Analysis of the Role of Endothelial Nitric Oxide in Regulating the Tone and Responses of Pulmonary Artery Rings to Drugs

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By

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CONTENTS

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ABBREVIATIONS

AC	Adenylate cyclase
ACh	Acetylcholine
AD	Adenosine
ADP	Adenosine 5'-diphosphate
Ang II	Angiotensin II
AT	Atropine
ATP	Adenosine 5'-triphosphate
BPA	Branch pulmonary artery
Cyclic AMP/cAMP	Adenosine-3' : 5' cyclic monophosphate
CARB	Carbachol
Cyclic GMP/cGMP	Guanosine-3' : 5' cyclic monophosphate
CCRC	Cumulative concentration response curve
CPRA	Chlorpheniramine
CRC	Concentration response curve
CSE	Cigarette smoke extract
FRC	Frequency response curves
HIST	Histamine
5-HT	5-hydroxytryptamine
IBMX	3-Isobutyl-1-methylxanthine
KCl	Potassium chloride
KB	Krebs buffer
L-Arg	L-arginine
L-NAME	Nω-nitro-L-arginine methyl ester
L-NOARG	N^G-nitro-L-arginine
MPA	Main pulmonary artery
MLCK	Myosin light-chain kinase
NA	Noradrenaline

HbO	Oxy-haemoglobin
PA	Pulmonary artery
PHE	Phenylephrine
PRAZ	Prazosin
PDE	Phosphodiesterase
SNP	Sodium nitroprusside
TXA_2	Thromboxane A_2
TTX	Tetrodotoxin

SUMMARY

The work presented here represents an examination of the effects of various drugs on the tone of vascular smooth muscle in isolated pulmonary artery rings mainly from Wistar rats and an investigation of contribution of endothelium-derived relaxing factor (EDRF, NO).

The results obtained in these studies are summarised below:

1) The vasoconstrictors, 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M), phenylephrine (PHE, 10^{-9} to 10^{-4}M), noradrenaline (NA, 10^{-10} to 10^{-6}M), angiotensin II (Ang II, 10^{-11} to 10^{-7}M) and the thromboxane A_2 (TXA_2) analogue, U46619 (10^{-9} to 10^{-6}M), induced concentration-dependent contractions in main (MPA) and branch (BPA) pulmonary artery rings from Wistar rats. BPA rings were more responsive than MPA rings to PHE (10^{-7} to 10^{-5}M), NA (10^{-9} to 10^{-7}M) and Ang II (10^{-9}M), whereas, there were no significant differences between MPA and BPA rings in responses to 5-HT or the thromboxane A_2 mimetic, U46619. In some experiments, prior treatment with Nω-nitro-L-arginine methyl ester (L-NAME, 5×10^{-4}M, a competitive inhibitor of L-arginine in endothelial cells) or mechanical removal of the endothelium by rubbing, potentiated responses of MPA and BPA rings to 5-HT or PHE. Cocaine (10^{-6}M) potentiated responses to NA (10^{-9} to 10^{-8}M) in MPA rings but had no effect in BPA rings .

2) Exposure of MPA and BPA rings for 30min or 1 hour to the concentration of 5-HT (10^{-4}M), that produced the maximum response caused a marked desensitisation to 5-HT. The desensitisation to 5-HT was most marked in intact rings. Prior treatment with L-NAME (5×10^{-4}M, for 15min) or mechanical removal of the endothelium, increased contractile responses to 5-HT and

reduced this desensitisation to 5-HT. Desensitisation to 5-HT in these rings, that had an intact endothelium, was reversed by oxidised haemoglobin (HbO, 1mg/ml) or L-NAME, but this did not occur in endothelium denuded rings or in rings pretreated with L-NAME. This effect of L-NAME was inhibited by L-arginine (L-Arg, 2×10^{-4}M). For comparison, exposure of these tissues for 2 hours to a high concentration of phenylephrine (PHE, 10^{-5}M) also produced a desensitisation to PHE but this was less than occurred after exposure to 5-HT. Also, repeated application of the concentration of Ang II (10^{-7}M) that produced the maximum response, induced rapid desensitisation to Ang II in MPA and BPA rings. Pretreatment of the rings with a subthreshold concentration of KCl (10^{-2}M, for 5min) but not with L-NAME (2×10^{-4}M) reversed the Ang II-induced desensitisation.

3) The endothelium-dependent vasorelaxants, ACh (10^{-9} to 3×10^{-6}M) or carbachol (CARB, 10^{-8} to 10^{-4}M) induced concentration-dependent relaxations in MPA rings and BPA rings, which had been precontracted with PHE (EC_{70}, 1.2×10^{-7}M). BPA rings were more responsive than MPA rings to ACh and to high concentrations of CARB (10^{-5} to 10^{-4}M). Pretreatment of these rings with the nitric oxide synthesis inhibitor, L-NAME (5×10^{-4}M, for 10min) or mechanical removal of the endothelium abolished ACh-induced relaxations to (10^{-9} to 10^{-6}M) of ACh. L-NAME, also abolished CARB-induced relaxations to low concentrations of CARB (10^{-8} to 10^{-6}M), but responses to high concentration (10^{-5} to 10^{-4}M) of CARB persisted.

4) The endothelium-independent vasorelaxant, sodium nitroprusside (SNP, 10^{-12} to 10^{-5}M) induced concentration-dependent relaxations in MPA and BPA rings, which had been precontracted with PHE (EC_{70}, 1.2×10^{-7}M) or 5-HT (EC_{50}, 10^{-5}M). MPA rings, which had been precontracted with PHE, were more responsive than BPA rings to low concentrations of SNP (10^{-11}M)

and also to high concentrations of SNP (10^{-7} to 10^{-6}M). There was a significant difference between responses to SNP (10^{-7}M) in MPA and BPA rings, which had been precontracted with 5-HT. Pretreatment of these rings with L-NAME (5×10^{-4}M, for 20min) did not affect responses to SNP in rings precontracted with either PHE or 5-HT.

Comparison of concentration-response curves to ACh and SNP in MPA rings and BPA rings, precontracted with PHE, indicated that SNP was more potent than ACh.

5) The purine adenine nucleotides, ATP and ADP induced concentration-dependent relaxations (10^{-8} to 10^{-5}M) in MPA and BPA rings, which had been precontracted with PHE (10^{-7}M). There were no significant differences between MPA rings and BPA rings in their responses to ATP but there were differences in responses to high concentrations (10^{-6} to 10^{-5}M) of ADP. Pretreatment of these tissues with L-NAME, (2×10^{-4}M, for 10min) or mechanical removal of the endothelium suppressed responses to all concentrations of ATP or ADP. Adenosine (AD, 3×10^{-6} to 10^{-4}M) induced concentration-dependent relaxations in MPA and BPA rings precontracted with PHE (10^{-7}M). There were no significant differences between the responses to AD in MPA rings and BPA rings. Pretreatment of the preparations with L-NAME (2×10^{-4}M, for 10min) depressed the responses in BPA rings to AD (10^{-5} to 5×10^{-5}M) and in MPA rings to the lowest of AD (5×10^{-6}M). In MPA rings, the mechanical removal of the endothelium by rubbing, depressed the response to (3×10^{-6}M) of AD.

6) Histamine (HIST, 10^{-6} to 10^{-3}M) induced concentration-dependent relaxations in MPA and BPA rings, which had been precontracted with PHE (10^{-7}M). Pretreatment of the preparations with L-NAME (2×10^{-4}M, for 10min) or with a potent H_1-receptor antagonist, chlorpheniramine (CPRA, 10^{-6}M, for

10min), inhibited the responses to all concentrations of HIST. BPA rings were more responsive than MPA rings to HIST (10^{-5} to 10^{-4}M).

7) The β-adrenoceptor agonist, isoproterenol (ISO, 10^{-10} to 10^{-5}M), induced concentration-dependent relaxations in MPA and BPA rings precontracted with PHE (10^{-7}M). Pretreatment of these rings with L-NAME (2×10^{-4}M, for 10min) depressed responses to low concentrations of ISO (MPA: 10^{-10} to 10^{-7}M; BPA: 10^{-9} to 10^{-8}M). Also, mechanical removal of the endothelium by rubbing, depressed the responses to low concentrations of ISO (MPA: 10^{-9} to 10^{-7}M; BPA: 10^{-9} to 10^{-8}M). MPA rings with an intact endothelium were more responsive than BPA rings to ISO, but there was no significant difference between MPA and BPA in their sensititivity to ISO when the endothelium was removed.

8) 5-HT (10^{-4} to 5×10^{-4}M) induced concentration-dependent relaxations in MPA and BPA rings, which had been precontracted with PHE (EC_{75}, 1.2×10^{-7}M). Pretreatment of the preparations with L-NAME (5×10^{-4}M, for 15min), or with the β-adrenoceptor antagonist, propranolol (PROP, 10^{-6}M, for 10min) and/or mechanical removal of the endothelium by rubbing, had no effect on 5-HT-induced relaxations.

9) The muscarinic-receptor antagonist, atropine (AT, 10^{-8} to 5×10^{-6}M) induced concentration-dependent relaxations in MPA and BPA rings, which had been precontracted with PHE (10^{-7}M) or 5-HT (10^{-5}M) but not with KCl 3×10^{-2}M). Pretreatment of the preparations with the nitric oxide synthase inhibitors, L-NAME (5×10^{-4}M, for 10min) or Nω-nitro-L-arginine (L-NOARG, 10^{-4}M, for 10min) or with a combination of oxy-haemoglobin (HbO, 1mg/ml, for 10min) and L-NAME (5×10^{-4}M, for 10min) together and/or mechanical removal of the endothelium, did not inhibit this effect of atropine. Also,

pretreatment of these rings with β-adrenoceptor antagonist, propranolol (10^{-6}M, for 10min) or with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 10^{-8}M, for 3min) had no effect. In intact endothelial rings but not in rings without endothelium, the vasorelaxant effect of atropine (5×10^{-6}M) was inhibited by L-NAME (5×10^{-4}M) and this effect of L-NAME was reversed by L-arginine (L-Arg, 2×10^{-4}M). MPA rings were more responsive to atropine than BPA rings.

10) Electrical field stimulation (EFS) induced frequency-dependent contractions in MPA rings. Pretreatment of these rings with L-NAME (2×10^{-4}M) enhanced the maximum responses to EFS.

11) In MPA and BPA rings, which had been precontracted with PHE; pretreatment with standard solutions of cigarette smoke extract (CSE, 1ml, for 10-15min), depressed vasorelaxant responses to ACh (10^{-9} to 10^{-5}M) but had no effect on relaxant responses to SNP (10^{-11} to 10^{-5}M). Also, pretreatment of MPA and BPA rings with CSE (2ml, for 10-15min), depressed contractile responses to PHE (10^{-8} to 10^{-5}M).

12) Noradrenaline (NA) and 5-hydroxytryptamine (5-HT) induced concentration-dependent contractions in MPA and BPA rings of sham operated and heart failure rabbits. There were no significant differences between responses of blood vessels from sham operated and HF rabbits to these agonists. Acetylcholine (ACh) induced concentration-dependent relaxations in these rings from sham operated and HF rabbits. There were significant differences between the responses in BPA rings only at the lowest concentration of ACh (10^{-9}M).

Chapter 1

INTRODUCTION

1 INTRODUCTION

The pulmonary circulation serves many important functions. Of these, the most vital is the maintenance of gas exchange through the constant flow of mixed venous blood through the pulmonary arterial circulation to the pulmonary capillaries, and the return of oxygenated blood through the pulmonary venous circulation to the left atrium (Guyton, 1992).

1.1 General Structure of Blood Vessels

All blood vessels have a number of structural features in common. Blood vessels are structurally adapted according to physiological requirements. Therefore, pulmonary arteries which supply a low-pressure system have thinner walls than do systemic arteries, such as the carotid or renal arteries, which supply a high-pressure system. Blood vessels form a network of tubes that carry blood away from the heart, transport it to the tissues of the body, and then return it to the heart.

1.1.1 *Arteries*

Arteries are the blood vessels that conduct blood away from the heart to the organs and tissues. They resist changes in blood pressure in their initial portions and regulate blood flow in terminal portions. It is customary to classify arteries into elastic (conducting) arteries and muscular (distributing) arteries. The walls of arteries are thick and strong and consist of three coats (Snell, 1984):

I) The tunica intima (internal coat) consists of a layer of endothelial cells lining the vessel's interior surface.

II) The tunica media (middle coat) consists chiefly of concentric layers of helically-arranged smooth muscle cells. Interposed among the smooth muscle

cells are variable amounts of elastic and reticular fibres and proteoglycans. In arteries the media is separated from the intima by an internal elastic lamina.

III) The tunica adventitia (outer coat) consists principally of longitudinally-oriented collagen and elastic fibres. The adventitial layer gradually becomes continuous with the enveloping connective tissue of the organ through which the vessel is running (Fig. 1.1).

1.1.2 *Pulmonary arteries*

The pulmonary trunk arises from the infundibulum of the right ventricle through the orifice of the pulmonary valve. The pulmonary trunk passes upward and backward into the concavity of the aortic arch, where it divides into the right and left main pulmonary arterial branches. The right main pulmonary arterial branch is slightly larger and longer than the left main pulmonary arterial branch (Murray & Nadel, 1988). The pulmonary artery is thin, with a wall thickness one-third that of the aorta. The main pulmonary arterial branches are all very short. However, all the pulmonary arteries, even the smaller arteries and arterioles, have much larger diameters than their counterpart systemic arteries. Since the vessels are very thin and distensible, the pulmonary arterial tree is very compliant. This large compliance allows the pulmonary arteries to accommodate the stroke volume output of the right ventricle.

Three types of pulmonary arteries can be identified in the normal adult human lung (Heath, 1969):

I) Elastic or conducting pulmonary arteries contain distinctive layers of elastic fibres embedded in a coat of muscle cells. The pulmonary artery trunk, its main branches, and all extralobular pulmonary arteries are of the elastic type.

II) Muscular or distributing pulmonary arteries have a thin medial layer of muscle sandwiched between well-delimited internal and external elastic laminae. Muscular pulmonary arteries lie within lung lobules and, hence accompany bronchioles.

III) Pulmonary arterioles are the terminal branches of the pulmonary arterial system; at their origin from muscular arteries they contain a partial layer of muscle that gradually disappears until the vessel wall consists only of endothelium and elastic lamina. Pulmonary arterioles supply alveolar ducts and alveoli.

1.2 Innervation

The pulmonary vessels are capable of reacting, by contraction or relaxation of vascular smooth muscle to neurogenic influences. Anatomical and histochemical techniques have demonstrated both adrenergic and cholinergic innervation of pulmonary arteries and adrenergic innervation of pulmonary veins (Kadowitz *et al.*, 1976; Knight *et al.*, 1981).

