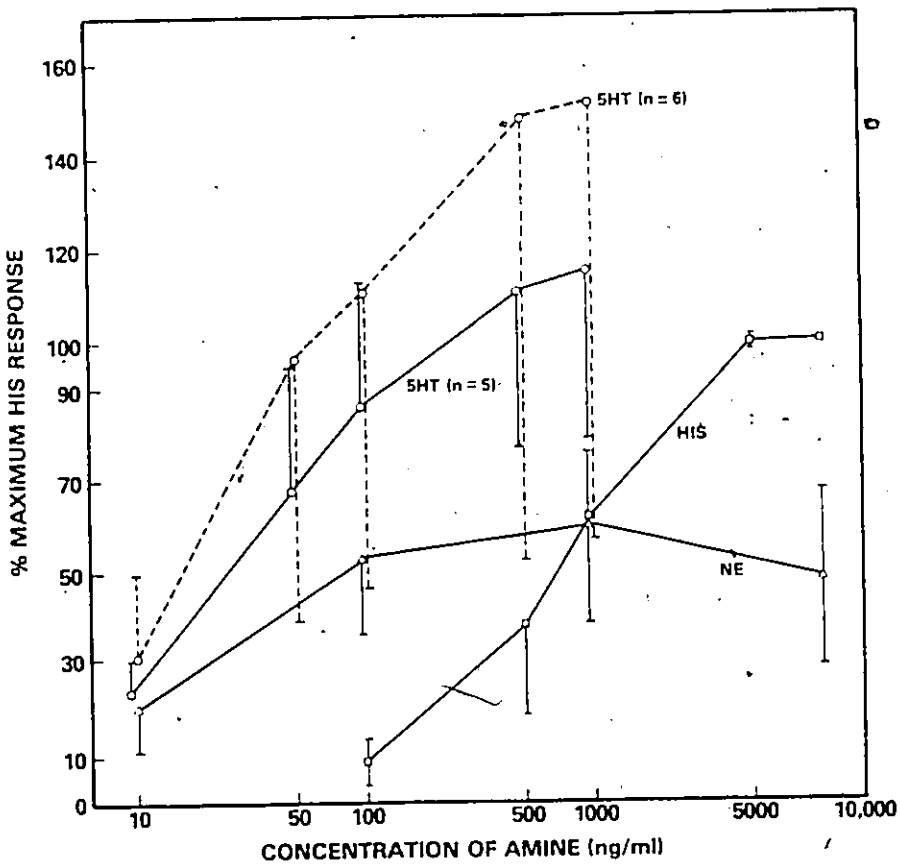


Fig 2-4. Mean responses (+ 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to 5HT, HIS (n = 6) and NE (n = 5).

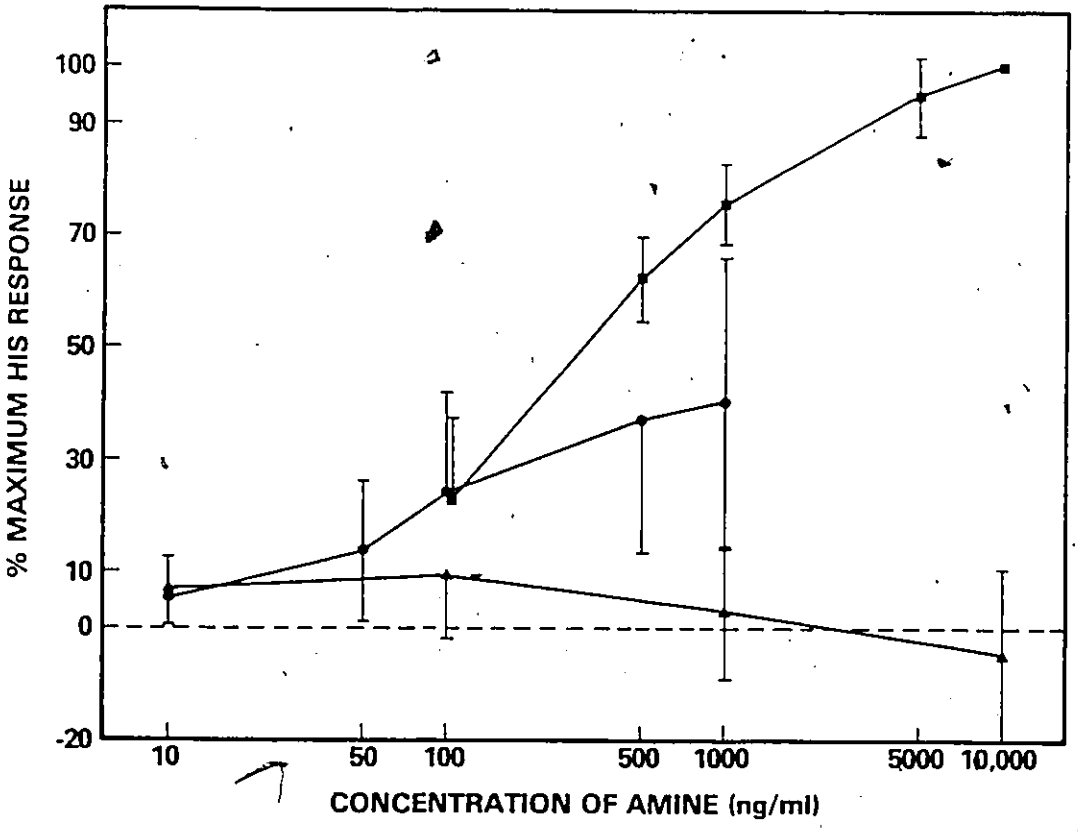


Fig 2-5. Mean responses (± 1 S.D.) of the distal segment of rabbit intrapulmonary artery to 5HT (●), HIS (■) and NE (▲). (n = 5).

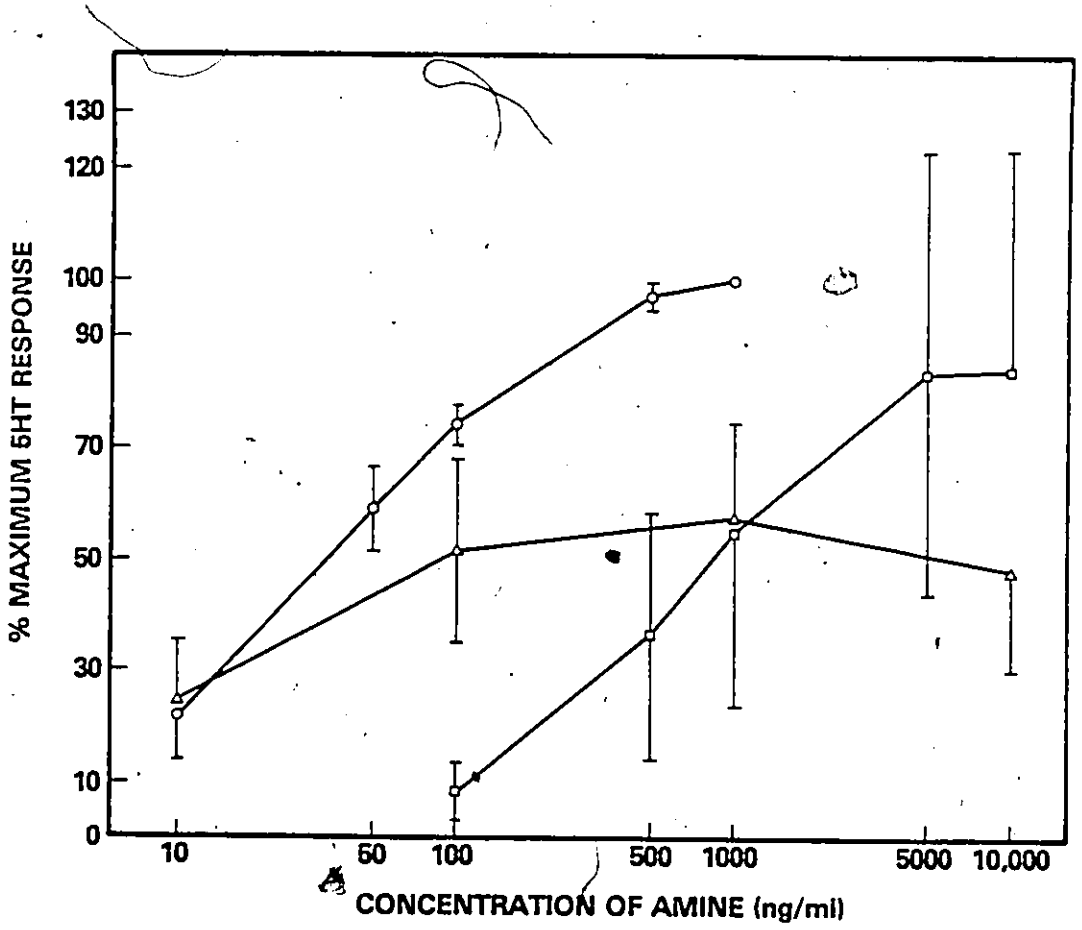


Fig 2-6. Mean responses (± 1 S.D.)of rabbit PIPA segment to 5HT (○), HIS (□), and NE (△). (n = 6).

as previously mentioned. Data from this experiment yielded markedly increased mean tensions and variability for 5HT and NE responses when analyzed relative to the HIS maximum response. For the purpose of graphically displaying data, the 5HT (n=5, solid line) and NE curves, (illustrated in fig. 2-4), represent data from five experiments excluding the poorly HIS responsive tissue. However, the 5HT (n=6, broken line) and HIS responses shown in fig. 2-4, include data from all experiments. Although the magnitudes of the mean HIS responses are somewhat depressed the relative shapes and positions of the other agonist dose response curves have been little changed, except that the mean responses and standard deviations have been much reduced by excluding the data from the poorly HIS responsive experiment, as seen by comparing the 5HT curves in fig. 2-4. However, given the exclusion of some data, no statistical analysis of this data was undertaken to determine the significance of the differences in the mean amine responses.

Examination of the proximal segment responses in fig. 2-4 reveals that while the relative magnitudes of the 5HT and HIS responses were comparable, the maximal response to NE was much less than the HIS and 5HT (n = 5) responses, (about 60% of the HIS maximal response). Figure 2-6, which also illustrates proximal responses to amines, but relative to the maximum 5HT response, includes data from all experiments. The relative magnitudes of the contractile responses to the amines are similar to those seen in fig. 2-4. The difference between the maximum 5HT and NE response was significant. In the distal

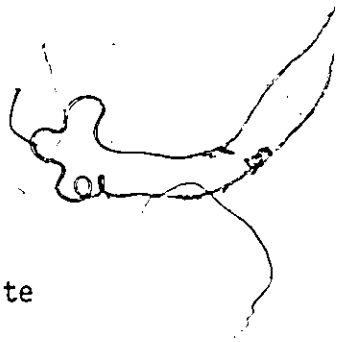
segments, (see fig. 2-5) the maximal response to 5HT was significantly less than the maximal response to HIS, (about 40% of the HIS response). The poor responsiveness of the distal segment to NE is clearly seen in fig. 2-5. The mean maximal response to NE did not exceed 10% of the maximum response to HIS.

Comparison, between proximal and distal segments, of the magnitudes of the amine responses relative to HIS revealed that distal 5HT responses were reduced relative to proximal responses. The proximal segment response to NE was reduced relative to 5HT and HIS responses while DIPA segments were very poorly responsive to NE. The relative sensitivity to the amines, however, was similar in both proximal and distal segments. The large variability and the observed differences in responses to the various amines indicated further investigations were required. Repetition of these trials, in part, was undertaken in Group 3 experiments.

The results of this series of experiments suggested some changes in the experimental protocol. Owing to rapid response to each of the drugs applied, the time of exposure to doses of amines was reduced from five to two minutes. The nature of the NE dose response curve indicated an incomplete dose range, consequently further experiments undertaken in this group included doses from 5 to 5000 ng/ml.

Repetitive Amine Dose Response Trials

To determine if tissue responsiveness was altered by repetitive exposure to the same drug, 5HT, HIS and NE, on separate occasions, were



administered to both proximal and distal segments at 30 minute intervals to obtain three or more consecutive dose response curves. The results of these experiments are illustrated in the graphs following, the data expressed as a percent of the maximum response obtained with the initial exposure to the amine. Only experiments in which both proximal and distal segments contracted in response to the amines were included in this study. The lack of reactivity to NE and the occasional relaxant nature of the distal response prevented analysis of its response in the manner described above. While proximal strip baseline tensions were usually well maintained, it was frequently observed that distal strips exhibited small and continuous declines in baseline tension throughout the duration of experiments. Declines in baseline tension were often associated with increased active tension in response to doses of amine.

5HT

Figure 2-7 illustrates the responses of the proximal and distal segments (n=6) to repetitive 5HT dose response trials. Both segments contracted to 5HT and the initial dose response curves obtained resemble those from the initial series of amine experiments seen in figures 2-1 and 2-2. In the proximal segment, repeated exposure to 5HT produced a decrease in the mean response at each dose. The decrease in mean tension relative to the initial dose response curve was significant for all doses with the third 5HT dose response trial. In

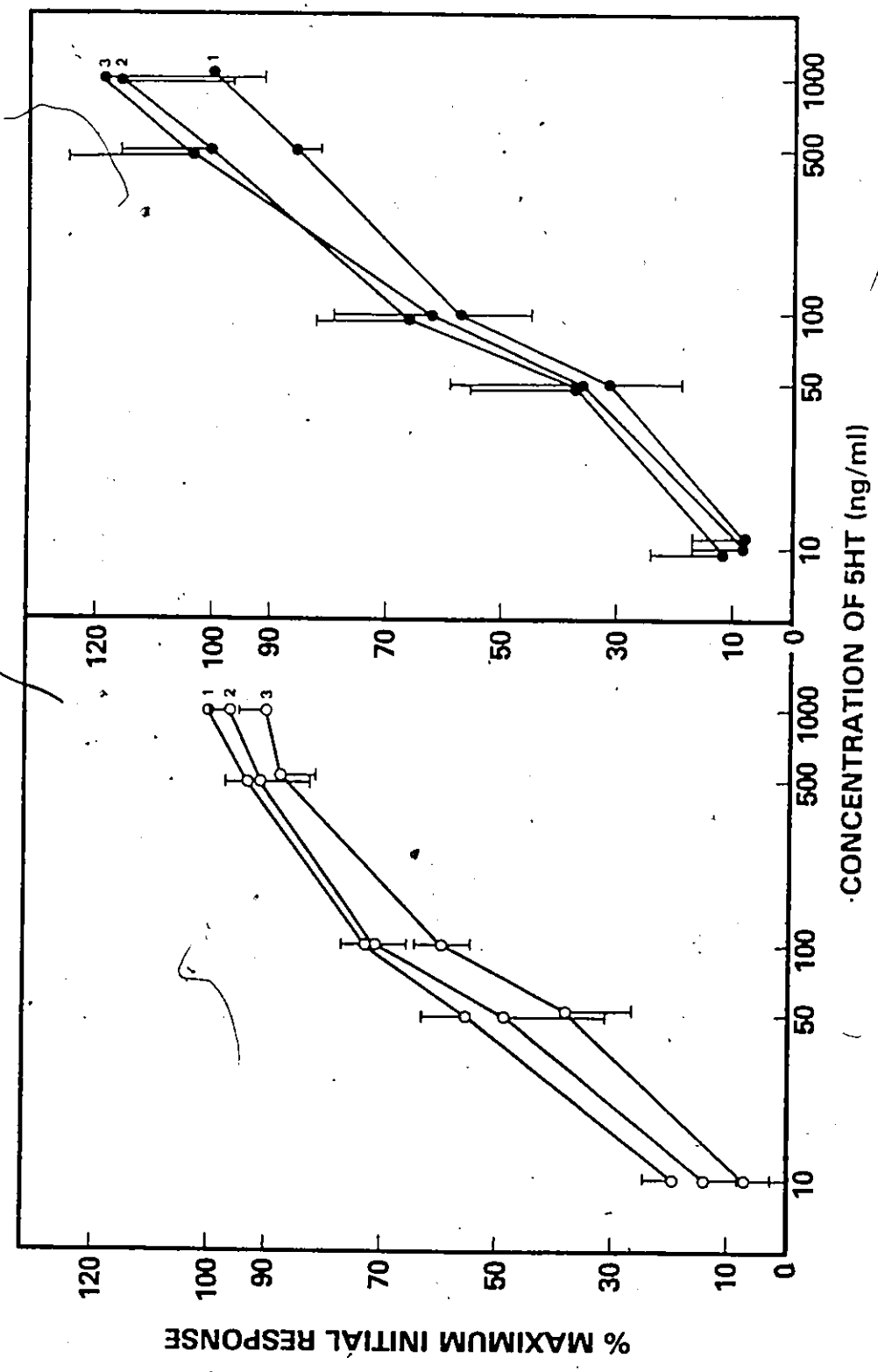


Fig. 2-7. Effect of repetitive 5HT dose-response trials on rabbit intrapulmonary artery. Numbering on graph refers to order of trials for proximal (○) and distal (●) segments. Each point represents the mean ± 1 S.D. of 6 experiments.

two experiments, two additional dose response curves were obtained and those exhibited further small declines in responses. When determined relative to the maximum tension for each trial, (not illustrated), differences in responses were significantly less than the initial responses at doses of 10, 50 and 100 ng/ml with the third dose response curve. Doses of 5 µg/ml 5HT when applied on two occasions to PIPA segments did not increase the maximal response to the amine when tested on the first, third and fifth exposures.

In contrast to the proximal segment, distal strips responded to 5HT with an increase in the mean response, however, the difference relative to the initial responses was significant only at high doses i.e. 0.5 µg/ml 5HT with the 2nd and 3rd trials and 1.0 µg/ml with the 2nd trial. However when tested in three experiments, the magnitude of responses began to decline with additional dose response trials. The addition of 5 µg/ml 5HT to the dose regimen usually produced small increments in active tension. When determined as a percent maximum tension for each trial, differences in dose response curves were not significant.

HIS

Proximal responses to HIS diminished with successive dose response trials as illustrated in fig. 2-8. The decline in active tension relative to the initial response was significant for the response to 500 ng/ml with the second dose response trial and for all

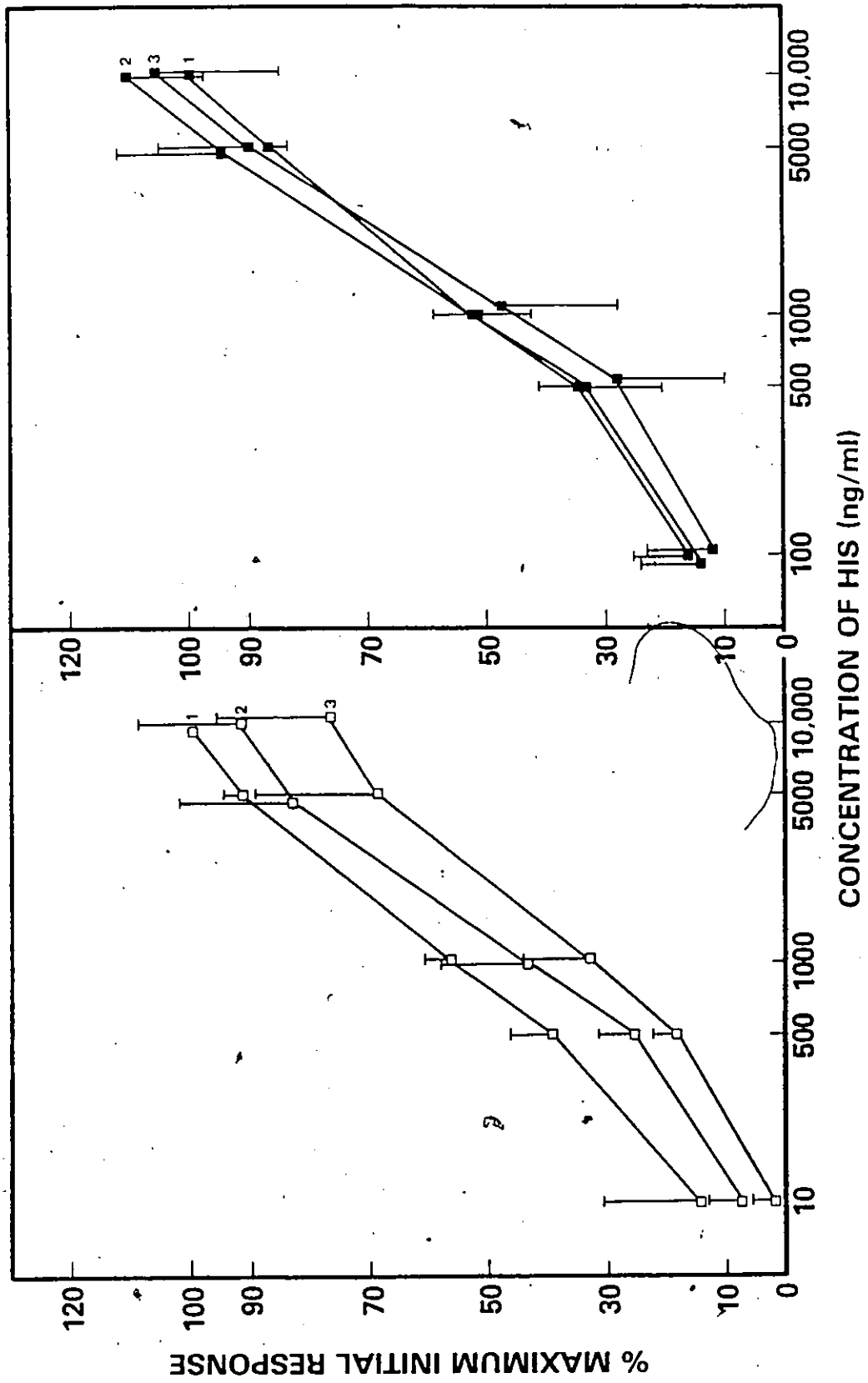


Fig. 2-8. Effect of repetitive HIS dose-response trials on rabbit intrapulmonary artery. Numbering on graph refers to order of trials for proximal (□) and distal (■) segments. Each point represents the mean ± 1 S.D. of 6 experiments.

responses except the lowest dose with the third exposure to HIS. Although the mean decline in maximal tension relative to the initial maximum was 24% with the third dose response trial, maximal tension declined only 5% in one experiment but 50% in another. Further declines in responses to doses of HIS were seen with additional dose response trials, (up to five). In the one experiment in which proximal responses to HIS were markedly attenuated with repetitive dose response trials, HIS 50 µg/ml increased the maximal tension generated. When determined as a percent of maximum tension, differences in responses relative to the initial responses were significantly less at doses of 500 and 1000 ng/ml for the second and third dose response trials.

The distal segment responses to HIS were well maintained with small but not significant, increments in responses with repetitive trials. In a single experiment the maximal response to HIS on third exposure declined 24% relative to the initial maximal response, however, this was offset by increased responses in other tissues. When determined as a percent of maximum tension, the responses obtained with the second and third dose response trials were reduced relative to initial responses.

NE.

The proximal segment responses to NE were well maintained with repetitive dose response trials (see fig. 2-9). The NE responses obtained resembled those seen in fig. 2-1 although differences were apparent due to the use of a more complete dose range. Relative to

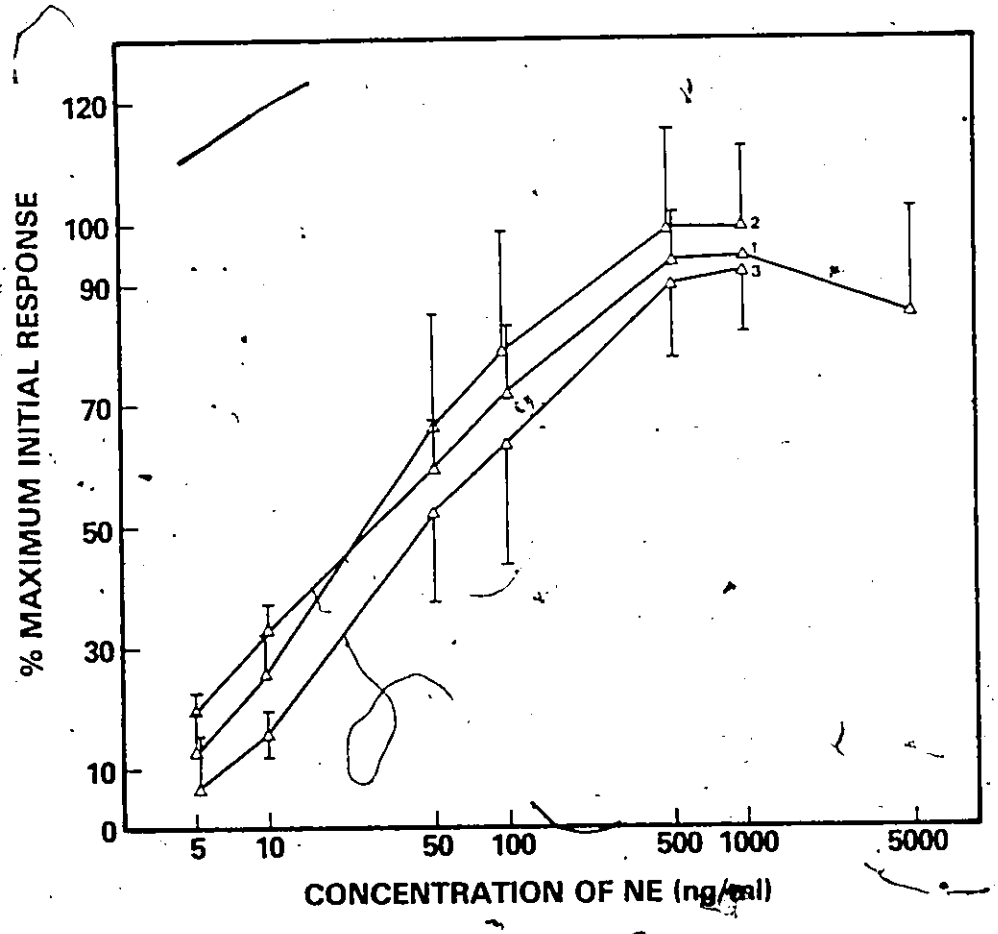


Fig 2-9. Effect of repetitive NE dose-response trials on the proximal segment of rabbit IPA. Each point represents the mean ± 1 S.D. of 4 experiments.

initial responses, small but significant decreases in tension were evident at doses of 5 and 10 ng/ml NE with the third exposure to the amine. The mean maximal response to NE did not change significantly, even with the fifth dose response trial, (five NE dose response trials were obtained in each experiment).

Owing to the small and variable nature of the distal segment response to NE it was difficult to assess the effect of repetitive trials. However, no clear or consistent change in response to NE was evident with the third exposure to the amine. With the fifth dose response trial some loss of reactivity was apparent.

Summary

Thus, while 5HT responses exhibited small but significantly diminished responses with repetitive dose response trials, the PIPA segment responses to NE were well maintained. Proximal segment HIS responses exhibited more pronounced decreases in responses than 5HT with repeated trials, and in one experiment were characterized by marked tachyphylaxis (defined as the development of decreased responsiveness with repeated administration of a drug). Distal segment responses were well maintained, indeed, responses often increased with repetitive dose response trials. An influence of baseline tension on arterial responsiveness was apparent, particularly in distal segments since increased active tension was often related to a proportional decline in baseline t[illegible]n prior to agonist stimulation.

High Flow Effects

To determine whether the rate of flow of superfusate influenced tissue responsiveness to drugs, on four occasions graded dose response curves to 5HT (n = 3) and NE (n = 1) were obtained initially at the normal flow rate of 10-12 ml/min and subsequently at an increased flow rate of about 20-25 ml/min. In both proximal and distal segments, the amine responses obtained were consistent with results previously described for repetitive dose response trials. The poor responsiveness of the distal segment to NE was unchanged by the increased flow rate. Thus a flow rate of 10 to 12 ml/min was judged to be adequate and increased flow rates were considered to be without influence on tissue responsiveness.

Hypoxia

To ascertain if oxygen tension influenced tissue responsiveness the effects of the use of anoxic gas (95% N_2, 5% CO_2) to aerate the superfusate on baseline tension and responses to HIS were examined in a separate series of three experiments. The experimental format consisted of control responses to HIS obtained in the presence of oxygenated PSS followed by two HIS trials in the presence of anoxic PSS while subsequent HIS responses were obtained in oxygenated PSS. In a single experiment the following parameters were obtained: oxygenated (95% O_2, 5% CO_2) PSS: pH - 7.43, P_{CO2} - 36 mmHg, P_{O2} - 530 mmHg; anoxic PSS: pH - 7.43, P_{CO2} - 35 mmHg, P_{O2} - 60 mmHg. It should be noted that the superfusion was not done in a

closed system thus permitting equilibration of the superfusate gas tensions with the atmosphere during cascade over the tissues.

The results of these experiments indicate that hypoxia caused no change in baseline tension. Most of the changes in active tension observed were consistent with results obtained from repetitive dose response trials to HIS. A notable exception was an increased proximal segment response to HIS in the presence of oxygenated PSS following two hypoxic trials in which a marked diminishment of the HIS response had been observed. It is of interest to note that the other proximal segment responses appeared to be unusually well maintained in the presence of anoxic gas. However, because of the limited number of trials it is difficult to attribute any specific effect on active tension of exposure to hypoxia.

Influence of Arterial Side Branches

In a single experiment, two strips were obtained from the proximal segment by cutting down its middle. The number of side branches was counted and found to be 16 in one strip and 11 in the other. The strips were then stimulated with K^+, 5HT and HIS to determine if the bulk of smooth muscle contained in the stumps of severed side branches significantly influenced the magnitude of responses. The contractions obtained were remarkably similar in magnitude differing by only 10 to 15 mg relative to maximal effects of about 125 mg. Thus vascular strip responses appeared to be unrelated to the number of arterial side branches present.

Extrapulmonary Compared with Proximal Intrapulmonary Responses

Given the differences in the proximal and distal intrapulmonary arterial responses, a brief examination of extrapulmonary versus intrapulmonary responses was undertaken. In a series of 6 experiments the effects of 5HT and HIS on isometric tension generation in extrapulmonary and proximal intrapulmonary arterial segments were compared[1]. Illustrated in figures 2-10 and 2-11 are the data from these experiments expressed as a percent of the maximum response to 5HT. In the proximal segment (fig. 2-10) the sensitivity to 5HT was greater, although the mean maximal response to HIS was significantly greater than the response to 5HT. These responses were similar with respect to sensitivity to those observed in fig. 2-6 taken from the initial amine experiments. In contrast, the variability of the HIS response was reduced and the maximal response to HIS was greater than the 5HT response in this series of experiments. (Recall that the mean HIS responses were reduced by a single poorly responsive proximal segment in the initial amine experiments.)

In extrapulmonary segments (fig. 2-11) the relative sensitivity of the amines was unchanged. However, in contrast to the proximal segment, the magnitude of the extrapulmonary maximal response to HIS was significantly less than the maximal response to 5HT. In general,

[1]The proximal segment of intrapulmonary artery employed in these trials was identical in size and origin to previously used proximal strips. The extrapulmonary artery was obtained in a similar manner. A longitudinal strip of the same dimensions was taken from the artery between the bifurcation of the main pulmonary artery and its insertion into the left lung.

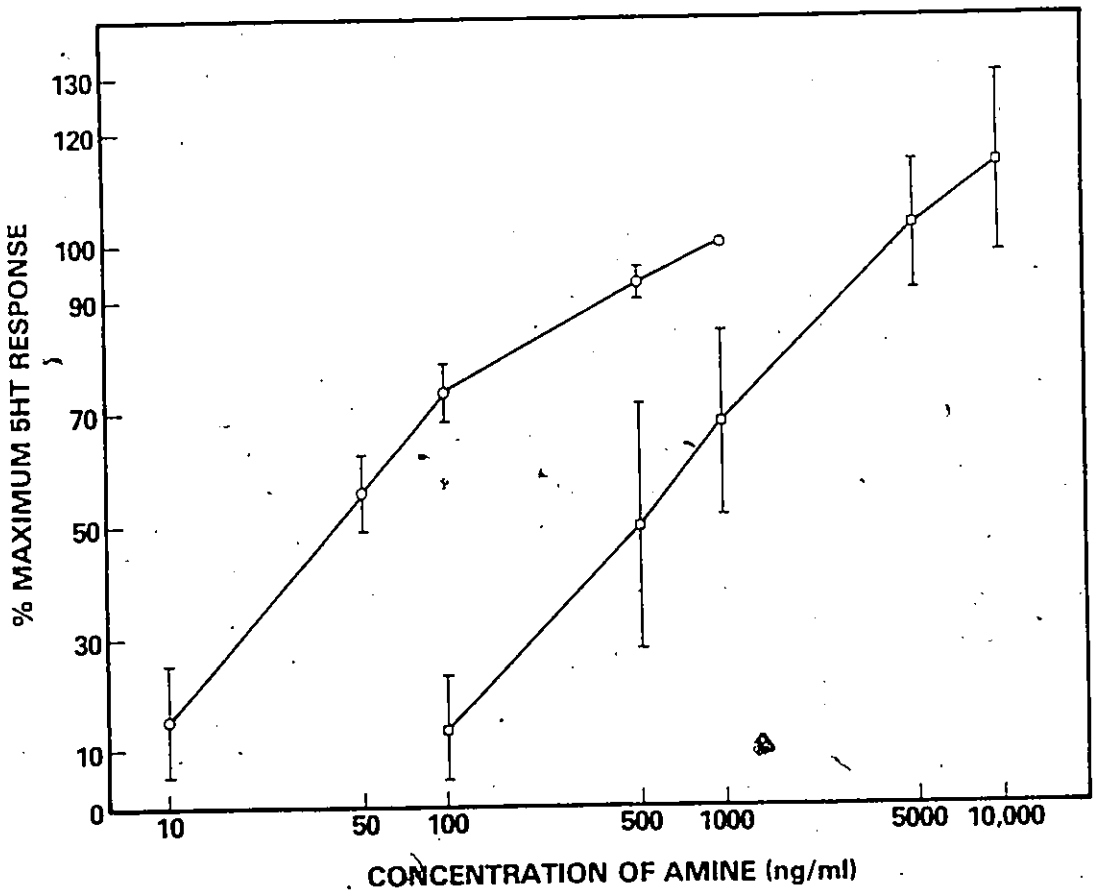


Fig 2-10. Mean responses (+ 1 S.D.) of rabbit proximal intrapulmonary artery to 5HT (○) and HIS (□). (n = 6).

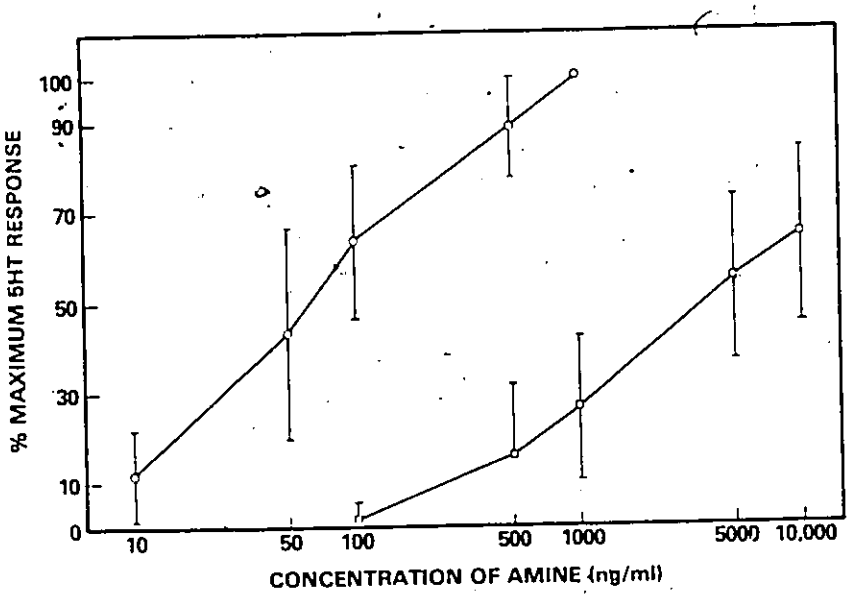


Fig. 2-11. Mean responses (+ 1 S.D.) of rabbit extrapulmonary artery to 5HT (○) and HIS (□). (n = 6).

the mean HIS responses relative to the 5HT response were much reduced in comparison to proximal responses. Also the response to 5HT was marked by much greater variability in extrapulmonary segments. Thus it appears that there are significant differences between intra- and extrapulmonary responses to HIS.

$PGF_{2\alpha}$ and PGE_2

In a series of six experiments the effects of $PGF_{2\alpha}$ and PGE_2 on PIPA and DIPA segments were examined, the dose ranges having been established in a preliminary trial. In a single experiment, the distal segment responded poorly, for unknown reasons (but perhaps related to tissue damage), with an increase of only 10 mg in response to high doses of $PGF_{2\alpha}$ despite a normal proximal response. Consequently data from this experiment was not employed in the determination of the mean responses. Quantities of ethanol, equal to those employed in the preparation of 10^{-5}M prostaglandins failed to elicit any response.

$PGF_{2\alpha}$ contracted both proximal and distal arterial segments in a dose related manner. The threshold for the $PGF_{2\alpha}$ response was about 10^{-8}M while maximal responses were obtained at 10^{-5}M in both segments. From fig. 2-12, the log concentration - percent maximum tension graph it can be seen that distal dose response curve was almost parallel but displaced to the right of the proximal curve indicating that the sensitivity of the two segments to the prostaglandin was

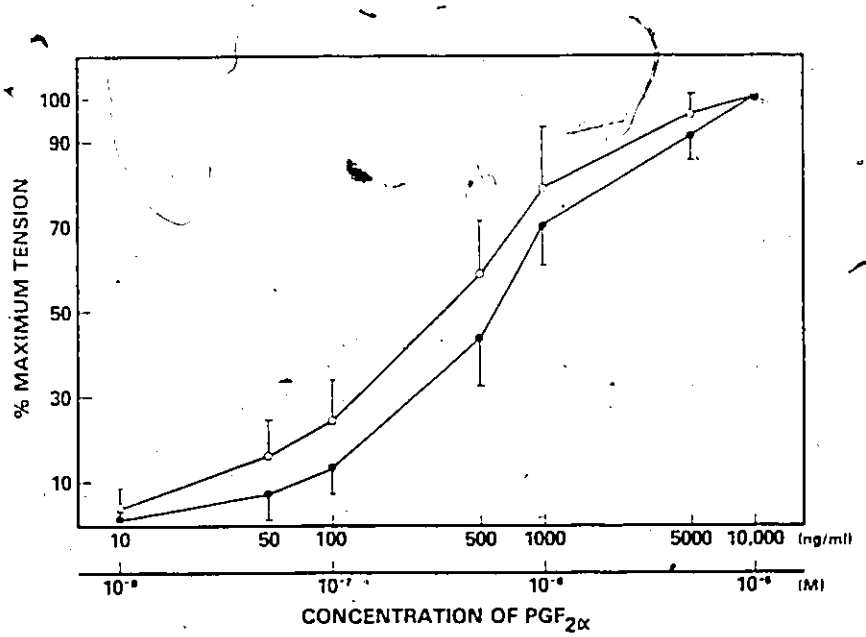


Fig 2-12. Mean responses (± 1.S.D.) of proximal (○) and distal (●) segments of rabbit IPA to $PGF_{2\alpha}$. (n = 4)

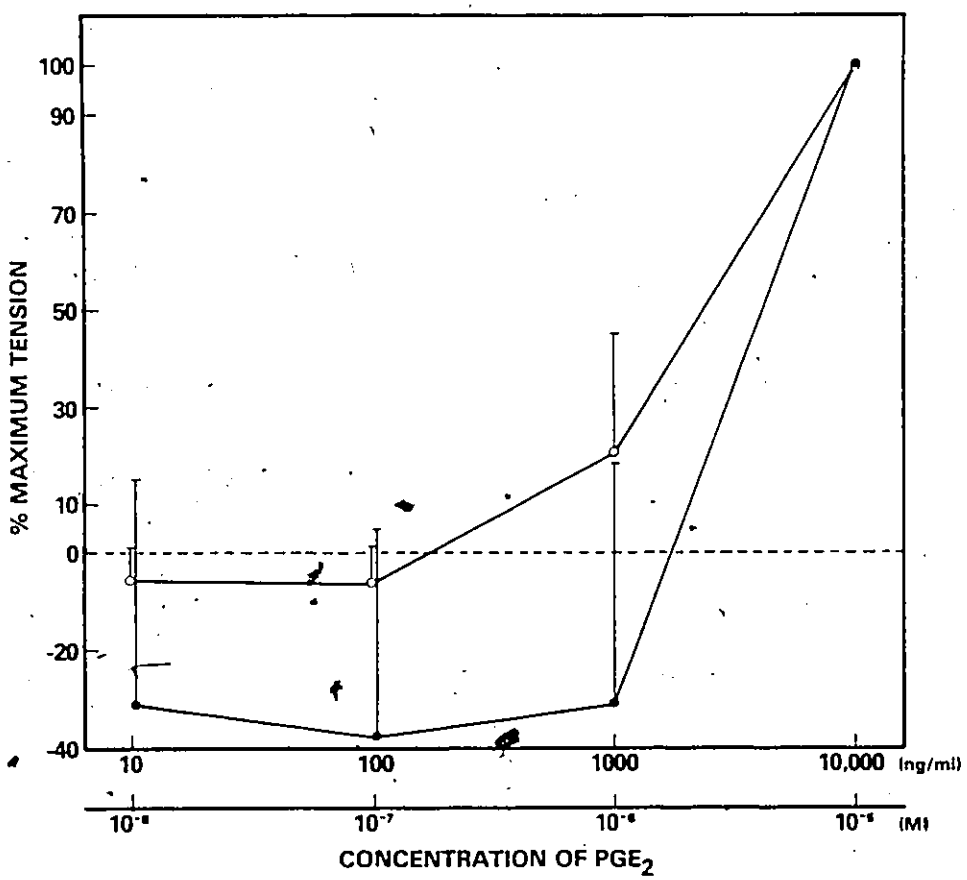


Fig. 2-13. Mean responses (± 1.S.D.) of proximal (○) and distal (●) segments of rabbit IPA to PGE_2. (n = 4)

similar. The difference between proximal and distal segment mean responses was significant only at a concentration of 100 ng/ml.

The effect of of PGE_2 is illustrated in fig. 2-13. PGE_2 elicited relaxant responses in both strips at concentrations less than 10^{-6}M, however, the mean response to 10^{-6}M was relaxant in distal but contractile in proximal segments. At 10^{-5}M both strips contracted to PGE_2. The differences in mean responses, relative to maximum tension, between proximal and distal segments were not significant. Fig. 2-14 illustrates the proximal and distal responses in PGE_2 in mg of tension. It can be seen that relaxant responses were small and of similar magnitude in the two segments. However, proximal contractile responses to PGE_2 at high doses in general exceeded distal responses (thus accounting for the more pronounced distal relaxant activity seen in the log concentration-percent maximum tension graph). In the three experiments in which both prostaglandins were applied to the intrapulmonary arterial segments, the magnitude of maximal $PGF_{2\alpha}$ responses exceeded maximal PGE_2 responses.

Proximal and distal segment responses to a number of prostaglandins including $PGF_{2\alpha}$ are illustrated in fig. 2-15.

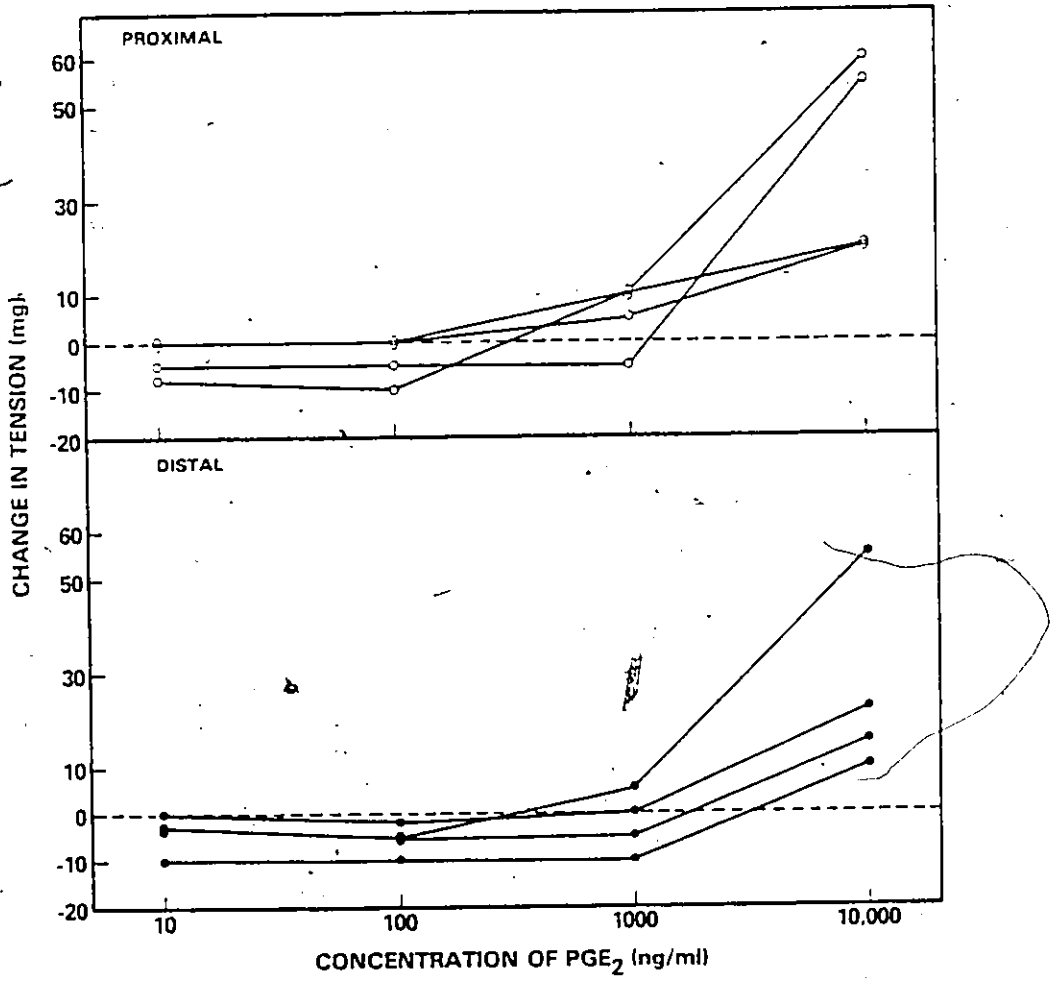


Fig. 2-14. Proximal and distal rabbit intrapulmonary arterial segment responses to PGE_2. The results from 4 experiments are shown.

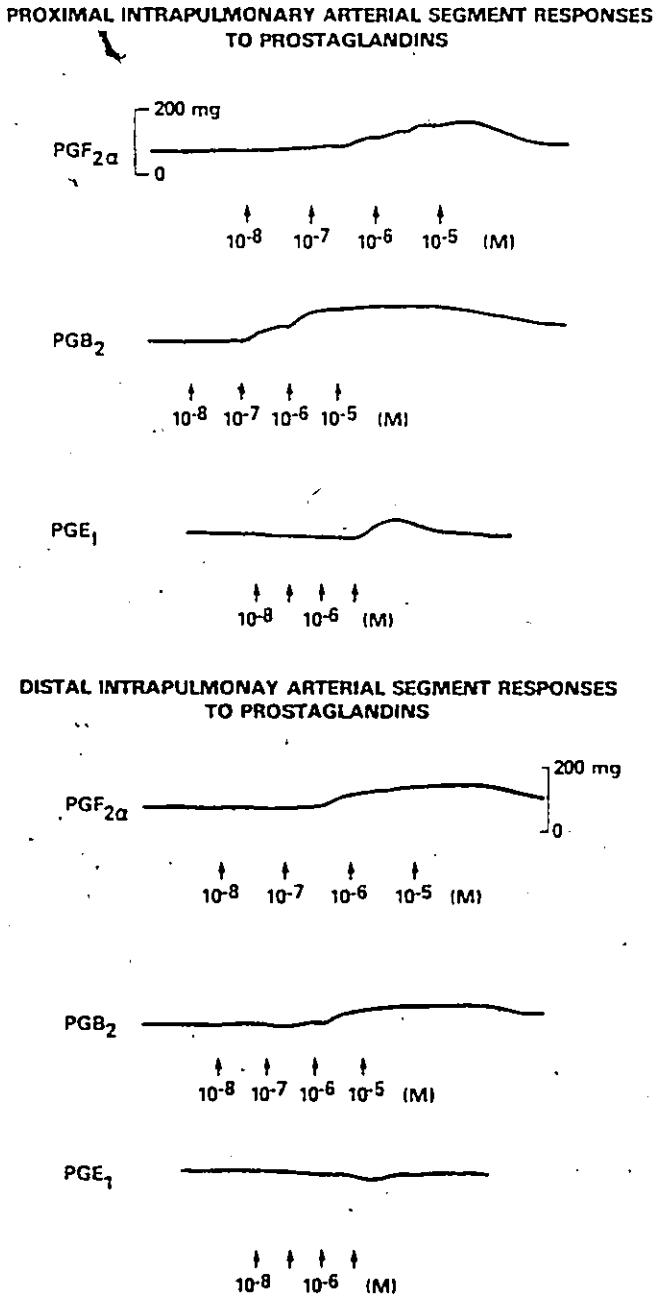


Fig. 2-15. Physiograph tracings illustrating responses of rabbit intrapulmonary artery to prostaglandins $F_{2\alpha}$, and B_2 and E_1.

CHAPTER 4

Group 2 - Prostaglandin Dose-Response Trials

In this second series of experiments the effects of prostaglandins A_1, B_1, B_2 and E_1 on isometric tension of proximal and distal segments were examined following a reference response to K^+ 123mM in PSS. Owing to a limited supply of prostaglandins, only partial dose response curves (4 points) were obtained. Responses to 10^{-5}M concentration of prostaglandins were considered maximal despite a lack of confirmatory evidence. Baseline tensions were initially set at about 300 mg in contrast to the wider range of tensions (250 - 350) employed in the Group 1 experiments. Ethanol, in concentrations used to prepare prostaglandins failed to elicit responses.

While proximal segments contracted in response to K^+, distal segment responses were frequently biphasic. Tissues responding to K^+ in such a fashion exhibited poor responses to prostaglandins, i.e. small and highly variable. Thus, accurate interpretation of the distal prostaglandin responses was not possible. Consequently distal segment responses were not quanitatively analysed but rather a brief qualitative description is given. In general, responses to prostaglandins were seen to develop more slowly than amine responses and thus a three minute time of exposure to each PG dose was used.

Proximal Segment Responses

PGA_1

The effect of PGA_1 on proximal segments is illustrated in fig. 2-16 and 2-17. At doses less than 10^{-6}M, small contractions, very slight relaxant activity or no responses were observed, while the response to 10^{-6} PGA_1 was predominantly contractile. At 10^{-5}M, PGA_1 produced marked contractile activity comparable to the response to K^+ resulting in a rapid and large generation of tension which continued for one to two minutes following withdrawal of the prostaglandin from the superfusing medium. (A rigid time cycle of 3 minutes per PG dose had been maintained.) Consequently, maximal tension was found following drug perfusion.

Prostaglindins B_1 and B_2

Prostaglandins of the B series elicited graded contractile activity (see fig. 2-18). A tracing of the physiograph recording of the response to PGB_2 is provided in fig. 2-15. The threshold for response to both prostaglandins was about 10^{-8}M with maximal responses at 10^{-5}M. The mean responses to PGB_2 exceeded those to PGB_1 and relative to maximum tension, the diferences in responses were significant at 10^{-7} and 10^{-6}M. Relative to the reference response (see fig. 2-19) responses to PGB_2 were greater than those to PGB_1, however, with the exception of the response to 10^{-7}M PG, the

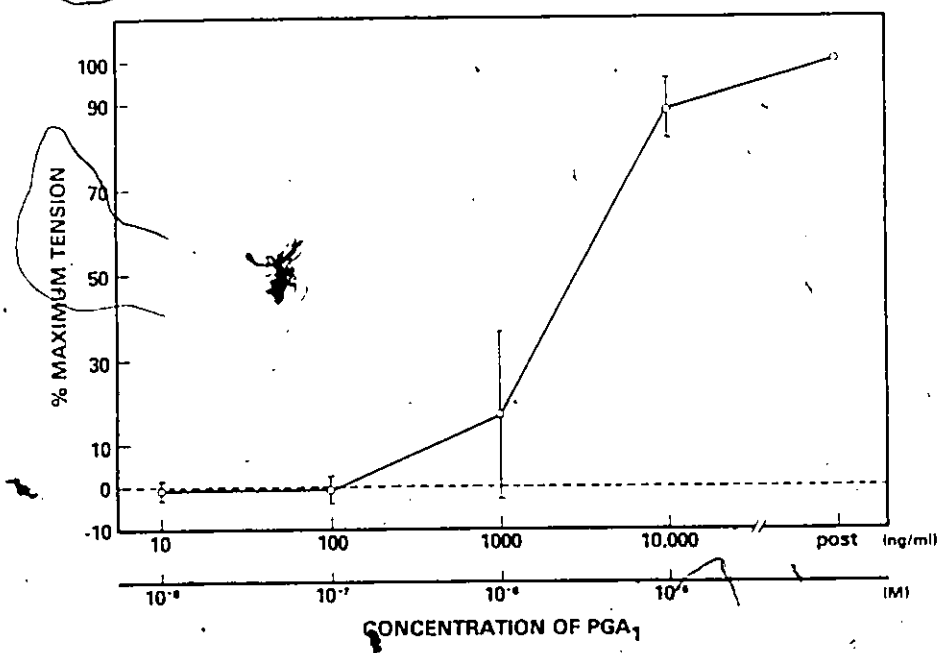


Fig 2-16. Mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to PGA_1 (n = 7). The response determined following withdrawal of PGA_1 from the superfusate is designated as "post".

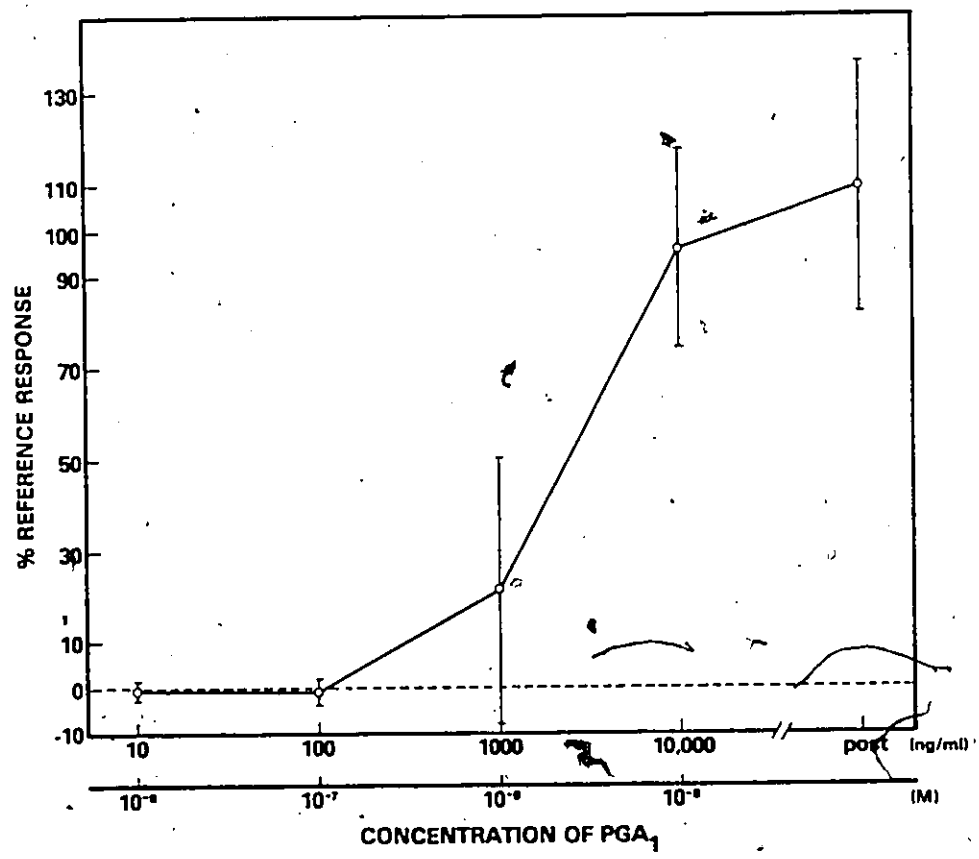


Fig. 2-17. Mean responses (± 1 S.D.) of the proximal segment of rabbit IPA to PGA_1 (n = 7). See fig. 2-16 for explanation of "post" response.

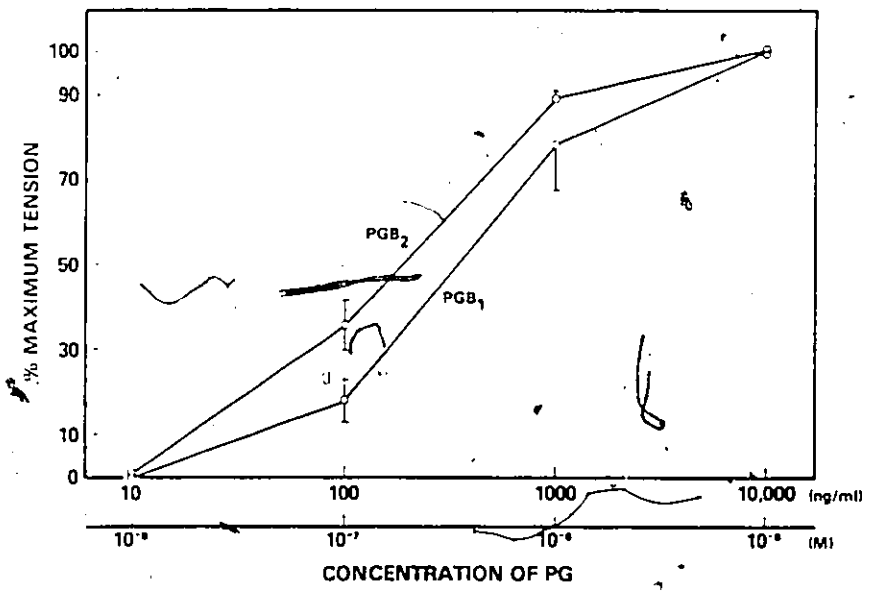


Fig 2-18. Mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to prostaglandins B_1 and B_2. (n = 5).

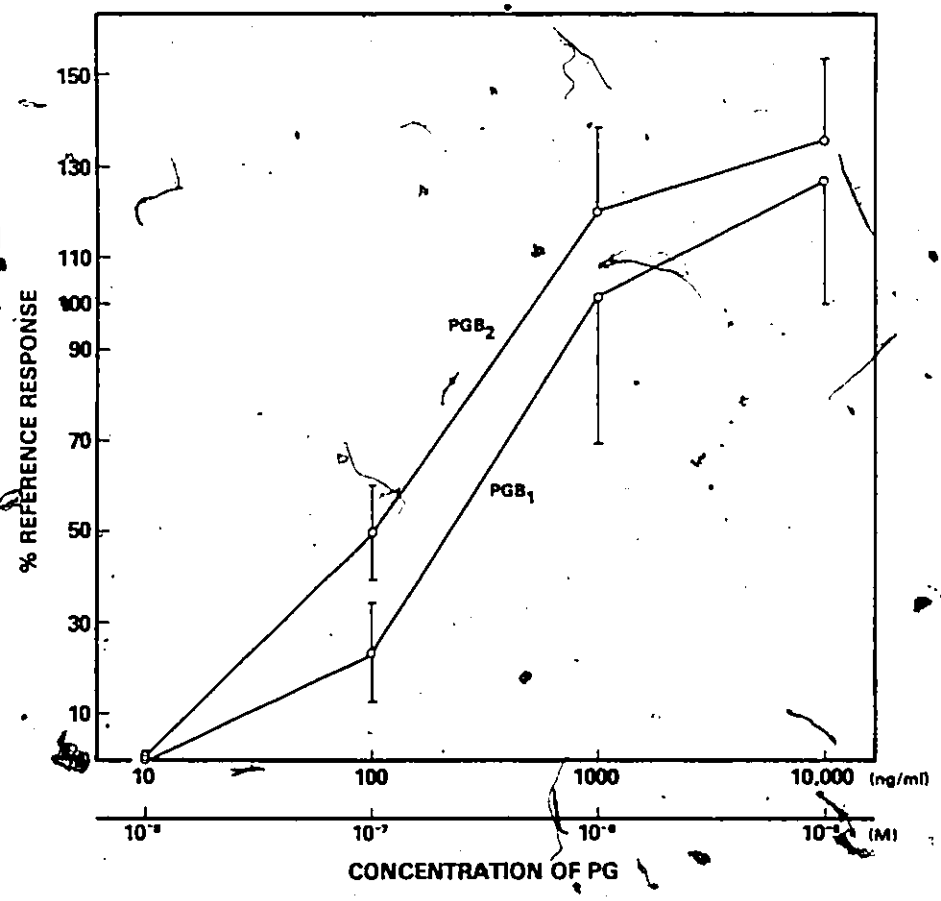


Fig. 2-19. Mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to prostaglandins B_1 and B_2. (n = 5).

differences were not significant. The mean maximal response to both prostaglandins was greater than the reference response to K^+.

PGE_1

A tracing of the physiograph recording of the response to PGE_1 is illustrated in fig. 2-15. PGE_1 produced either small contractile responses, slight relaxant responses or no response at all at concentrations less than or equal to 10^{-6}M, (see figures 2-20 and 2-21). At 10^{-5}M, moderate contractile activity to PGE_1 was obtained, the maximal response having been about 40% of the reference response.

Summary of PG Responses

Figures 2-22 and 2-23 summarize intrapulmonary arterial segment responses to prostaglandins. All prostaglandins tested caused contraction of PIPA segments at high concentrations. While both PGA_1 and PGE_1 produced small or no contractile responses or slight relaxant activity at doses less than or equal to 10^{-6}M, the B series of prostaglandins produced considerable contractile activity. Although PGA_1 produced maximal contractile effects comparable to but less than the maximal responses to prostaglandins of the B series, PGE_1 produced only moderate contractile activity of less than half the magnitude of other prostaglandin responses. The responses of the B series of prostaglandins were similar to the responses to $PGF_{2\alpha}$, while the response to PGE_1 most closely resembled the response to PGE_2.

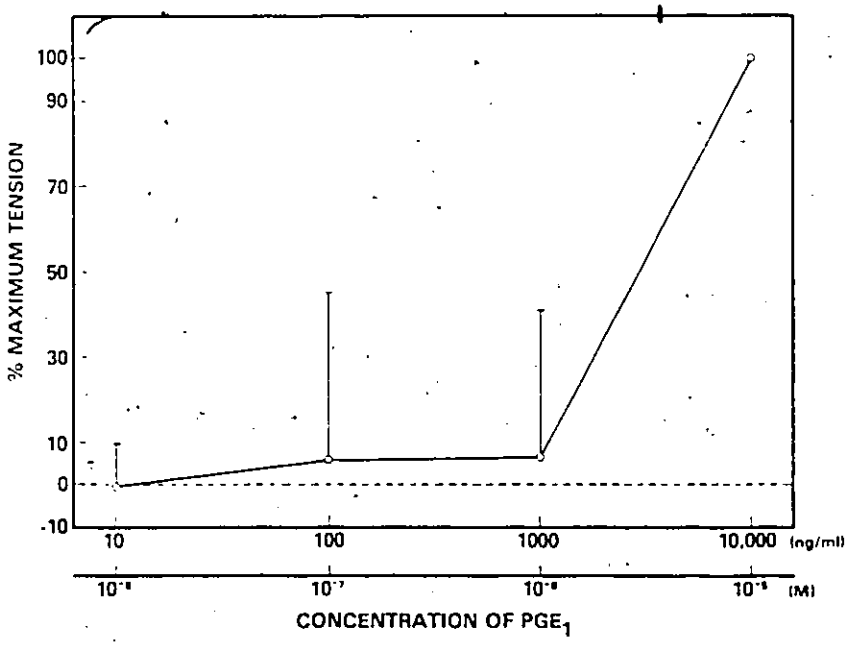


Fig 2-20. Mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to PGE_1. (n = 7).