1.2.1 *Sympathetic nerves*

The pulmonary blood vessels are innervated by sympathetic fibres, but more sparsely than systemic vessels (Berne & Levy, 1988). The density of adrenergic innervation and the degree of penetration of the nerve terminals into the smooth muscle layers are of great strategic significance. In pulmonary arteries, the adrenergic nerve terminals are localised around the outside of the tunica media at the adventitia-medial junction, whereas in veins they innervate the deep medial smooth muscle layers (Bevan & Su, 1973; Ljung *et al.*, 1975). The principal neurotransmitter released on electrical stimulation of adrenergic nerves is noradrenaline (NA) which induces pulmonary vasoconstriction. Sympathetic stimulation usually causes pulmonary vasoconstriction but

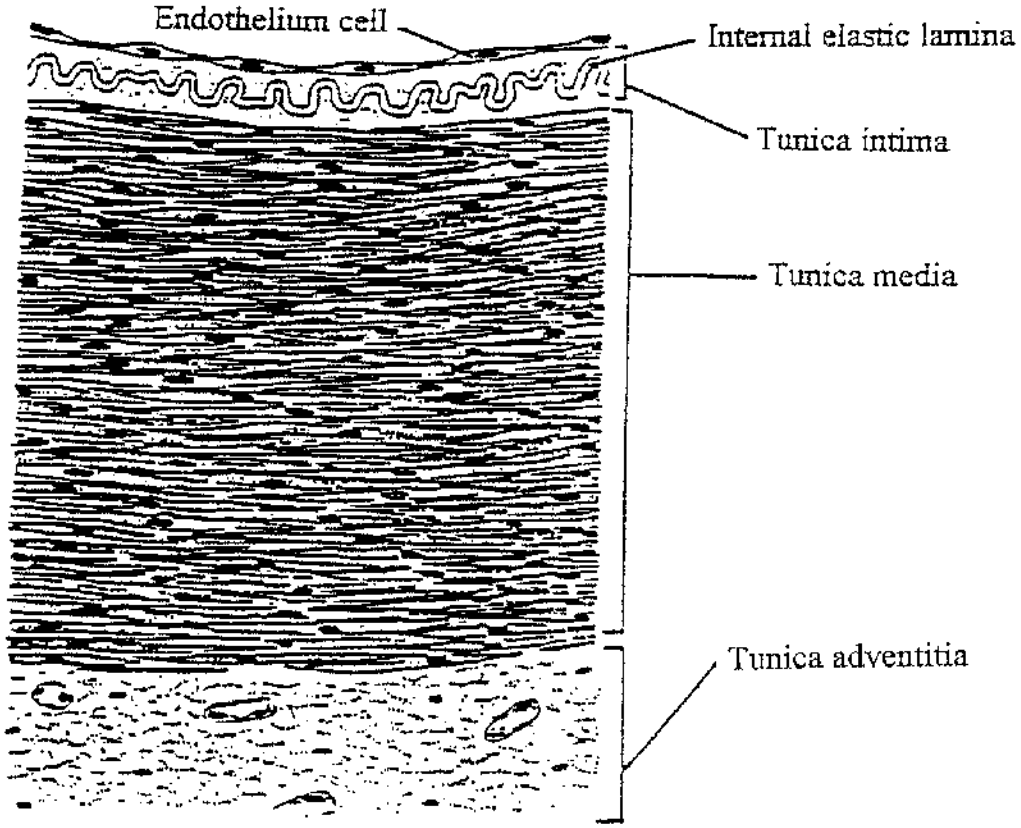

Fig. 1.1
Diagram showing general structure of a muscular artery

sometimes leads to a vasodilatation which is not abolished by atropine. Vasodilatation abolished by atropine occasionally followed vagal stimulation. Pharmacological studies indicate that α_1, α_2 and β_2-adrenoceptors are present in pulmonary blood vessels (Hyman *et al.*, 1981; Hyman & Kadowitz, 1985). Pre-junctional α-adrenoceptors and post-junctional α-adrenoceptors differ in their pharmacology and function. Pre-junctional α-adrenoceptors mediate the regulation of transmitter release from adrenergic nerve endings, whereas post-junctional α-adrenoceptors mediate contraction of vascular smooth muscle (Somlyo & Somlyo, 1970; Stjärne & Gripe, 1973; Strake *et al.*, 1975). Pre-junctional α-adrenoceptors were originally classified as α_2-adrenoceptors and post-junctional α-adrenoceptors may be either α_1 or α_2-adrenoceptors or of both types (Langer, 1974; Starke *et al.*, 1975; Wikberg, 1979).

1.2.2 *Parasympathetic nerves*

Unlike most systemic vessels, the pulmonary blood vessels receive some parasympathetic innervation. Cholinergic receptors respond to parasympathetic nerve stimulation by causing vasodilation of a previously constricted vascular bed (Nandiwada *et al.*, 1983). Cholinergic agonists vasodilate through the action of an intermediary substance, endothelium-derived relaxant factor (EDRF), which is produced by endothelial cells and acts on adjacent smooth muscle in the vessel wall. (Vanhoutte *et al.*, 1986). Although acetylcholine (ACh) is the principal parasympathetic neurotransmitter, there is no evidence that it is normally released in blood vessels to cause them to relax. Furthermore administration of the muscarinic receptor antagonist, atropine, to cat cerebral arteries does not block the neurogenic vasodilation induced by transmural nerve stimulation (Lee, 1980). Additionally, this model of neurogenic vasodilation does not require an intact endothelium, whereas ACh-mediated vasodilation is endothelium dependent (Lee *et al.*, 1984).

1.2.3 *Non-adrenergic non-cholinergic (NANC) nerves*

In the early 1960s Burnstock and his colleagues were able to show that when the responses to both adrenergic and cholinergic nerve stimulation had been blocked with guanethidine and atropine in guinea-pig intestinal preparations, large transient hyperpolarizations and relaxation were produced. Since these responses were abolished by tetrodotoxin, they were established as inhibitory junction potentials resulting from stimulation of NANC neurones. At the end of the 1960s, non-adrenergic non-cholinergic nerves were recognized in a wide variety of organs including the respiratory and cardiovascular systems (Burnstock, 1969). Experiments carried out to identify the transmitter of the non-adrenergic non-cholinergic neurones that supply to smooth muscle of the gastrointestinal tract showed that among many substances examined, including catecholamines, 5-hydroxytryptamine (5-HT), adenosine, etc., adenosine triphosphate (ATP) is the principal transmitter in these nerves (Burnstock, 1986). In 1971, nerves utilizing ATP as the principal transmitter were termed "purinergic" (Burnstock, 1971). Adenyl compounds cause dilatation of most blood vessels, but they have been found to cause both vasoconstriction of lung vessels and vasodilation, especially after previous vasoconstriction with 5-hydroxytryptamine. The nature of the response is dose-dependent. Gaddum and Holtz (1933) showed that ATP, adenosine and AMP caused vasodilatation in low doses, but caused vasoconstriction in higher doses. Hauge and colleagues (1966) found that the initial response of the perfused rabbit lung to ATP injected into the pulmonary arterial tubing was vasodilation, while after 10 minutes to 2 hours, this response changed to vasoconstriction.

1.2.4 *Co-transmission*

Some nerve cells store and release more than one transmitter (Burnstock, 1969). There is now considerable experimental support for the co-existence of peptides or purine nucleotides together with classical neurotransmitters

(Burnstock *et al.*, 1984; Burnstock, 1985; 1986). ATP and NA are released as co-transmitters from the sympathetic nerves supplying some blood vessels (Burnstock *et al.*, 1984).

1.3 Regulation of Pulmonary Vascular Tone

Vascular tone is determined by the degree of contraction maintained by the blood vessel (Morgan, 1987) and is influenced by a multiplicity of factors (Vanhoutte, 1978). The high resistance of the systemic circulation is largely caused by the muscular arterioles, which control the distribution of blood flow to various organs of the body. The pulmonary circulation does not have such thick walled small vessels. Pulmonary resistance, however, is not constant, but varies widely under a variety of conditions:

1.3.1 *Passive Factors:*

1.3.1.1 *Vascular pressure*

Pulmonary vascular resistance decreases as the pressure within the vessels rises. As pulmonary artery pressure rises, some capillaries that are usually closed, begin to conduct blood, thus lowering the overall resistance (Berne & Levy, 1988). In addition, as the vascular pressure rises, more and more capillary segments become distended. Because the distension of the pulmonary vascular bed has a limit, the effects of pulmonary arterial pressure changes on resistance are greater when left atrial pressure is low (Berne & Levy, 1988).

1.3.1.2 *Lung volume*

With large lung volumes the resistance of the extra-alveolar vessels decreases. In contrast, the resistance of the alveolar vessels increases. Because of the opposing effects of lung volume on the calibre of alveolar and extra-alveolar

vessels, total pulmonary vascular resistance usually is minimal at functional residual capacity (Berne & Levy, 1988).

1.3.1.3 *Left atrial pressure*

When the left side of the heart fails, blood beings to dam up in the left atrium. As a result, the left atrial pressure rises. Pulmonary arterial pressure rises when the left atrial pressure increases (Guyton, 1992).

1.3.2 *Active Factors:*

1.3.2.1 *Neural effects*

The pulmonary vasculature is innervated by both adrenergic and cholinergic systems (Dawson, 1984). Neurogenic alterations in vascular tone are predominatly produced by changes in the activity of sympathetic adrenergic nerves (Bevan & Su, 1973). It has been established that the pulmonary vascular bed is innervated by the adrenergic system and that stimulation of the adrenergic nerves increase pulmonary vascular resistances and decreases pulmonary vascular compliance (Ingram *et al.*, 1968; Daly *et al.*, 1970; Kadowitz & Hyman, 1973). The pulmonary vascular bed is also innervated by the parasympathetic system (Kadowitz *et al.*, 1976; Knight *et al.*, 1981), but the influence of parasympathetic nerve stimulation is uncertain. Pulmonary vasodilation has been demonstrated when the distal vagosympathetic trunk was stimulated (Daly & Hebb, 1952). Despite this innervation, there is little evidence that the autonomic nervous system controls vascular tone in the normal lung.

1.3.2.2 *Humoral effects*

In addition to the adrenergic and cholinergic innervation, the lung itself is a source of a large number of other vasoactive substances, which influence

pulmonary vasomotor tone when released (Bergofsky, 1980). Neuroepithelial cells in the airways contain various vasoactive substances (Keith *et al.*, 1981); and there are vasoactive peptides produced and/or stored within the lung (Said, 1982). The lungs are able to convert arachidonic acid into a number of pulmonary vasoactive substance, including various prostaglandins, their intermediates and metabolites, thromboxane A_2, and leukotrienes (Anggard & Samuelsson, 1965). In general, the potential effects of this large number of endogenous vasoactive substances on pulmonary vasomotor tone are inferred from their effects on the pulmonary vascular bed when infused as exogenous substances. Knowledge about their roles in the physiological responses of the pulmonary vasculature is relatively sparse. In some cases their potential effects on the pulmonary circulation may be complex. For example, 5-hydroxytryptamine (5-HT) is a potent pulmonary vasoconstrictor and blood contains a considerable amount of the 5-HT, but the normal plasma concentrations are low because most of the 5-HT is contained in the platelets (Paasonen, 1956). The effect of the 5-HT on the pulmonary vascular bed may also depend on the condition of the pulmonary endothelial cells which are normally capable of inactivating 5-HT (Gillis & Pitt, 1982). The 5-HT is metabolized in the endothelial cell of the lung by amine oxidases (Aviado, 1960; Gillis, 1973). Histamine is generally considered to be a pulmonary vasoconstrictor, but it causes vasodilation when the pulmonary vascular tone is abnormally elevated (Tucker *et al.*, 1976). Type H_1 and H_2 histamine receptors mediate pulmonary vasoconstrictor and vasodilator responses, respectively (Tucker *et al.*, 1975). Bradykinin can also be a pulmonary vasoconstrictor (Hauge *et al.*, 1966) or dilator (Levine *et al.*, 1973). In the rat bradykinin appears to be a pulmonary vasoconstrictor (Hauge, 1968), and in humans with pulmonary hypertension it is a vasodilator (De Freitas *et al.*, 1966). A major precursor of prostaglandins, arachidonic acid, is an active pulmonary vasoconstrictor (Okpako, 1972; Wicks *et al.*, 1976). $PGF_{2\alpha}$ is a

pulmonary vasoconstrictor. The mechanisms of action of $PGF_{2\alpha}$ have not been completely defined, but in the rodent, the excitatory action of this agent is associated with depolarization and reduction of smooth muscle cell membrane resistance (Kitamura *et al.*, 1976). PGE_1 is a vasodilator in lung and PGE_2 seems to be a vasoconstrictor (Kadowitz *et al.*, 1975). PGH_2 and thromboxane A_2 (TXA_2) are pulmonary vasoconstrictors. The pulmonary endothelium is a source of prostacyclin (PGI_2; Gryglewski *et al.*, 1978), which is a pulmonary vasodilator (Hyman & Kadowitz, 1986). Cyclo-oxygenase inhibition increases pulmonary vascular resistance and increases tone in isolated pulmonary arteries (Hadhazy *et al.*, 1983), thus there is speculation that prostacyclin (PGI_2) may be involved in maintenance of the normal low pulmonary vasomotor tone. Polypeptides, including angiotensin II formed from angiotensin I by the endothelial cell-converting enzyme, is a pulmonary vasoconstrictor (Aviado, 1960). Blood vessels are innervated by unmyelinated and thinly myelinated sensory neurons containing substance p (SP), neurokinin A (NKA), and calcitonin gene-related peptide (CGRP). Each of the sensory neuropeptides induces relaxation of blood vessels. The effect is mediated through receptors coupled to different second-messenger system, such as cyclic AMP (cAMP) and cyclic GMP (cGMP) and/or the release of endothelium-derived relaxing factor (EDRF). SP induces in precontracted rings of guinea-pig pulmonary a prompt but transient relaxation. In rings, in which the endothelium is removed beforehand, SP evokes a transient, small contraction and CGRP causes endothelium-independent relaxation (Maggi *et al.*, 1990). Vasoactive intestinal peptide (VIP) induces relaxation in isolated pulmonary blood vessels (Hand *et al.*, 1984).

1.3.2.3 *Hypoxia*

The response of pulmonary vasculature to hypoxia has been investigated extensively, and it is clear that in most species, hypoxia results in an increase in

total pulmonary vascular resistance. Hypoxic pulmonary vasoconstriction (HPV) is an important regulatory mechanism in matching regional blood flow and ventilation. Although hypoxic pulmonary vasoconstriction (HPV) has been demonstrated in large and small pulmonary arteries, the primary site of action of HPV appears to be the small muscular arteries (Voelkel, 1986). Despite the observation that hypoxia directly stimulates contraction of pulmonary vascular smooth muscle, the cellular mechanisms by which alveolar hypoxia causes HPV are still poorly understood. The pulmonary endothelium has been suggested to act as a hypoxia sensor (Holden & McCall, 1984), and both the endothelium-derived relaxing factor (EDRF) and contracting factor (EDCF), as well as other vasoactive mediators have been shown to play a modulatory role in HPV (Holden & McCall, 1984; Rubanyi & Vanhoutte, 1985a; Voelkel, 1986). Bioassay studies indicated that both the hypoxic relaxation in the systemic circulation and contraction in the pulmonary circulation are mediated by diffusable factor(s) released from the endothelium (Busse *et al.*, 1983; Rubanyi & Vanhoutte, 1985a). Indomethacin, a cyclo-oxygenase inhibitor had no effect on these responses (Rubanyi & Vanhoutte,1985a). Methylene blue, an inhibitor of soluble guanylate cyclase and nitro-L-arginine, an inhibitor of nitric oxide synthase, inhibited both hypoxia-induced relaxations and contractions (Rubanyi & Gräser, 1991). In rings without endothelium, the exogenous donor of nitric oxide, SIN-1, nitroglycerin, and dibutyryl cGMP inhibited hypoxic contractions (Gräser & Vanhoutte,1991). Hypoxia significantly decreased cGMP levels only in vascular preparations with endothelium (Gräser & Vanhoutte, 1991). These results suggest that hypoxic relaxation is due to the release of EDRF (NO) from the endothelium, whereas hypoxic contraction is the consequence of inhibition of the effect of endothelium-derived nitric oxide in vascular smooth muscle. It has been suggested that ATP and 5-HT may be released in response to hypoxia from endothelial cells (Hopwood *et al.*, 1986; Burnstock *et al.*, 1988). These substances may also contribute to the stimulated

release of EDRF (NO) during hypoxia. Hypoxia can also change smooth muscle tone by modulating adrenergic neurotransmission via the release of K^+ in certain vascular preparation (Borda *et al.*, 1980). There is now evidence that in some species, especially in certain age groups, capillaries (Hakim *et al.*, 1983; Mazzone, 1984) and veins (Rivera-Estrada *et al.*, 1958; Morgan *et al.*, 1968) may also constrict. The pulmonary vein also contracts in response to hypoxia (Zhao *et al.*, 1993).

1.4 Vascular Smooth Muscle (VSM)

1.4.1 *Contraction*

Smooth muscle of pulmonary arteries are able to develop and maintain maximal contraction in response to hormones and neuromediators. The onset of the contraction is linked to an increase in cytosolic calcium concentration $[Ca^{2+}]_i$ and subsequent formation of a calcium-calmodulin-myosin light chain kinase (MLCK) complex leading to the phosphorylation of myosin light chains (Adelstein & Hataway, 1979; Murphy *et al.*, 1983). In contrast, the mechanism responsible for the maintenance of tone which occurs together with a decrease in both $[Ca^{2+}]_i$ and phosphorylation of myosin light chains (MLC), remains unclear (Jiang & Morgan, 1989). The agonists produce a host of physiological responses in their target tissue as a result of their interaction with specific cell surface receptors. In addition to receptors that regulate cyclic AMP (cAMP) formation, there is another major type of cell surface receptor which mediates their effects via an increase in cytosolic Ca^{2+}. Such receptors are referred to as Ca^{2+}-mobilizing receptors which affect intracellular Ca^{2+} levels via hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). There has been increasing interest in the polyphosphoinositides (PPI) as a result of two major findings: ***(a)*** that their hydrolysis by phospholipase C (PLC) is coupled to the activation of

Ca^{2+}-mobilizing receptors such as cholinergic muscarinic receptors (Abdel-Latif *et al.*, 1977; Akhtar & Abdel-Latif, 1984), α_1-adrenoceptors (Abdel-Latif *et al.*, 1978), vasopressin receptors, etc., and ***(b)*** that the products of the phospholipase C (PLC) hydrolysis, namely DAG and IP_3, may serve as intracellular second messengers. Further degradation of DAG by specific lipases also leads to the release of arachidonic acid (AA), the precursor of prostaglandins (PGs), thromboxanes and leukotrienes. There is now growing experimental evidence which suggests that IP_3 and DAG synergistically mediate signal transduction in receptor systems linked to Ca^{2+}-mobilization. IP_3 has been shown to be involved in the release of Ca^{2+} from intracellular stores, such as the endoplasmic reticulum (ER), sarcoplasmic reticulum (SR), and DAG activates the phospholipid sensitive protein kinase C (PKC), which phosphorylates specific proteins. Mechanisms that increase $[Ca^{2+}]_i$ include either Ca^{2+} influx, such as occurs with potassium chloride (KCl), or mobilization of intracellular calcium as a result of PI-hydrolysis and generation of the second messengers IP_3 and DAG (Berridge, 1981). Both of these pathways cause phosphorylation of the contractile proteins.

1.4.1.1 *The phosphatidylinositol pathway*

Hokin and Hokin (1955; 1958) reported that phospholipids, later identified as phosphatidylinositol (PI) and phosphatidic acid (PA), may have an important role in receptor-mediated cell responses. Several years later, Michell (1975) listed a number of tissues which showed a PI effect in response to various stimuli, and since then, more tissues including blood vessels have been added to the list (Takhar & Kirk, 1981; Villalbos-Molina *et al.*, 1982; Zeleznikar *et al.*, 1983). The coupling factor linking receptor and phospholipase C is probably a GTP-binding protein. There is good experimental evidence for the involvement of GTP-binding proteins in the coupling of various Ca^{2+}-mobilizing receptors to PIP_2 hydrolysis and Ca^{2+} mobilization. Thus, in permeabilized cells,

non-hydrolyzable analogues of GTP introduced into and then trapped within the cytosol are able to substitute for external ligands in stimulating histamine release, which is a well-defined Ca^{2+}-dependent process (Gomperts, 1983). These GTP analogues also stimulate serotonin release and DAG formation in permeabilized platelets (Haslam & Davidson, 1984). Enhanced PI or PPI turnover is involved in Ca^{2+}-gating at the plasma membrane. In 1975, Michell suggested that PI metabolism is triggered only by those receptors that control a rise in $[Ca^{2+}]_i$ which then acts as the second messenger for stimulating the functional response of the cell. He also suggested that PI breakdown precedes the entry of Ca^{2+} into cells and could therefore be a universal biochemical event intrinsic to the Ca^{2+}-gating mechanism (Michell, 1975; Michell & Kirk, 1981). Agonist-stimulated breakdown of polyphosphoinositides regulates the plasma membrane Ca^{2+}-pump. It has been suggested that IP_3 might act on a Ca^{2+}-channel in the plasma membrane (Kuno & Gardner, 1987). Irvine and Moor (1986) proposed that IP_4 has a role in regulating Ca^{2+} entry. They reported that the Ca^{2+}-releasing action of IP_3 is insufficient to activate the sea urchin eggs fully, and that Ca^{2+} entry due to IP_4 is necessary as well. Because IP_4 did not activate the eggs when injected alone, they speculated that the release of Ca^{2+} by IP_3 is a necessary prelude to the action of IP_4.

1.4.1.2 *Protein kinase C (PKC) pathway*

Another primary product of the stimulated PPI hydrolysis is diacylglycerol (DAG), which has an important second messenger function (Nishizuka, 1984; Hirasawa & Nishizuka, 1985; Kikkawa *et al.*, 1986). DAG, which is formed transiently at the plasma membrane, stimulates C-kinase (PKC) activity, which is dependent on Ca^{2+} and acidic phospholipids, particularly phosphatidylserine, to catalyze the phosphorylation of serine and threonine residues of various cellular proteins (Nishizuka,1984; Hirasawa & Nishizuka, 1985; Cockcroft & Gomperet, 1985). The Ca^{2+}/phospholipid-dependent protein kinase was first

purified from brain as a cyclic nucleotide-independent protein kinase which could be activated by a Ca^{2+}-dependent protease also present in brain (Takai, *et al.*, 1977). Without proteolysis, activation of the enzyme required phospholipids. A protein kinase which required Ca^{2+}, phosphatidylserine, and DAG for maximum activity has been demonstrated and named C-kinase (Kishomoto *et al.*, 1980). The enzyme is both cytosolic and membranous. Phorbol esters, such as phorbol-12-myristate-13-acetate (PMA) and 12-0-tetradecanoyl-phorbol-13-acetate (TPA), which are potent cell activators and co-carcinogens, are able to substitute for DAG at extremely low concentrations and directly activate C-kinase both *in vivo* and *in vitro* (Castagna *et al.*, 1982; Niedel *et al.*, 1983; Yamanishi *et al.*, 1983). This circumvents the physiological pathway of DAG production via receptor activation of phosphoinositide hydrolysis. DAG is formed transiently in the stimulated cell, and under resting conditions, its concentration in the plasma membrane is too low to be detected. The lack of DAG accumulation in the plasma membrane is due both to its rapid phosphorylation to phosphatidic acid (PA) by DAG kinase and to its further degradation by lipases. In spite of this rapid metabolism, changes in DAG levels have been reported on stimulation of phosphoinositide breakdown in smooth muscle (Yousufzai & Abdel-Latif, 1984). PKC is present in high concentration in vascular smooth muscle (Kuo *et al.*, 1980), and induces contraction of vascular smooth muscle by phosphorylation of MLC at a different site from that of MLCK (Nishikawa *et al.*, 1984; Ikebe *et al.*, 1985; Itoh, *et al.*, 1986).

1.4.2 *Relaxation*

Vascular muscle relaxes when intracellular Ca^{2+} falls below a threshold level. The removal of Ca^{2+} from the cytoplasm is brought about by a combination of Ca^{2+} accumulation into the sarcoplasmic reticulum and extrusion from the cell across the plasma membrane. The major route of Ca^{2+} removal is probably

the uptake of Ca^{2+} into the sarcoplasmic reticulum (Kargacin & Fay, 1991), which is accomplished by a Ca^{2+}, Mg^{2+}-ATPase (Ca^{2+}-pump) that undergoes Ca^{2+}-dependent phosphorylation (Sumida *et al.*, 1984). The activity of the pump is regulated by phospholamban, a protein that is localized to the sarcoplasmic reticulum (Ferguson *et al.*, 1988) and is modulated by cyclic nucleotide-dependent phosphorylation (Eggermont *et al.*, 1988a). The plasma membrane contains a distinct Ca^{2+}, Mg^{2+}-ATPase, which extrudes Ca^{2+} from the cell (Eggermont *et al.*, 1988b). This ATPase binds calmodulin so that its activity is stimulated when cytoplasmic levels of Ca^{2+} rise. Smooth muscle membrane preparations have also been shown to contain a Na^{+}-Ca^{2+} exchanger (Grover *et al.*, 1983). This may contribute to Ca^{2+} extrusion in some blood vessels (Aaronson *et al.*, 1991), but it is still debated how much it contributes in others. The relative contribution of each of these Ca^{2+}-removal processes may well vary among different vascular bed. The most important agents that control vascular relaxation are the cyclic nucleotides; cAMP and cGMP, which act through activation of cAMP-dependent protein kinase (PKA), and cGMP-dependent protein kinase (PKG), respectively and through phosphorylation of targets such as MLCK.

1.4.2.1 *Cyclic AMP (cAMP)*

Following the discovery of the role of the cyclic AMP by Sutherland and Rall (1957), cyclic AMP has been of major importance in helping to understand many of the mechanisms underlying the effects of neurotransmitters, hormones and drugs on their target tissues. Cyclic AMP is synthesised within a cell by the action of the enzyme adenylate cyclase (AC) on ATP, following agonist-receptor interaction. However, because receptors lie on the outer surface of the membrane, a G-protein which lies within the membrane is necessary to link the receptor to adenylate cyclase (AC) which faces inside the cell. The cyclic nucleotide acts within the cell on cyclic AMP-dependent protein kinase (PKA)

and is degraded by phosphodiesterases (PDE). Elevation of cyclic AMP produces its effect by four different methods:

(a) cyclic AMP promotes Ca^{2+} extrusion from the cell by activating pumps in the membrane, **(b)** it promotes uptake of Ca^{2+} into intracellular stores, **(c)** it decreases the sensitivity of myosin light chain kinase (MLCK) to Ca^{2+} , and **(d)** it reduces Ca^{2+} entry into the cell by inactivating voltage-operated channels (VOC). Many smooth muscle relaxants produce their effects by stimulating adenylate cyclase activity with a subsequent increase in the level of cAMP and a reduction in $[Ca^{2+}]_i$. These agents include adenosine (Burnstock & kennedy, 1985), vasoactive intestinal peptide (VIP, Hand *et al.*, 1984), histamine (Tucker *et al.*, 1975), prostaglandins (Bolton, 1979), and forskolin (Seamon & Daly, 1986), as well as the β-adrenoceptor agonists such as isoprenaline (Bolton, 1979; Hardman, 1981). One of the actions of cyclic AMP to reduce free Ca^{2+} is by promoting extrusion mechanisms as seen in the isoprenaline-induced relaxation of smooth muscle (Marshall & Kroeger, 1973). This Ca^{2+} extrusion could be due to activation of Na^+-Ca^{2+} exchange resulting from a cyclic AMP-dependent increase in Na^+-K^+-ATPase (Scheid *et al.*, 1979). Relaxation of smooth muscle by reduction in free $[Ca^{2+}]_i$ can be achieved not only by Ca^{2+} extrusion from the cell but also by uptake of Ca^{2+} into intracellular stores. Cyclic AMP along with cyclic AMP-dependent protein kinase have been implicated in the accumulation of Ca^{2+} in storage sites in smooth muscle, mainly the sarcoplasmic reticulum. The third method by which cyclic AMP produces relaxation involves the suppression of Ca^{2+} binding by the contractile proteins, actin and myosin (Rüegg *et al.*, 1981). More recently, a fourth method by which the second messenger, cyclic AMP, can induce relaxation has been noted. Cyclic AMP can modulate ion channels and is known to inhibit Ca^{2+} entry through voltage-operated ion channels (Meisheri & van Breemen, 1982). Relaxation mediated by cyclic AMP is produced by many signal pathways, all of which involve reduction in $[Ca^{2+}]_i$ within the cell.

1.4.2.2 *Cyclic GMP (cGMP)*

The actions of cyclic GMP are much less clear than those of cyclic AMP, although cyclic GMP is known to promote relaxation by impairing MLCK activity and accelerating Ca^{2+} extrusion from the cells. However, the synthesis and function of cyclic GMP has been studied and extensively reviewed by Waldman and Murad (1987). A relationship between raised levels of cyclic GMP and relaxation in response to drugs and inhibitory NANC stimulation has been noted in many smooth muscles (Bowman & Drummond, 1984). Rises in cyclic GMP also accompany relaxation produced by a number of drugs in a variety of vascular and non-vascular smooth muscles (Rapoport & Murad, 1983). A group of drugs, collectively termed nitrovasodilators, has been helpful in elucidating the mechanisms by which cyclic GMP may be involved in smooth muscle relaxation. These drugs have been used since the nineteenth century following the synthesis of amylnitrite (Balard, 1844). Since then, this group has grown to include nitroprusside, nitroglycerin, hydroxylamine and sodium nitrate, which produce relaxation by activation of guanylate cyclase and raised levels of cyclic GMP (Schultz *et al.*, 1977). Nitrovasodilators generate nitric oxide either spontaneously or enzymatically and this free radical is the proximal activator of guanylate cyclase (Murad & Aurbach, 1977; Murad *et al.*, 1981). It has been shown that EDRF and probably the inhibitory factor released from some inhibitory NANC nerves (Moncada *et al.*, 1988; Gillespie & Sheng, 1988; Martin *et al.*, 1988) are nitric oxide or a closely related substance. EDRF and the inhibitory factor produce increased levels of cyclic GMP in their target organs. This suggests that the actions of nitric oxide are indeed mediated by cyclic GMP. Furthermore agents which inhibit guanylate cyclase activity and hence the production of cyclic GMP, including methylene blue, cyanide and ferricyanide also inhibit relaxation induced by nitrovasodilators (Katsuki *et al.*, 1977; Holzmann, 1983). The effects of cyclic GMP were recently proposed to

be mediated via cyclic nucleotide kinases with subsequent protein phosphorylation.

1.5 The Vascular Endothelium

The vascular endothelium comprises a single layer of cells, resting on a basement membrane, which lines the luminal side of every blood vessel within the body, as well as the chambers of the heart. In addition to regulating vascular smooth muscle tone by a number of vasoactive agents which are released from cells into the bloodstream from nerve terminals in the vessel wall, the endothelium is also able to secrete a number of vasoactive substances, including two vasodilator substances, endothelium-derived relaxing factor (EDRF; Furchgott & Zawadzki, 1980b), and prostacyclin (PGI_2; Moncada *et al.*, 1976), and the endothelins, a group of vasoconstrictor peptides (Yanagisawa *et al.*, 1988). The endothelium also provides a non-thrombogenic lining to the blood vessel both due to the nature of the endothelial cell surface and through the release of EDRF and prostacyclin (PGI_2) which inhibit platelet aggregation (Azuma *et al.*, 1986; Radomski *et al.*, 1987a). Additionally, EDRF has been shown to inhibit platelet adhesion (Radomski *et al.*, 1987b), whilst prostacyclin (PGI_2) has been shown to inhibit the adhesion only of activated platelets (Fry *et al.*, 1980).