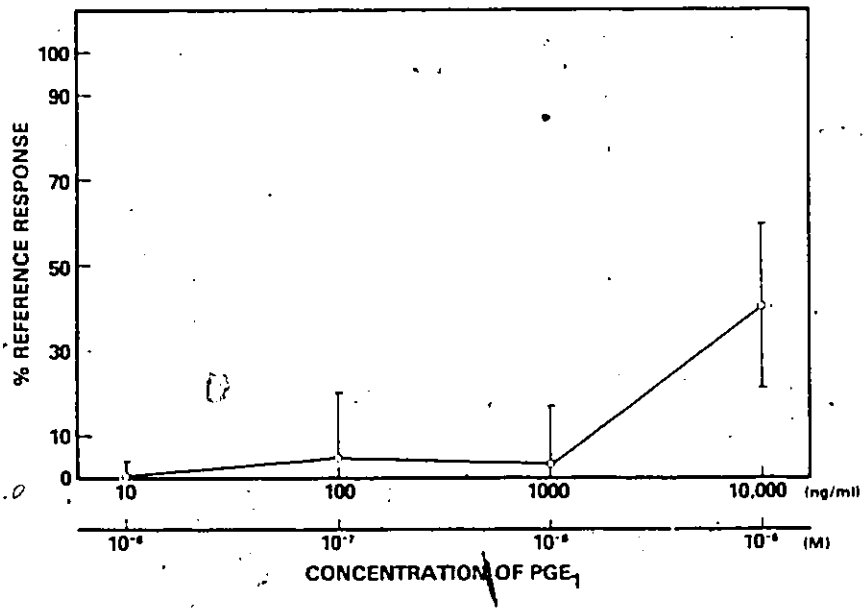


Fig. 2-21. Mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to PGE[illegible]. (n = 5).

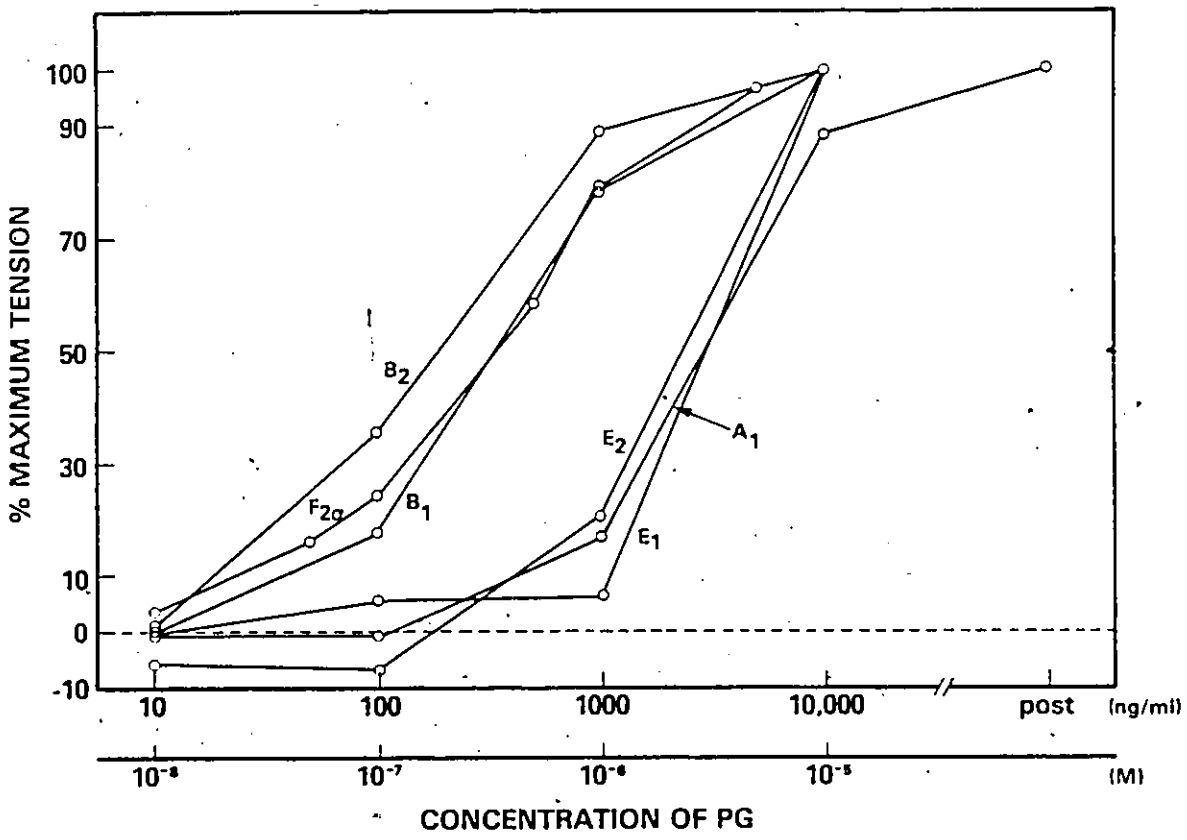


Fig 2-22. Summary of mean responses of the proximal segment of rabbit intrapulmonary artery to prostaglandins A_1 (n = 7), B_1 (n = 5), B_2 (n = 5), E_1 (n = 5), E_2 (n = 4) and $F_{2\alpha}$ (n = 4). Error bars have been omitted for the sake of clarity.

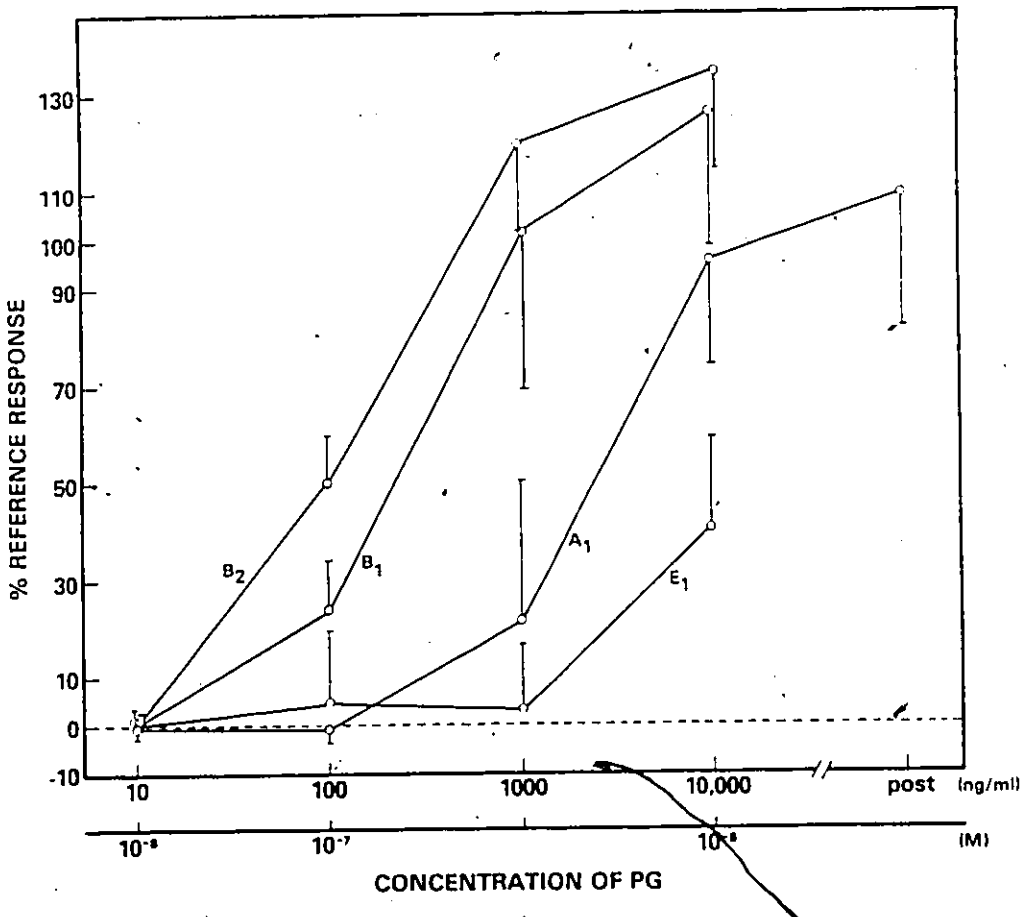


Fig 2-23. Summary of mean responses (± 1 S.D.) of the proximal segment of rabbit intrapulmonary artery to prostaglandins A_1 (n = 7), B_1 (n = 5), B_2 (n = 5) and E_1 (n = 5).

Distal Segment Responses

Distal segments appeared to respond to increasing doses of PGA_1, B_1 and B_2 with contractile activity. The distal response to PGB_2, illustrated in fig. 2-15, was typical of the activity of these prostaglandins. However, concentrations of less than 10^{-5}M of all prostaglandins tested often elicited either no response or slight and highly variable responses i.e. contractions, relaxations or biphasic responses. While in most instances 10^{-5}M concentrations of PGA_1, B_1 and B_2 elicited contractile responses, 10^{-5}M PGE_1 produced either more pronounced relaxations (as shown in fig. 2-15) in two of five experiments or slight biphasic activity, predominated by contraction. Owing to the frequent occurrence of biphasic reference responses, these interpretations of distal segment prostaglandin responses must be cautiously regarded.

Poorly Responsive Arterial Segments

In 3 of 10 PGA_1 trials, 1 of 6 PGB trials and 4 of 9 PGE_1 trials representing 6 separate experiments the proximal segment responses to the respective PGs were negligible despite good responses to K^+ (and amines when tested). In the case of the PGA_1 trials, the maximal response ranged from 0 to 11% of the reference response, while with respect to PGE_1 the maximal tensions elicited ranged from - 4.62 to 6.25% of the K^+ response. Since these data were uncharacteristic of the other experimental results they were considered separately and not included in the determination of mean responses. It

would appear that some rabbit intrapulmonary arterial segments may be characterized as poorly responsive to prostaglandins.

Relaxant Effects of Prostaglandins

Prostaglandins A_1, E_1 and E_2 all produced some relaxant effects in proximal segments. However, the magnitude of these relaxant responses was small and occasionally no relaxant responses were obtained. The application of sodium nitroprusside (10^{-5}M) on a single occasion produced relaxation of only 8 and 10 mg in proximal and distal segments. The inability of these tissues to produce more pronounced relaxant effects suggests that they possess little intrinsic muscle tone. In order to confirm that prostaglandins were indeed capable of producing relaxation of strips, PGE_2, which had produced the most pronounced relaxant responses at low doses, was applied to a proximal and distal segment, in the presence of $PGF_{2\alpha}$ 10^{-6}M, which had been used to submaximally contract the tissues. The results of this experiment are illustrated in fig. 2-24. Control responses in both segments were relaxant (to 10 mg) at doses less than 10^{-5}M and contractile at 10^{-5}M. With the addition of PGE_2, 10^{-8}M, the contractile response of $PGF_{2\alpha}$ was abruptly inhibited. The relaxant response to 10^{-8}M PGE_2 was maximal in the proximal strip and near maximal in the distal segment. The magnitude of this relaxation was greater than in the control response. The relaxant effect was maintained until the introduction of 10^{-5}M PGE_2 which produced a contractile effect that was more pronounced in proximal segments.

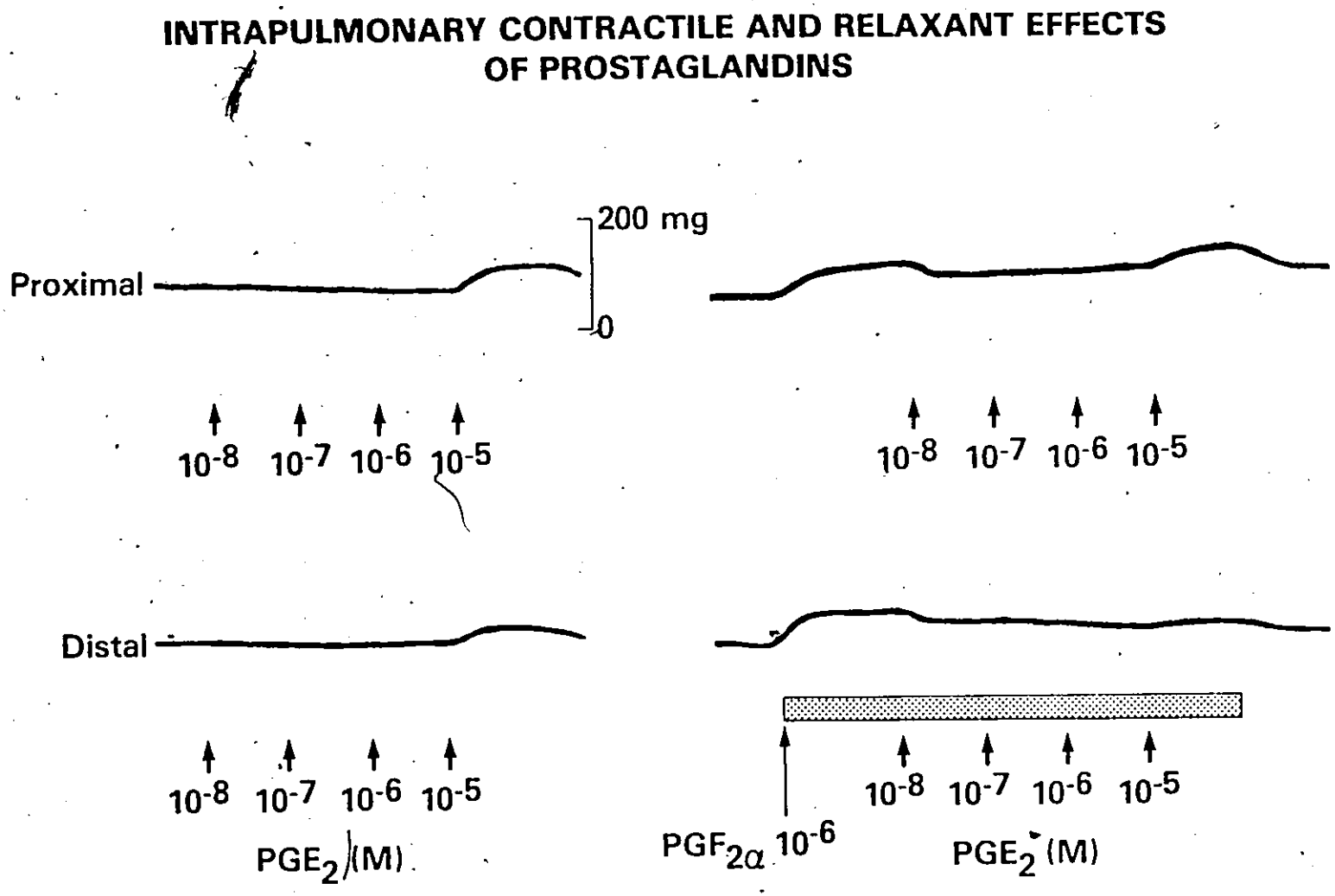


Fig. 2-24. Physiograph tracing illustrating control responses of rabbit intrapulmonary artery to PGE_2 and the responses of the contracted artery to PGE_2.

CHAPTER 5

Lenth-Tension and Smooth Muscle Fixation Experiments

Biphasic Response

Numerous trials with HIS, K^+ and infrequently 5HT have demonstrated the ability of the distal strip to respond with either contractile or so called biphasic responses. Even the proximal strip responded on two occasions in a biphasic manner, although the decline in tension was very small. The ability to respond in such a fashion appeared to be a characteristic of some tissue strips rather than specifically related to particular agonists. The unusual nature of the response and its occasional appearance prompted closer examination.

Following a number of amine and prostaglandin experiments, tissues were exposed to K^+ and unsuccessful attempts were made to block the relaxant phase of the biphasic response with propranolol and atropine. A single repetition of the biphasic response failed to alter the nature of the response, although, on four of twenty occasions, in which multiple responses to K^+ were examined, the biphasic responses initially produced were found to be changed to contractile responses with repetitive exposures. However, in a single experiment following a number of biphasic responses to K^+, baseline tension was increased to 1000 mg and a large, solely contractile response to K^+ was obtained.

Consequently the effect of baseline tension on the response to agonists (K^+ and HIS) was examined in a series of length-tension experiments.

Length-tension Experiments

In light of the change of the biphasic response with elevated baseline tension and in order to characterize some of the contractile properties of the pulmonary vascular smooth muscle, four length tension experiments were conducted. All distal segments responded to the reference response in a biphasic manner while proximal strips contracted well. When reduced to the minimal length permitting adequate superfusion, baseline tensions were about 100 mg or less. Upon stimulation at low baseline tensions, it was observed that the tissues often responded by curling on themselves which suggested that at low baseline tensions the orientation of the smooth muscle was not parallel to the long axis of the strip but rather helical.

All of the distal segments responded to agonist stimulation, at minimal lengths, in a biphasic manner. The nature of the change in the distal segment biphasic response associated with increments in length is illustrated in fig. 2-25. In general, the distal segment responded to increments in length with an increase in passive tension and both a decrease in the magnitude of the abrupt decline in tension (relaxant phase) and an increase in the contractile component of the biphasic response. With several increments of length the response became solely contractile.

At the minimal length in proximal segments, two strips contracted while the two other strips responded in a biphasic manner.

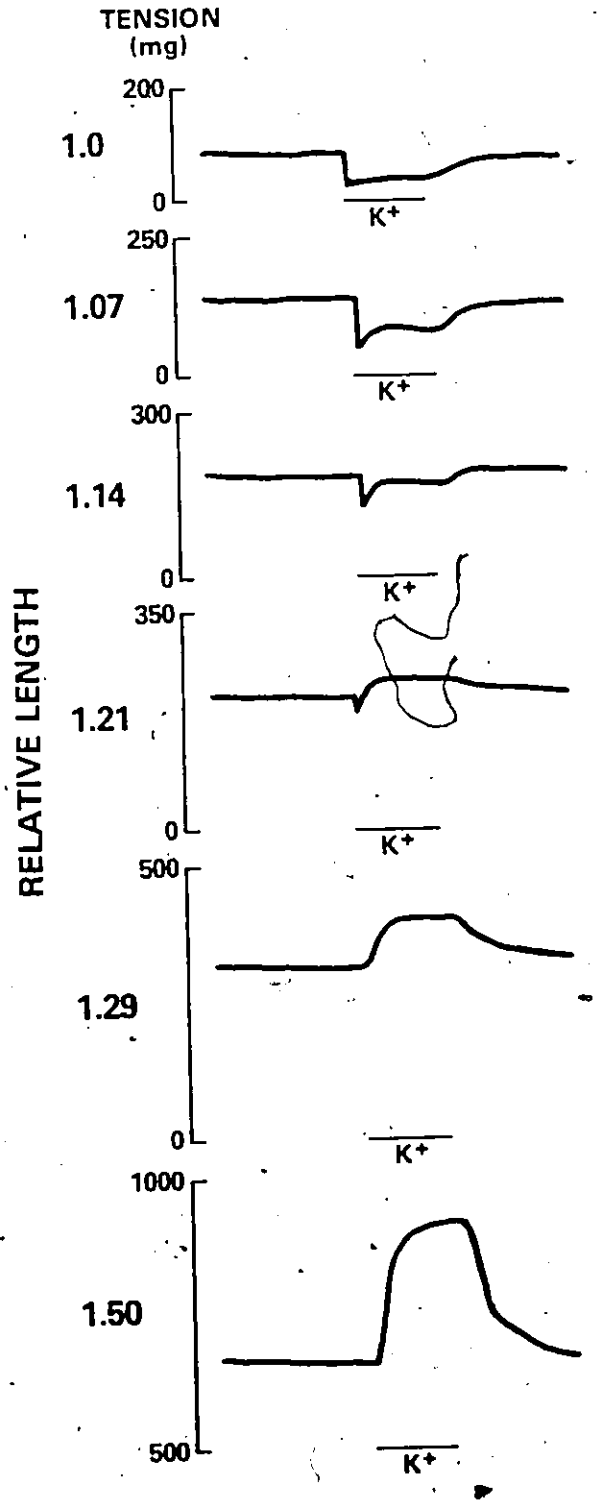


Fig. 2-25. The changes in the response of the distal segment to high K^+ associated with increments in strip length (and hence passive or baseline tension).

However, the magnitude of the decline in tension was small, only 10 mg. Further stimulation, following increments in length, contracted all segments, the magnitude of the response being increased with each trial.

Figures 2-26 and 2-27 illustrate changes in passive (developed in response to stretch) and active (developed in response to a stimulus) tension that accompanied increments in the length of a proximal and a distal segment. The passive tension of the proximal segment increased in a linear fashion with increments in tissue length. Although the changes in passive tension were similar in both segments at smaller lengths, the passive tension of the distal strip rose sharply, in an exponential manner at greater lengths. Both segments responded to increments in length with increased active tension that reached a maximum and subsequently declined. No attempt was made to continue experiments to ensure that maximal responses, rather than a plateau of responses, had been achieved in order to avoid tissue strain and tearing with possible damage to force transducers. The change in active tension per increment of length was much greater in distal segments.

Fig. 2-28 represents the composite data from the four experiments and illustrates the relationship between length and tensions in proximal and distal segments. Tension development in all strips, both passive and active, was a function of length. The development of passive tension in proximal segments appeared to be linearly related to length while the changes in passive tension with length in the distal segment progressively increased. Active tension

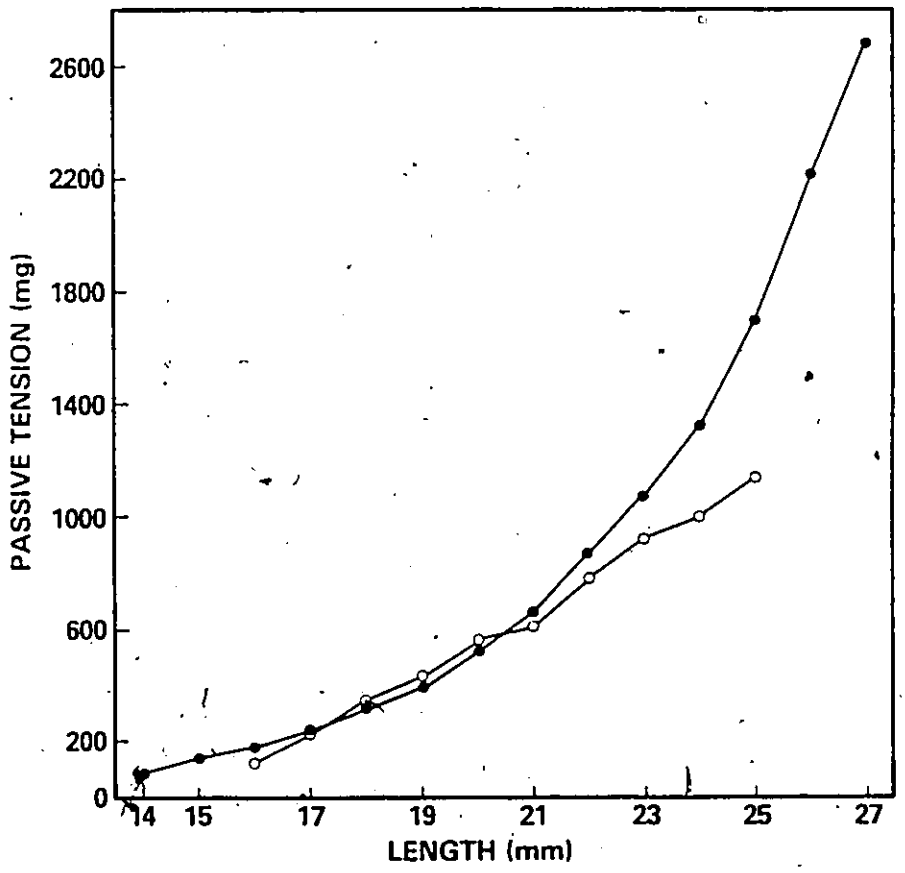


Fig 2-26. Passive isometric tension - length relationship for a proximal (O) and distal (●) segment of rabbit IPA.

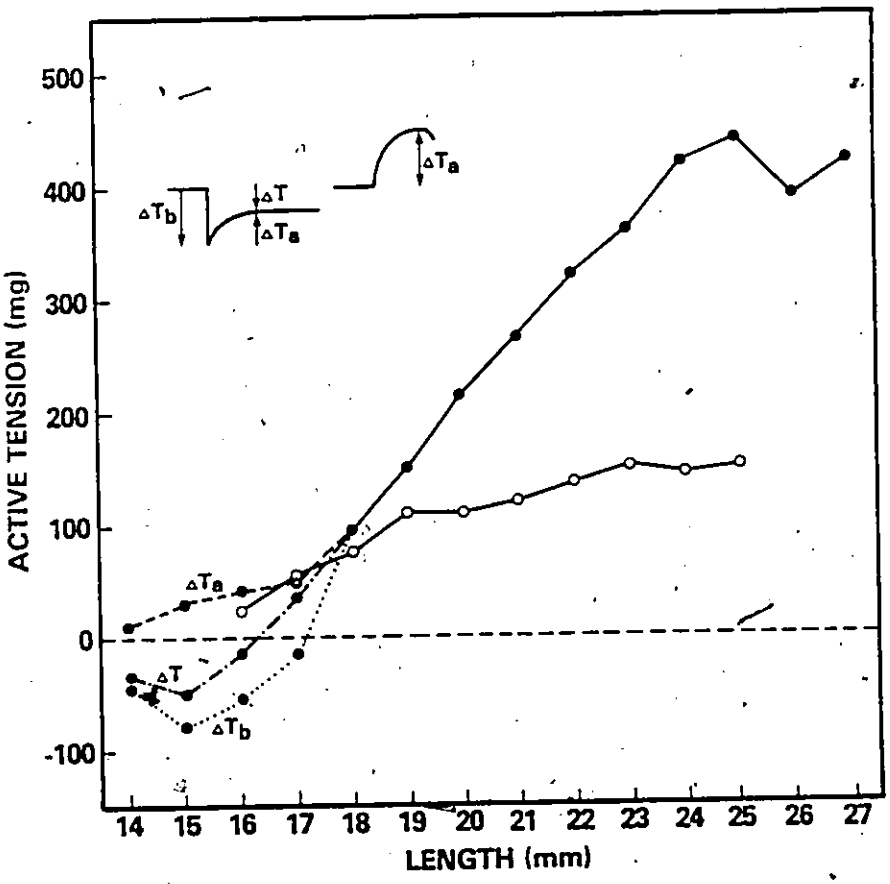
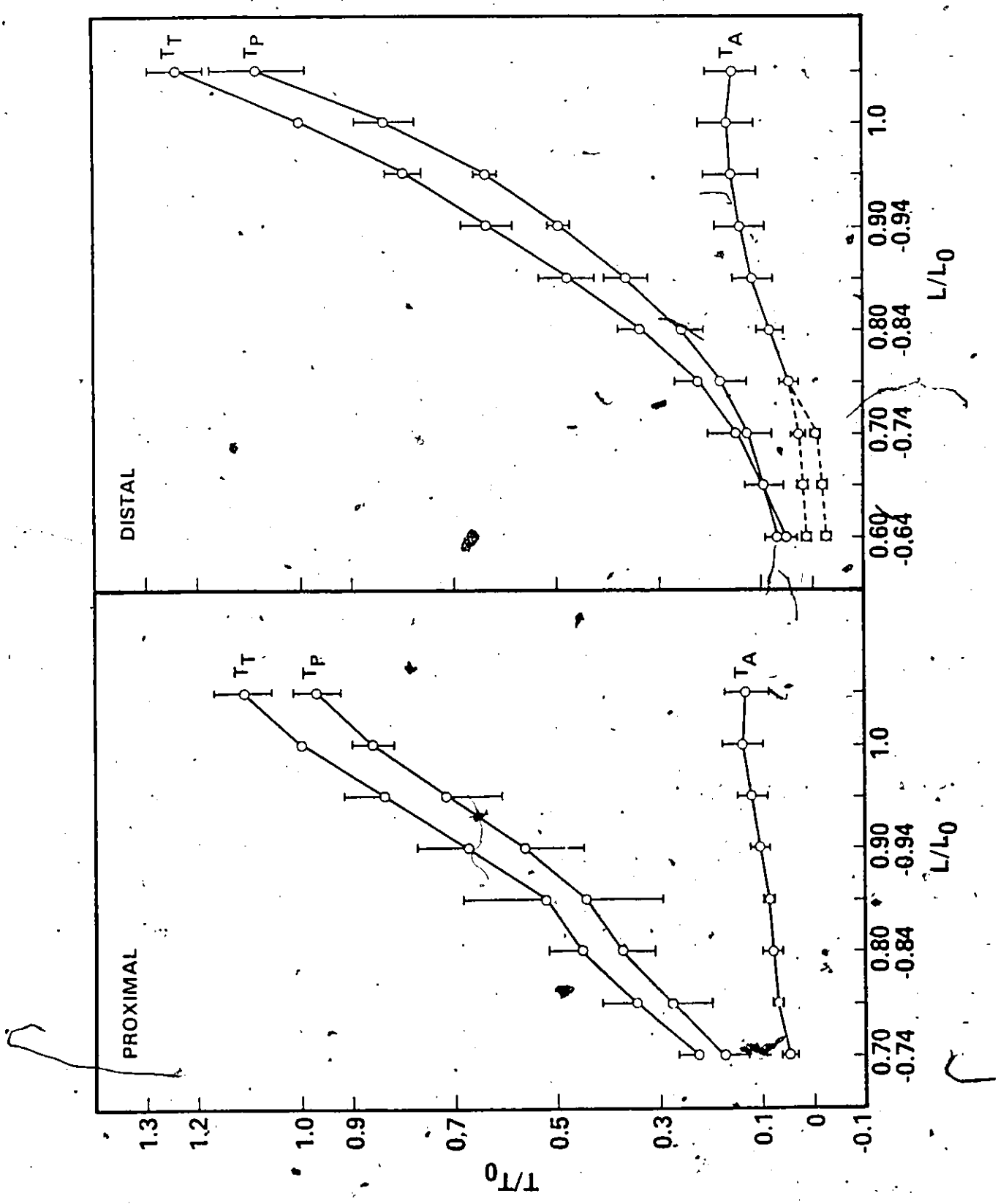


Fig 2-27. Active isometric tension - length relationship for a proximal (○) and distal (●) segment of rabbit IPA. The biphasic response of the distal segment is plotted as T_b referring to the magnitude of the decline in baseline tension (relaxant phase), T_a referring to the active tension generated (contractile phase) and T referring to the absolute change in tension relative to the initial baseline tension.

Fig 2-28. Overleaf: Length-tension relationship for proximal and distal segments of pulmonary arteries (n = 4). Length and tension values have been normalized as a fraction of optimal length (L_0) at which maximal tension (T_0) was developed. To accommodate comparison of responses, data has been grouped according to length and mean tension values (± 1 S.D.) determined. T_T-total tension. T_p-passive tension. T_A-active tension. This method of analysis is based upon a similar graph constructed by Herlihy and Murphy, 1973.


PROXIMAL
DISTAL
T_T
T_P
T_A
T/T0
1.3
1.2
1.0
0.9
0.7
0.5
0.3
0.1
0
-0.1
0.70
-0.74
0.80
-0.84
0.90
-0.94
1.0
L/L0
0.60
-0.64

increased with elongation of both segments up to a maximun response (at the optimal length, L_0) and then subsequently declined with a further increase in length. The magnitude of active tension, relative to passive tension was small.