1.5.1 *Endothelium-derived relaxing factor (EDRF) and nitric oxide (NO)*

Furchgott and Zawadzki (1980b) demonstrated that acetylcholine-induced relaxation of pre-constricted rabbit aortic strips was dependent on the presence of the endothelium. Since that time, endothelium-dependent vasodilation has been elicited in vessels from a variety of mammalian species, by a number of pharmacological agents. These agents, including bradykinin, adenosine

triphosphate (ATP), adenosine diphosphate (ADP), thrombin, histamine, substance P (SP), arachidonic acid, platelet activating factor (PAF), 5-hydroxytryptamine (5-HT), vasopressin and calcium ionophore, A23187 (Furchgott, 1984; Griffith *et al.*, 1984; Vanhoutte *et al.*, 1986) stimulate the endothelium to release a very labile, humoral factor referred to as endothelium-derived relaxing factor (EDRF). Some of these substances exhibit endothelium-dependent relaxation only or predominantly in some vascular beds and not in others. Endothelium-dependent relaxation of vascular smooth muscle has also been shown to be induced by hypoxia, increased blood flow and electrical stimulation (see Moncada *et al.*, 1988, for review). There is increasing evidence that EDRF is nitric oxide (Palmer *et al.*, 1987, Ignarro *et al.*, 1987b) or a closely related nitrosothiol (Myers *et al.*, 1990). Several lines of evidence indicate that L-arginine (L-Arg) is the physiological precursor for the formation of nitric oxide in vascular tissues (Moncada *et al.*, 1989; Palmer *et al.*, 1988a; Sakuma *et al.*, 1988), following activation of the enzyme nitric oxide synthase. Nitric oxide appears to be derived from the terminal guanido nitrogen of L-Arg by a stereospecific enzymatic process (Palmer *et al.*, 1988b; Schmidt *et al.*, 1988). The synthesis of NO is Ca^{2+}-dependent, in particular, extracellular Ca^{2+} (Singer *et al.*, 1982; Griffith *et al.*, 1986). NO synthase was found to be a calmodulin-requiring enzyme, thereby explaining numerous reports of a crucial role for calcium in endothelial-dependent smooth muscle relaxation (Palmer & Moncada, 1989; Bredt & Snyder, 1990; Bredt *et al.*, 1991). Neurones in the peripheral (Gillespie *et al.*, 1989) and central nervous system (Garthwaite *et al.*, 1988), are able to produce nitric oxide, which modulates the activity of other neurones. Furthermore, cells of the immune system, most notably macrophages, can produce massive quantities of nitric oxide (Stuehr & Marletta, 1987; Iyengar *et al.*, 1987), following activation by bacterial endotoxin or cytokines such as interleukin-1, tumour necrosis factor and interferon-γ, and use this substance not as an intracellular messenger, but as a

means of killing invading microorganisms. Smooth muscle cells in the vascular wall have a similar capacity to synthesize large quantities of nitric oxide following such immunological stimulation, and this is believed to account for the profound fall in vascular tone associated with endotoxin shock (Kilbourn *et al.*, 1991; Gray *et al.*, 1991). EDRF directly activates soluble guanylate cyclase of vascular smooth muscle causing an increase in guanosine 3', 5'-cyclic monophosphate (cGMP) and thereby causing relaxation of the vascular smooth muscle. (Förstermann *et al.*, 1986; Furchgott & Zawadzki, 1980b). Selective inhibitors of cGMP phosphodiesterase, such as M&B 22948 (Zaprinast) and 2MY 5445, have been shown to potentiate endothelium-dependent relaxation (Martin *et al.*, 1986). Structural modification at one of the guanidino nitrogens of L-arginine has led to the development of a number of compounds, N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME; Rees *et al.*, 1989a; 1990; Moore *et al.*, 1990), that competitively inhibit nitric oxide synthase. These agents inhibit endothelium-dependent vasodilation *in vitro* and have a powerful hypertensive effect *in vivo*, indicating an important role for nitric oxide in the regulation of systemic blood pressure (Rees *et al.*, 1989b).

1.6 History of 5-Hydroxytryptamine (5-HT)

The endogenous vasoconstrictor substance serotonin (5-hydroxytryptamine) was demonstrated by Steven and Lee (1884) and later investigated by Brodie (1900), who proved that the serum obtained after blood clotting increased vascular tone. In 1933, Vialli and Erspamer found the presence of *enteramine* in the gastrointestinal tract (GIT). Rapport and colleagues (1947) reported the existence in serum of a vasotonic substance, which was originally called *serotonin.* The latter substance was identified as 5-HT (Rapport *et al.*, 1948; Rapport, 1949), and was found to be identical with enteramine. 5-HT was

subsequently found in the CNS (Bogdanski *et al.*, 1956; Brodie & Shore, 1957). It is believed that 5-HT plays an important role as a neurotransmitter and as a local hormone in the peripheral vascular system. At the peripheral level, 5-HT affects smooth muscle fibres, causing constriction or relaxation (Vanhoutte, 1985; Saxena & Villalon, 1991). 5-HT is involved in numerous physiological events, it affects various functions of the CNS, including. sleep, thermoregulation, learning and memory, sex and other functions (review by Zifa & Fillion, 1992). 5-HT is thought to play a role in various types of pathological conditions (review by Zifa & Fillion, 1992).

1.6.1 5-HT receptors

The effects of 5-HT in the cardiovascular system differ from species to species; obviously this results from activation of a number of different mechanisms, involving more than on type of 5-HT receptor (Ruffolo *et al.*, 1979). The 5-HT receptors have been divided into four classes: 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors (Bradley *et al.*, 1986; Fozard, 1990; Bockaert *et al.*, 1992). The 5-HT_1 class itself is heterogeneous and has been subclassified into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes which exhibit distinct pharmacological profiles and are coupled to different second messenger systems (Hoyer, 1989).
In the vascular system, 5-HT activates distinct 5-HT receptors to elicit either contraction or relaxation of vascular smooth muscle. Contraction of isolated vessels is mediated by 5-HT_2 receptors, which are blocked by the 5-HT_2 receptor antagonist ketanserin, or by 5-HT_1-like receptors which are characterized by a high agonist activity of 5-carboxamidotryptamine (5-CT) (Bradley *et al.*, 1986; Hamel & Bouchard, 1991; Bax *et al.*, 1992). The 5-HT-induced relaxation of vessels with intact endothelium or denuded of endothelium was shown to involve 5-HT_1-like receptor subtypes (Cocks & Angus 1983; Molderings *et al.* 1989; Mylecharane 1990).

1.6.2 *Desensitisation of the 5-HT_2 receptors*

Homologous desensitisation is a widespread phenomenon in which initial exposure to an agonist results in decreased cellular responsiveness on second exposure. 5-HT receptors, like many other receptors, are susceptible to agonist induced desensitisation. Thus, 5-HT_2 receptors located on cortical neurons (Leysen *et al.*, 1989), cerebellar granule cells (Dillon-Carter & Chuang, 1989), facial motoneurons (Aghajanian, 1990) and platelets (Kagaya *et al.*, 1990) undergo desensitisation. As with other receptors leading to activation of protein kinase C (PKC), desensitisation of 5-HT_2 receptors results from feedback inhibition mediated by protein kinase C (Roth *et al.*, 1986; Aghajanian, 1990). Such inhibition may serve (i) to protect cells from over stimulation, (ii) as a modulatory substrate by other regulators of cellular activity.

A second mechanism leading to loss of receptor mediated responsiveness is internalization or sequestration of receptors by which hydrophilic agonists are prevented access to receptors. Sequestration of β-adrenoceptors has been extensively studied (Waldo *et al.*, 1983; Toews *et al.*, 1984; Kassis *et al.*, 1986). Internalization of β-adrenoceptors occurs in the presence of high concentrations of agonist (Waldo *et al.*, 1983). Sequestration of $5HT_4$ receptors has been recently demonstrated (Ansanay *et al.*, 1992). Recently, multiple mechanisms of 5-HT_2 receptor desensitisation by Rahman and Neuman (1993) have been studied. They investigated mechanisms underlying desensitisation of 5-HT_2 receptors in rat sensory-motor cortex and concluded that 5-HT_2 receptors both induce and undergo several forms of desensitisation.

1.7 Cigarette Smoking

Cigarette smoking is one of the major risk factors for the development of atherosclerosis (Jacobs *et al.*, 1993). One of the most important health consequences of cigarette smoking in human subjects is the development of diffuse vascular injury. In the past, despite the fact that cigarette smoking caused alterations in the intima of the muscular pulmonary arteries and fibrosis and wall thickening of the small pulmonary arteries and arterioles, cigarette smoke (CS)-related research has mainly focused on the airways and alveoli. Nevertheless CS has been implicated in cardiovascular disease, but little research has been carried out to determine its implications on the pulmonary vasculature. This is surprising since the constituents of inhaled CS have immediate access to the pulmonary circulation and it is likely that these are the vessels most likely to be affected at an early stage. The pulmonary veins will be exposed to the highest concentration of CS at its site of absorption. Thereafter, with dilution in and diffusion from the vascular compartment and distribution to other sites, the concentration of CS attained in other blood vessels will be much lower. Therefore, it is reasonable to assume that if CS causes cardiovascular damage, impairment of pulmonary vascular function will be seen. Indeed, morphological abnormalities in endothelial cells have been observed in human subjects who have been smokers for fairly long periods of time (Rubinstein *et al.*, 1991). The chemical composition of CS is complex and it is difficult to determine which compound or compounds may be involved in vascular injury. It is known that CS contains approximately 10^{-14} free radical per inhalation (Noronha-Dutra *et al.*, 1993), mainly NO derived radicals in the gas phase and quinone radicals in the tar phase, as well as various tars, the vasoactive substance, CO, as well as acrolein, acetaldehyde and nicotine. Nicotine, being the major pharmacologically-active component of CS, has been considered as a mediator of vascular injury (Kershbaum *et al.*, 1961).

However, several studies have failed to demonstrate any consistent and significant vascular effects due to nicotine exposure. This project will seek to determine if smoking has any detrimental effects in the cardiovascular system. The lungs function to oxygenate the blood, but they also have a protective role by removing vasoactive substances from the bloodstream. Therefore, any impairment of lung vascular functioning caused by CS may have serious implications for the heart's functioning, since, the harmful vasoactive substances will not be removed and instead they will be transported directly to the heart with hazardous consequences, not only for the heart, but potentially for the rest of the systemic circulation.

1.8 Chronic Heart Failure (CHF)

Heart failure in man has been described as *" the inability to deliver blood because of structural changes from diverse causes "* (Stead, 1967). The most common cause is ischaemic heart disease although hypertension and genetic factors are also important. Heart failure may be acute or chronic. Congestive heart failure is the clinical syndrome which usually results from chronic heart failure (CHF). The congestion is caused by a build up of fluid in the cardiovascular system as the body attempts to compensate for a reduced blood pressure from the failing heart. This will often manifest itself as oedema, engorgement of vascular beds such as the liver, and distension of peripheral veins, especially in the neck. The site of the congestion depends on which side of the heart is affected.

In left sided heart failure (HF), fluid will accumulate in the pulmonary circulation, leading to pulmonary oedema. This causes dyspnoea, and this is most noticeable on exertion. This is the classical first symptom of CHF. Right sided failure, which often follows on from left sided failure, gives more generalized systemic oedema (Davidson, 1964). The body's natural reaction to

CHF is to increase sympathetic activity, which triggers a general neurohormonal excitation (Ferguson, 1993), leading to peripheral vasoconstriction, so increasing the total peripheral resistance. Unfortunately, as far as every other organ in the body is concerned, this is a maladaptive response, as it only serves to put further strain on the failing heart. Some of the effects of the neurohormonal responses to HF include an increase (i) in plasma levels of angiotensin II (Ang II), (ii) in aldosterone, (iii) in antidiuretic hormone (ADH) and (iv) in atrial natriuretic factor (ANF). Increased sympathetic activity also places extra strain on the heart by promoting fluid retention, and is responsible for the congestion and oedema. The modern treatment of choice for CHF is the angiotensin converting enzyme (ACE) inhibitors, such as captopril and enalapril. These drugs are indeed, amongst the only drugs which have been proven to prolong the life of CHF sufferers (Cohn *et al.*, 1986). Ang II is elevated during heart failure because the increased sympathetic activity activates β_1-adrenoreceptors on the juxtaglomerular cells of the kidney, which in turn secrete renin to increase, eventually the production of Ang II, which itself is a potent vasoconstrictor that will also stimulate aldosterone secretion from the adrenal cortex. Aldosterone acts on the kidney to promote Na^+, and therefore water, retention. Both of these actions put increasing strain on the failing heart.

1.9 The Aims of this study, which were:

i) Investigation of mechanical responses of rat isolated pulmonary artery rings to both vasoconstrictor and vasorelaxant drugs.

ii) Investigation of the role nitric oxide (NO) in the 5-hydroxytryptamine-induced desensitisation in rat isolated pulmonary artery rings.

iii) Investigation of the effects of cigarette smoke on the responsiveness of the pulmonary vasculature of rat to both vasoconstrictor and vasorelaxant drugs.

iv) Investigation of the responses of rat isolated pulmonary artery rings to electrical field stimulation.

v) Investigation of the effects of vasoconstrictor and vasorelaxant drugs in isolated pulmonary artery rings of sham operated and HF rabbits.

Chapter 2

MATERIALS & METHODS

2 MATERIALS & METHODS

2.1 Experimental Rats

The rats used for research purposes were kept in a control area. Their food was obtained from Labsure CRM diet and ordinary tap water was used for their drinking. The temperature and light of the animal house were controlled. The temperature was maintained between 18-22°C and the lighting of the area was regulated in a cycle of twelve hours light (i.e. 6:30-18:30 h) and twelve hours dark.

2.2 Preparation of Artery Rings and Tension Recording

Male Wistar rats weighing 250-300g were killed by stunning and exsanguination. The heart and lungs were excised together and pinned on silicone rubber in a petri dish containing Krebs buffer (0°C) in an orientation that facilitated identification of the pulmonary arteries. The pulmonary arterial tree was rapidly dissected from the lung parenchyma with a small scissors and forceps. Segments of, the main pulmonary artery (MPA) and its first branches (BPA) were carefully cleared of adhering tissue and cut to obtain three rings (3-4mm in length); one MPA ring of approximately 3mm in diameter, and two BPA rings of approximately 1-2mm in diameter were transferred to fresh ice-cold Krebs buffer. Generally, the right BPA was found to be of a slightly larger diameter than the corresponding left BPA. Endothelial cells were removed from some rings by gently rubbing the intimal surface with a moist wooden stick for 30-60s (Furchgott & Zawadski, 1980a). The effectiveness of this procedure was subsequently investigated using acetylcholine (ACh, 10^{-6}M) which normally relaxed artery rings but had no such effect in rubbed rings (Furchgott & Zawadski, 1980a). Artery rings then were mounted under 1g

resting tension onto two 0.2mm shaped stainless steel wire hooks and/or one shaped and one triangular, (to prevent the tissue slipping off the holder, especially during stimulation) gently inserted into the lumen to avoid damage to the endothelium in 25ml organ baths containing Krebs-Henseleit solution maintained at 37°C, and containing (mM) NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24; Glucose, 11; gassed with mixture 95% O_2 and 5% CO_2, which gave a pH of 7.3-7.4. The top hook was connected by cotton [surgical silk, black braided, silk suture, EP2 (US 3/0), non absorbable - non sterile, 1001-40 Lot 92/43/9356] to a Grass FT03 isometric transducer while the bottom hook was connected to a stable glass tissue holder. A diagram of the experimental apparatus is shown in figure (2.1) Tension responses were recorded isometrically with Grass FTO3 transducers and were displayed on a Grass model 7 polygraph. Before commencing each experiment, artery rings were allowed to equilibrate for 1 hour, during which time a steady resting tension was achieved. In some of the experiments, during the equilibration period the bathing fluid was changed regularly (every 15min) and tension was reset to 1g if necessary. In some of experiments, the optimal preload was determined for each segment by measuring the maximal contractile response to 30mM KCl after incremental stretch. After each experiment, the tissues were then washed by changing the bathing Krebs solution 37°C three times over a 15min period before further increasing the degree of stretch of the tissue. In addition, when noradrenaline (NA) was used, the Krebs solution also contained 23×10^{-6}M ethylene diaminetetra-acetic acid (EDTA) in order to reduce degradation of the noradrenaline. At the end of each day's experiments the artery strip preparations were gently blotted, dry and weighed on a microbalance. The contractile force was expressed in terms of mg tension per mg weight of tissue.