The maximum active tension in kg per sq cm cross section at length L_0 was calculated by dividing the maximum active tension by the cross sectional area (see Methods). The mean tension development was 0.035 ± 0.015 kg/cm^2, n = 3, ($3.5 \times 10^4 \pm 1.5$ dynes/cm^2) for proximal segments and 0.17 ± 0.11 kg/cm^2, n = 3, ($17 \times 10^4 \pm 11$ dynes/cm^2) for distal segments. This difference was not significant which may reflect the large variability in the distal response and the small sample size. (Data from the fourth experiment was not utilized since tissue mass was not obtained owing to equipment failure.)

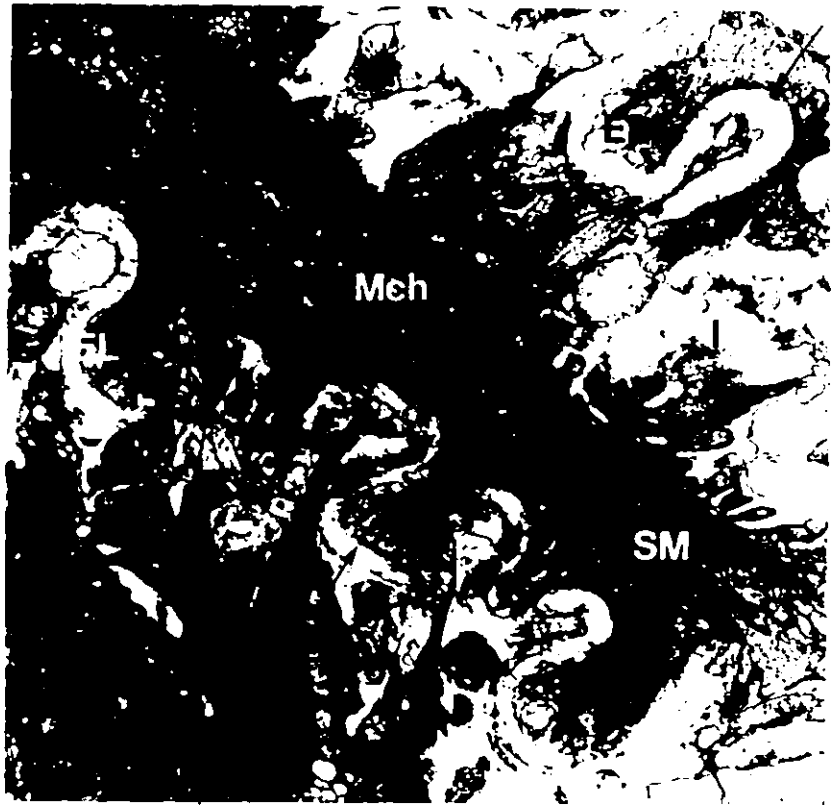
The results of this series of experiments suggested changes in the experimental protocol. Since biphasic responses were not obtained at passive tensions exceeding 400 to 500 mg, it was decided to re-adjust the experimental baseline tension. Passive tensions associated with maximal tension development ranged from 350 - 1200 mg in proximal segments and 1200 - 2400 mg in distal segments. However, the stretching required to produce these passive tensions was often great, particularly in distal segments occasionally requiring up to a 90% increase in length relative to the minimal or resting length. Although the absolute magnitude of the active response was increased at high passive tensions, relative to the passive tension the magnitude of the active tension actually declined. Consequently baseline tensions in both segments were henceforth initially set at 500 mg.

Smooth Muscle Analysis

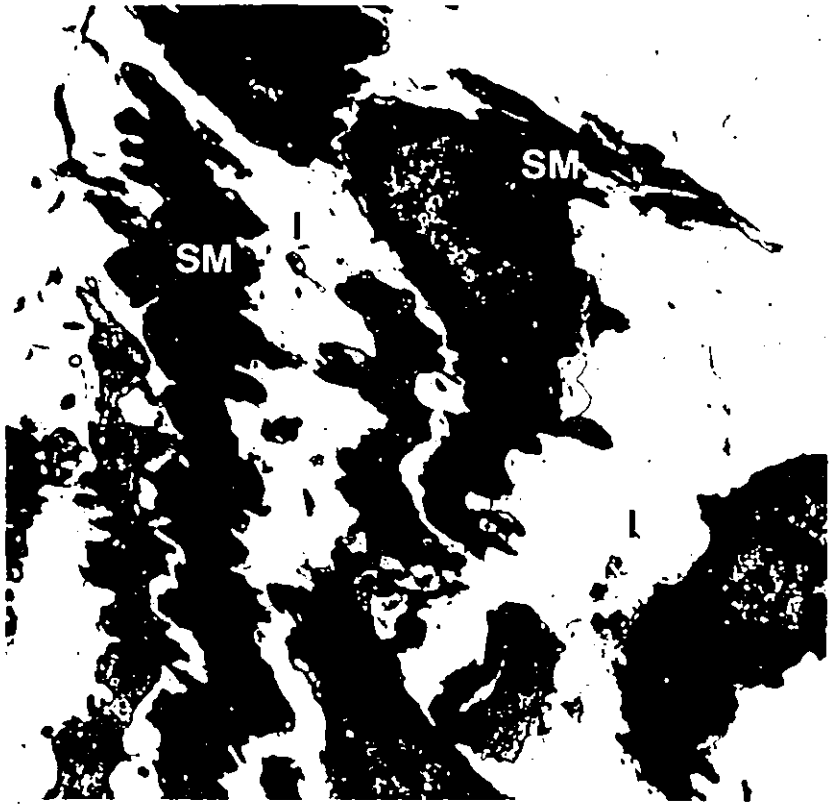
A histological analysis of the smooth muscle content and orientation of both proximal and distal arterial segments was undertaken to determine structural differences. Both arterial segments were fixed while contracted. Electron micrographs of cross sectional areas of a proximal and distal segment are shown in fig. 2-29. In the proximal segment parallel elastic laminae were present. In addition to the internal and external elastic laminae as many as 14 layers of elastin were visible in the vessel wall of the proximal segment with smooth muscle cells well interdigitated into the folded bands of elastin. The orientation of the smooth muscle cells was helical. The distal segment, on the other hand, had both an internal and external elastic lamina but only 4-6 layers of elastin in the media which consisted of loosely arranged smooth muscle cells. The orientation of fibres was predominantly helical, however, there were indications of some longitudinal smooth muscle cells near the adventitial surface. Further investigation of this finding has been undertaken by other investigators from this laboratory. The appearance of the proximal segment was typical of a large elastic artery while that of the distal segment resembled the structure of a small elastic artery beginning to undergo transition into a muscular artery.

Fig. 2-29. Overleaf. Electron micrographs of a proximal and distal segment of rabbit IPA. EL - elastin, I - Interstitium, Mch - mitochondria and SM - smooth muscle.

RABBIT INTRAPULMONARY ARTERIES



PROXIMAL SEGMENT



DISTAL SEGMENT

CHAPTER 6

Group 3 - Dose Response Trials

In this third group of experiments, the effects of 5HT, HIS and NE were re-examined and dose response curves for arachidonic acid and isoproterenol were obtained. Specific antagonists were employed (see table 1) to demonstrate pharmacological blockade and to unmask responses to agonists stimulating more than one type of receptor. Baseline tensions initially set at 500 mg, were seen to relax especially in distal segments. A reference response to K^+ was obtained at the onset of each experiment and in the event of a biphasic response, baseline tension was readjusted as outlined in the section on methods. Agonist effects have been expressed as a percent of maximum tension, relative to the reference response and, in some antagonist trials, as a percent of control responses.

5HT

The effects of 5HT on proximal and distal intrapulmonary arterial segments are illustrated in figure 3-1. Small contractile responses occurred at about 10^{-9}M 5HT and increased in a stepwise manner with increasing concentrations of the amine. Maximal effects of 5HT were obtained at a concentration of about 10^{-6}M. Log concentration-percent maximum tension curves constructed from mean proximal and distal responses are shown in figure 3-2. The distal

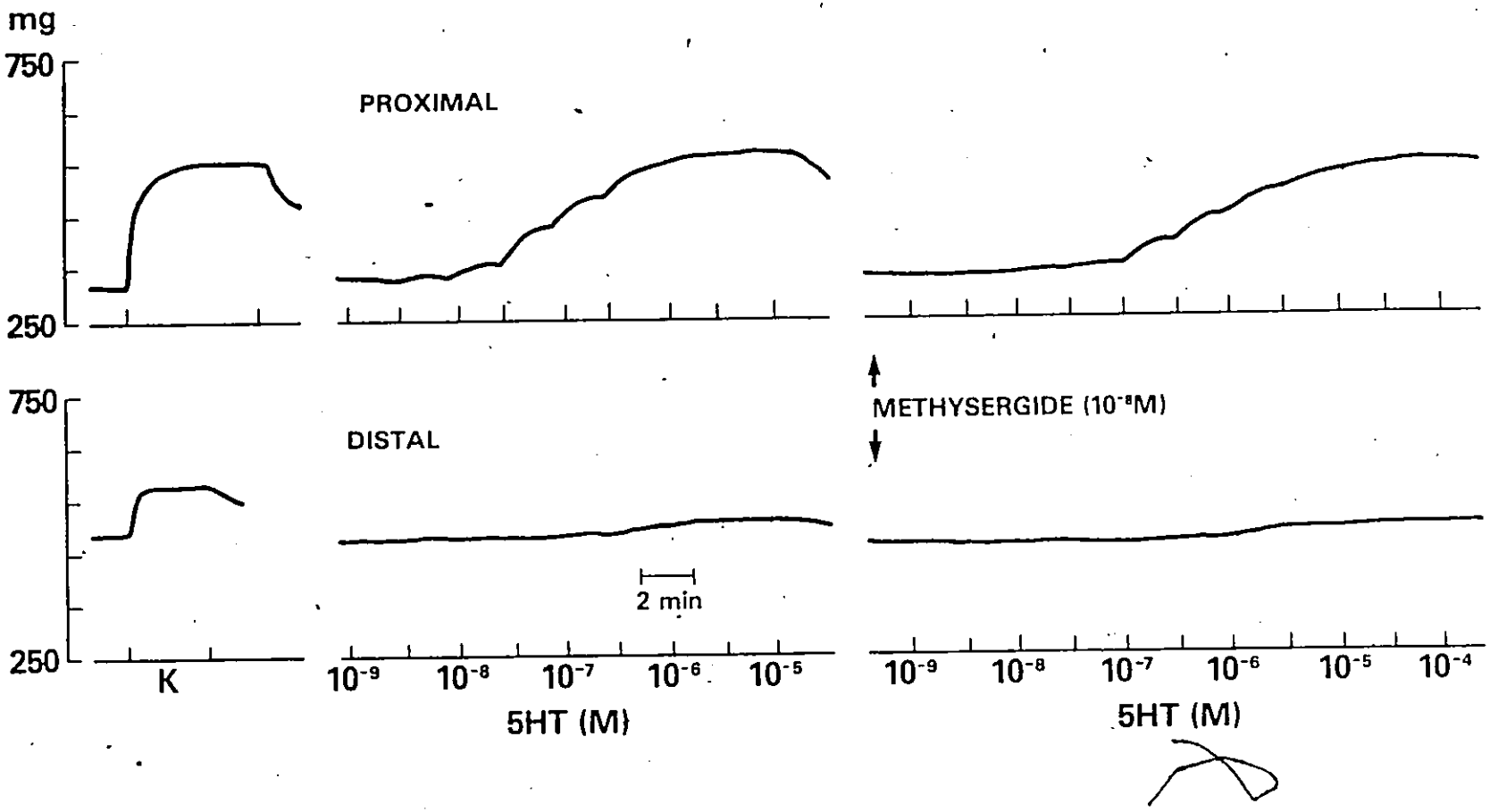


Fig. 3-1. Physiograph tracing illustrating control responses of rabbit intrapulmonary artery to 5HT and the effect of 10^{-8} M methysergide on the 5HT responses.

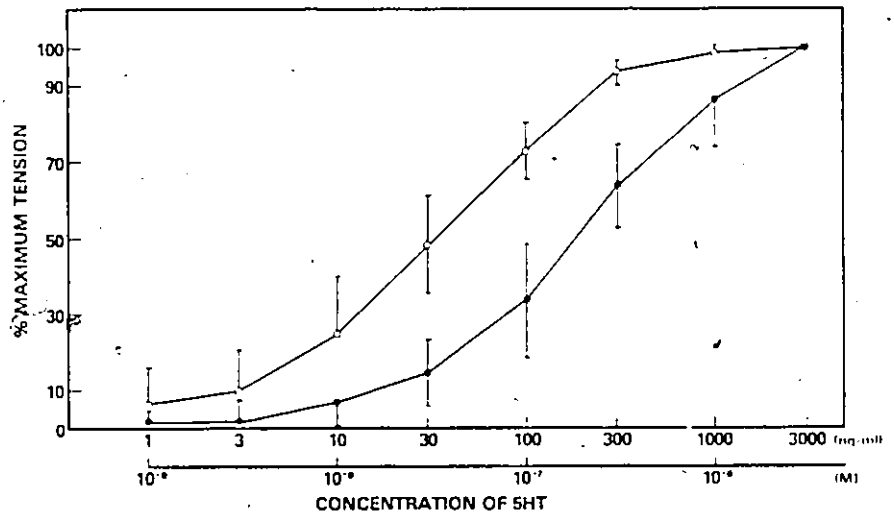


Fig 3-2. Mean responses (± 1 S.D.) of proximal (o) and distal (●) segments of rabbit IPA to 5HT. (n = 5).

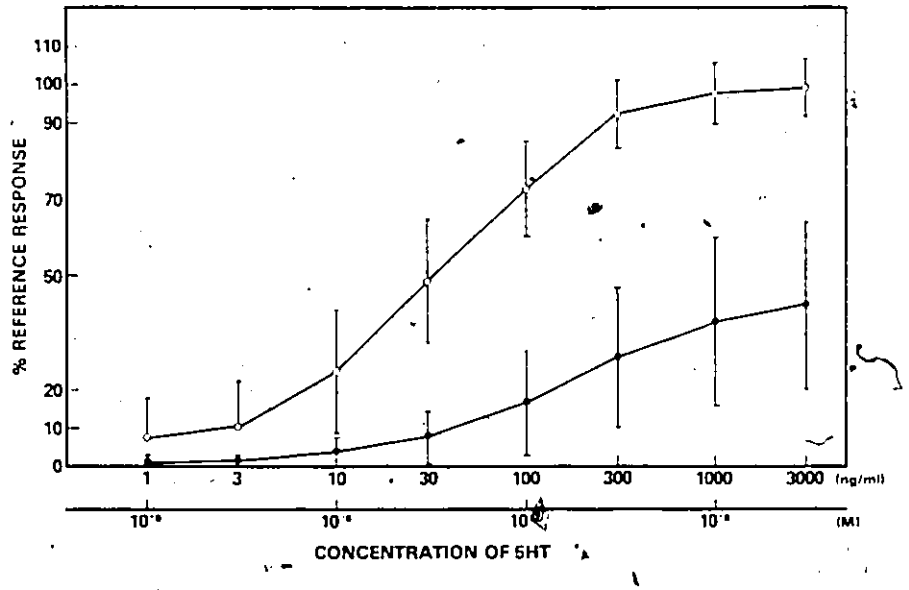


Fig. 3-3. Mean responses (± 1 S.D.) of proximal (o) and distal (●) segments of rabbit IPA to 5HT. (n = 5).

segment dose-response curve can been set to the right of the proximal curve indicating a significant difference in proximal and distal sensitivity to the amine. The ED_{50} for the distal segment ($5.22 \times 10^{-7}M \pm 3.21$) was significantly greater than that for the proximal segment ($8.93 \times 10^{-8}M \pm 4.46$). (See table 2). From figure 3.3 it is evident that relative to the reference response, distal segment responses to 5HT were significantly less than proximal responses at all doses greater than 3 ng/ml. The maximum tension produced by the distal strip in response to 5HT was about 43% of the proximal response.

Methysergide $10^{-8}M$, which had no effect on the baseline or resting tension, antagonized contractile responses to 5HT ($n = 4$) in both proximal and distal segments (see fig. 3-1), which produced a shift to the right (relative to the control) of the 5HT dose response curves as seen in figures 3-4 and 3-5. Proximal and distal ED_{50} values for 5HT in the presence of methysergide were significantly greater than their respective control values (see table 3). In the presence of methysergide, the maximum tension produced in response to 5HT, relative to the reference response, was reduced significantly when compared to the control maximum response in proximal segments. In distal segments, although the maximum response was reduced in the presence of methysergide, the difference was not significant. In two experiments an increased concentration of methysergide, ($10^{-7}M$) was used to antagonize 5HT effects and produced an even greater inhibition of contractile responses.

TABLE 2 ED_{50} Values for Contractile Agonists

Agonist	Intrapulmonary Artery Proximal	Distal	No. of expts.
5HT	$8.93 \times 10^{-8} \pm 4.46$ *	$5.22 \times 10^{-7} \pm 3.21$	5
HIS	$5.77 \times 10^{-6} \pm 0.99$ *	$3.49 \times 10^{-6} \pm 0.82$	7
NE	$3.79 \times 10^{-8} \pm 1.5$		10
AA	$3.96 \times 10^{-8} \pm 1.21$	$2.87 \times 10^{-8} \pm 1.50$	4

*proximal-distal difference signficant ($p < 0.05$) by Student's t-test

TABLE 3 Effects of Antagonists on ED_{50} Values for Contractile Agonists

	Intrapulmonary Artery Proximal	Distal	No. of Expts.
5HT (control)	$1.03 \times 10^{-7} \pm 0.38$	$5.88 \times 10^{-7} \pm 3.29$	4
5HT + Methysergide (10^{-8}M)	$6.63 \times 10^{-7} \pm 2.85$ *	$7.41 \times 10^{-6} \pm 6.38$ *	
HIS (control)	$6.23 \times 10^{-6} \pm 1.12$	$3.70 \times 10^{-6} \pm 1.08$	4
HIS + Cimetidine (10^{-5}M)	$5.21 \times 10^{-6} \pm 1.15$	$3.78 \times 10^{-6} \pm 0.66$	
HIS + Cimetidine (10^{-5}M) + Mepyramine (10^{-9}M)	$1.65 \times 10^{-5} \pm 0.39$ *	$1.02 \times 10^{-5} \pm 0.25$ *	
NE (control)	$4.14 \times 10^{-8} \pm 1.76$		4
NE + Propranolol (10^{-7}M)	$2.14 \times 10^{-7} \pm 1.61$ *		
NE (control)	$3.82 \times 10^{-8} \pm 1.72$		3
NE + Rogitine (10^{-8}M)	$1.39 \times 10^{-7} \pm 0.51$ *		

*significantly different from control ($p < 0.05$) by Student's t-test

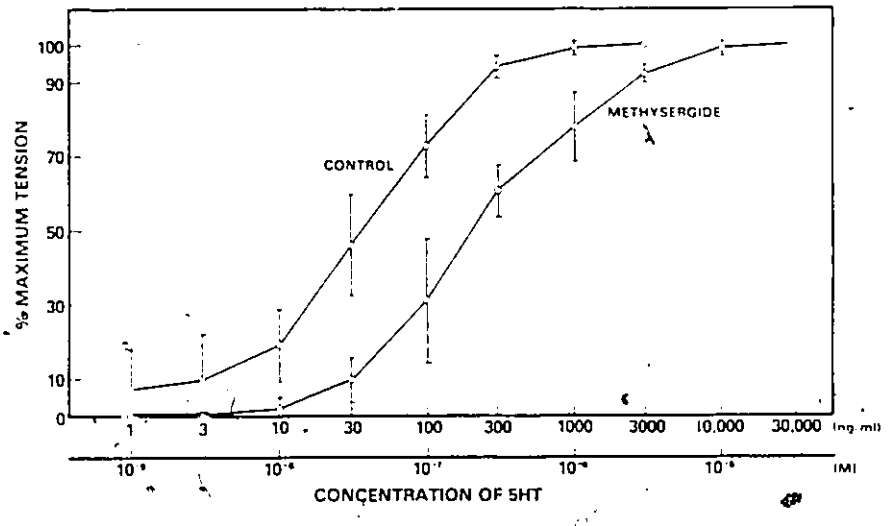


Fig 3-4. Effect of 10^{-8}M methysergide on responses to 5HT in proximal segments of rabbit IPA. (n = 4).

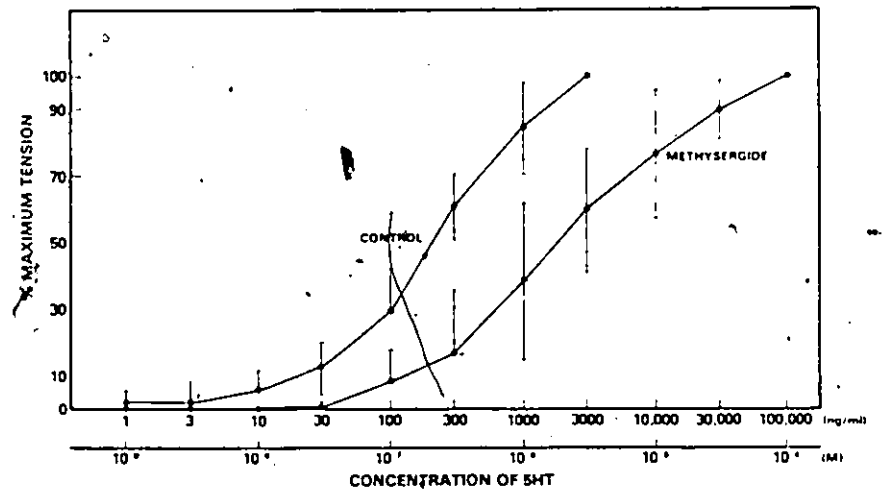


Fig. 3-5. Effect of 10^{-8}M methysergide on responses to 5HT in distal segments of rabbit IPA. (n = 4).

The specificity of action of methysergide was also studied. Partial dose response curves to HIS were obtained and then re-examined in the presence of methysergide (10^{-8}M) in two experiments. Although there was no effect on the threshold for response in either segment, methysergide did appear to depress the maximum tension elicited in proximal segments. In distal segments the maximum tension was decreased on one occasion but increased on the other.

H_1 - HIS Receptor Activity

Proximal and distal strips contracted to HIS in a dose related fashion (see fig. 3-6). The observed threshold for the HIS response was about 10^{-7}M while maximal effects were elicited at 10^{-4}M (see fig. 3-7). There was a small but significant difference in the estimated ED_{50} values, the distal strip having been more sensitive to the effects of HIS (see table 2). Relative to maximum tension, distal responses exceeded proximal segment responses at all concentrations between 30 and 30,000 ng/ml. The difference in responses was significant at doses of 300, 1000 and 3000 ng/ml. Relative to the reference response, distal responses were greater than proximal responses at all doses greater than 30 ng/ml (see fig. 3-8). However the differences were significant only at concentrations of 300 and 1000 ng/ml.

Figures 3-9 and 3-10 give the results of four experiments in which the effects of both cimetidine and cimetidine plus mepyramine on

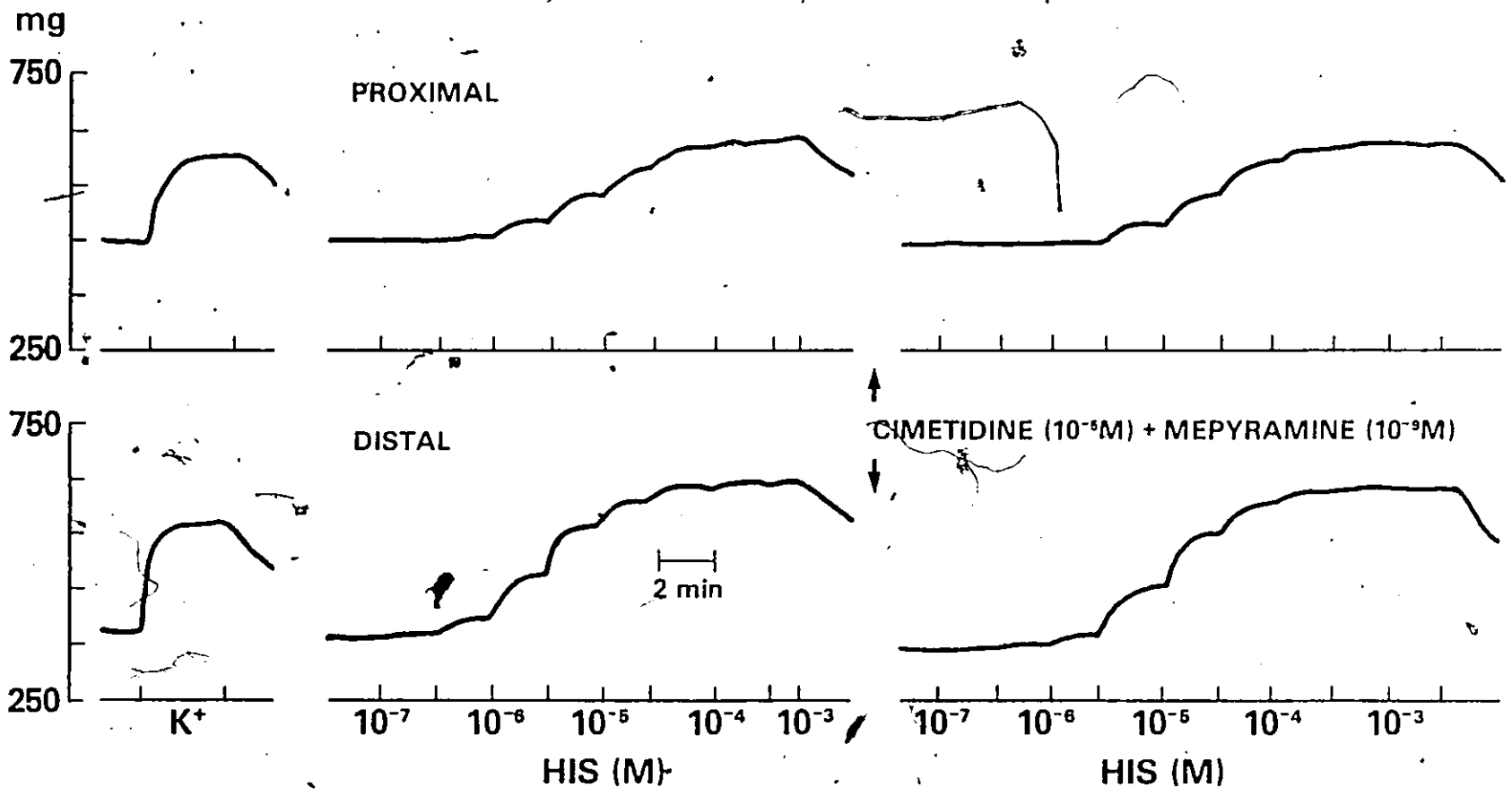


Fig. 3-6. Physiograph tracing illustrating control responses of rabbit intrapulmonary artery to HIS and the effect of 10^{-5}M cimetidine plus 10^{-9}M mepyramine on the HIS responses.

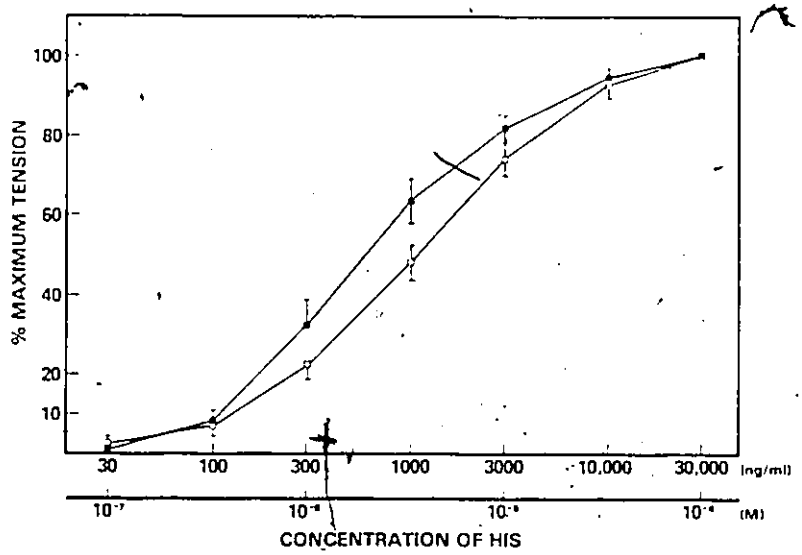


Fig 3-7. Mean response (± 1 S.D.) of proximal (□) and distal (■) segments of rabbit IPA to HIS. (n = 7).

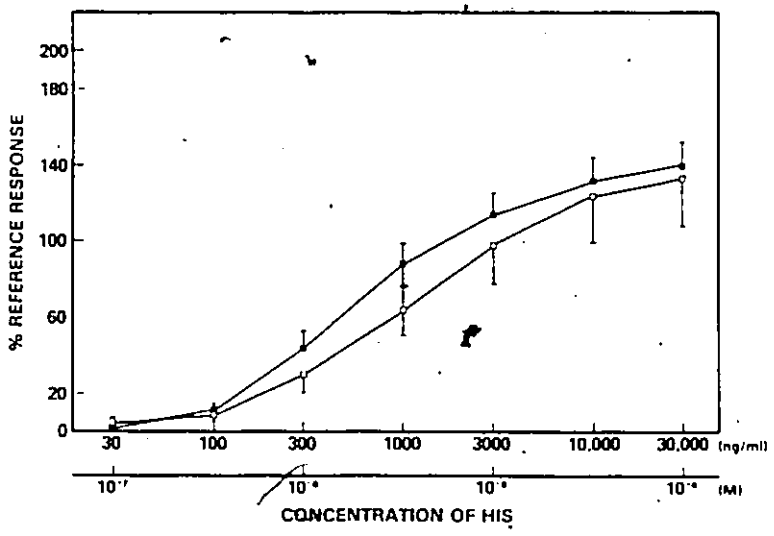


Fig. 3-8. Mean responses (± 1 S.D.) of proximal (□) and distal (■) segments of rabbit IPA to HIS. (n = 7).

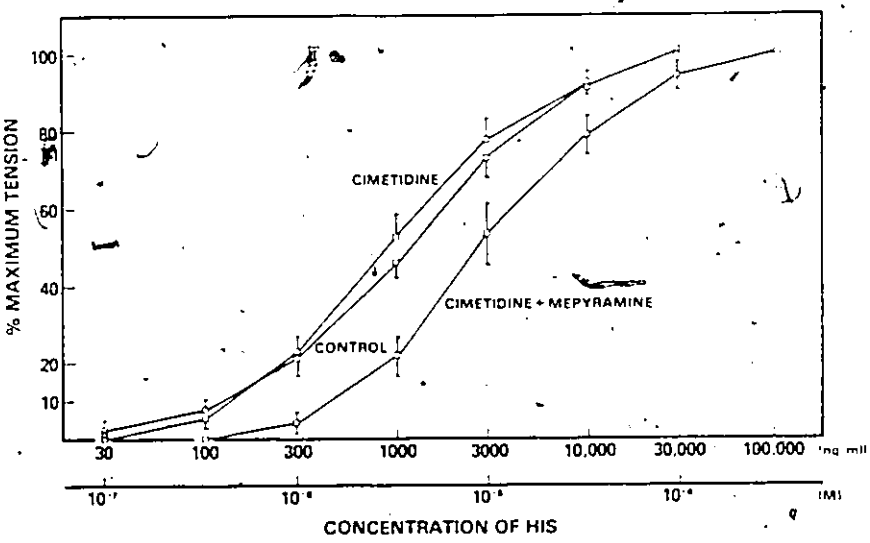


Fig 3-9. Effect of 10^{-9}M mepyramine and 10^{-5}M cimetidine on responses to HIS in proximal segments of rabbit IPA. (n = 4).