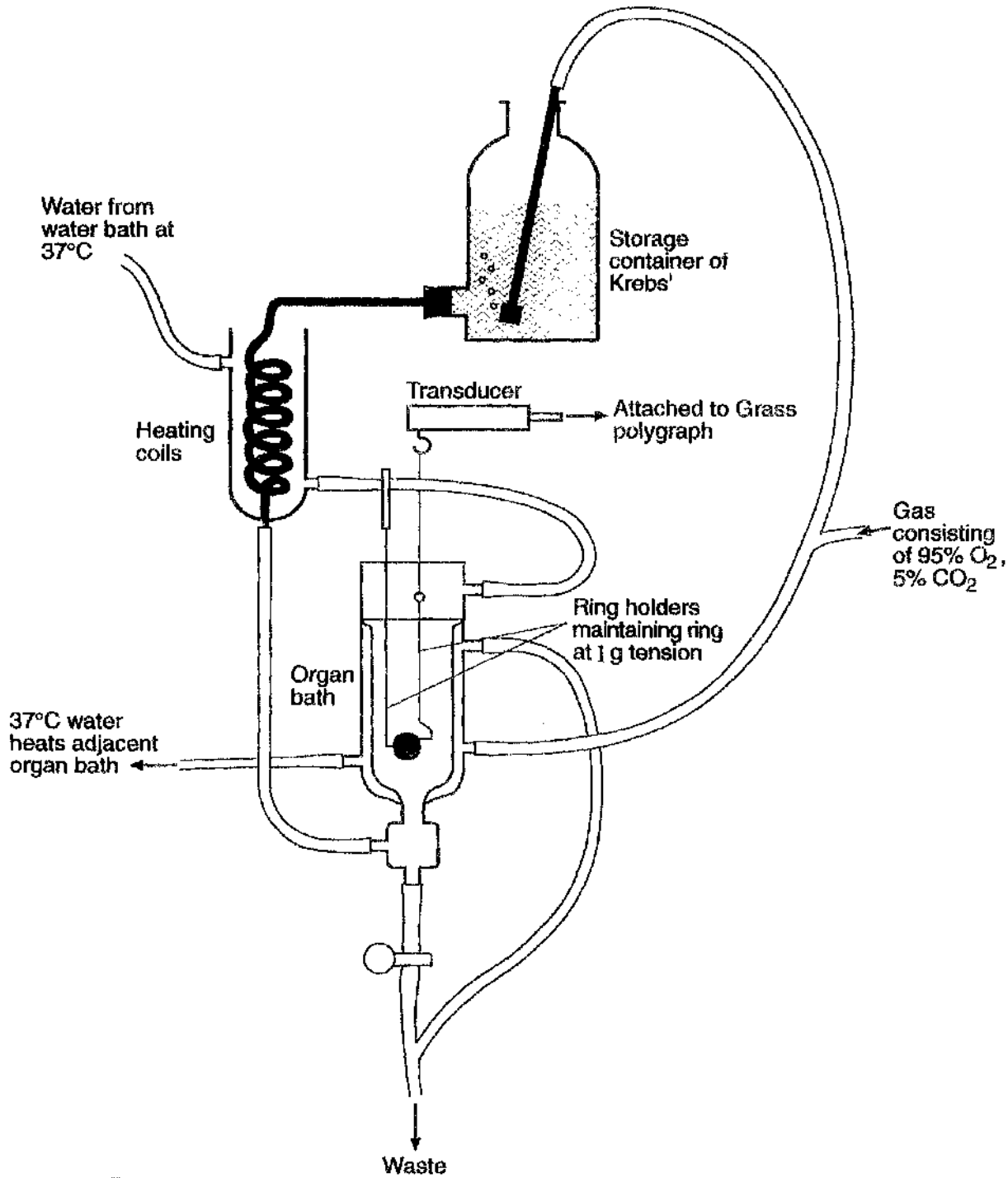

Fig.2.1.
Diagram of apparatus used to measure tension of preparation rings

2.3 Preparation of Cigarette Smoke Extract

A standard cigarette smoke extract (CSE) was freshly prepared in Krebs-Henseleit buffer (KHB, 4 cigarettes/100ml) for each day's experiment and was used within two hours of preparation. Smoke extract from the cigarettes ("Players ": 1mg nicotine, 14mg tar per cigarette) was obtained using a simple piston smoking machine (Fig. 2.2) which "inhaled" the smoke and then pumped it into a Woulfe bottle, where it was bubbled through the Krebs buffer (room temperature) to dissolve the water soluble constituents of cigarette smoke (CS). The smoking machine had a calibrated piston, which allowed the volume of smoke inhaled per puff to be controlled, and altered to a volume of between 1 and 10ml. The rate at which the machine smoked the cigarette could also be controlled. Thus the rate of smoking could be varied from 20 "inhalations" /minute to 60 "inhalations"/minute. A rate of 20 "inhalation"/minute was selected for this experiment. There was a valve between the inlet and the outlet tubes so that there could be no blow back of air through the cigarette. A Scinta glass bubbler was attached to the end of the outlet tube and this was securely held and sealed in the Woulfe bottle below the level of the Krebs buffer. Therefore, when smoke was exhaled down the outlet tube, a very large number of tiny bubbles were produced with each exhalation, providing a large surface area for dissolving the water soluble constituents of the smoke.

2.4 Effect of Electrical Field Stimulation (EFS)

Some experiments were carried out in order to determine whether there was any evidence of the existence of a neuronally-mediated response and if so, what was the nature of the transmitter(s) and receptor(s) involved. Electrical field stimulation (EFS) was applied by two parallel wire electrodes, (one of silver; 0.5mm, thickness and the other stainless steel; 0.2mm, thickness) positioned at

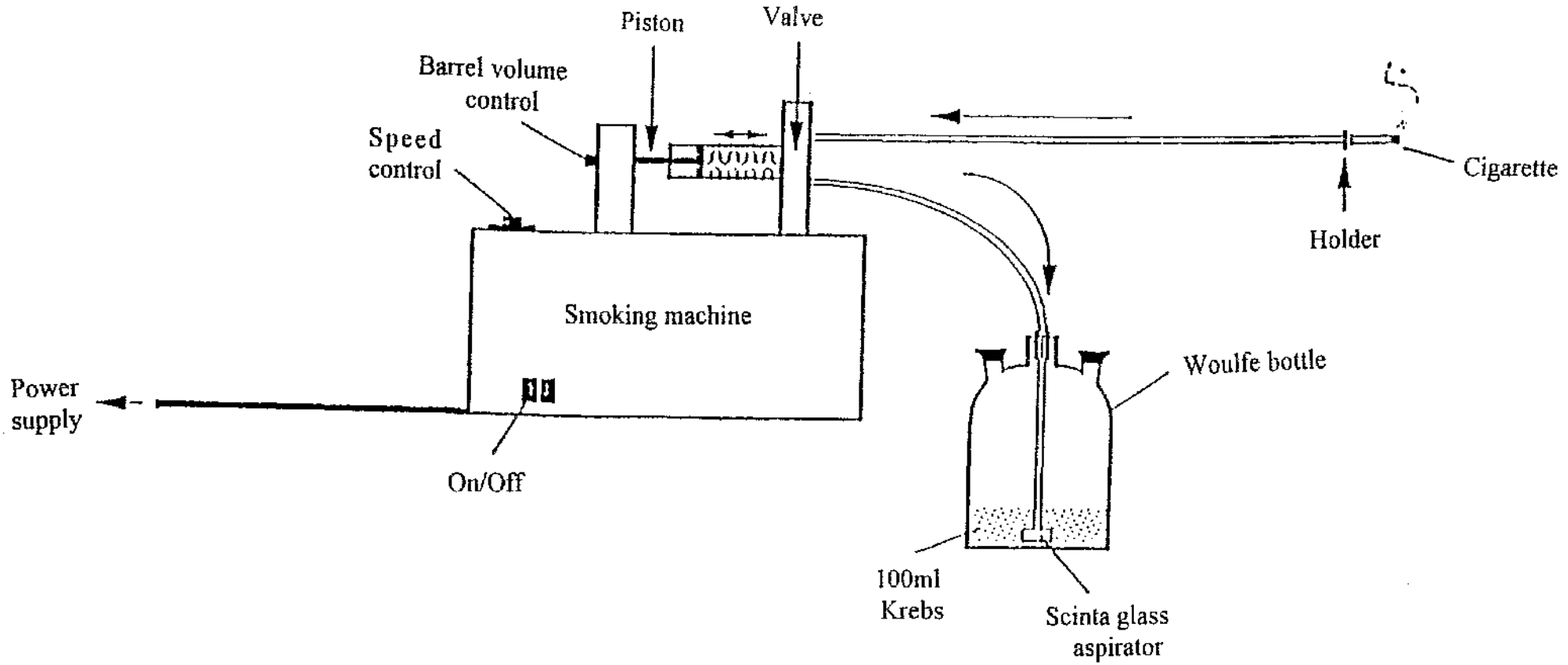

Fig. 2.2
Diagram showing smoking machine used for preparing the cigarette smoke extract

each end of the vessel rings and connected to a Grass S44 stimulator. To activate the intramural nerves without inducing a myogenic response, voltage-response curves were obtained in the absence and presence of 10^{-7}M tetrodotoxin. The supramaximal voltage was determined by field stimulation with the following parameters: 4, 8, 16 and 32 Hz for 1, 5 and 10 seconds train, with a pulse width of 0.1 ms at voltages of 10, 20, 30, 40, 50, 60 and 70V. After determining the supramaximal voltage (70V) in a preliminary study, control frequency-response curves (FRC), were obtained for each preparation, in a frequency range of 4, 8, 16 and 32 Hz, each stimulation being applied for 10 seconds. Repetitive stimulation was applied, once every 5min, until constant responses were achieved. In separate experiments, to determine the origin of the contractile responses to EFS, the artery rings were incubated with tetrodotoxin (10^{-7}M; for 10-20min) or prazosin (PRAZ, 10^{-9}M, for 15min) and stimulated at 4 to 32 Hz or 16 to 32 Hz. To determine whether endogenous nitric oxide (NO) had any effect on the EFS-induced responses, the artery rings were pretreated with NO synthase inhibitor, L-NAME (10^{-4}M, for 10min) before EFS.

2.5 Experimental Model of Heart Failure

Heart failure was induced by coronary artery ligation in male New Zealand rabbits (Denvir, 1994). This procedure produced an area of ischaemia, and myocardial infarct. In this respect it is very similar to a coronary atheroma, which is a very common cause of chronic heart disease (CHD). The operation on the rabbits was carried out by surgeons at the Royal Infirmary in Glasgow. Male New Zealand white rabbits weighing 2.5-3.0 kg were premedicated with intramuscular (i.m) Hypnorm [0.3 mg/kg fluansione (10mg/ml): fentanyl citrate (0.315 mg/ml)]. The animal was further sedated with intravenous (i.v.) midazolam (0.25-0.5 mg/kg) to allow endotracheal intubation. The animal then

was ventilated (0.3 - 0.4 l/min/kg). The rabbits were anaesthetized with an inhaled mixture of 1% halothane/ 1% nitrous oxide (N_2O)/ 20% oxygen (O_2). Left lateral thoracotomy was performed to expose the heart, the pericardium opened and a tie placed through the cardiac apex to allow easier manipulation of the heart. The left marginal branch of the left circumflex coronary artery was ligated using a 6/0 Ethicon suture at midpoint between the atrioventicular groove and the cardiac apex. The infarcted area rapidly became blue, and the position of the suture could be altered proximally or distally to obtain a satisfactory size of infarction. The rabbits were injected with intravenous quinidine (15mg/kg) into the marginal ear vein 5 minutes prior to ligation to reduce the incidence of arrhythmias. After ensuring that the lungs were properly inflated, the chest wall was closed and post-operatively, antibiotics were administered by intra-muscular injection (ampicillin 25mg/kg and cephalexidine 15mg/kg). Post operative analgesia was administered (0.2 mg/kg intra-muscular buprenorphine) at 30 minutes and 6-8 hours, and as considered to be required. The animals were then allowed to develop chronic heart failure (CHF) over the next 8 weeks. Sham operated animals underwent a similar procedure but the ligature was not tied round the coronary artery. At the end of 8 weeks, a catheter was inserted via the femoral artery to measure left ventricular ejection fraction to assess the extent of heart failure. The preparation of the animals was done by surgeons at Glasgow Royal Infirmary. The heart failure rabbits were killed at 8 weeks after ligation by a lethal intravenous injection of pentobarbitone into the ear vein. The sham-operated rabbits were killed at 8 weeks by the same method. The animals were number coded and the experiments were performed *blind*; i.e. the codes were not broken until after each experiment. The dissection of the artery rings and the way they were set up in the organ baths, were the same as previously described, with the difference that from each rabbit two or three MPA rings

(3-4 mm in length) and four or six BPA rings (3-4 mm in length) were obtained.

2.6 Experimental Protocols

2.6.1 *Effect of contractile agonists*

In these experiments, cumulative concentration response curves (CCRCs) to some of contractile agonists, such as phenylephrine (PHE), 5-hydroxytryptamine (5-HT), noradrenaline (NA), angiotensin II (Ang II) and thromboxane A_2 mimetic, U46619 were obtained on endothelium-intact or endothelium-denuded rings and also in the absence and presence of Nω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase from Wistar rat main pulmonary artery and its first branch rings. Agonists were cumulatively added in volumes 0.1ml (the final dilution in the organ bath) over the full concentration range. When the effect of L-NAME was to be examined on basal release of nitric oxide, it was added 10-20min before the addition of any contractile agonist. Also in experiments in which CCRCs to NA were obtained, cocaine was added at least 10min prior the onset of a second CCRCs. EC_{75} values were calculated as the concentration of drug required to elicit 75% of the maximum response.

2.6.2 *Effect of relaxant agonists*

In these experiments, CCRCs were obtained in the presence of the EC_{75} or the EC_{70} for PHE or the EC_{50} for 5-HT to vasorelaxants, such as the endothelium-dependent relaxants, acetylcholine (ACh), carbachol (CARB), histamine (HIST) or the endothelium-independent relaxants, sodium nitroprusside (SNP) or purinoceptor agonists, such as adenosine (AD), adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) or the β-adrenoceptor agonist, isoprenaline. These vasorelaxant responses were obtained in the absence or

presence of L-NAME or in the presence of antagonists or in endothelium denuded rings. Vasorelaxant agonists were added cumulatively in volumes of 0.1ml (the final dilution in the organ bath), when the contractions to PHE (EC_{75} or EC_{70}) or 5-HT (EC_{50}) reached a stable plateau. When the effects of L-NAME or antagonists were to be examined, these drugs were added 10-20min before the addition of any relaxant agonists. Relaxant effects were expressed as a percentage (%) relaxation of the PHE- or 5-HT-induced tone.

2.6.3 *Desensitisation to 5-hydroxytryptamine (5-HT) or phenylephrine (PHE)*

In these experiments, pulmonary artery rings and its first branch rings were exposed either to 5-HT (10^{-4}M, for 30min or 1 hour) or to PHE (10^{-5}M, for 2 hours), which produced desensitisation. Responses to standard submaximal concentrations of 5-HT and PHE or concentration response curves (CRC) were then obtained and compared with control responses obtained prior to the desensitisation. In some experiments, the part played by the endothelium in agonist-induced desensitisation was examined using rings in which the endothelial cells had been removed by rubbing. In addition, the role of NO in desensitisation was examined by studying the effects of L-NAME, L-arginine (L-Arg) and oxy-haemoglobin (HbO), which were administered according to two protocols. First, HbO or L-NAME was added after desensitisation had been induced by prolonged exposure either to 5-HT or PHE. The reversibility of the effect of L-NAME was then investigated by adding L-arginine. In the second protocol, the L-NAME was added before the desensitisation procedure and again after desensitisation had been induced.

2.6.4 *Effects of cigarette smoke extract*

After having obtained control concentration-response curves for PHE, concentration-response curves to ACh and SNP were also obtained. In subsequent experiments, increasing amounts of cigarette smoke extract (CSE) in Krebs buffer were administered to the baths as a pretreatment (10-15min), ensuring that a final volume of 25ml was maintained after each CSE addition, and the concentration-response curves to PHE, ACh and SNP repeated. The contractile response curves to PHE and also the relaxant response curves to ACh and SNP were obtained in the absence and presence of CSE. The contractile response curves to PHE were expressed as tension mg mg $^{-1}$ tissue and the dilator responses to ACh and SNP were expressed as % inhibitions of the contractile response to the EC_{70} of the precontracting agonist PHE (10^{-7}M).