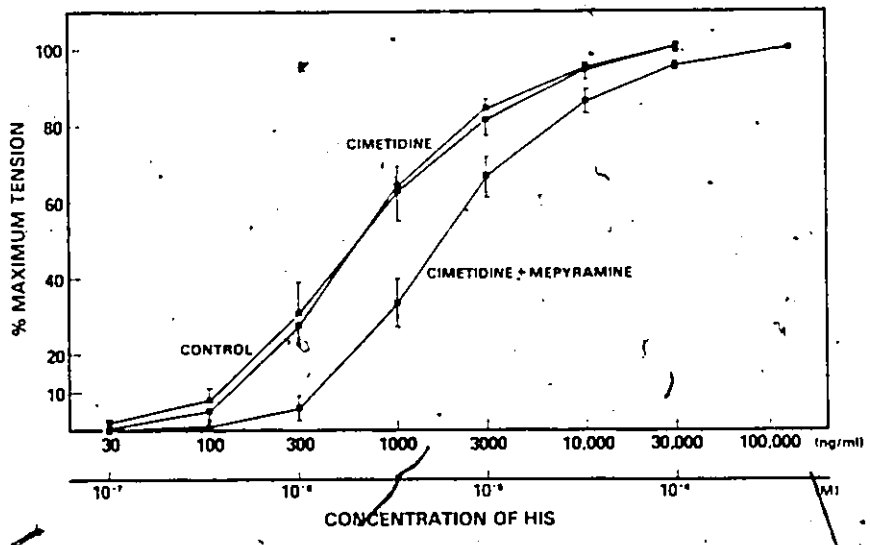


Fig. 3-10. Effect of 10^{-9}M mepyramine and 10^{-5}M cimetidine on responses to HIS in distal segments of rabbit IPA. (n = 4).

responses to graded doses of HIS were examined. Cimetidine (10^{-5}M) did not significantly alter responses to HIS relative to their maximal effects nor was the ED_{50} changed (see table 3). However, as a percent of the reference response, HIS responses were increased in both segments relative to control responses at all doses greater than 300 ng/ml. The increase was significant at a dose of 1000 ng/ml in the proximal segment and at doses greater than 1000 ng/ml in distal segments. The addition of mepyramine (10^{-9}M) produced significant inhibition of the HIS responses and shifted the dose response curve to the right in both segments. Proximal and distal ED_{50} values for the HIS dose response curve in the presence of mepyramine were significantly greater than the control ED_{50} values. (See table 3). Relative to the reference response there was no significant difference between the maximum tension produced by HIS in the presence of mepyramine and cimetidine and that produced in control responses.

In two of the above experiments, strips were exposed to an additional trial in which HIS responses were antagonized by 10^{-8}M mepyramine. The increased concentration of the antagonist produced a further inhibition of HIS responses. Neither cimetidine nor mepyramine appeared to affect baseline tensions.

In a single experiment the order of antagonists was reversed and mepyramine was introduced first and then cimetidine was added. While the effect of the mepyramine was similar to previous trials, cimetidine appeared to produce, in both segments, substantial increments in HIS contractile responses antagonized by mepyramine,

relative to both the maximum tension and the reference response, between doses of 0.1 and 100 µg/ml.

NE

The effect of NE on isometric tension of proximal and distal segments is illustrated in fig. 3-11. The proximal segment contracted to NE in a concentration dependent manner while the distal segment responded poorly with slight contractile or relaxant responses. The threshold for NE activity was at a concentration of about 10^{-9}M while maximal effects were obtained at concentrations of 10^{-7} to 10^{-6}M. Higher concentrations of NE, in both segments, usually produced a decline in active tension, or relaxation, which was reversed immediately following washing of the tissues. This response was identical in nature to that seeen in the initial amine experiments.

When plotted as a percent of maximum tension, as seen in fig. 3-12 the proximal segment responses were greater than distal responses ($n = 10$). Despite the great variability that characterized distal segment responses, the differences between proximal and distal segment responses to NE were significant at all doses other than 1, 30 and 100 ng/ml. Proximal segments were quite sensitive to the effects of NE: the ED_{50} as shown in table 2, was 3.79×10^{-8}M $\pm$ 1.5. Determination of distal segment ED_{50} values for NE was not possible by linear regression analysis owing to the considerable lack of uniformity in response. To illustrate, maximal effects in DIPA segments were

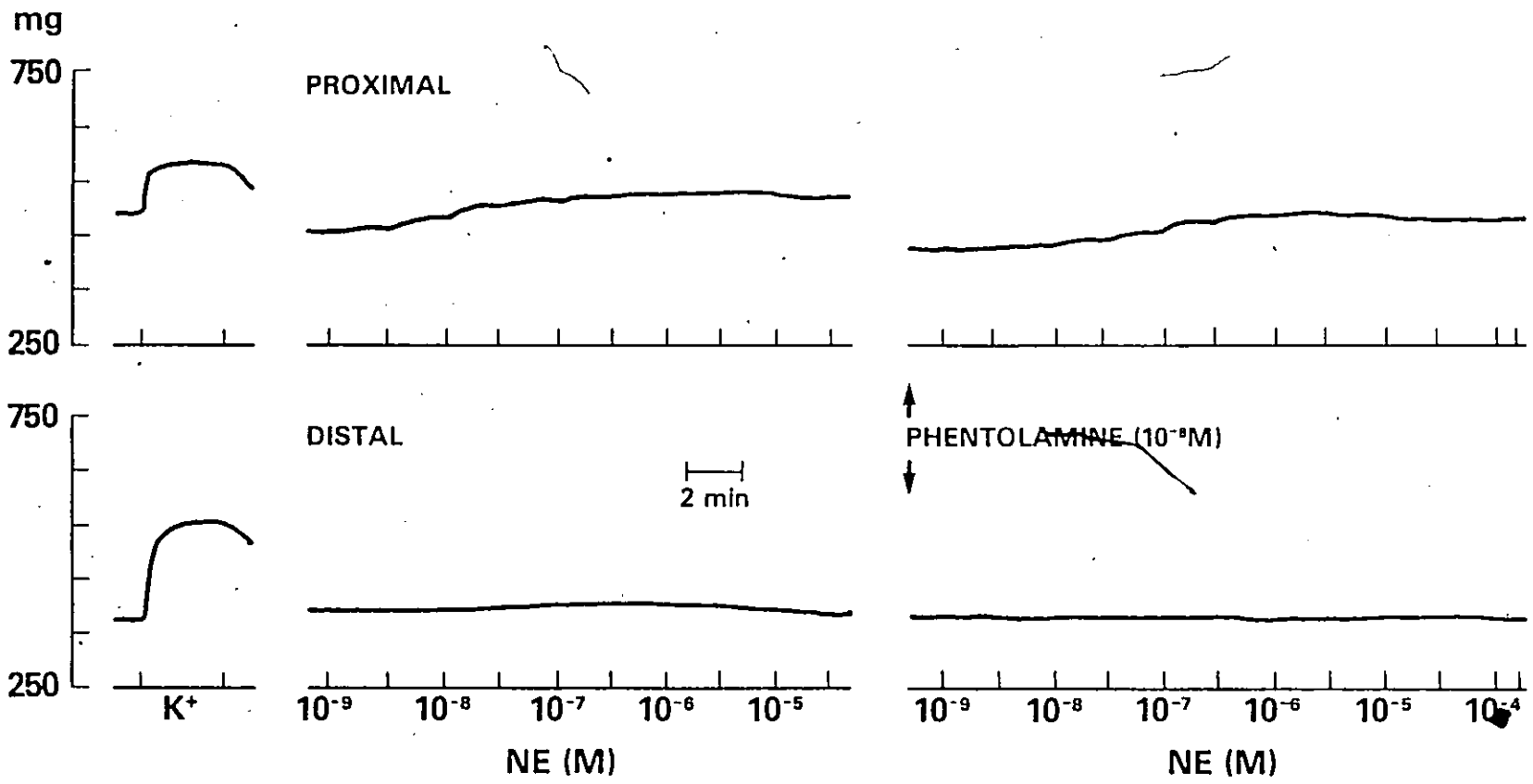


Fig. 3-11. Physiograph tracing illustrating control responses of rabbit intrapulmonary artery to NE and the effect of 10^{-8}M phentolamine on the NE responses.

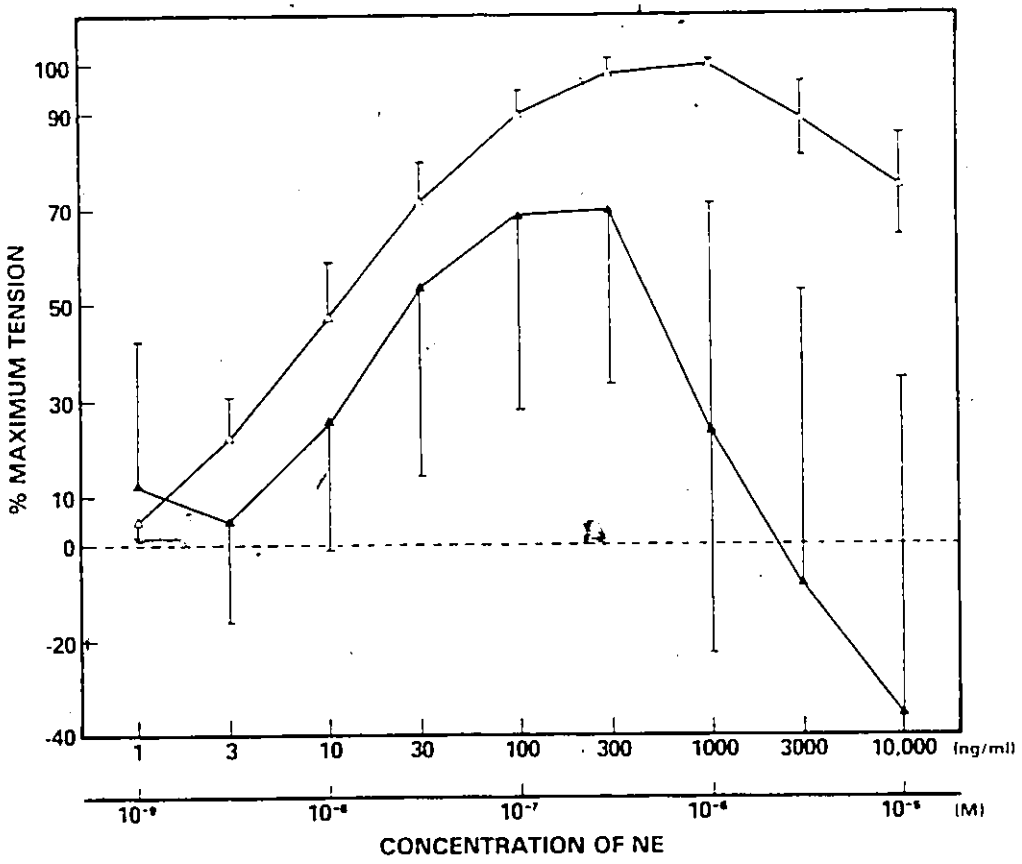


Fig 3-12. Mean responses (± 1 S.D.) of proximal (△) and distal (▲) segments of rabbit IPA to NE. (n = 10).

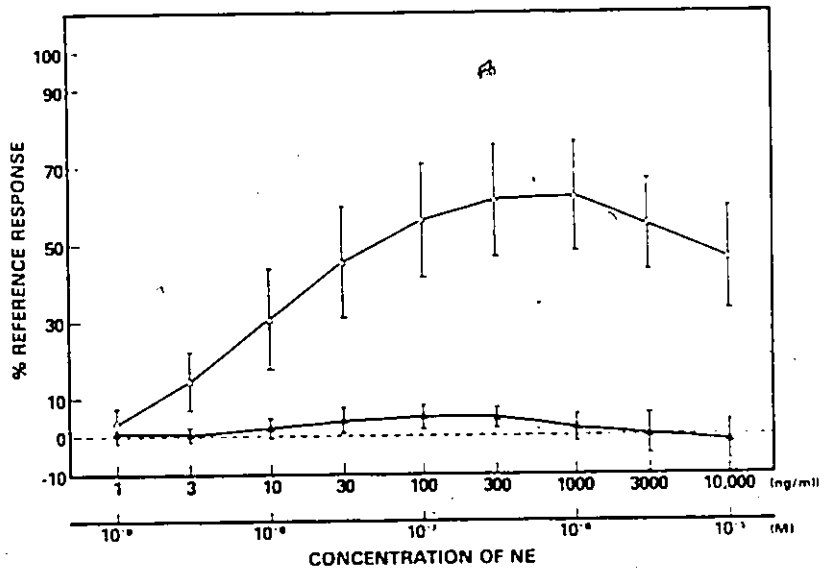


Fig. 3-13. Mean responses (± 1 S.D.) of proximal (△) and distal (▲) segments of rabbit IPA to NE (n = 10).

obtained at concentrations ranging from 1 ng/ml to 3000 ng/ml. The slight decline in mean distal tension between doses of 1 and 3 ng/ml in fig. 3-12 was attributable to a single experiment in which the distal strip responded maximally at its lowest dose. A rough estimation of the distal segment ED_{50} for NE may be obtained graphically from fig. 3-12 and was found to be about 9×10^{-8}M.

Significant differences between proximal and distal segment responses to NE are clearly seen in fig. 3-13 in which the NE responses are given as a percent of the reference response. The mean maximum response of the proximal segment was about 60% of the reference response while the distal segment response was significantly less, only about 5%. The very small magnitude of distal contractile and relaxant responses is easily seen.

The effects of phentolamine and propranolol (alpha and beta adrenoreceptor blockers respectively) on responses to graded doses of NE were examined in both proximal and distal segments. In proximal segments, following a control response to NE, the effect of propranolol was examined in 4 experiments. As seen in fig. 3-14, propranolol (10^{-7}M) produced a significant inhibition of responses to NE. The ED_{50} for NE was significantly increased in the presence of propranolol relative to the control response (see table 3). In contrast to the control response, in the presence of propranolol, high concentrations of NE, did not produce a decline in active tension. There was no significant difference in the magnitudes of the mean maximum response to NE in control and propranolol trials, (although

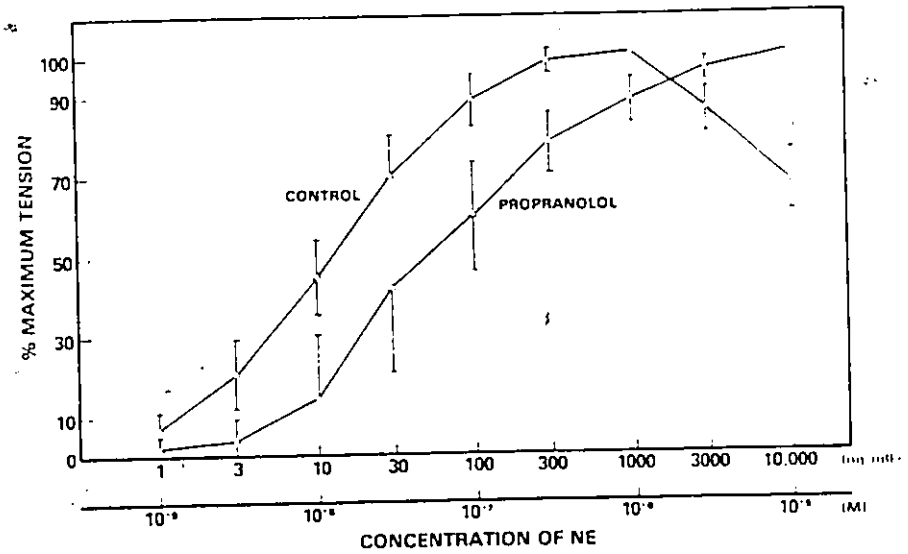


Fig 3-14. Effect of 10^{-7}M propranolol on responses to NE in proximal segments of rabbit IPA. (n = 4).

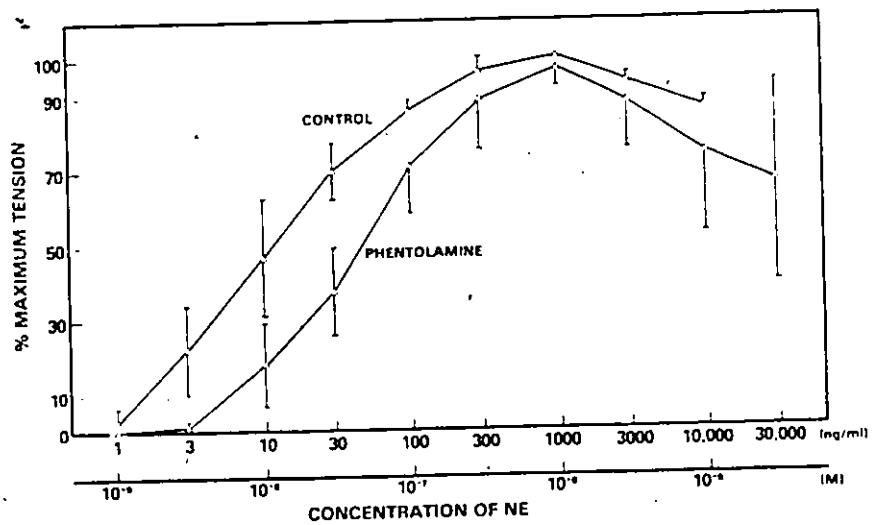


Fig. 3-15. Effect of 10^{-8}M phentolamine on the response to NE in proximal segments of rabbit IPA. (n = 3).

in one experiment, in the presence of propranolol, the maximal response to NE increased, from a control value of 67%, to 95% relative to the reference response). Phentolamine, 10^{-9}M, failed to alter significantly responses to NE (n = 3). However, 10^{-8}M phentolamine produced a significant inhibition of the NE responses in proximal segments (n = 3) as illustrated in fig. 3-15. This inhibition was significant at doses between 1 and 100 ng/ml. The antagonism of the NE response by phentolamine produced a significant increase in the ED_{50} relative to the control (see table 3). The magnitude of the maximum response to NE in the presence of phentolamine was reduced, but not significantly, relative to the control. Phentolamine had no effect on the decline in active tension at high doses of NE.

Distal segment responses to antagonists are shown in fig. 3-16 as a percent of the reference response, rather than as a percent of maximum tension, for the sake of clarity. The decline in active tension that accompanied increased doses of NE was blocked by propranolol (10^{-7}M) and instead contractile responses were obtained (n = 4). The responses in the presence of propranolol exceeded the control responses to NE at doses greater than 10 ng/ml. The differences in magnitude of responses were significant at doses greater than 300 mg/ml. This ability of propranolol to block the decline in active tension in response to high doses of NE suggests that this relaxant effect is mediated by NE stimulation of beta adrenoreceptors. Although phentolamine 10^{-9}M had little effect on responses to NE, it

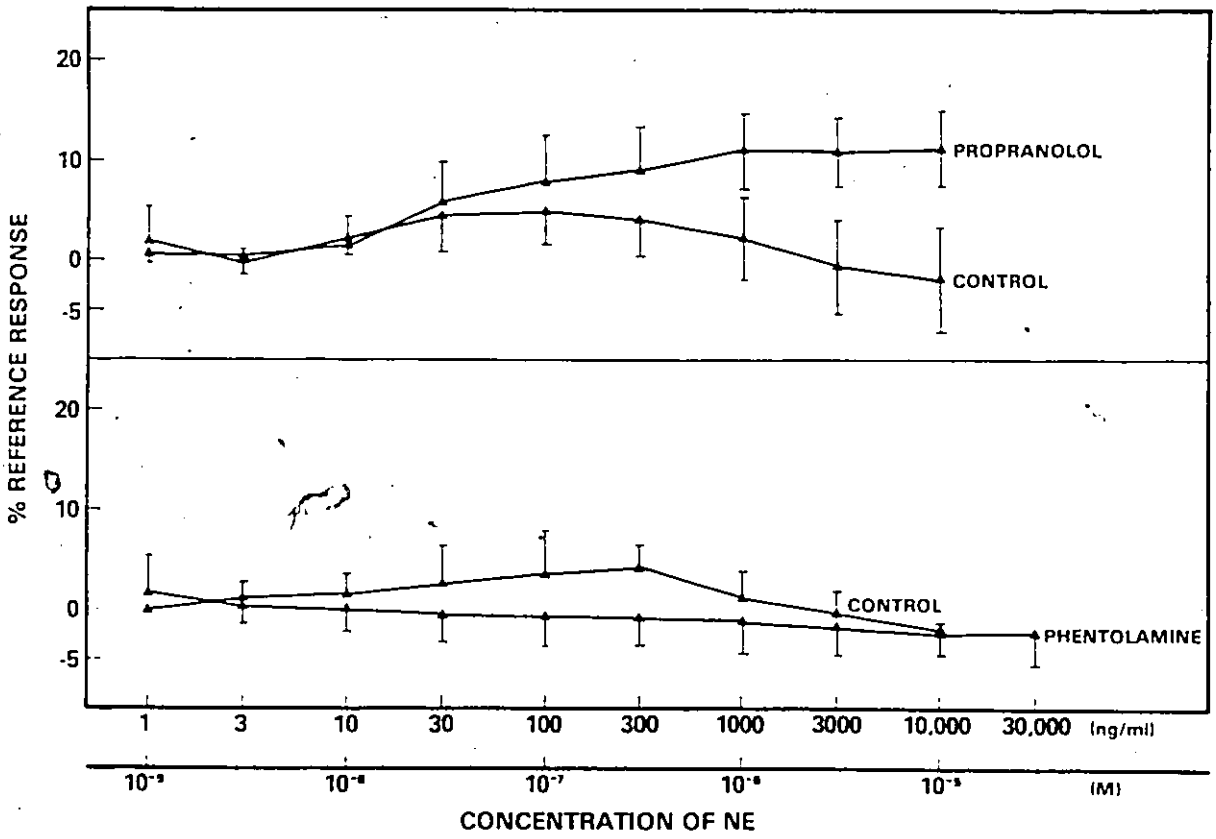


Fig 3-16. Effects of 10^{-7} propranolol (n = 4) and 10^{-8}M phentolamine (n = 3) on responses to NE in distal segments of rabbit IPA.

can be seen in fig. 3-16, that the distal segment responses to NE were inhibited in the presence of phentolamine 10^{-8}M. Despite the great variability in responses, this difference was significant at a dose of 300 ng/ml. Phentolamine, 10^{-8}M, was sufficient to produce complete inhibition of contractile responses in two of three experiments accounting for the predominantly relaxant mean responses in the presence of this antagonist. Neither propranolol nor phentolamine appeared to affect baseline tensions of proximal and distal segments.

Phenoxybenzamine, another alpha adrenoreceptor antagonist was added following propranolol trials in two experiments and produced a similar inhibition of NE contractile responses. Propranolol was added to the superfusate following phentolamine, in two experiments, to determine the influence of the combination of the two antagonists. In proximal segments, compared to phentolamine trials, the addition of propranolol appeared to produce some further antagonism of NE responses at lower doses, however, the magnitude of the maximal responses was increased on both occasions. In contrast to the control and phentolamine trials, in the presence of both antagonists no decline in active tension was seen despite the use of concentrations of NE of up to 0.1 mg/ml suggesting that this relaxant effect may be mediated by beta adrenoreceptors. In distal segments in the presence of the two antagonists, NE produced virtually no response at all.

In two each of the propranolol and phentolamine experiments, dose response trials to HIS were alternated with NE dose response

trials to determine any effect of the adrenoreceptor antagonists on HIS responses. Although neither propranolol (10^{-7}M) nor phentolamine (10^{-8}M), appeared to substantially alter maximal responses to HIS, phentolamine did appear to produce some slight inhibition of HIS responses in both segments but this effect was not consistently observed. Mixed effects of propranolol on HIS responses were seen in both segments.

Comparison of Amine Responses

Proximal and distal segment responses to 5HT, HIS and NE are compared relative to maximum tensions in figures 3-17 and 3-18 and relative to the reference response in figures 3-19 and 3-20. Proximal and distal strip sensitivity was greatest for NE and 5HT (in that order) and least for HIS. The results in fig. 3-17 and 3-18 are similar to those reported in the initial amine experiments as shown in fig. 2-1 and 2-2. In proximal segments, the maximal response to HIS was significantly greater than the maximal response to 5HT which closely resembled the magnitude of the reference response to K^+. The maximal response to NE was significantly less than the response to 5HT, about 60% of the reference response. In distal segments the response to HIS exceeded the magnitude of the response to K^+ and was similar to the proximal response. The maximal response to 5HT was less than 50% of the reference response and significantly less than the proximal response, while NE maximal effects were only about 5% of the reference

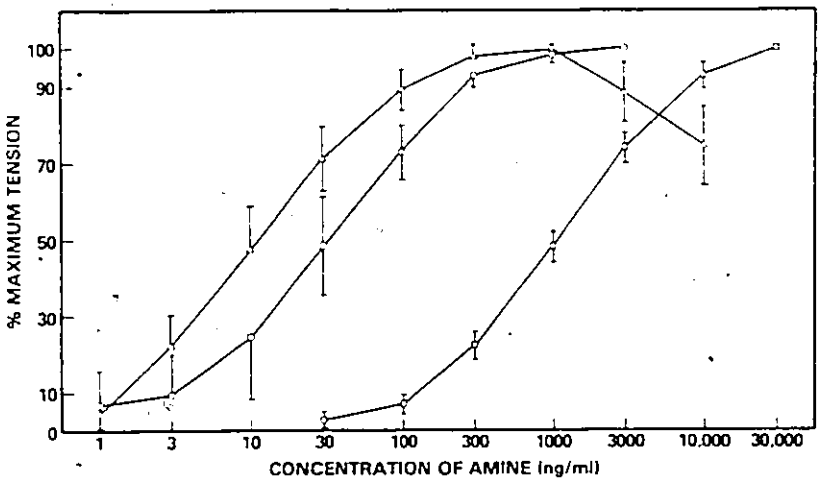


Fig 3-17. Summary of mean responses (± 1 S.D.) of the proximal segment of rabbit IPA to 5HT (○ , n = 5) HIS (□ , n = 7) and NE (△ , n = 10).

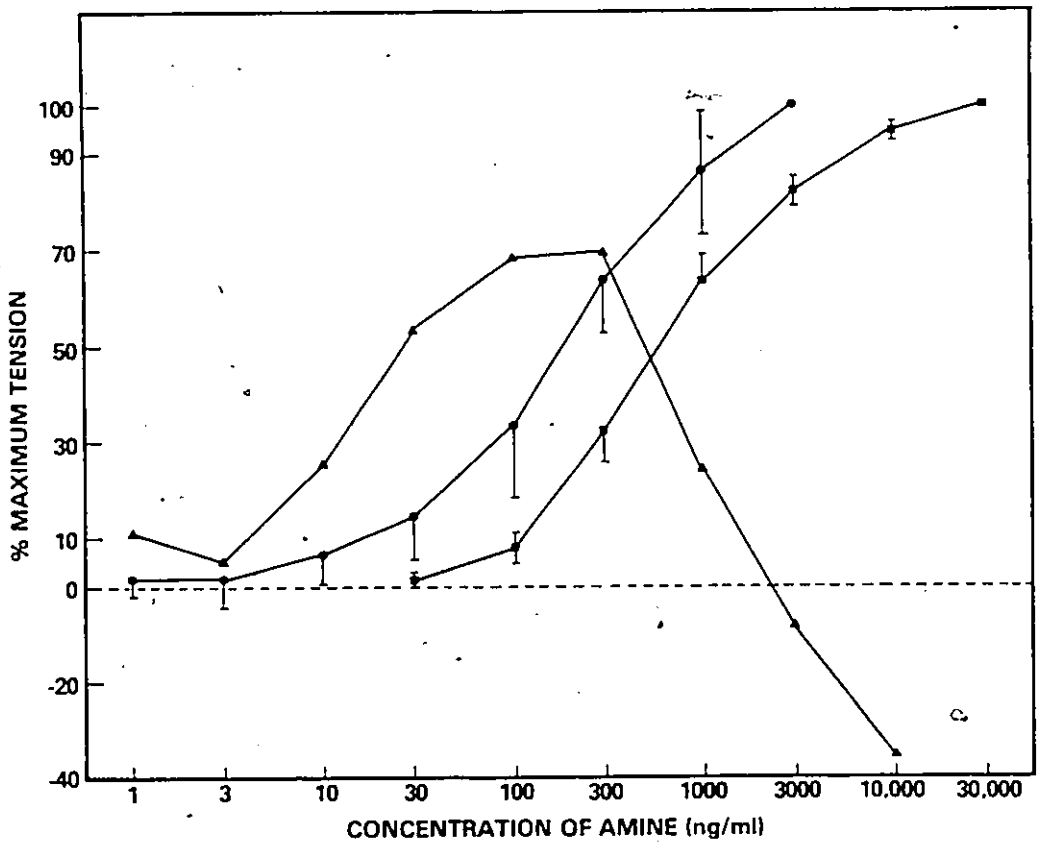


Fig. 3-18. Summary of mean responses (± 1 S.D.) of the distal segment of rabbit IPA to 5HT (● , n = 5), HIS (■ , n = 7) and NE (▲ , n = 10). Error bars for the response to NE have been omitted for the sake of clarity.

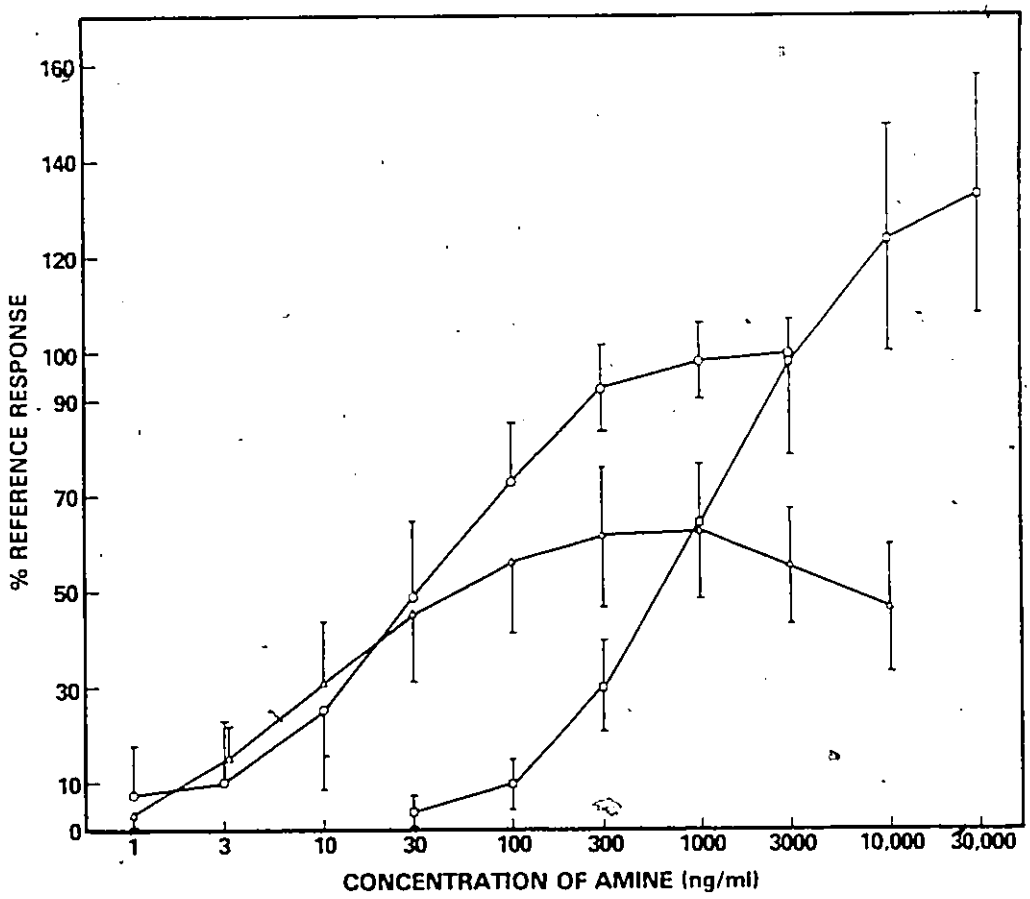


Fig 3-19. Summary of mean responses (+ 1 S.D.) of the proximal segment of rabbit IPA to 5HT (○ , n = 5), HIS (□ , n = 7) and NE (△ , n = 10).

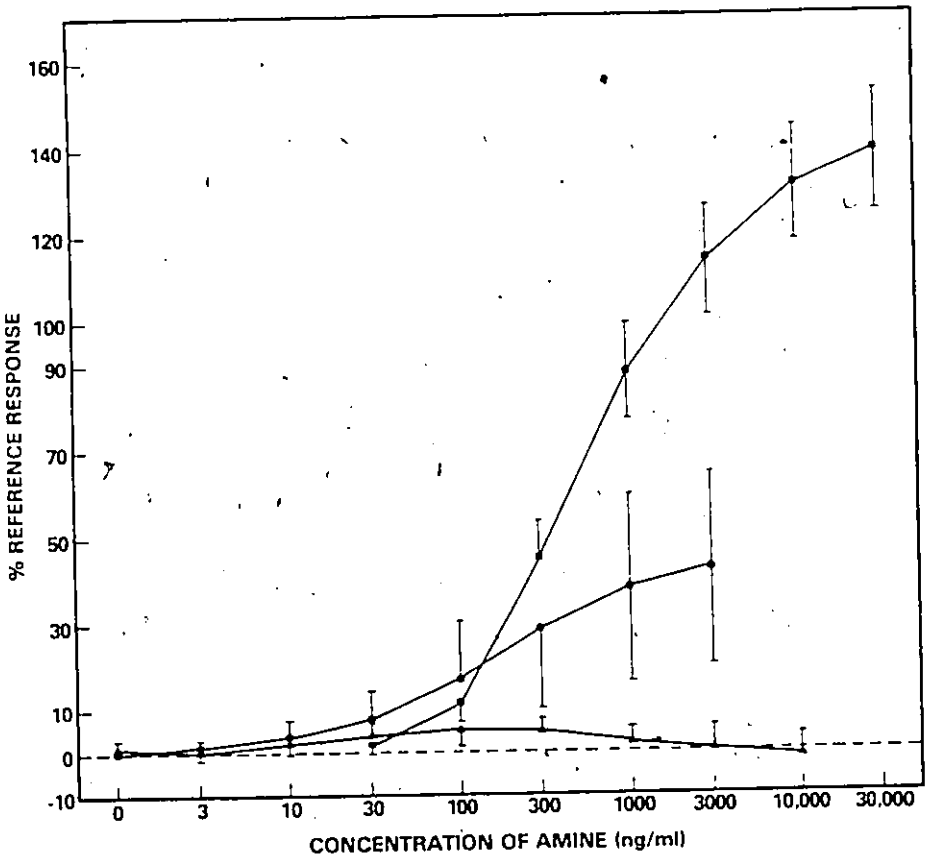


Fig 3-20. Summary of mean responses (+ 1 S.D.) of the distal segment of rabbit IPA to 5HT (● , n = 5), HIS (■ , n = 7) and NE (▲ , n = 10).

response or 10% of the proximal response. Thus it can be seen that there exist important and significant differences between proximal and distal responses with respect to sensitivity and maximal effects of the amines.

Arachidonic Acid

AA contracted both proximal and distal segments in a dose dependent manner as illustrated in figs. 3-21 and 3-22. Both strips responded strongly to the lowest dose applied (3 ng/ml), indicating a much lower threshold, while maximal effects were obtained at 300 - 1000 ng/ml. There was no signficant difference in the maximum response of both segments to AA, nor were the maximum responses significantly greater than the reference responses. Sensitivity to AA was great and was similar in both proximal and distal strips as can be seen from the ED_{50} values in Table 2. Concentrations of vehicle used in the preparation of AA produced no response. However, it is of interest to note that quantities of AA stored at room temperature and exposed to light for one or more days retained considerable contractile activity.

In 2 preliminary experiments, AA, in increasing doses from 3 to 300 ng/ml, was applied at 30 minute intervals for 3 consecutive trials. Marked tachyphylaxis was evident with repeated exposure, however, dose related contractile activity persisted. In proximal segments, the second exposure to AA produced maximum responses of about 70% and 55% of the control maximum response while the third exposure yielded responses of 50% and 35% respectively. In distal segments the maximum

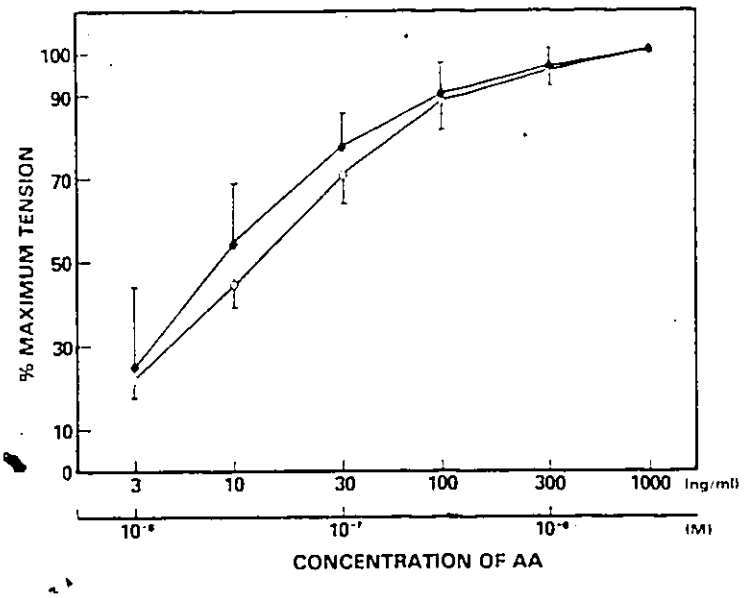


Fig 3-21. Mean responses (+ 1 S.D.) of proximal (◊) and distal (♦) segments of rabbit IPA to AA. (n = 4).

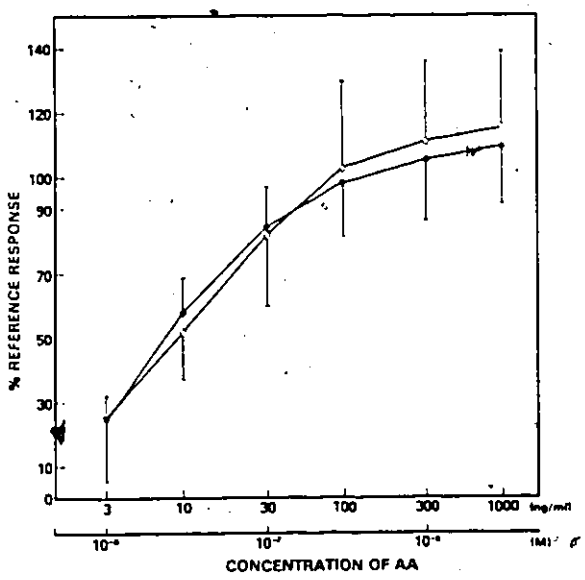


Fig. 3-22. Mean responses (+ 1 S.D.) of proximal (◊) and distal (♦) segments of rabbit IPA to AA. (n = 4).

response to the second exposure was 40% of the control maximum response in the first instance and 85% in the other, similarly with the third exposure to AA, the maximum responses were 20% and 60% of the control maximum response.

The effects of the cyclo-oxygenase inhibitor indomethacin on AA and $PGF_{2\alpha}$ induced contractions of PIPA and DIPA segments (n = 4) are shown in figs. 3-23 and 3-24 respectively. The responses to AA in both PIPA and DIPA segments were affected similarly by indomethacin. At a concentration of 10^{-5}M, indomethacin produced a significant inhibition of the partial dose response curve to AA, the maximal response being reduced to about 20 - 25% of the control maximum. Almost complete blockade of the contractile response to AA was produced by 10^{-4}M indomethacin, only a slight response being produced by 1000 ng/ml of AA. Following withdrawal of indomethacin from the superfusate, AA produced contractile responses of large but highly variable magnitude in both segments of artery. Indomethacin had no apparent effect on baseline isometric tension.

A single dose of $PGF_{2\alpha}$ (1000 ng/ml) capable of eliciting a contractile response of about 75% of maximum, as determined from fig. 2-21, was applied to both proximal and distal strips following responses to AA and the effect of indomethacin on this response was examined. In the presence of 10^{-5}M indomethacin, the prostaglandin response in both segments was not significantly changed. However, indomethacin 10^{-4}M produced a significant but incomplete inhibition

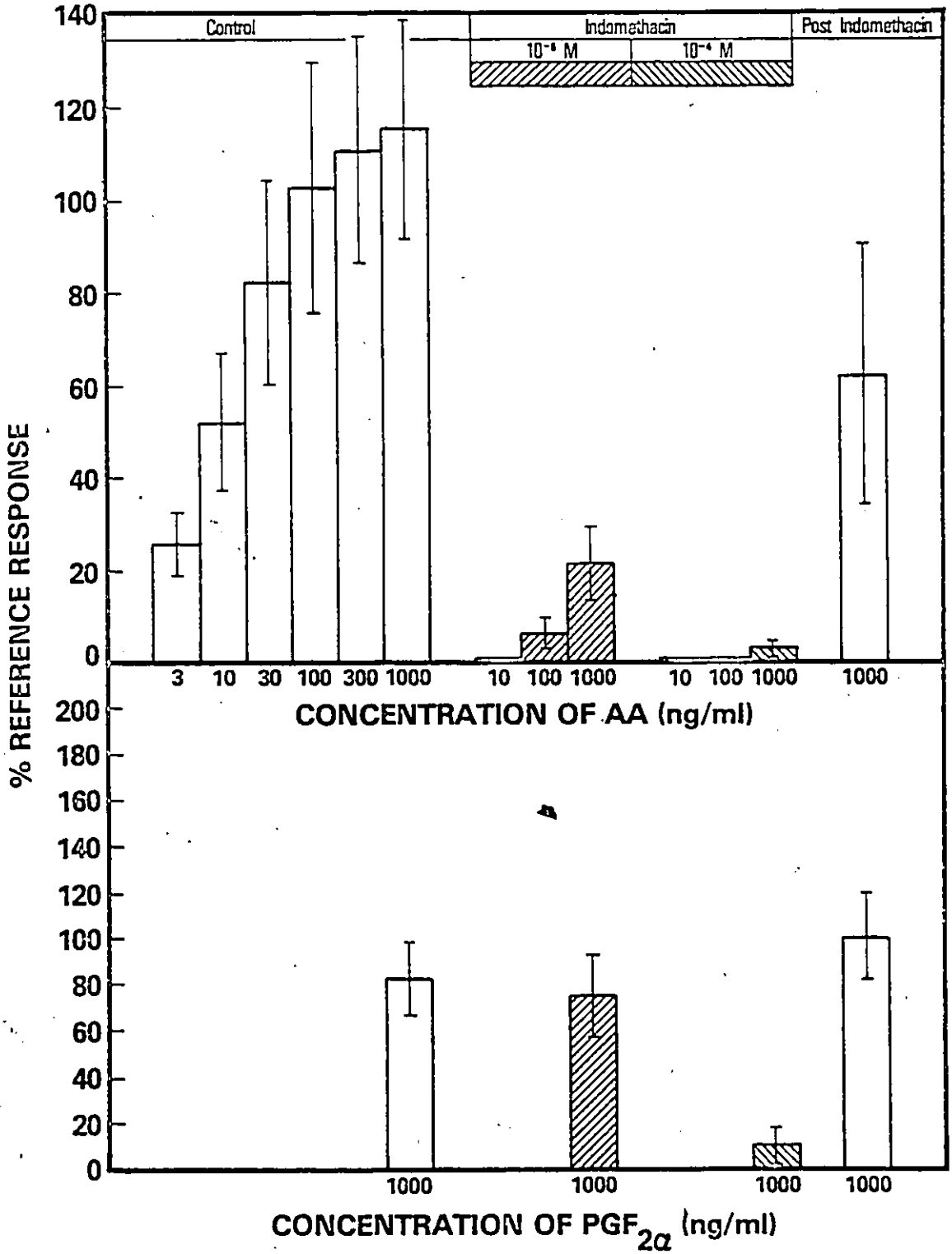


Fig. 3-23. Effects of 10^{-5}M and 10^{-4}M indomethacin on responses (mean ± 1 S.D., n = 4) to AA and $PGF_{2\alpha}$ in proximal segments of rabbit IPA.

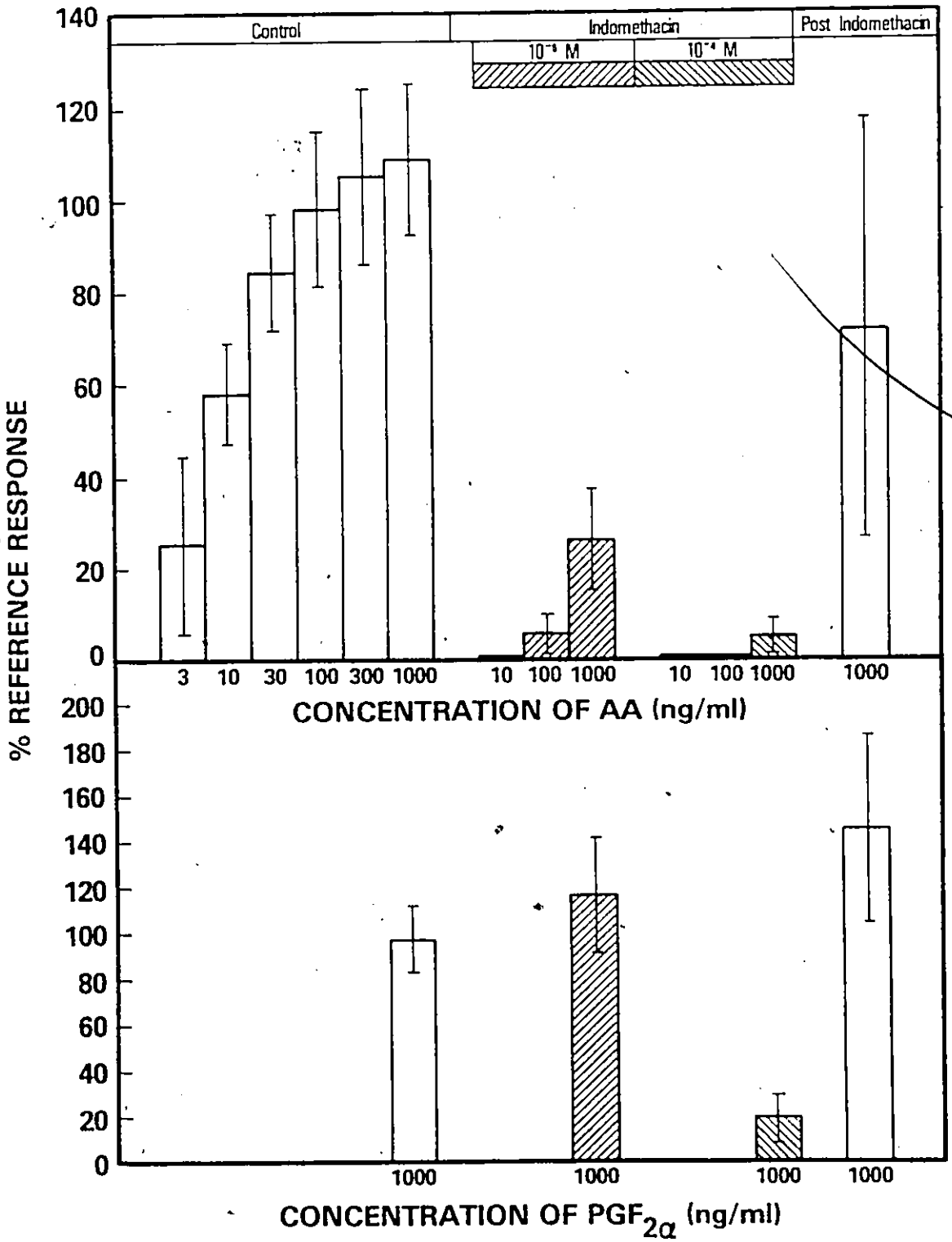


Fig. 3-24. Effects of 10^{-5}M and 10^{-4}M indomethacin on responses (mean ± 1 S.D., n = 4) to AA and $PGF_{2\alpha}$ in distal segments of rabbit IPA.

of the $PGF_{2\alpha}$ response. Following washing of the tissue after withdrawal of indomethacin, application of $PGF_{2\alpha}$ resulted in a contractile response greater than the control response. The difference was significant in distal segments.

In a single experiment from this series, both proximal and distal segments responded very weakly to AA and $PGF_{2\alpha}$. AA produced maximal effects, relative to the reference response, of only 7% in proximal and 30% in distal segments. The response to $PGF_{2\alpha}$ 1 μg/ml was 8% in proximal and 21% in distal segments relative to the reference response. Data from this experiment was not utilized in the determination of mean responses. Attempts to demonstrate relaxant effects, by precontracting these segments with HIS 3 μg/ml and adding graded doses of AA and $PGF_{2\alpha}$ were unsuccessful.

Isoproterenol

Since IsoP had produced slight relaxations or no responses at low doses accompanied by contractile effects at higher doses in all proximal and two of three distal segments when initially studied, its relaxant effects were re-examined using vessel strips that were partially contracted with $PGF_{2\alpha}$. The prostaglandin was used to induce a submaximal level of contraction in order that relaxant effects might be observed since these arteries possess little intrinsic muscle tone. The dose of $PGF_{2\alpha}$ used, 500 ng/ml, was chosen since it gave a response of about 50% of maximum as determined graphically from fig.

2-12. The response to the prostaglandin was well maintained, when examined for duration of time that it was required to partially contract the tissue for IsoP dose-response trials although, small and gradual declines in active tension related to the time of exposure were occasionally seen. These studies were conducted concurrently with NE - propranolol trials by alternating dose response trials to these agonists.

In both proximal and distal strips (n = 5), the addition of IsoP in graded doses produced dose dependent relaxation (see fig. 3-25), the magnitude of which greatly exceeded that seen in unstimulated strips. The relaxant action of IsoP was similar in both proximal and distal segments (see fig. 3-26). Maximal relaxant effects were obtained at a concentration of 10^{-6}M in proximal strips and 10^{-5}M IsoP in distal strips. Concentrations of 10^{-5}M IsoP produced less pronounced relaxant activity in proximal strips. The differences between proximal and distal mean relaxant response were significant at doses greater than 10^{-8}M. Fig. 3-27 illustrates the responses of $PGF_{2\alpha}$ and IsoP relative to the reference response. The distal segment mean response to $PGF_{2\alpha}$ (500 ng/ml), was significantly greater than the proximal responses in this series of experiments. Differences between proximal and distal segment relaxant responses, relative to the reference response, were not significant at doses greater than 1 ng/ml. The action of IsoP did not produce relaxations of greater magnitude than the PG induced contractions.

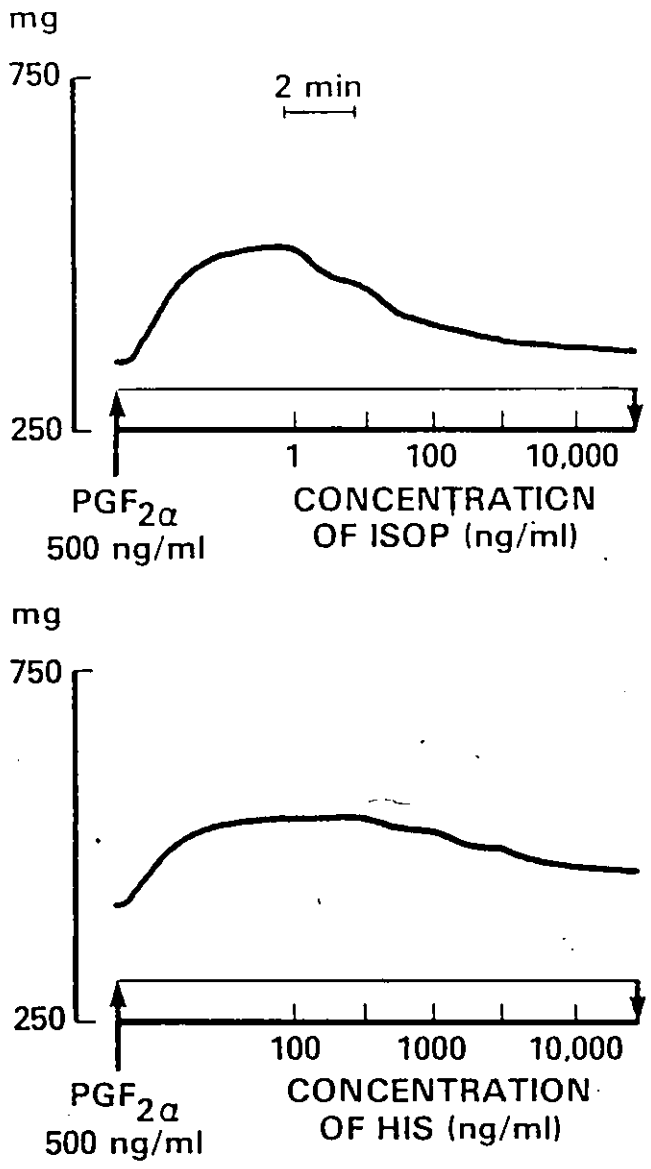


Fig. 3-25. Physiograph tracings illustrating relaxant responses of contracted rabbit intrapulmonary artery to IsoP and HIS. The HIS relaxant response was obtained in the presence of 10^{-7}M mepyramine.

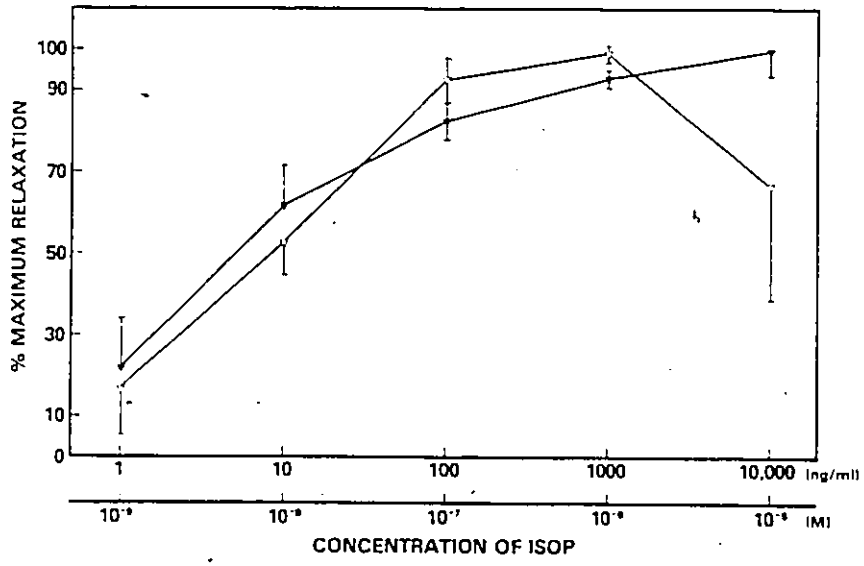


Fig 3-26. Mean responses (± 1 S.D.) of partially contracted proximal (▽) and distal (▼) segments of rabbit IPA to IsoP. (n = 5).

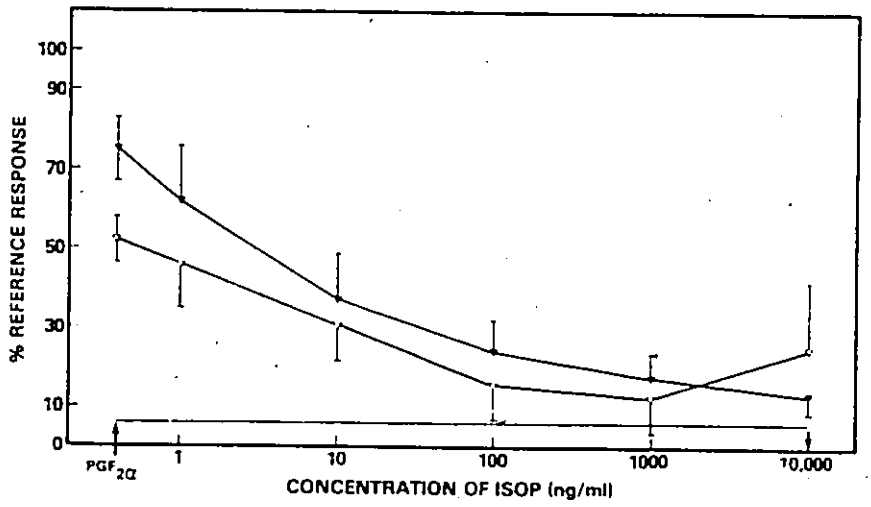


Fig. 3-27. Mean responses (± 1 S.D.) of partially contracted proximal (▽) and distal (▼) segments of rabbit IPA to IsoP. (n = 5).

In two experiments, proximal responses to $PGF_{2\alpha}$ 500 ng/ml were 3 and 13% relative to the reference response while in distal segments responses were 18 and 23% respectively. Since these tissues produced uncharacteristically poor prostaglandin responses, similar to previously noted experiments, the data was not included in the analysis of IsoP responses. However, relaxant responses to IsoP were observed in these experiments in both segments and tensions did relax to below baseline levels.

Propranolol antagonized the relaxant action of IsoP in both proximal (fig. 3-28) and distal (fig. 3-29) segments. Although relaxant activity was completely blocked at lower doses of IsoP resulting in slight contractile responses, higher doses of IsoP did produce relaxant responses.

In a single experiment the effects of HIS H_1 and H_2 receptor antagonists on the response to IsoP of strips partially contracted to $PGF_{2\alpha}$ were examined. Mepyramine did not appear to affect the response to IsoP while cimetidine failed to block its relaxant activity.

H_2-HIS Receptor Activity

The variable nature of the distal responses to HIS seen in the original amine experiments suggested that HIS stimulated both contractile and relaxant activity. However, the results of the length tension experiments demonstrated that the nature of the distal segment response i.e. contractile or biphasic, was dependent on the magnitude

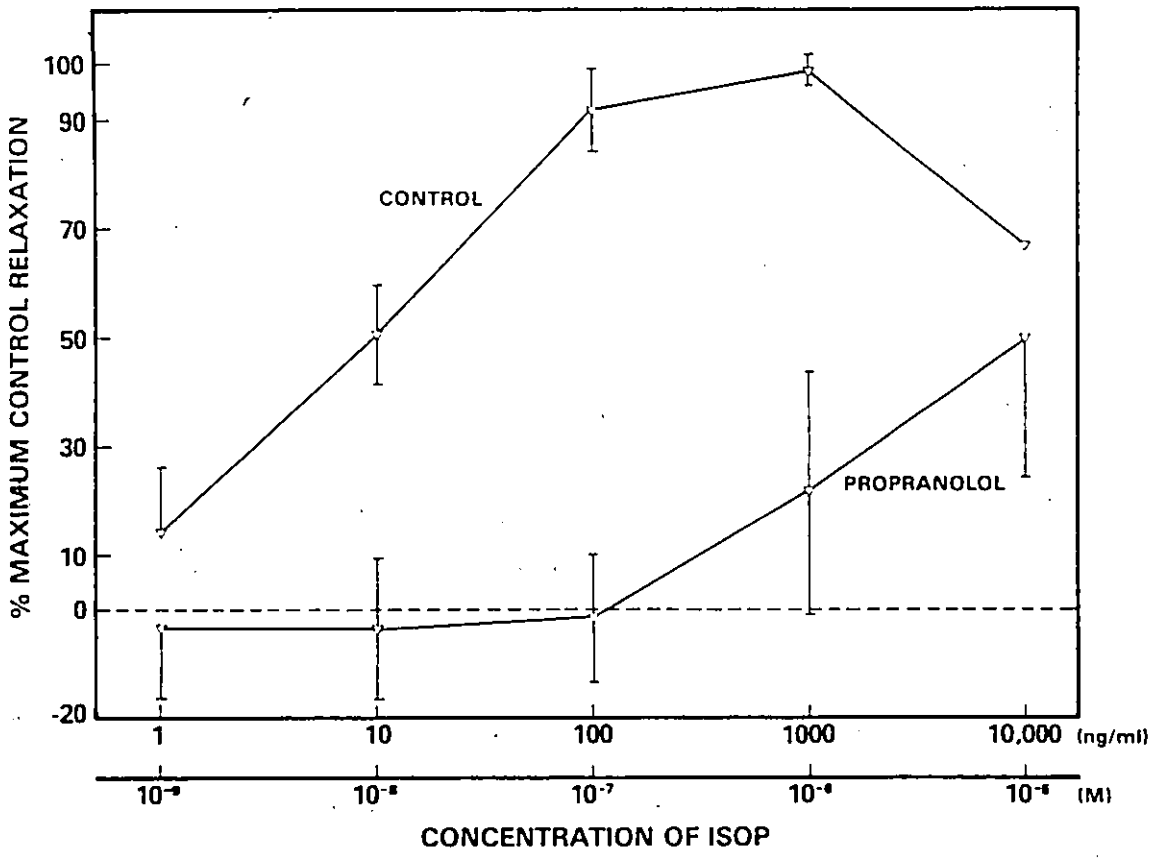


Fig 3-28. Effect of 10^{-7}M propranolol on responses to IsoP in proximal segments of rabbit IPA (n = 3).

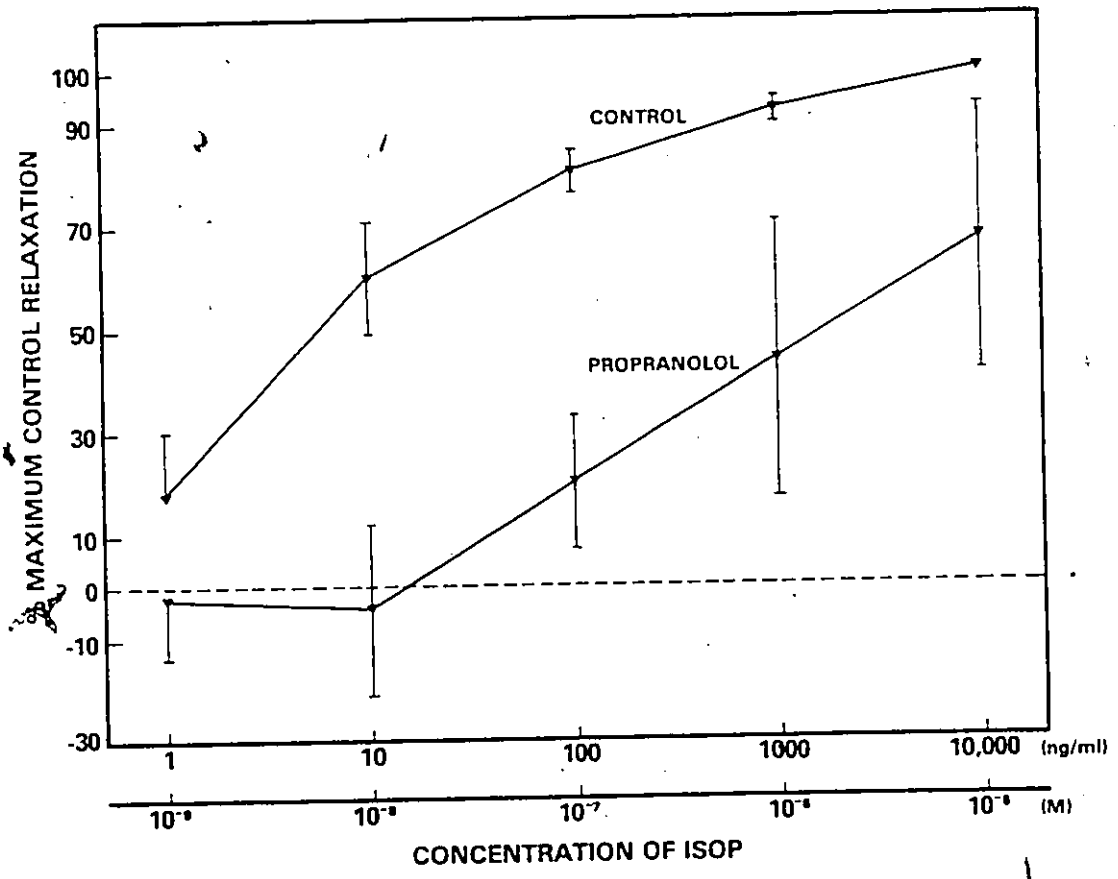


Fig 3-29. Effect of 10^{-7}M propranolol on responses to IsoP in distal segments of rabbit IPA (n = 3).

of baseline tension applied to the strips. To determine if HIS, in fact, was capable of producing relaxant responses mediated by H_2-HIS receptors, the effect of HIS applied to vessel strips in the presence of mepyramine was examined. Since the tissues possessed little intrinsic muscle tone and consequently any relaxant effects would be minimal, tissues were precontracted with $PGF_{2\alpha}$ 500 ng/ml. The concentration of mepyramine used (10^{-7}M), greater than that employed previously, was chosen to produce significant H_1 blockade over the range of HIS doses employed.