2.7 Drugs

5-hydroxytryptamine creatine sulphate, L-phenylephrine hydrochloride, acetylcholine chloride, carbamylcholine chloride (carbachol), Nω-nitro-L-arginine methyl ester (L-NAME), L-arginine HCl, sodium nitroprusside (SNP), haemoglobin, DL-propranolol HCl, atropine sulfate, (–)-isoprenaline, adenosine, adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), angiotensin II, mianserin HCl, indomethacin, histamine, prazosin HCl, theophylline, cocaine HCl, (–)-arternolol, (+)-chlorpheniramine, bradykinin, idazoxan HCl, substance P, U46619, 3-isobutyl-1-methylxantine (IBMX), Nω-nitro-L-arginine (L-NOARG), glybenclamide (glyburide), were obtained from Sigma (Dorset); di sodium ethylendiaminetetra-acetate from M&B (May & Baker LTD); potassium chloride from Fisons; sodium chloride from BDH. All drugs were dissolved in saline (0.9%) or in Krebs buffer.

2.8 Statistical Analysis

The results are expressed as means ± standard errors of the means (S.E.M). Comparisons were made using the paired Student's t-test and where appropriate, the unpaired t-test were used. A probability of less than 0.05 was considered to be significant.

Chapter 3

RESULTS

3 RESULTS

3.1 Investigation of Contractile Responses to Agonists in Isolated Main Pulmonary Artery (MPA) Rings and in its First Branches (BPA) from Wistar Rats

3.1.1 *Contractile responses to 5-hydroxytryptamine (5-HT)*

5-Hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M), administered non-cumulatively, produced concentration-dependent contractions in Wistar rat isolated main pulmonary artery (MPA) rings and in its first branches (BPA) (Fig. 3.1 to 3.2). The MPA and BPA rings were equally responsive to 5-HT (10^{-7} to 10^{-4}M), but BPA rings were more responsive to high concentrations (10^{-3}M) of 5-HT than MPA rings ($0.05 > P > 0.01$; Fig. 3.3). In endothelium-intact rings, Nω-nitro-L-arginine methyl ester (L-NAME, 5×10^{-4}M) caused a slowly-developing increase in resting tone but not in all of the preparations (data not shown) and potentiated responses in both MPA and BPA rings to high concentrations (10^{-5} to 10^{-3}M) of 5-HT (Fig. 3.1 to 3.2), but not in endothelium-denuded rings. In comparison between contractile response curves to 5-HT, in intact rings pretreated with L-NAME, BPA rings were more responsive than MPA rings to 5-HT (10^{-5}M) ($0.05 > P > 0.01$; Fig. 3.4). The mechanical removal of the endothelium by rubbing potentiated concentration-response curves to 5-HT in these rings. The effects of incubation with L-NAME (5×10^{-4}M, for 20min) and removal of the endothelium in MPA rings on the concentration response curves to 5-HT were very similar (Fig. 3.1 to 3.2. In endothelium-denuded rings, L-NAME was without effect on either resting tone or on the contractile responses to 5-HT. The EC_{50} values and the maximum contractile responses to 5-HT, in the absence and presence of L-NAME, are summarized in Tables 3.1 and 3.2. L-NAME did not affect the

EC_{50} of 5-HT. There were no significant differences between the EC_{50} values to 5-HT, in MPA and BPA rings, whether in the absence or presence of L-NAME (Table 3.2). In one series of the experiments, cumulative response curves were obtained in the absence and presence of 5-HT_2-antagonist, mianserin (10^{-6}M; for 10min). Mianserin completely inhibited cumulative response curves to 5-HT (10^{-7} to 10^{-4}M).

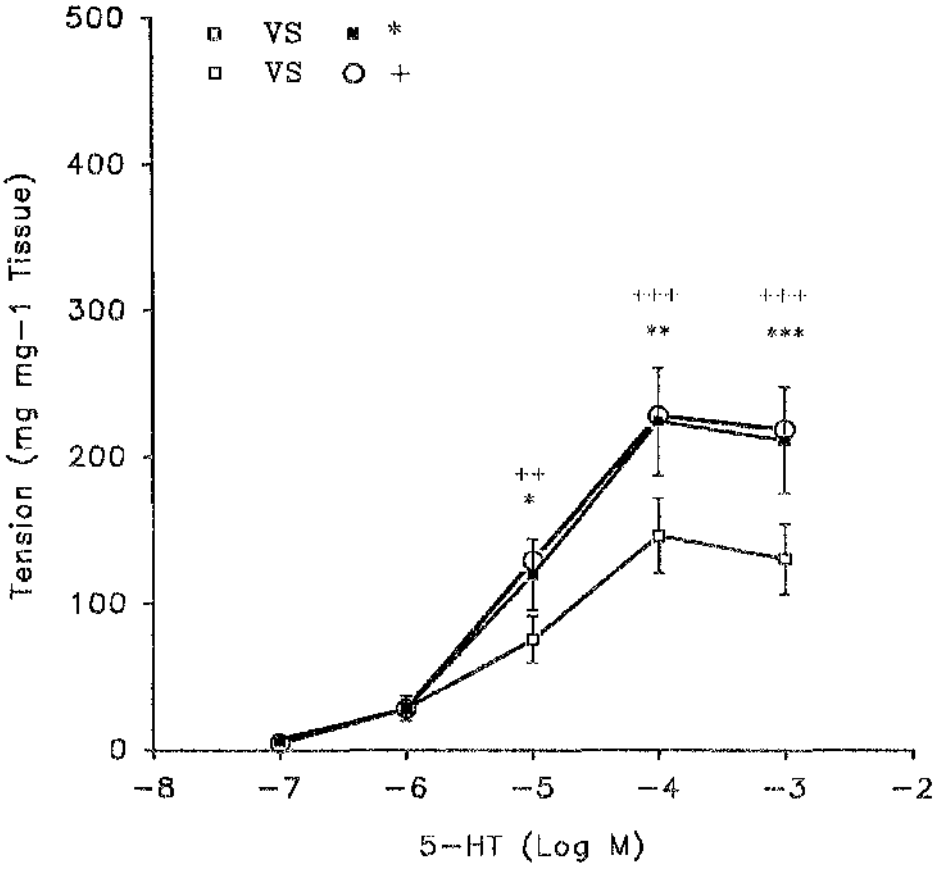

Fig. 3. 1

Non-cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M) in the absence (open square) and presence (closed square) of L-NAME (5×10^{-4}M) in MPA rings and/or in endothelium-denuded (open circle) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=8 rings from 8 animals. The vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: * $P < 0.05$; ** $P <$ or $^{++} P < 0.01$; *** $P <$ or $^{+++} P < 0.001$ (paired and unpaired Student's t test).

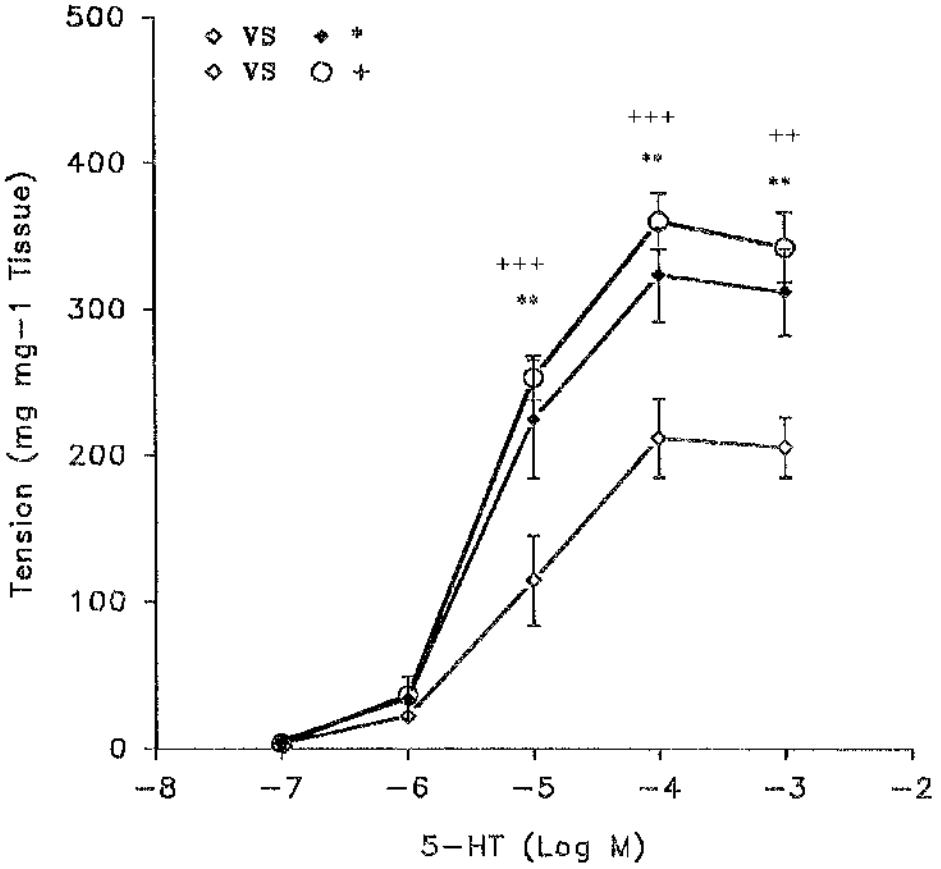

Fig. 3. 2

Non-cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M) in the absence (open diamond) and presence (closed diamond) of L-NAME (5×10^{-4}M) in BPA rings and/or in endothelium-denuded (open circle) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=8 rings from 6 animals. The vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: ** $P <$ or $^{++}$ $P < 0.01$; $^{+++}$ $P < 0.001$ (paired and unpaired Student's t test).

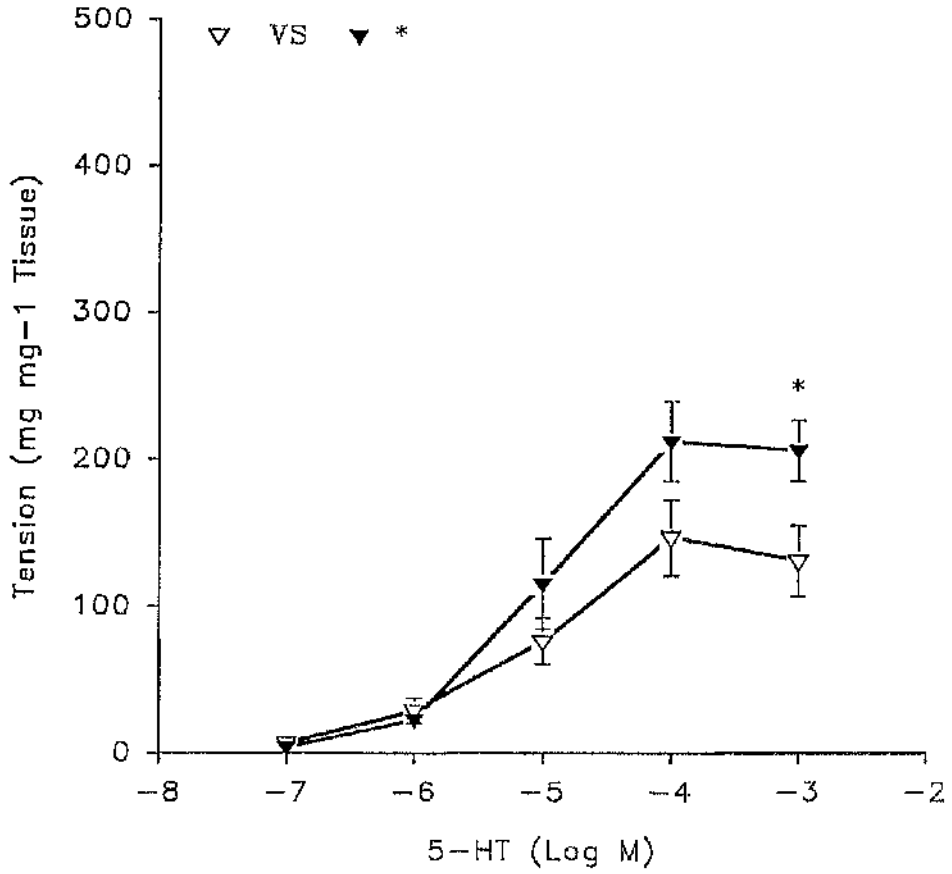

Fig. 3. 3

Comparison between non-cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M) in MPA (open symbol) or BPA (closed symbol) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and 5-HT induced concentration-dependent contractions were obtained in these preprations. The results are expressed as means of n=6-8 rings from 6-8 animals. The vertical bars show ± S.E.M. Asterisk indicates significant difference between means by *p value:* * $P < 0.05$ (unpaired t test).

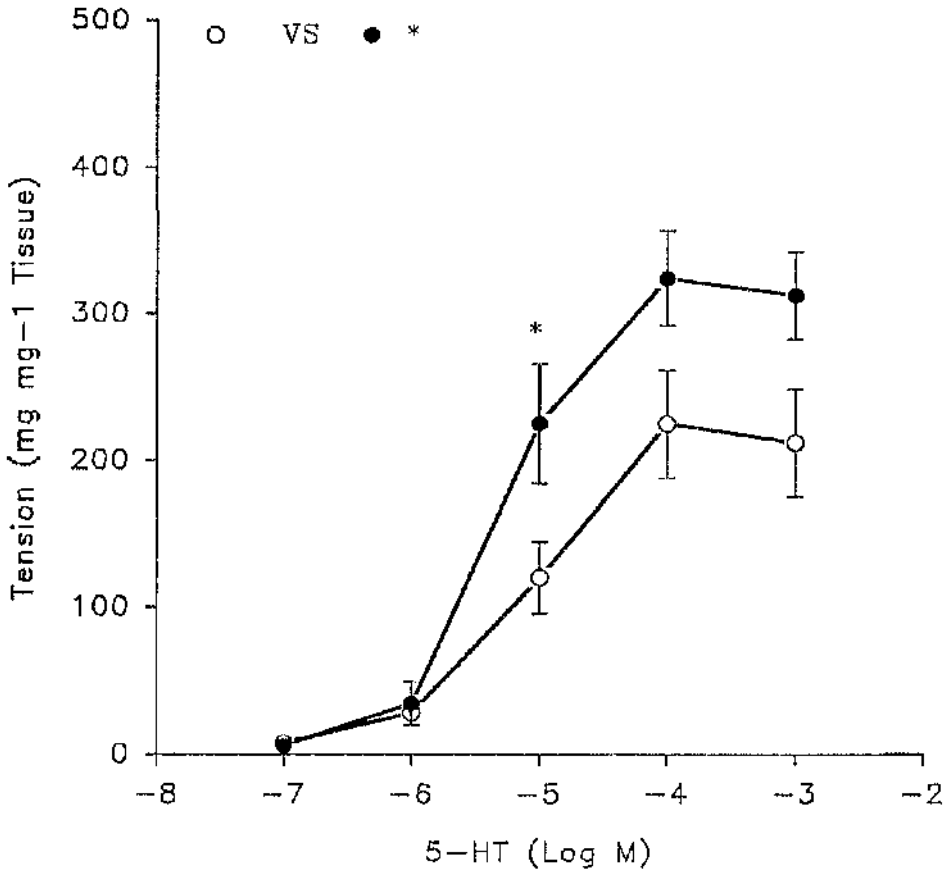

Fig. 3. 4

Comparison between non-cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M) in the presence of L-NAME (5×10^{-4}M) in MPA (open symbol) or in BPA (closed symbol) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and 5-HT induced concentration-dependent contractions were obtained in these preprations. The results are expressed as means of n=6-8 rings from 6-8 animals. The vertical bars show ± S.E.M. Asterisk indicates significant differences between means by: *p value* * $P < 0.05$ (unpaired t test).

Pulmonary Artery	The E_{max} (mg mg^{-1} tissue)	P values
MPA (control) V MPA (treated)	146.60±25.80 V 224.70±36.74	0.01 > ** P > 0.001
BPA (control) V BPA (treated)	212.13±26.98 V 324.08±32.28	0.01 > ** P > 0.001
MPA (control) V BPA (control)	146.60±25.80 212.13±26.98	P > 0.05
MPA (treated) V BPA (treated)	224.70±36.74 V 324.08±32.28	P > 0.05
(+EC) MPA (treated) V (-EC) MPA (control)	224.70±36.74 V 228.27±4.28	P > 0.05
(+EC) BPA (treated) V (-EC) BPA (control)	324.08±32.28 V 360.62±19.31	P > 0.05

Table 3.1
Comparison between the maximum responses (E_{max}) induced by 5-HT in the absence (control) and presence (treated) of L-NAME (5×10^{-4}M, for 20min) in MPA and BPA rings or in endothelium-denuded (-EC) rings and/or in MPA rings versus (V) BPA rings from Wistar rats. The results are expressed as means of n=8 rings from 6-8 animals. Asterisks indicate significant differences between means. *p values*: ** $P < 0.01$ (Student's t test).

Pulmonary Artery	The EC_{50} (Molar)	P values
MPA (control) V MPA (treated)	10.61×10^{-6}M V 9.38×10^{-6}M	P > 0.05
(+EC) MPA (treated) V (-EC) MPA (control)	9.38×10^{-6}M V 9.22×10^{-6}M	P > 0.05
(+EC) BPA (treated) V (-EC) BPA (control)	6.29×10^{-6}M V 5.65×10^{-6}M	P > 0.05
BPA (control) V BPA (treated)	9.37×10^{-6}M V 6.29×10^{-6}M	P > 0.05
MPA (control) V BPA (control)	10.61×10^{-6}M V 9.37×10^{-6}M	P > 0.05
MPA (treated) V BPA (treated)	9.38×10^{-6}M V 6.29×10^{-6}M	P > 0.05

Table 3.2
Comparison between the EC_{50} values for 5-HT in the absence (control) and presence (treated) of L-NAME (2×10^{-4}M) in MPA and BPA rings or in endothelium-denuded (-EC) rings and/or in MPA rings versus (V) BPA rings from Wistar rats. The results are expressed as means of n=8 rings from 6-8 animals.

3.1.2 *Contractile responses to phenylephrine (PHE)*

Phenylephrine (PHE; 10^{-9} to 10^{-4}M) administered non-cumulatively also produced concentration-dependent contractions in isolated MPA rings and in BPA rings of rat vasculature (Fig. 3.5 to 3.6). L-NAME (5×10^{-4}M, for 20min) also potentiated responses of MPA to all of concentrations of PHE (Fig. 3.5), as well as potentiating responses of BPA to low concentrations (10^{-8} to 10^{-6}M) of PHE (Fig. 3.6). The BPA rings were more responsive to all of concentrations of PHE than were MPA rings. Also, BPA rings pretreated with L-NAME were more responsive than MPA rings to PHE (10^{-7} to 10^{-4}M) (Fig. 3.7 to 3.8). The mechanical removal of the endothelium by rubbing potentiated concentration-response curves to PHE in MPA rings and in BPA rings. The effects of incubation with L-NAME or removal of the endothelium in MPA rings on the PHE concentration response curves were very similar (Fig. 3.5 to 3.6). L-NAME had no effect on either resting tone or contractile responses to PHE in endothelium denuded rings of MPA. The EC_{50} values and the maximum contractile responses to PHE in the absence and presence of L-NAME, are summarized in Tables 3.3 and 3.4. In comparison between the maximal contractile responses to PHE, BPA rings were more responsive than MPA rings, whether rings were pretreated with L-NAME or not (Table, 3.3). In comparison between the EC_{50} values to PHE, L-NAME increased the sensitivity of rings to PHE (respectively, MPA: 10.43 fold; BPA: 6.57 fold). BPA rings were more sensitive than MPA rings when the rings were not pretreated with L-NAME (1.9 fold; Table 3.4).

In other experiments, prazosin (10^{-8}M, for 10min) inhibited contractile responses to PHE. In comparisons between the contractile responses to PHE and 5-HT, expressed as percentages of the maximum response, the former was more potent than the latter in both MPA and BPA rings (Fig. 3.9 to 3.10).

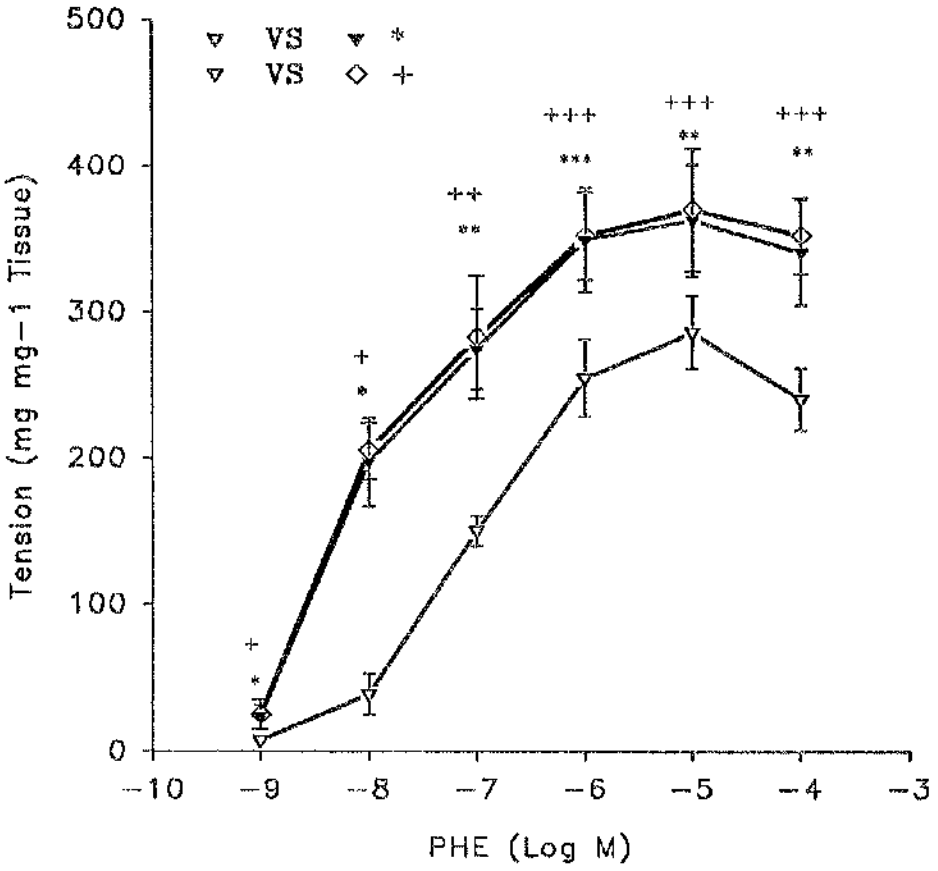

Fig. 3. 5

Non-cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M) in the absence (open triangle) and presence (closed triangle) of L-NAME (5×10^{-4}M) in MPA rings and/or in endothelium-denuded (open diamond) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and PHE-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: * $P <$ or $^{+} P < 0.05$; ** $P <$ or $^{++} P < 0.01$; *** $P <$ or $^{+++} P < 0.001$ (paired and unpaired Student's t test).

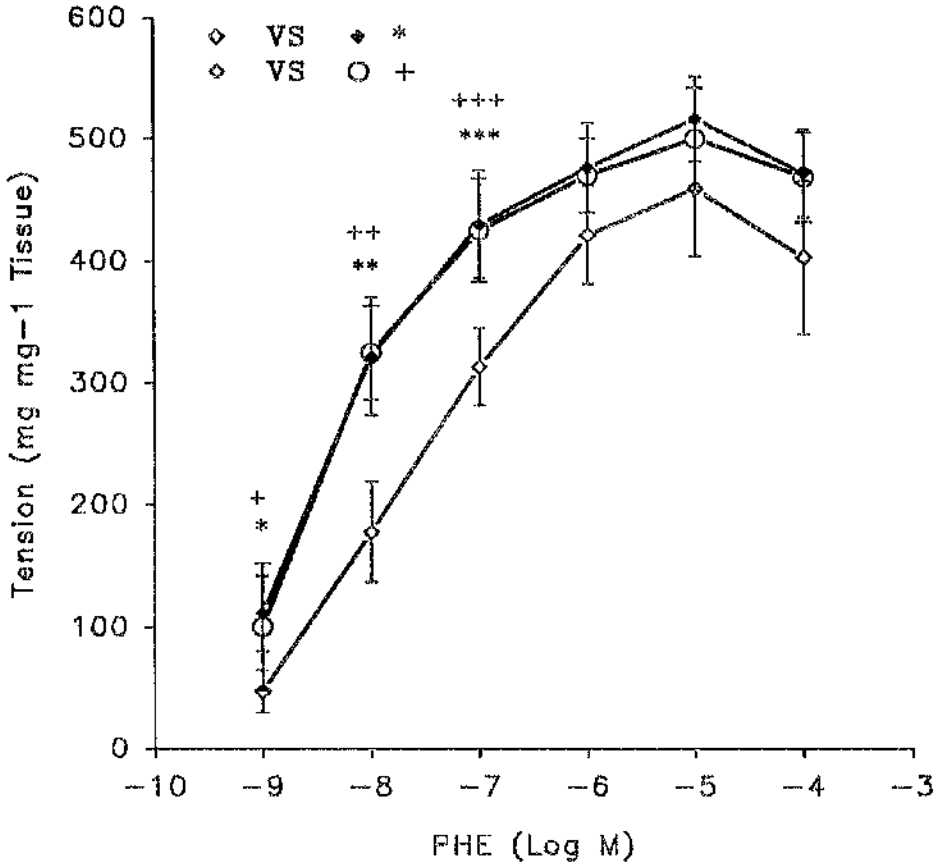

Fig. 3. 6

Non-cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M) in the absence (open diamond) and presence (closed diamond) of L-NAME (5×10^{-4}M) in BPA rings and/or in endothelium-denuded (open circle) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and PHE- induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: * $P <$ or $^{+} P < 0.05$; ** $P <$ or $^{++} P < 0.01$; *** $P <$ or $^{+++} P < 0.001$ (paired and unpaired Student's t test).

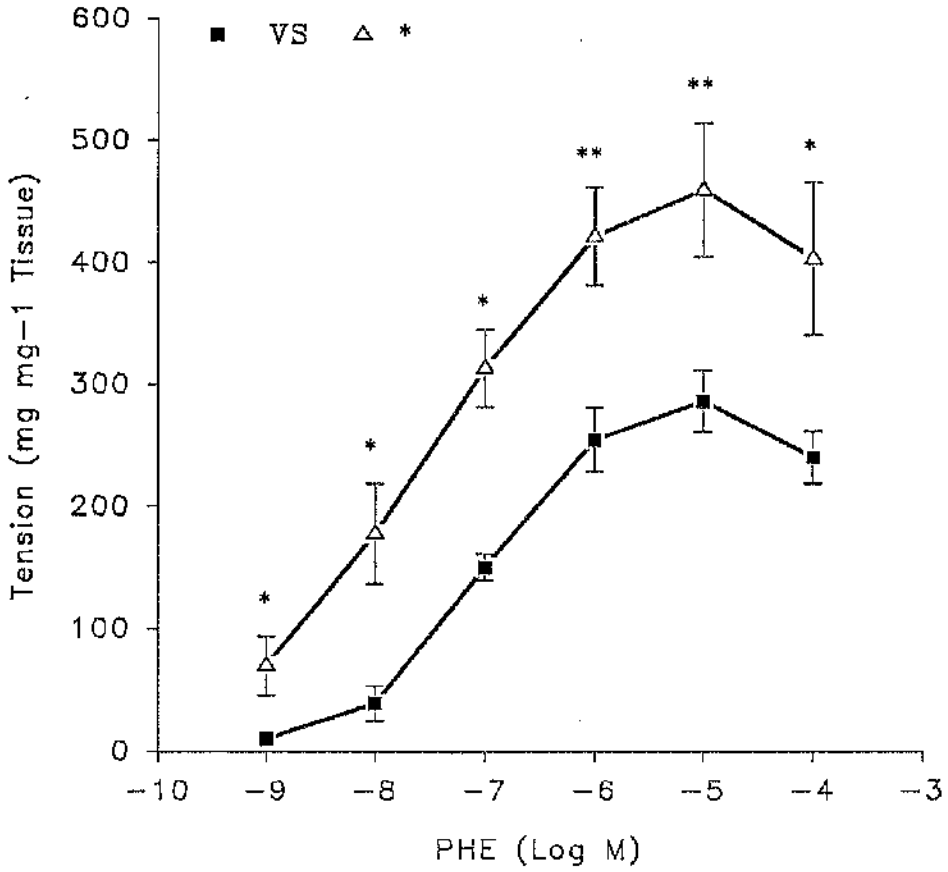

Fig. 3. 7

Comparison between non-cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M) in MPA (closed symbol) or BPA (open symbol) rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and PHE-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$(unpaired t test)

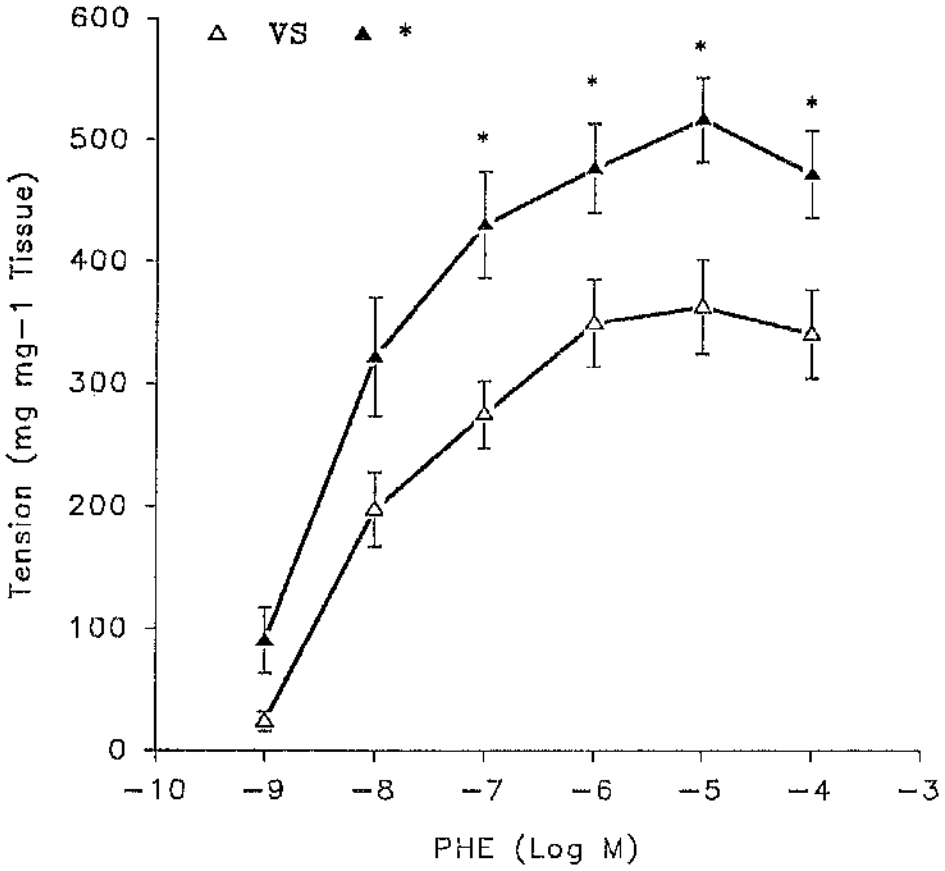

Fig. 3. 8

Comparison between non-cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M) in the MPA (open symbol) or BPA (closed symbol) rings pretreatment with L-NAME (5×10^{-4}M). Tension was recorded (mg mg^{-1} tissue) and PHE-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p values:* * $P < 0.05$ (unpaired t test).