When precontracted with $PGF_{2\alpha}$ in control trials, the addition of graded doses of HIS elicited dose related contractions (see figs. 3-25 and 3-30). In the presence of mepyramine (10^{-7}M) stimulation with HIS of the precontracted strips produced dose dependent relaxation. The magnitude of maximal relaxation relative to the induced contraction was not significantly different in proximal and distal strips nor did the maximal relaxation exceed the prostaglandin induced contraction. The addition of cimetidine (10^{-5}M) antagonized the relaxant responses to HIS (small relaxations at low doses were present in distal strips) resulting in contractile HIS responses which were substantially and significantly reduced relative to control responses.

Fig. 3-31 illustrates the responses to HIS, previously described, relative to maximal contractile and relaxant effects. The relaxation to HIS in the presence of mepyramine and the antagonism of this response with the addition of cimetidine are clearly shown.

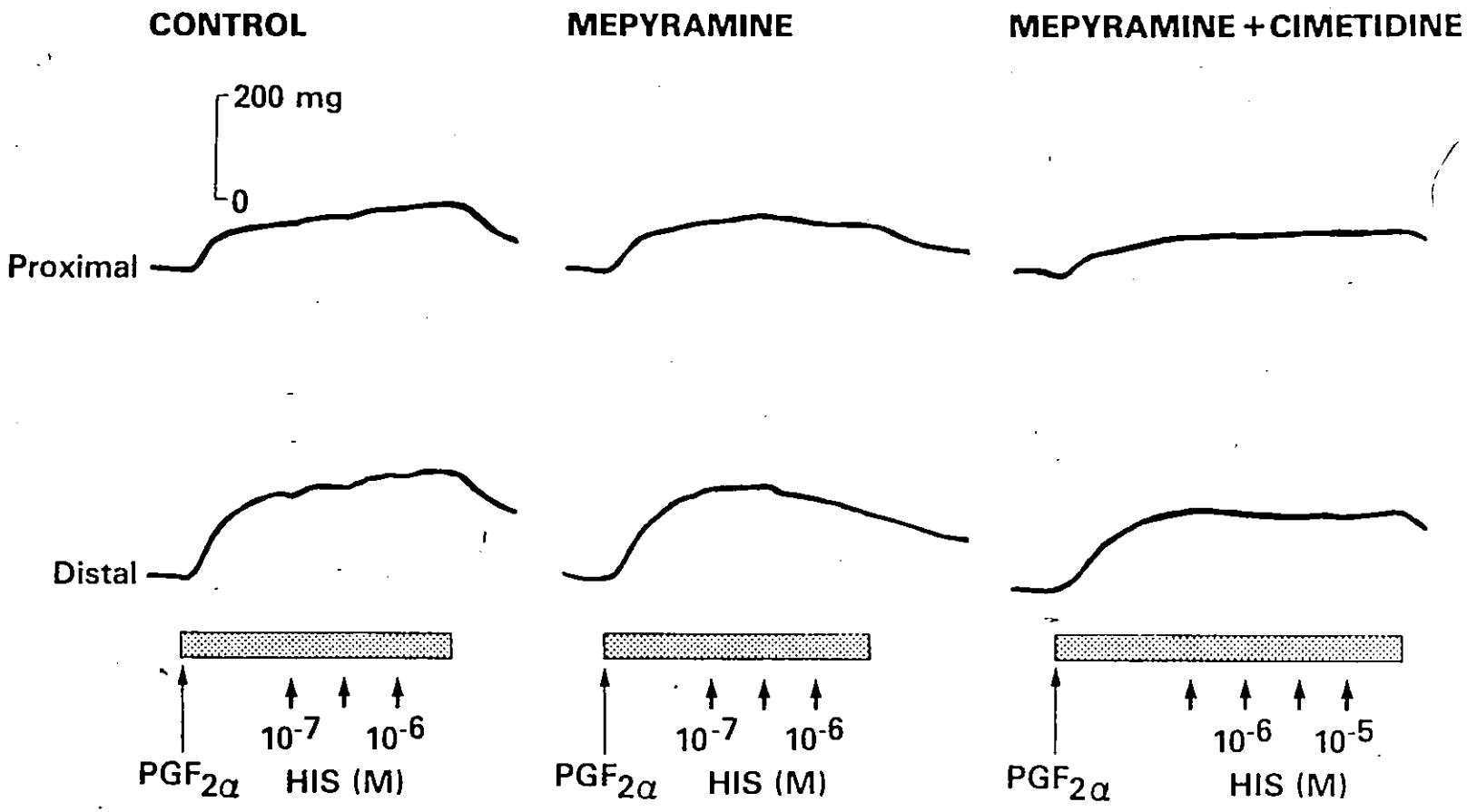


Fig. 3-30. Physiograph tracing illustrating control responses of contracted rabbit IPA to HIS, the relaxant response of contracted IPA to HIS in the presence of 10^{-7}M mepyramine and the effect of 10^{-5}M cimetidine plus 10^{-7}M mepyramine on the HIS relaxant response. Data taken from a preliminary experiment.

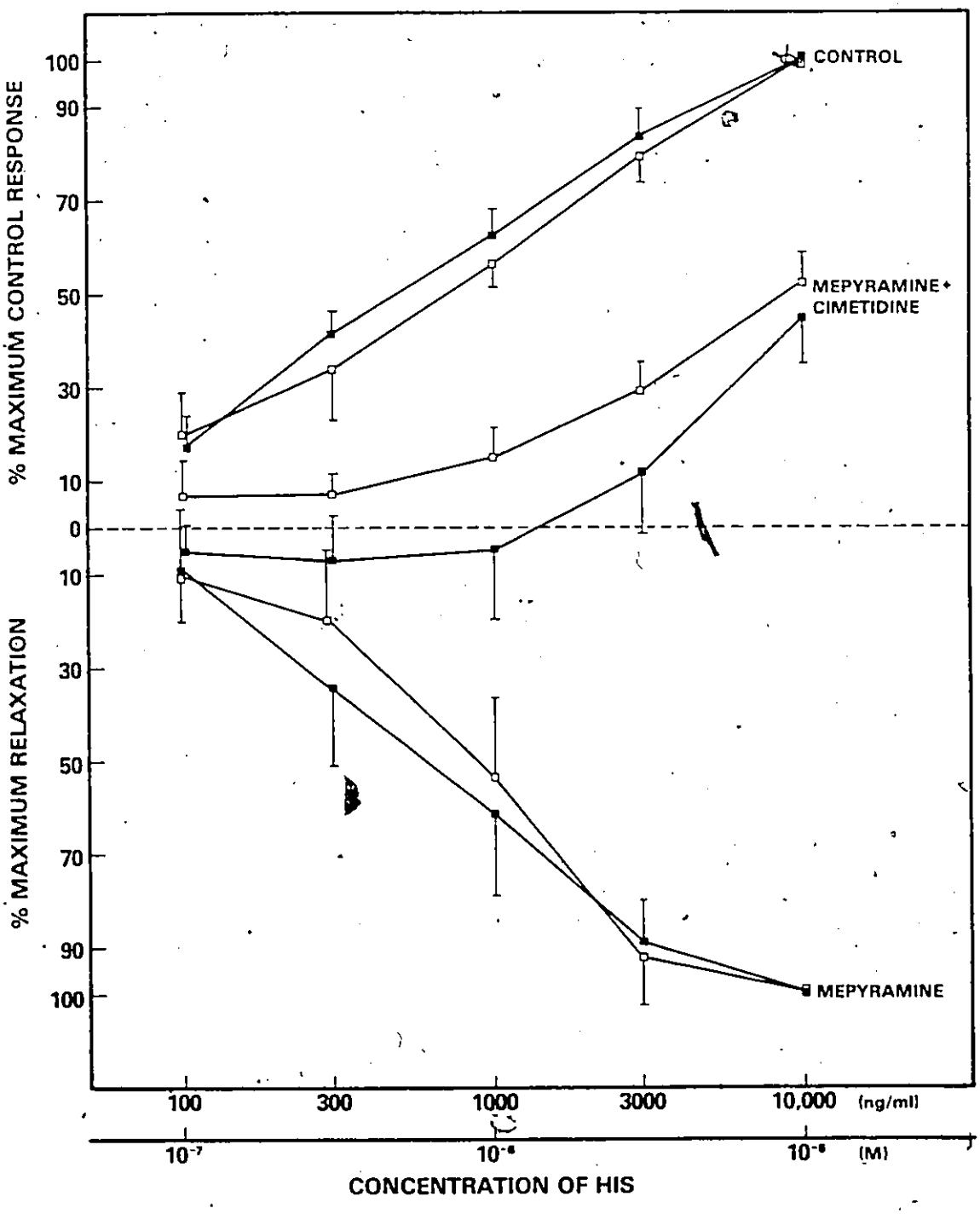


Fig. 3-31. Effects of 10^{-7}M mepyramine and 10^{-5}M cimetidine on responses to HIS in partially contracted proximal (□) and distal (■) segments of rabbit IPA. (n = 6).

Proximal and distal responses, relative to the reference response are shown in figures 3-32 and 3-33 respectively. Although no significant difference between proximal and distal segments was found for the mean response to $PGF_{2\alpha}$ (500 ng/ml), the prostaglandin response was characterized by great variability. This contrasts with the previous use of the same dose and agonist in the IsoP trials in which there was little variability in response and a significantly greater distal segment response. The great variability may be attributed to two experiments in which the tissues responded poorly to the prostaglandin. Data from these experiments were retained, however, since the responses did exceed 25% of the reference response in both segments. Despite this variability, in both segments the relaxant response to HIS in the presence of mepyramine was found to be significantly less than the control responses at doses greater than 100 ng/ml and less than the responses antagonized by cimetidine and mepyramine at doses greater than 300 ng/ml. The magnitude of the contractile response to HIS in the presence of mepyramine and cimetidine was significantly less than control responses at doses of 3 µg/ml in both segments and 10 µg/ml in proximal segments only. The antagonism, produced by cimetidine, of the HIS relaxant response in the presence of mepyramine clearly supports the hypothesis that the relaxant effect is produced by HIS H_2 receptor stimulation.

The results obtained from preliminary trials (n = 4), conducted to establish the dose range of HIS and to determine suitable

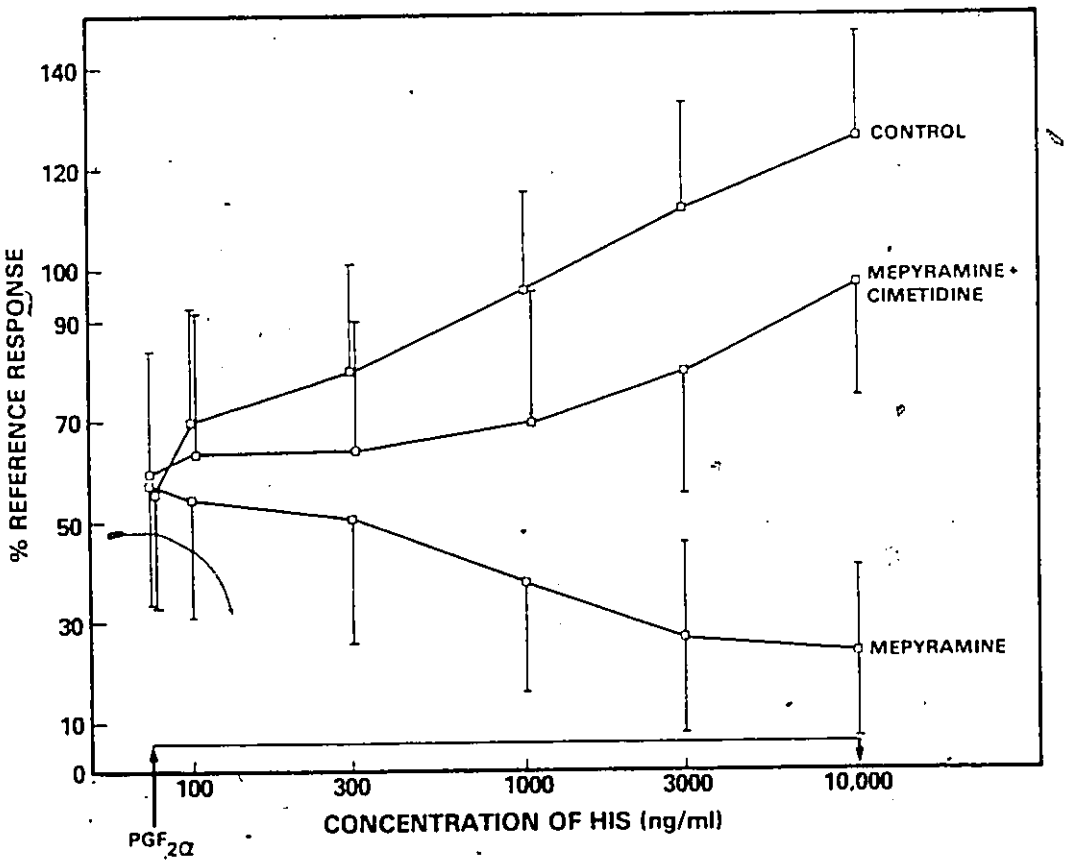


Fig 3-32. Effects of 10^{-7}M mepyramine and 10^{-5}M cimetidine on responses to HIS in partially contracted proximal segments. (n = 6)

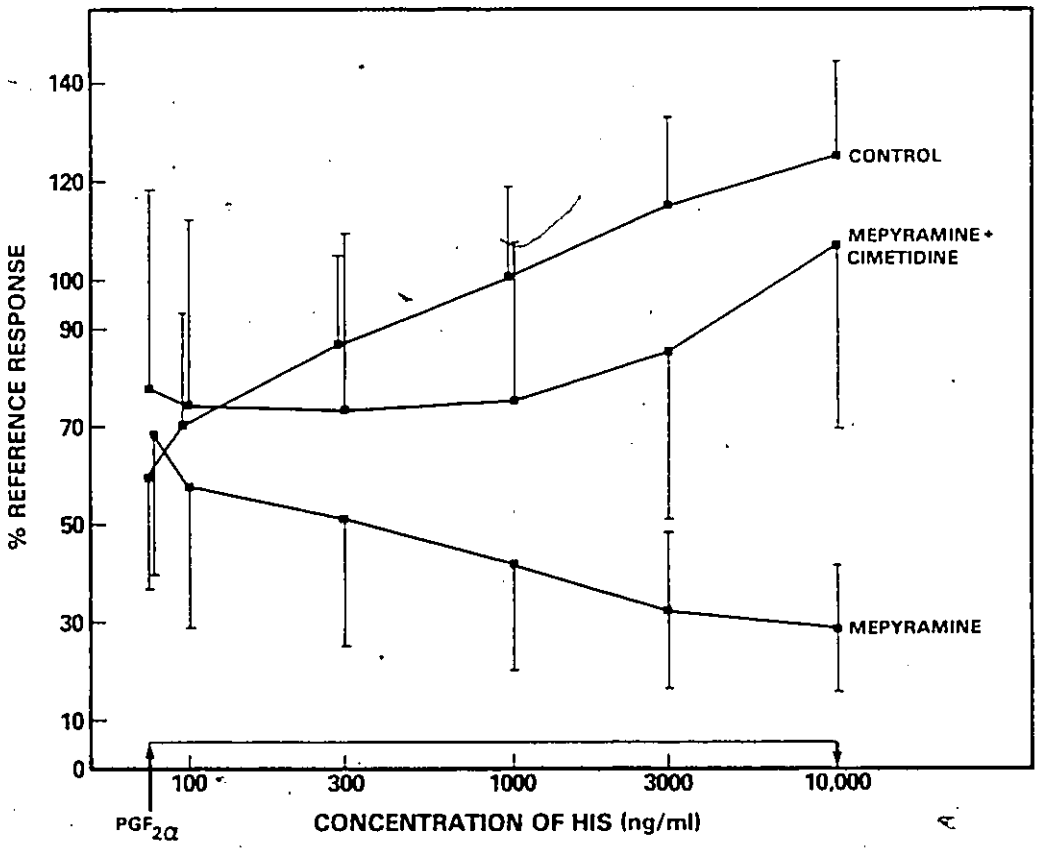


Fig 3-33. Effect of 10^{-7}M mepyramine and 10^{-5}M cimetidine on responses to HIS in partially contracted distal segments (n = 6).

concentrations of antagonists, supported the hypothesis that the relaxant effects were produced by H_2 receptor stimulation. A single HIS dose response trial, obtained in the presence of mepyramine (10^{-7}M) without precontracting strips, produced graded relaxant activity of low magnitude (about 20 mg) in both segments. Exposure to doses of HIS greater than 10 µg/ml produced diminished relaxant responses, (that is, contractile effects). Repetition of the HIS relaxant trial in precontracted strips produced no change in the responses with the second exposure. It was demonstrated that the HIS relaxant effects were not related to the use of PGs to partially contract the strips since the use of 5HT in its place yielded similar results. Metiamide, (10^{-6}M) another H_2-HIS recptor antagonist also produced blockade of the relaxant response to HIS.

In four experiments, following the relaxant response to HIS in the presence of mepyramine and prior to the addition of cimetidine, propranolol was added to the superfusate and the partially contracted strips were stimulated with HIS. Indomethacin was then used to replace propranolol and the procedure was repeated. These steps were undertaken to confirm that the relaxant effect was specifically a function of HIS receptor stimulation and not, in part, a beta adrenergic receptor effect, and to determine if this relaxant effect was attributable to HIS receptor mediated prostaglandin synthesis. Propranolol, at a concentration of 10^{-7}M which was sufficient to produce significant inhibition of the relaxant response to IsoP (see

figs. 3-28 and 3-29) failed to block the relaxant action of HIS. Although relative to the reference response, the mean HIS responses in the presence of mepyramine plus propranolol were greater than control responses, these differences were not significant and more likely related to the increased (not significant) prostaglandin response in the presence of propranolol. HIS relaxant responses in the presence of indomethacin 10^{-5}M (which had previously been demonstrated to produce considerable inhibition of AA responses), were not significantly antagonized in distal segments and only at high doses of 3 and 10 μg/ml in proximal segments were mean responses significantly less, but only by a small amount, than control responses. However, relative to the magnitudes of the PG induced contractions, (see fig. 3-34), there were no significant differences in the mean HIS relaxant responses indicating that both propranolol and indomethacin do not significantly antagonize the H_2-HIS receptor.

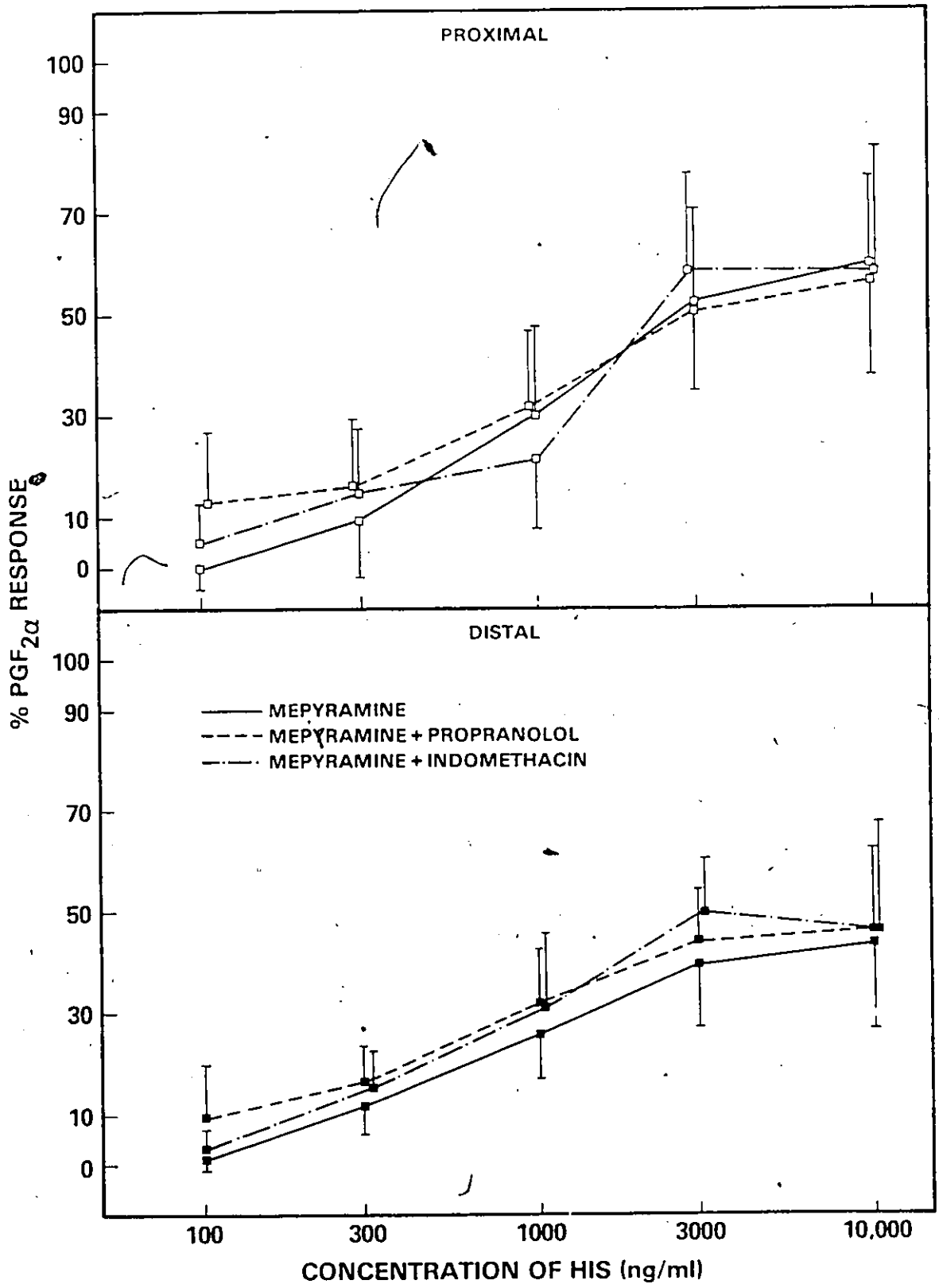


Fig. 3-34. The effects of 10^{-7}M propranolol and 10^{-5}M indomethacin on HIS relaxant activity in the presence of 10^{-7}M mepyramine in rabbit IPA. (n = 4).

CHAPTER 7

Summary of Major Findings

1. A method of preparing longitudinal strips from intrapulmonary arteries of the rabbit is given. Strips of proximal and distal segments of rabbit intrapulmonary artery responded to several agonists. No sex related differences in responses were seen.
2. At low baseline tensions the DIPA segment frequently responded in a biphasic manner characterized by an abrupt decline in tension (relaxant phase) followed by an increase in tension (contractile phase).
3. Passive and active isometric tension development was a function of length in both proximal and distal segments. There were, however, some differences in length-tension characteristics between the two segments.
4. The histological structure of the proximal segment was typical of a large elastic artery with many layers of elastin with smooth muscle cells interposed while the distal segment possessed fewer elastic laminae and loosely arranged smooth muscle cells in the media.
5. 5HT contracted both PIPA and DIPA segments in a dose dependent manner. Proximal segment maximal effects and sensitivity were significantly greater than those of the distal segment.

Methysergide (10^{-8}M) antagonized responses of both segments to 5HT.

6. HIS contracted both PIPA and DIPA segments in a dose dependent manner. Some strips exhibited marked tachyphylaxis in response to repetitive HIS dose-response trials. Although maximal effects were similar in both segments, the sensitivity of the distal segment was greater by a small but significant amount. Cimetidine (10^{-5}M) potentiated some responses to high doses of HIS. Mepyramine (10^{-9}M) antagonised responses to HIS, indicating that HIS contractile effects are mediated by HIS H_1 receptor stimulation.

7. In the presence of high doses of mepyramine, HIS elicited dose dependent relaxation of precontracted segments of proximal and distal intrapulmonary artery. Propranolol (10^{-7}M) and indomethacin (10^{-5}M) failed to block this relaxant action of HIS. However, cimetidine (10^{-5}M) did antagonize this relaxation indicating that HIS relaxant effects are mediated by H_2-HIS receptor stimulation.

8. PIPA segments contracted in a dose dependent manner in response to NE while the distal segment responded poorly with slight contractile and relaxant effects. At high doses the response to NE was characterized by a decline in active tension. Propranolol (10^{-7}M) blocked the decline in active tension to high doses of NE producing dose dependent contractile activity in both segments. However, PIPA segment responses

to NE in the presence of propranolol were inhibited except at high doses. Phentolamine (10^{-8}M) inhibited contractile responses to NE.

9. The presence of beta receptors in both PIPA and DIPA segments was demonstrated by the addition of IsoP to partially contracted segments which produced dose dependent relaxation of similar magnitude in both segments. Inhibition of relaxant responses with high doses of IsoP was seen in proximal segments. Small relaxant effects at low doses and contractile effects at high doses of IsoP were observed in unstimulated strips. Propranolol (10^{-7}M) antagonized responses to IsoP.

10. Prostaglandins B_1, B_2 and $F_{2\alpha}$ produced dose dependent contractile activity in PIPA segments. Prostaglandins A_1, E_1 and E_2 produced little or no contractile responses or slight relaxant activity in unstimulated PIPA strips at low doses, however, at higher concentrations moderate contractile activity was produced by prostaglandins of the E series while PGA_1 produced contractile effects comparable to PGB_1 and PGB_2. In distal segments $PGF_{2\alpha}$ produced graded contractile activity while prostaglandins A_1, B_1, B_2, E_1 and E_2 appeared to produce little or no contractile responses or slight relaxant activity at low doses but at higher concentrations contractile activity was seen.

Indomethacin (10^{-4}M) inhibited responses to $PGF_{2\alpha}$ (1 μg/ml). Some intrapulmonary arteries were very poorly responsive to prostaglandins.

11. AA contracted both PIPA and DIPA segments in a dose dependent manner. Maximal effects and ED_{50} values were similar in both segments. Indomethacin (10^{-5}M) produced significant inhibition of AA responses while 10^{-4}M indomethacin produced almost complete blockade.

CHAPTER 8

Discussion

Introduction.

The experimental results confirm that rabbit intrapulmonary arteries are responsive to a number of vasoactive agents present in lung tissue or circulating blood. Their contractile and relaxant activity suggest that the main lobar branches may play a significant contributory role in altering pulmonary vascular resistance and in modifying blood volume by altering pulmonary vascular capacity. The observed differences in isolated proximal and distal segment responses support the original hypothesis that some regional differences in arterial responsiveness exist in the pulmonary vasculature.

The results presented here confirm the adequacy of superfusion in the analysis of isolated intrapulmonary vascular strip responses. The more traditional alternative, submersion of the tissue strip in a bath, is equally suitable for the experimental work described. Certain advantages and disadvantages may be attributed to either approach to studying vessel strip responses. However, superfusion was employed primarily in consideration of future experimental work since it offers the potential for concurrent measurement of mechanical responses and neurotransmitter release or endogenous production of vasoactive substances.

It is evident from the experimental results that the isolated longitudinal intrapulmonary arterial strips are capable of developing significant isometric tension. In vivo, activation of vascular smooth muscle may cause constriction or isometric contraction which is produced by generating active stress at constant vessel dimensions (21). Dobrin (21) describes arterial motion, in vivo, as occurring predominantly in the circumferential direction with minimal movement in the longitudinal direction during each cardiac cycle. The constancy of vessel length, in situ, results from the local constraints provided by perivascular connective tissue and by arterial side branches and opposes longitudinal wall stress developed with smooth muscle activation (21).

The use of a longitudinal segment of intrapulmonary artery in the present study contrasts with the previous use of helical and, infrequently, ring segments. In general, vascular smooth muscle cells of the tunica media are arranged in a low pitch helix (11), however, the angle of the helix and any changes in it with decreasing lumenal diameter are poorly described. As well, excised arteries retract to about two-thirds of their in situ length permitting the walls to thicken and intramural structures to retract and become reoriented (21). Consequently dissection of a helical segment offers little assurance that the bulk of smooth muscle is oriented along the longitudinal axis of the prepared strip. In contrast to helical segments, longitudinal segments are more easily prepared, particularly

in smaller arteries, and require less handling which may enhance their potential reactivity. Furthermore, reorientation of vascular wall constituents including smooth muscle in vessel strips along the axis of stretching is thought to occur (21). The use of ring segments, as described by Su and coworkers (89) appears eminently suitable for comparison of vascular responses from vessels of varying diameter, however, it is unlikely that superfusion could be easily adapted to such a preparation.

The results of the various experiments indicate a relatively high degree of variability in response which may reflect an inherent property of pulmonary arteries, perhaps specifically related to differences in bulk and/or orientation of pulmonary vascular smooth muscle. Damage to smooth muscle fibres from transverse cutting and orientation of smooth muscle at oblique angles to the axis of force measurement may contribute to this variability. Isolation of tissues may differentially alter arterial responsiveness and also account for the need to employ large agonist doses which, no doubt, exceed physiological levels. Tissue responsiveness did not appear to be influenced by rate of perfusion nor presence of side branches. Tachyphylaxis was not a significant problem although evidence of tachyphylaxis, in some tissues, to 5HT and particularly HIS was apparent when repetitive dose-response curves were examined. The lack of a consistent effect of hypoxic exposure suggests that the level of oxygen tension, over the range examined, is not critical to isolated tissue responsiveness at least over short periods (two hours) of time.

Length-Tension and Smooth Muscle Characteristics

The results of the length-tension experiments reveal that both passive and active tension is a function of arterial strip length. In this respect the pulmonary artery resembles other arterial strip preparations (9,30,36,77,81,82). However, in contrast to other systemic vascular strips (30,36,81,82) the pulmonary arterial segments were not capable of substantial tension development at short lengths, active tension relative to passive tension was small and the maximum active isometric tension per cross-sectional area was low. These discrepancies may reflect intrinsic differences in contractility between systemic and pulmonary arteries and/or result from the methodology employed.

The maximum active tensions developed in proximal and distal strips were $3.5 \times 10^4 \pm 1.5$ dynes/cm^2 and $17 \times 10^4 \pm 11$ dynes/cm^2 respectively. Although these values are less than 1/10 of those reported for systemic arteries (see Murphy, 1976) they compare favorably with the range of maximum active isometric stress values previously reported for dog (77) and rabbit (9) pulmonary artery which were $6.28 - 16 \times 10^4$ dynes/cm^2 (n = 11) and 3.5×10^4 dynes/cm^2 respectively. (Minor variations in experimental design and data calculation may contribute to differences in maximal values.) Dobrin (21) has suggested that this difference between systemic and pulmonary arteries may be attributed to the low smooth muscle content of the pulmonary artery which may be 25% or less of the wall volume (9) or alternatively to a true physiological difference in contractility.

Other contributing factors may include the helical orientation of smooth muscle cells relative to the axis of force measurement and possible impairment of smooth muscle function under in vitro conditions.

The differences in the proximal and distal length-tension relationships may be related to the observed differences in their histological structure since the various viscous elastic properties of the components of the vascular wall, that is, elastin, collagen and smooth muscle, do influence active and passive length tension relationships (2,21,43). Furthermore, it has been suggested that segmental differences in the relative content and arrangement of smooth muscle and elastin alter the accessibility of vasoactive agents and influence agonist reactivity (94). The appearance in the media of thick wave like elastic laminae with interposed helically oriented smooth muscle cells is similar to previous histological descriptions of pulmonary artery (25,35). The disappearance of many of the elastin bands in the segment of distal artery indicates a relatively rapid transition into muscular arteries. The arrangement and abundance of elastin in the proximal segment may offer significantly more resistance to extension and thus account for the linear nature of the passive tension-length relationship and the generally lower active tension development in this strip. The linear nature of the proximal segment curve and the magnitude of the active responses relative to passive tensions is similar to that obtained in ring segments of rabbit main

pulmonary artery (9). The concave appearance of the passive tension-length curve of the distal segment is typical of many systemic arteries (30,36,81,82). The presence of longitudinal smooth muscle, as noted in the distal segment of artery, has been previously described in canine main pulmonary artery (77).

Biphasic Response

It is difficult to account for the biphasic nature of agonist responses at low baseline tensions frequently seen in distal segments. The contractile phase of the response is essentially normal both in time course and relationship to length. On the other hand, the relaxation is unusual because of both its rapidity and presence only at short lengths. It would appear that at short lengths, the activation of smooth muscle may not be homogeneous throughout the vascular wall and consequently the layers of smooth muscle that do initially respond contract in such a manner that the active isometric force recorded externally is rapdily diminished. This might be accomplished if this smooth muscle was oriented perpendicular to the longitudinal axis of the strip. The subsequent development of tension (the second component of the biphasic response) must reflect force development in smooth muscle with a longitudinal component of orientation. Realignment of the vascular wall constituents along the longitudinal axis of the strip, with stretching would account for the loss of the relaxant phase. Structural features of myofilament arrangements in vascular

smooth muscle have prompted speculation that different myofilament units may contract at different rates and at different times (20).

5-Hydroxytryptamine (Serotonin)

5HT contracted proximal and distal rabbit intrapulmonary arterial segments in a dose related manner. This action of 5HT is the same as that described previously for dog (12,45,58), human (38), cat (5) and rabbit (12,58,89) intrapulmonary arteries[1]. The inhibition of 5HT responses by methysergide is consistent with previous in vitro findings with rat main pulmonary arteries (4) and canine intrapulmonary arteries (45). The results of the present study indicate that both the sensitivity and maximal effects of 5HT in proximal segments is greater than in distal segments.

Table 4 compares ED_{50} values for contractile effects obtained for 5HT, HIS, NE and AA in this and some other studies of pulmonary arterial responses to amines. The ED_{50} for the proximal segment response to 5HT very closely resembles that obtained by Su and coworkers (89) for rabbit IPA responses but is less than other values reported for canine IPA and rat MPA. However, Su and coworkers (89) reported that rabbit intrapulmonary arterial sensitivity for 5HT did not change with vessel calibre in contrast to the significant

[1]For each of the agonists, comparison of pulmonary vascular strip responses, both between species and with vessels taken from other vascular beds in the same species, must be cautiously interpreted since although similar responses may be observed, suggesting that the drug induced activity is similar, the same mechanisms of action need not apply.

Agonist	Present Work Proximal (mean ± S.D.)	Present Work Distal (mean ± S.D.)	Su et al (89) rabbit IPA (mean ± 95% C.I.)	Joiner et al (45) canine IPA (mean ± S.E.M.)	Park et al (69) rat MPA (mean ± S.E.M.
5HT	8.93×10^{-8} ± 4.46 n = 5	5.22×10^{-7} ± 3.21 n = 5	7.8×10^{-8} (2.2×10^{-8} - 2.7×10^{-7}) n = 18	1.1×10^{-7} ±0.01 n = 7	2.82×10^{-6} ± 1.13 n = 8
HIS	5.77×10^{-6} ± 0.99 n = 7	3.49×10^{-6} ± 0.82 n = 7	5.3×10^{-6} (1.5×10^{-6} - 1.8×10^{-5}) n = 21	not tested	no response
NE	3.79×10^{-8} ± 1.5 n = 10	(9×10^{-8})* n = 10	2.4×10^{-8} (7×10^{-9} - 8×10^{-8}) n = 23		2.58×10^{-8} ± 0.93 n = 8
AA	3.96×10^{-8} ± 1.21 n = 4	2.87×10^{-8} +1.50 n = 4			

*graphical estimation from mean dose response curve

TABLE 4 ED_{50} Values for Contractile Effects of 5HT, HIS, NE and AA on Pulmonary Arteries

difference in proximal and distal sensitivity to 5HT obtained in this study. However, it is not clear how Su and coworkers (89) determined ED_{50} values, whether by linear regression analysis as in the present study or by graphical estimation. Since linear regression analysis is less subject to observer bias, a difference in the method of ED_{50} determination could influence the precision of the results obtained. Despite no apparent influence of multiple agonist exposure, the data utilized in the present study for the determination of ED_{50} values was obtained only from the first exposure of a strip to an amine and subsequent trials with other agonists were not considered in the calculation of ED_{50} values, thus accounting for the relatively small sample sizes. It is unclear whether the same criteria were used by Su and coworkers (89). Perhaps as a result of the above described differences, in comparison to the present study, the statistical analysis of data by these workers expresses much greater variability.

There are a number of important similarities and differences between the results of the present study and those reported by Su and coworkers (89). The primary differences are the observations by Su and coworkers that maximal effects of 5HT, HIS and KCl are equal in all arterial segments and that the ED_{50} values of agonists did not change with arterial calibre. These findings contrast with the results of the present study which indicate a lower sensitivity and a reduction in maximal responses to 5HT in distal segments. (Of lesser importance are some other differences, to be discussed). The observation that the

distal segment response to 5HT is reduced was consistently obtained both in the initial series of experiments comparing amine responses using HIS as a reference response and in later trials utilizing a KCl standard. This difference in distal segment responsiveness to 5HT is unlikely to be attributable to smooth muscle damage or structurally determined since distal responses to other agonists (to be discussed) were comparable to proximal segment responses.

There are numerous methodological differences between the two studies including the use of ring segments in contrast to longitudinal segments and differences in passive stretch. It is possible that the geographic distribution of receptors is not uniform, for example, specific receptors may be clustered at sites of arterial branching and hence the type of preparation could influence the response of a segment to a particular agonist. There are differences between the two studies in passive stretch applied to segments, (passive stretch applied by Su and coworkers was 0.5 to 4.0 g depending on vessel size), however, it is difficult to appreciate how this difference could selectively inhibit distal 5HT responses without similiarly influencing distal responses to other agonists.

Alternatively, the observed differences in the 5HT response between the two studies may reflect an intrinsic difference in the strain of species studied. On the other hand, the presence of an inhibitory receptor for 5HT in distal segments could account for the decline in maximal effects and loss of sensitivity. Atypical

tryptamine receptors mediating relaxant effects which are not inhibited by methysergide, dibenamine or morphine have been described in sheep pulmonary veins but not arteries (24). Certainly, further investigation of the distal 5HT response is warranted in order to determine if the type of preparation is responsible for altering responsiveness. The significance of the distal segment responsiveness to 5HT is unknown, however, it would appear to offer some protection of the distal pulmonary circulation from platelet release of 5HT[1].

Histamine

The experimental results clearly show that HIS has a dual effect, eliciting both contraction and relaxation of isolated and superfused rabbit intrapulmonary arterial segments. The contractile effect of HIS in both proximal and distal arterial segments is mediated by HIS H_1 receptors while the relaxation induced by HIS, only after blockade of HIS H_1 receptors, is mediated by HIS H_2 receptors. The thresholds for responses, the concentrations yielding maximal effects and the maximal contractile and relaxant responses were similar in proximal and distal segments for both H_1 and H_2 HIS responses although the ED_{50} values for contractile responses exhibited a small but significant difference.

[1]The in vitro ability of an agonist to elicit a tissue response suggests a capacity for a similar action in vivo but does not indicate that such an action necessarily occurs.

Pulmonary contractile responses to HIS have been previously reported in numerous species both in vivo (see review by Owen, 1977) and in vitro. Human, (38), rabbit, (12,58,89,91), canine, (12,46,58,92), and cat (92) intrapulmonary arterial contractile responses to HIS have been described. Interestingly, in the rat, Thompson and coworkers (92) observed contractile responses to HIS while Park and coworkers (69) failed to obtain a response of the main pulmonary artery to HIS, despite few differences in methodology. Although contractions to HIS were consistently observed in the present study, Sundt and Winkelmann (91) described a lack of responsiveness in about 50% of small rabbit intrapulmonary arteries tested.

The ED_{50} values for HIS contractile responses (see Table 2) differed by a small but significant amount, the distal segment being more sensitive to the amine, however, both values resemble closely the ED_{50} reported by Su and coworkers (89), (see Table 4). This difference in proximal and distal ED_{50} values may reflect the small sample size relative to the other study in addition to the previously described differences in the determination of ED_{50} values. A significant difference in ED_{50} values does suggest a difference in affinity for the HIS receptor between the two segments which may reflect structural differences or the presence of H_2 HIS receptors. (The latter appears unsupported, however, by the observation that the HIS relaxant response was similar in both proximal and distal segments.)

The maximal developed contractile tension, relative to the reference response, was the same in both proximal and distal segments, thus agreeing with the results obtained by Su and coworkers (89). However, the HIS response in the present study exceeded both the reference reponse to K^+ and the maximal response to 5HT in both segments suggesting that HIS activitation of contractile units is more complete. This contrasts, for reasons unknown, with the observation that maximal contractile effect of 5HT, HIS and KCl were equal in all rabbit intrapulmonary arterial segments tested as reported by Su and coworkers (89).

It should be noted that in extrapulmonary segments the maximal response to HIS was significantly less than the response to 5HT, (the opposite of results obtained in proximal intrapulmonary segments). Relative to their respective 5HT maximal responses, the extrapulmonary contractile responses to all doses of HIS appeared reduced when compared to proximal intrapulmonary responses to HIS. The basis for this discrepancy was not further investigated, however, a plausible explanation may be a reduction in the number of H_1 HIS receptors, either in absolute numbers or relative to H_2 receptors, in extrapulmonary segments.

Although dilator responses to HIS have been previously demonstrated in intact and isolated lungs, (see review by Owen, 1977), only two previous reports (85,92) have described relaxant HIS reponses in isolated strips. Su (85) described a slight reduction in elevated

tone in response to HIS in rabbit MPA. Thompson and coworkers (92) reported that HIS caused relaxation of rat main pulmonary arteries during contractions caused by vasoactive agents (including HIS itself). However, relaxant effects were not consistently observed by Thompson and coworkers (92) nor were antagonists employed in either study to demonstrate that the response was due to HIS receptor stimulation. Of specific interest is that rabbit and cat pulmonary arteries were also tested but these failed to relax to HIS (92).

It has been suggested that HIS mediates relaxant effects by releasing epinephrine within the lungs or vascular wall producing beta adrenoreceptor mediated relaxation (92). In the present experiments the failure of propranolol to significantly inhibit HIS relaxant effects in the isolated and superfused rabbit intrapulmonary arteries suggests that the relaxation is a primary effect of HIS. Similarly the failure of indomethacin (10^{-5}M) to inhibit HIS mediated relaxation suggests that the relaxant response is not mediated by local prostaglandin synthesis.

The ability of mepyramine to inhibit HIS induced contractile responses is consistent with previous in vivo and in vitro findings. Joiner (46) has described blockade of isolated canine IPA arterial responses to HIS by the HIS H_1 receptor antagonist pyrilamine. The present study is the first to describe the effect of HIS H_2 receptor antagonists on HIS responses of isolated pulmonary arteries. Previous in vivo work has demonstrated HIS H_2 receptor antagonists effects

upon the intact or isolated pulmonary circulation in a number of species (see review by Owen, 1977). The observation that HIS is capable of eliciting both contractile and relaxant effects in the pulmonary circulation of the rabbit is consistent with the mechanisms described to account for the changes in systemic blood pressure of the rabbit in response to HIS, i.e. a balance between pressor responses due to HIS H_1 receptor mediated vasoconstriction and HIS H_2 receptor mediated vasodilation causing depressor responses (67).

The prior addition of cimetidine to the superfusate did not cause a consistently significant potentiation of the contractile effect of HIS at all doses. (As described previously some increases in the magnitude of response were evident at higher doses, especially in distal segments.) Such a potentiation might be expected since cimetidine blocks HIS H_2 receptors responsible for relaxation. However, a relatively high concentration of HIS H_1 antagonist was required to unmask the HIS H_2 receptor mediated relaxant response indicating that the contractile response is predominant. In the single experiment, previously described, in which mepyramine, the HIS H_1 receptor antagonist, was added to the superfusate prior to cimetidine, potentiation of the partially blocked contractile response to HIS was apparent. It is of interest to note that in a separate study examining HIS receptors, metiamide, another HIS H_2 receptor antagonist failed to potentiate the contractile effect of HIS in the rat stomach strip but not potentiate the response in aortic strips (22).

The physiological role of HIS in the lung is unclear. The results of the present experiments support the hypothesis previously proposed by Howard and coworkers (39) that HIS, because of its dual action on vascular smooth muscle, may play a role in the humoral regulation of pulmonary vascular tone. On the other hand, the weight of evidence suggests that HIS may regulate both local blood flow and ventilation since it possesses both bronchoconstrictor and vasoactive properties (92). Thompson and coworkers (92), however, have suggested that the distribution and proximity of mast cells, which contain a high proportion of lung HIS, to pulmonary vessels might enable their contents to affect the vessels without simultaneous action on airways.

Norepinephrine

The results of this study demonstrate that contractile responses to NE are restricted to the larger pulmonary arteries since the distal segments, of smaller lumenal diameter, responded poorly or not at all. The response of vessels downstream from the distal site to alpha adrenoreceptor stimulation is unknown. Previous experiments, in numerous species (8,38,65,69,77) have shown that isolated pulmonary arteries, contract in response to NE, however, most often the vascular segments studied were either of extrapulmonary origin or were intrapulmonary vessels of large diameter. Poor responsiveness of smaller intrapulmonary arteries was first described by Bohr, Goulet and Tanquini (12) using rabbit and canine vessels 0.2-0.3 mm in diameter. These workers obtained catecholamine responses only at very high

concentrations. Using a similar preparation of comparable size, Sundt and Winkelman (91) were unable to obtain responses to NE, E or IsoP in rabbits. However, these studies did not seek to determine regional differences in pulmonary responsiveness and thus comparison with responses of larger vessels, under the respective experimental conditions, was not undertaken.

Su and coworkers (89) did examine regional responses and found that the maximal contractile response to NE was comparable to that of KCl in arteries greater thatn 1.4 mm (outside diameter), only 30 - 40% of the KCl response in arteries 0.6 to 1.4 mm and small or absent in arteries less than 0.6 mm. It can be seen that in this respect that the results of the present study are in close agreement with those obtained by Su and coworkers. The proximal segment, from that portion of the intrapulmonary artery with a lumenal diameter of 1 mm or greater elicited a maximal response to NE of about 60% of the magnitude of the K^+ response while the distal segment, the mean lumenal diameter being less than 1 mm, elicited a maximal response of only 10%. The median effective dose (ED_{50}) obtained for proximal segment responses to NE was similar to that obtained by Su and coworkers (89) for all arterial preparations (see Table 4). The ED_{50} for distal segment responses estimated graphically, owing to a lack of responsiveness to NE by some segments, was of the same order of magnitude as the proximal segment.

Phentolamine inhibited contractile responses to lower concentrations of NE in both proximal and distal segments. The dose

response curve for the proximal strip was shifted to the right of the control response while the distal segment inhibition of contractions was more pronounced such that predominatly relaxant effects were observed. Previously, phentolamine has been shown to antagonize contractile responses in isolated dog (45,46) and toad intrapulmonary arteries (37).

In the proximal segment, at high concentrations, with increasing doses, the response to NE was found to decline indicating inhibition of the contractile response. The presence of phentolamine did not alter the nature of this response. Distal segments responded in a similar fashion although in the presence of phentolamine predominantly relaxant responses were obtained. These results suggest that in the rabbit intrapulmonary artery, NE, at high doses, can exert sufficient beta adrenoreceptor mediated relaxant activity to produce some inhibition of the alpha adrenergic contractile effect contributing to a lessening of its maximal effect. Somlyo and Woo (79) have previously demonstrated that beta adrenergic blockade can produce potentiation of NE induced contractile responses of the isolated chicken main pulmonary artery indicating autoinhibition of alpha adrenoreceptor mediated vasocontraction by beta adrenergic effects. Results obtained in vivo in the dog have shown that NE induced pulmonary vasodilatation after alpha adrenergic blockade (39).

In the present study, propranolol, (in a dose sufficient to antogonize beta adrenoreceptors), produced both inhibition of relaxant activity and potentiation of contractile responses at high doses of NE

in distal segments only. In proximal segments, although propranolol did inhibit relaxant activity, contractile effects were antagonized in three of four experiments, while in the fourth the maximum contractile response was potentiated. The basis of the propranolol mediated inhibition of contractile responses in proximal segments is unknown although one previous report (79) has described some depression of responses of isolated avian pulmonary artery at low doses only, (with potentiation at higher doses), in the presence of the beta adrenergic receptor antagonist pronethalol. Local anaesthetic properties have been attributed to propranolol suggesting that its inhibitory effect may be, in part, independent of receptor antagonism (73). Depression of systemic vascular strip responses to NE by pronethalol or propranolol have been described and attributed to non specific local anaesthetic effects and possibly some interaction with alpha adrenoreceptors (see Somlyo and Somlyo, 1970). However, the antagonism of NE was not consistently observed nor when tested was propranolol induced inhibition of $PGF_{2\alpha}$ or HIS contractile responses apparent. Consequently it is difficult to attribute to any specific mechanism, the observed inhibition of NE induced contractile responses by propranolol. Further investigation, employing a range of propranolol concentrations, would appear to be indicated in order to determine if this antagonist produces non-specific effects at the dose employed.

Thus the results of the present study confirm those obtained by Su and cowo[illegible]emonstrating regional differences in the response of the pulmonary artery to NE. The results suggest a variable

distribution of alpha adrenoreceptors in the pulmonary arterial tree, specifically a reduction in the number of receptors distally, although the nature of the responses of the vessels downstream to the distal site are unknown. Although a decrease in the number of alpha adrenoreceptors with diminuition of vessel size would account for the reduction in the magnitude of the contractile response to NE, alternate mechanisms to account for the poor distal segment reactivity include an alteration in the affinity of NE for its receptor or the presence of an inhibitory receptor for NE.

The significance of the poor reactivity of smaller arteries to NE is unknown. Whether it is specific to the rabbit pulmonary vascular bed has yet to be determined although Bohr et al (12) obtained similiar results with canine vessels. Su and coworkers (89) have speculated that the sympathetic control of pulmonary vascular tone is restricted to the relatively large arteries. Extensive arterial constriction of the pulmonary vasculature, through which the output of the right heart must pass during increased sympathetic activity may be undesirable. Clearly though the relative inability of the smaller pulmonary arteries to constrict in response to NE serves to undermine the suggestion that the catecholamines may be concerned with the regulation of pulmonary vascular tone (39) at least for the smaller (distal) vessels. However, the capacity for relaxant effects does suggest a possible role in local modulation of vessel calibre.

Isoproterenol

The results of this study indicate that IsoP produces relaxation of rabbit intrapulmonary arteries. However, in proximal segments using unstimulated strips, IsoP at high doses produced contractile activity and in partially contracted strips the relaxant response was diminished at the highest doses employed suggesting partial contractile activity. Although Su (85) and Park (68) have previously reported marked relaxation of precontracted rabbit EPA, Sundt and Winkelmann (91) failed to elicit any response of rabbit IPA to IsoP despite using high agonist doses. Contractile activity at high doses of IsoP has been previously described in isolated dog (77), cat (17), chicken (18) and bovine (23) pulmonary arteries. In the dog, Joiner and coworkers (46) observed relaxation of IPA at low doses of IsoP, and at higher doses, contractions; although in the presence of phentolamine, IsoP produced only relaxation. The ability of the beta adrenoreceptor antagonist, propranolol, to inhibit relaxant responses to IsoP confirms that the relaxation is mediated by beta adrenoreceptors present in rabbit pulmonary artery. Beta adrenoreceptor antagonists have been previously utilized to inhibit relaxant activity in rabbit (85), toad (37) and bovine (23) pulmonary arteries.

Thus IsoP appears to possess strong beta adrenergic and relatively weak alpha adrenergic effects. Given that the response to IsoP was similar in both proximal and distal segments the distribution of beta adrenoreceptors would appear to be uniform. The failure to

observe any contractile activity in distal strips is consistent with the hypothesis that there are few alpha adrenoreceptors present in this segment. The physiological significance of the role of beta adrenoreceptor mediated relaxation is unclear although it undoubtedly serves to modulate catecholamine induced vasoconstriction.

Prostaglandins

The proximal segment of rabbit intrapulmonary artery contracted to high concentrations of all prostaglandins tested. Prostaglandins B_1, B_2 and $F_{2\alpha}$ produced graded contractile activity over the range of doses applied to the strips while prostaglandins A_1, E_1, and E_2 at doses less than 10^{-5}M produced little or no contractile responses or slight relaxant activity. Although previous studies of the effects of prostaglandins on isolated intrapulmonary arteries have utilized other species, the actions of prostaglandins E_1, E_2 and $F_{2\alpha}$ on the main pulmonary artery of the rabbit have been previously described by Kitamura et al (56). These workers obtained contractile responses to $PGF_{2\alpha}$ but no contractions to PGE_1 or PGE_2 over a dose range of 10^{-9} to 10^{-6} g/ml, although, partial relaxation of precontracted arteries was obtained in response to PGE_1 & PGE_2 10^{-6} g/ml. In the present study, contractile responses to the E series of prostaglandins were obtained consistently at a dose of 10^{-5}M (10 μg/ml), however, concentrations of less than 10^{-5}M produced variable activity as previously described. On the single occasion that PGE_2 was applied to precontracted strips, partial

relaxation was obtained at low doses while 10^{-5}M PGE_2 increased isometric tension. Chand and Eyre (18) also observed biphasic responses, consisting of, slight relaxant effects at low doses and contractions at higher doses, to PGE_1 and PGE_2 in isolated chicken pulmonary artery. Similar biphasic effects in response to prostaglandins A_1, E_1, and E_2 have been reported for isolated skeletal muscle, mesenteric and renal arteries of the dog (84). In the isolated rabbit lung PGA_1 produced vasoconstriction (31), a response similar to that obtained in the present study at higher doses.

Comparison of the present results with those obtained in other species emphasizes the tremendous species variability that exists. Although the graded contractile response to $PGF_{2\alpha}$ is consistent with the effect of this prostaglandin on isolated intrapulmonary arteries of numerous species, the contractile effect of the B series of prostaglandins differs from previous reports of poor responsiveness to these prostaglandins in isolated dog intrapulmonary arteries (50). Responses to prostaglandins of the E series have been generally regarded as relaxant although contractile responses in monkey and human pulmonary arteries (51) have been described. PGA_1 has been previously tested only in canine pulmonary artery and produced relaxant responses (51).

Both interpretation and comparison of distal segment responses to prostaglandins A_1, B_1, B_2 and E_1 is hindered by the biphasic nature of the contractile response which was frequently obtained in these experiments. Little significance can be attached to the results

since the nature of the biphasic response was highly variable and its mechanism unknown. Thus it is not possible to accurately assess differences in segmental responses to prostaglandins and consequently repetition of these trials at a higher baseline tension might prove useful. Distal responses to $PGF_{2\alpha}$ and PGE_2 obtained in the first series of experiments were similar to proximal responses.

Doses of prostaglandins required to elicit maximal effects were very high but comparable to those employed by other investigators (47, 56). The observation that prostaglandin induced responses are slower to develop than amine responses has been previously noted (47). The ability of some of the prostaglandins (particularly PGE_2) to elicit relaxant activity at lower doses but contractions at high doses suggests the presence of mixed and dose dependent prostaglandin receptors. Alternatively the primary effect of the prostaglandin may be altered by secondary effects of prostaglandin exposure on the internal milieu of the tissue or on the endogenous prostaglandin production.

It was noted on a number of occasions that despite good responses to K^+ (and amines when tested), prostaglandin responses were unusually poor. This observation of poor responsiveness was supported by similar results obtained in a separate series of experiments at a later date, in which AA and $PGF_{2\alpha}$ only weakly stimulated the arterial segments. No relaxant effects in partially contracted strips could be demonstrated on that occasion. Similar observations have since been made by other investigators in this

laboratory. Thus some rabbit intrapulmonary arterial segments can be characterized as poorly responsive to prostaglandins and probably their precursors as well. No relationship to sex or weight of the rabbit nor length of dissection was apparent. A search of the literature has revealed no prior reference to any similar observation regarding isolated pulmonary vascular responses to prostaglandins. Whether the poor responsiveness to prostaglandins is an intrinsic property of the pulmonary arterial vasculature, e.g. a deficiency in prostaglandin receptors or whether it is due to environmental causes remains unknown.

The relatively high doses of prostaglandins required to produce effects upon the isolated tissues may reflect the fact that in the intact animal endogenous synthesis and release of prostaglandins produces primarily local effects (60). Consequently exogenous application of prostaglandins may not introduce sufficient quantities at intramural sites to effect rapid and marked tissue responses. Also it is possible that isolation of arterial strips or the preparation of prostaglandins with ethanol (see Altura and Altura, 1976) may markedly alter vascular reactivity to prostaglandins. Alternatively, other prostaglandins or intermediates of the prostaglandin biosynthetic pathway may play a more significant role in influencing pulmonary vascular tone. Prostaglandins have been implicated as mediators of local vascular tone and modulators of reactivity to other vasoactive agents (62) and the results of the present experiment support a potential role in these areas.

Arachidonic Acid

Both proximal and distal segments of rabbit IPA contracted in response to AA and there were no significant differences in the maximal response or median effective dose (see Table 2) between the two segments. Contractile responses to AA have been obtained by Gruetter and coworkers (32) using helical strips of rabbit IPA. However, a comparison of dose response curves obtained in the two studies, in which both utilized high KCl as a reference response, reveals much greater sensitivity and maximal contractile effects elicited in the present study. For example, the magnitude of the maximal contractile response to AA that Gruetter and coworkers (32) obtained was only about 50% of the reference response to KCl in constrast to a response of about 100% obtained in this study. It is not clear what methodological differences might account for this discrepancy in results, although it may be related to the use of an organ bath, by these workers, and the accumulation of a vasodilatory metabolite. In the same study by Gruetter and coworkers, AA failed to significantly contract bovine isolated IPA but did elicit a dose dependent relaxation of canine IPA indicating highly variable species specific responses to this prostaglandin precursor. In the present study marked tachyphylaxis was obtained with repetition of dose response curves to AA. Although Gruetter and coworkers (32) did not describe tachyphylaxis in their determination of pulmonary vessel strip responses, a similar tachyphylactic effect of AA, that was abolished by an activator of PG

synthetase, reduced glutathione, was observed using the longitudinal stomach strip of the rat (83).

The ability of 10^{-5}M indomethacin to significantly inhibit the action of AA on pulmonary arteries without antagonizing the response to $PGF_{2\alpha}$ suggests that the vascular contractile effects of AA require, at least in part, its enzymatic conversion to endoperoxides, thromboxanes, prostaglandins or other metabolites. However, indomethacin 10^{-4}M did significantly inhibit smooth muscle contractile responses to both AA and $PGF_{2\alpha}$ suggesting that high doses of indomethacin may possess a nonspecific effect. A similar inhibition by indomethacin, 10^{-4}M, of contractions to other agonists including 5HT and NE has been obtained in this laboratory by this and other investigators. An alternative explanation to account, in part, for indomethacin inhibition is that endogenous production of prostaglandins and/or their intermediates may be integral to contractile responses to some amines and exogenous prostaglandins.

The results of these experiments confirm that rabbit intrapulmonary arterial vascular smooth muscle exhibits the capacity, at least in vitro, for the enzymatic conversion of AA by cyclo-oxygenase to vasoconstrictor metabolites, probably intermediates or products of the prostaglandin biosynthetic pathway. It appears that there are no regional differences in the responsiveness of the arteries tested to AA. The ability of the rabbit pulmonary arterial vessels, downstream to those studied, to respond to AA in unknown, however, Gruetter and coworkers (32) were unable to obtain responses with

isolated rabbit intrapulmonary veins. The in vitro findings correlate well with studies in the intact rabbit which describe a marked pressor response to AA (see Hyman et al, 1978). Thus, the synthesis and release of vasoactive products of AA by the main lobar branches of intrapulmonary arteries may produce both local and downstream effects causing increased pulmonary vascular resistance.

Research Prosposals

The results of these experiments indicate that further investigations could prove fruitful. A thorough examination of the distal strip response to 5HT may reveal the basis for the poor reactivity of that segment. Further exploration of the pulmonary responses to catecholamines is essential for a more complete understanding of their role. Investigation of the effects of indomethacin and other cyclo-oxygenase inhibitors on arterial responses to vasoactive substances is indicated to determine if the cyclo-oxygenase pathway is integral to some drug induced activity. In addition, characterization of tissues poorly responsive to prostaglandins would be of value. A closer examination of the histology and length-tension characteristics could reveal important structural and/or functional properties. Finally, investigation for differential effects of other vasoactive agents including angiotensin and bradykinin is indicated.

Concluding Remarks

The pulmonary circulation is designed to serve the primary function of the lung which is gas exchange. Since the lungs receive the entire output of the right heart they are vulnerable to insult from the systemic release of humoral mediators and emboli. Furthermore pulmonary synthesis and/or release of vasoactive substances in pathophysiological episodes may also comprise pulmonary function. Given these considerations the following model attempts to explain some of the observed vascular responses to vasoactive agents.

The proximal segment of the left lower lobar artery controls the flow of blood to the entire lobe. Perhaps fulfilling a protective function it contracts maximally to 5HT and HIS, considered to be the principal mediators of a number of pathophysiological conditions, enabling blood flow to be diverted in part to an unaffected lobe. The submaximal response to alpha andrenoreceptor stimulation would reduce a generalized increase in pulmonary vascular resistance accompanying sympathetic discharge. On the other hand the distal segment of the intrapulmonary artery approaches the calibre of vessels concerned with the regulation of perfusion to discrete areas of parenchyma. Like the proximal segment, it responds maximally to HIS which also exhibits marked bronchoactive properties and may contribute at this level to the control of local blood flow and ventilation. The submaximal response to 5HT in the distal segment may protect blood flow allowing gas exchange. The very poor reactivity to NE may preserve efficient gas exchange in the presence of increased sympathetic activity. In

both segments the ability of H_2-HIS and beta adrenergic receptors to inhibit activity suggests a capacity for humoral modulation of vasoconstrictor responses. The vasoactivity of AA and the prostaglandins substantiates a possible role of these substances in the local regulation of pulmonary vascular tone and modulation of reactivity to other vasoactive agents in the pulmonary arterial circulation.

Thus in summarizing, rabbit intrapulmonary arteries respond to a wide number of vasoactive agents. The in vitro activity of these humoral substances and any regional differences in their arterial responsiveness have been described. It is possible that factors present in vivo may modify or alter their responses in the intact animal. Nevertheless the potential influence of these vasoactive agents on pulmonary vascular tone and reactivity is significant.

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APPENDIX

Results from these studies were presented at the annual meeting of the American Lung Association, May 1978. Abstracts of the experimental work may be found in the Am. Rev. Resp. Dis. 117 (4, part 2): 366, 1978 and 119 (4, part 2): 379, 1979.