Pulmonary Artery	The E_{max} (mg mg^{-1} tissue)	P values
MPA (control) V MPA (treated)	286.46±24.95 V 362.86±38.37	0.01 > ** P > 0.001
(+EC) MPA (treated) V (-EC) MPA (control)	362.86±38.37 V 370.36±42.10	P > 0.05
BPA (control) V BPA (treated)	459.56±54.90 V 516.49±34.82	P > 0.05
(+EC) BPA (treated) V (-EC) BPA (control)	516.49±34.82 V 500.25±42.10	P > 0.05
MPA (control) V BPA (control)	286.46±24.95 V 459.56±54.90	0.05 > * P > 0.01
MPA (treated) V BPA (treated)	362.86±38.37 V 516.49±34.82	0.05 > * P > 0.01

Table 3.3
Comparison between the maximum responses (E_{max}) induced by PHE in the absence (control) and presence (treated) of L-NAME (5×10^{-4}M, for 20min) in MPA and BPA rings or in endothelium-denuded (-EC) rings and/or in MPA rings versus (V) BPA rings from Wistar rats. The results are expressed as means of n=6 rings from 6 animals. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$ (Student's t test).

Pulmonary Artery	The EC_{50} (Molar)	P values
MPA (control) V MPA (treated)	9.5×10^{-8}M V 9.1×10^{-9}M	*** P < 0.001
(+EC) MPA (treated) V (-EC) MPA (control)	9.1×10^{-9}M V 9.28×10^{-9}M	P > 0.05
BPA (control) V BPA (treated)	5×10^{-8}M V 7.6×10^{-9}M	0.01 > ** P > 0.001
(+EC) BPA (treated) V (-EC) BPA (control)	7.6×10^{-9}M V 7.84×10^{-9}M	P > 0.05
MPA (control) V BPA (control)	9.5×10^{-8}M V 5×10^{-8}M	0.01 > ** P > 0.001
MPA (treated) V BPA (treated)	9.1×10^{-9}M V 7.6×10^{-9}M	P > 0.05

Table 3.4
Comparison between the EC50 values induced by PHE in the absence (control) and presence (treated) of L-NAME in MPA and BPA rings or in endothelium-denuded (-EC) rings and/or in MPA rings versus (V) BPA rings from Wistar rats. The results are expressed as means of n=6 rings from 6 animals. Asterisks indicate significant differences between means. p values: ** P < 0.01; *** P < 0.001 (Student's t test).

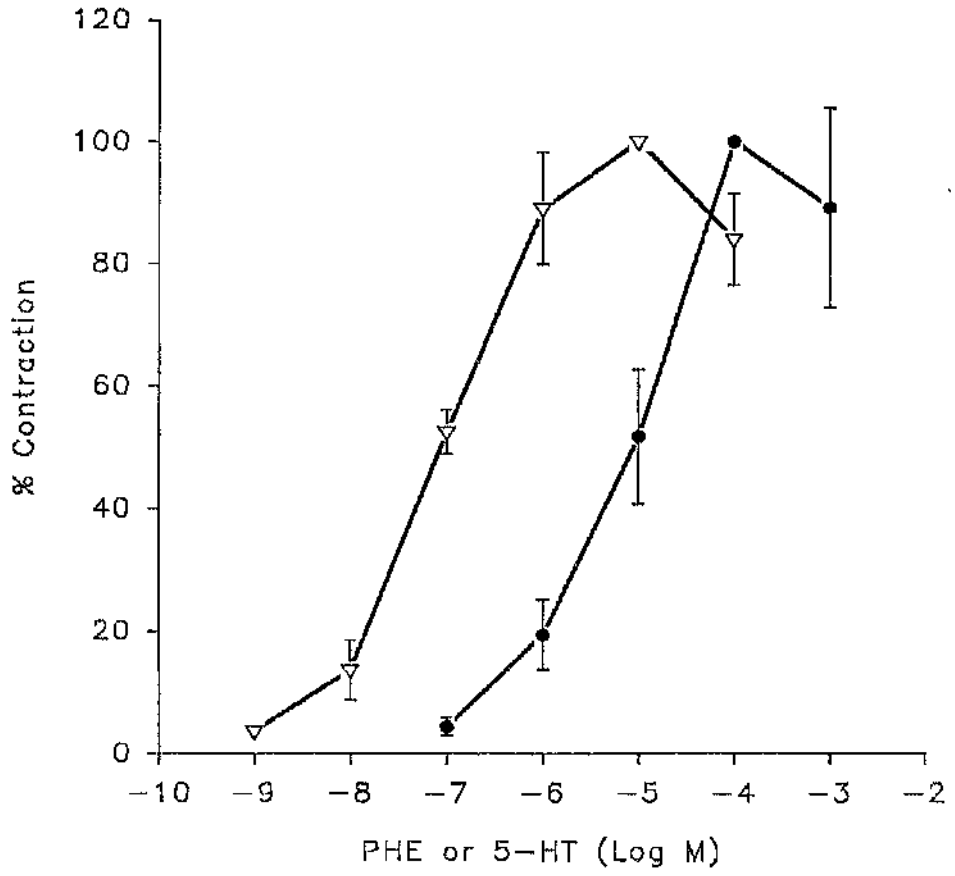

Fig. 3. 9
Comparison between concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M; open symbol) or 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M; closed symbol) in MPA rings of Wistar rats. PHE and 5-HT induced concentration-dependent contractions in these preparations. The results are expressed as means and as a percentage of the maximum response to PHE or 5-HT of n=8 rings from 8 animals. The vertical bars show ± S.E.M.

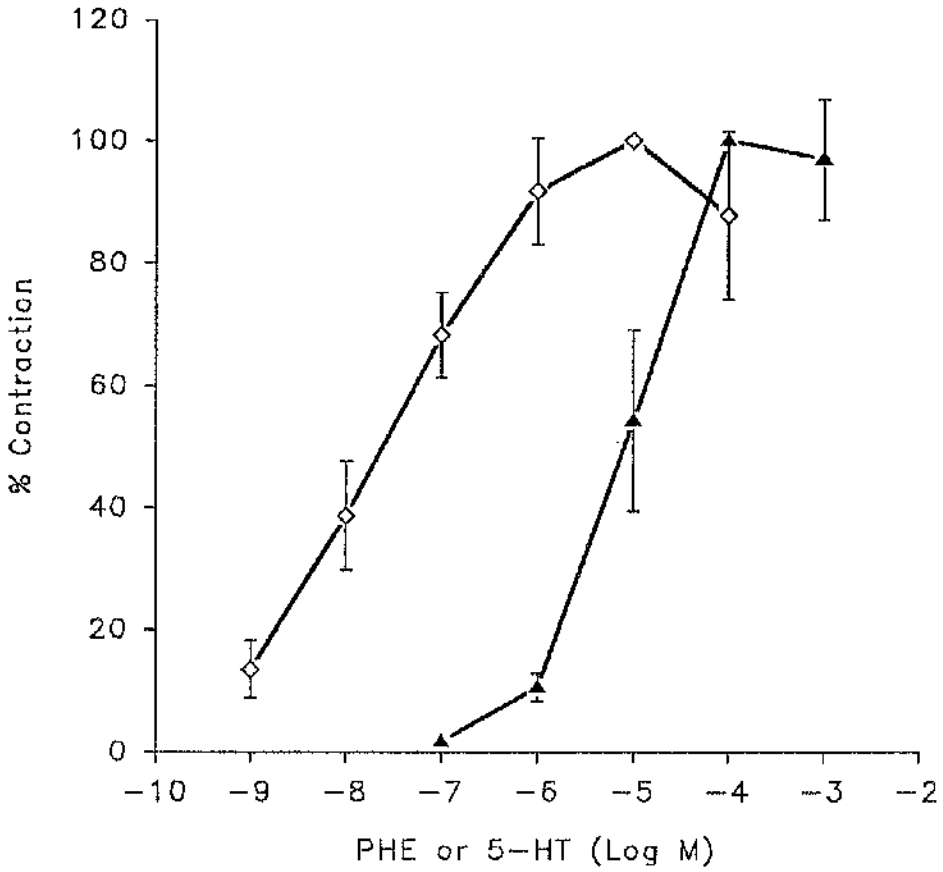

Fig. 3. 10

Comparison between concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-4}M; open symbol) or 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-3}M; closed symbol) in BPA rings of Wistar rats. PHE and 5-HT induced concentration-dependent contractions in these preparations. The results are expressed as means and as a percentage of the maximum response to PHE or 5-HT of n=8 rings from 8 animals. The vertical bars show ± S.E.M.

3.1.3 *Contractile responses to noradrenaline (NA)*

Noradrenaline (NA, 10^{-10} to 10^{-6}M), administered cumulatively, induced concentration-dependent contractions in MPA rings and in BPA rings (Fig. 3.11 to 3.12). The MPA rings were less responsive than the BPA rings to NA (10^{-9} to 10^{-7}M) (Fig. 3.13). Cocaine (10^{-6}M, for 10-15min) slightly enhanced responses to some concentrations (10^{-9} to 10^{-8}M) of NA in MPA rings, but had no effect in BPA rings (Fig. 3.11 to 3.13). Cocaine did not affect the maximum contractile responses to NA in either the MPA or BPA rings. The EC_{50} values and the maximum contractile responses to NA in the absence and presence of cocaine are summarized in Tables 3.5 and 3.6. There were no significant differences between the maximum responses to NA in MPA or BPA rings whether cocaine was absent or present. In comparisons between the EC_{50} values to NA, cocaine caused an increase in the sensitivity of MPA rings (1.75 fold) but not of BPA rings to NA (Table 3.6; Fig. 3.11 to 3.12). In other experiments, prazosin (10^{-8}M) completely inhibited contractile responses to NA (data not shown).

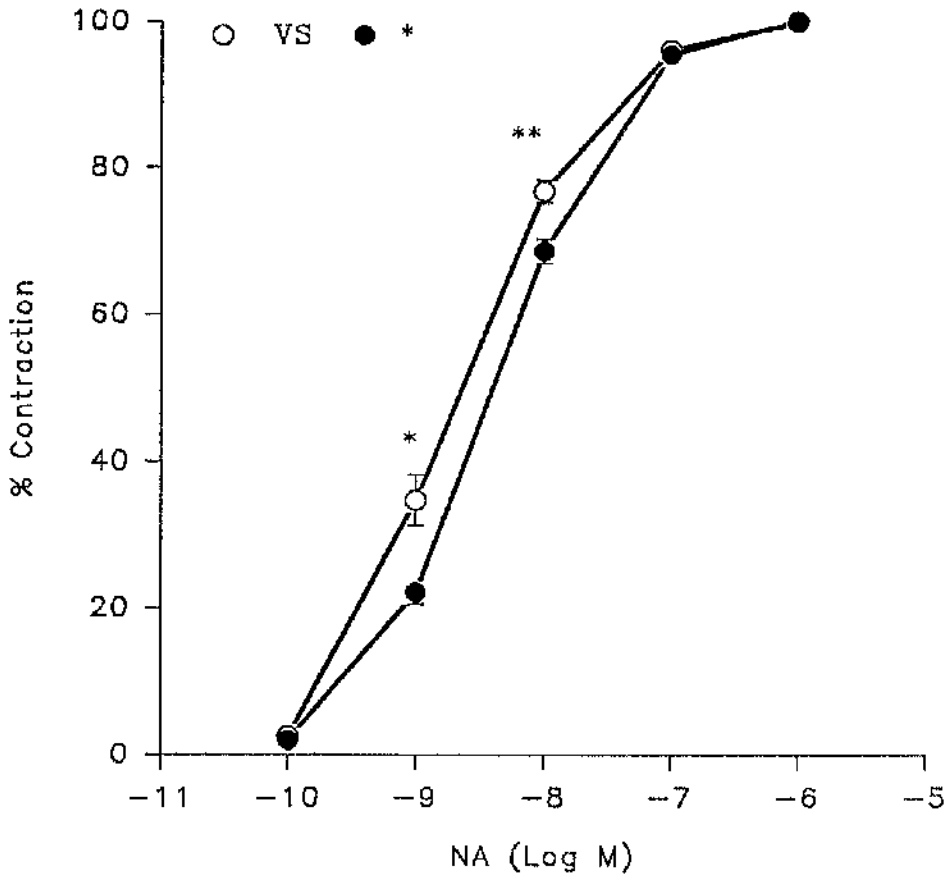

Fig. 3. 11

Concentration response curves to noradrenaline (NA, 10^{-10} to 10^{-6}M) in the absence (closed symbol) and presence (open symbol) of cocaine (10^{-6}M) in MPA rings of Wistar rats. NA induced concentration-dependent contractions in these rings. The results are expressed as means and as a percentage of the maximum response to NA of n=7 rings from 7 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$ (paired Student's t test).

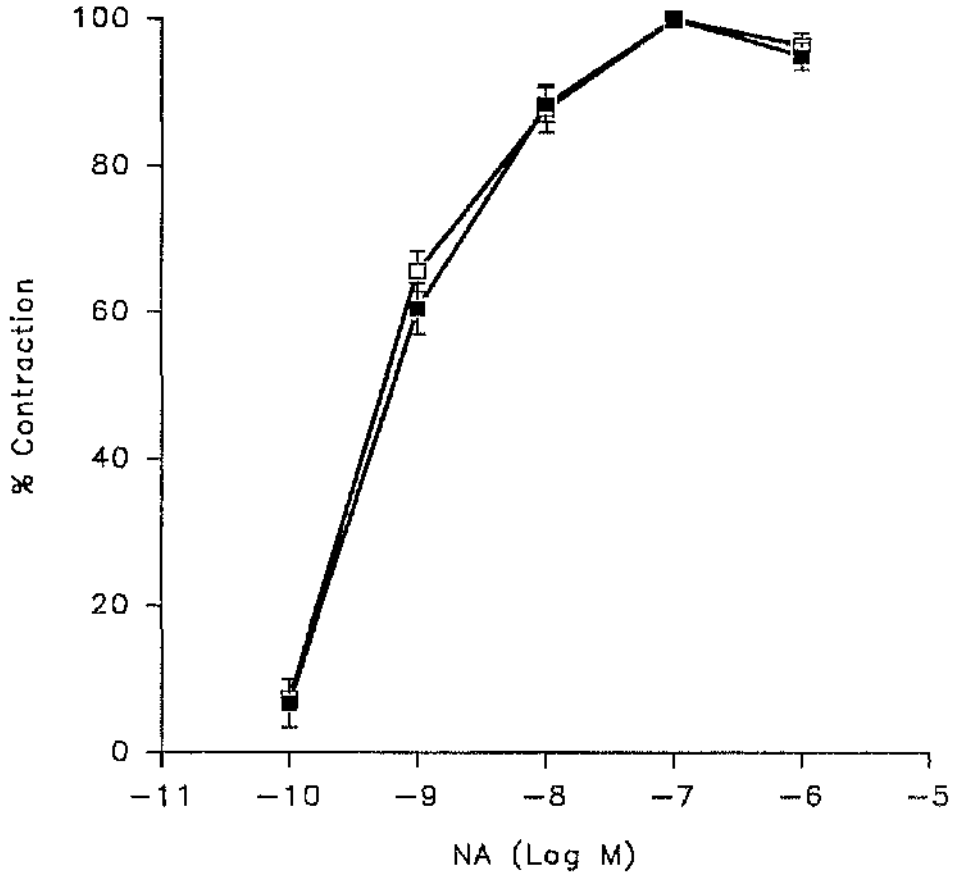

Fig. 3. 12
Concentration response curves to noradrenaline (NA, 10^{-10} to 10^{-6}M) in the absence (closed symbol) and presence (open symbol) of cocaine (10^{-6}M) in BPA rings of Wistar rats. NA induced concentration-dependent contractions in these rings. The results are expressed as means and as a percentage of the maximum response to NA of n=7 rings from 7 animals. The vertical bars show ± S.E.M.

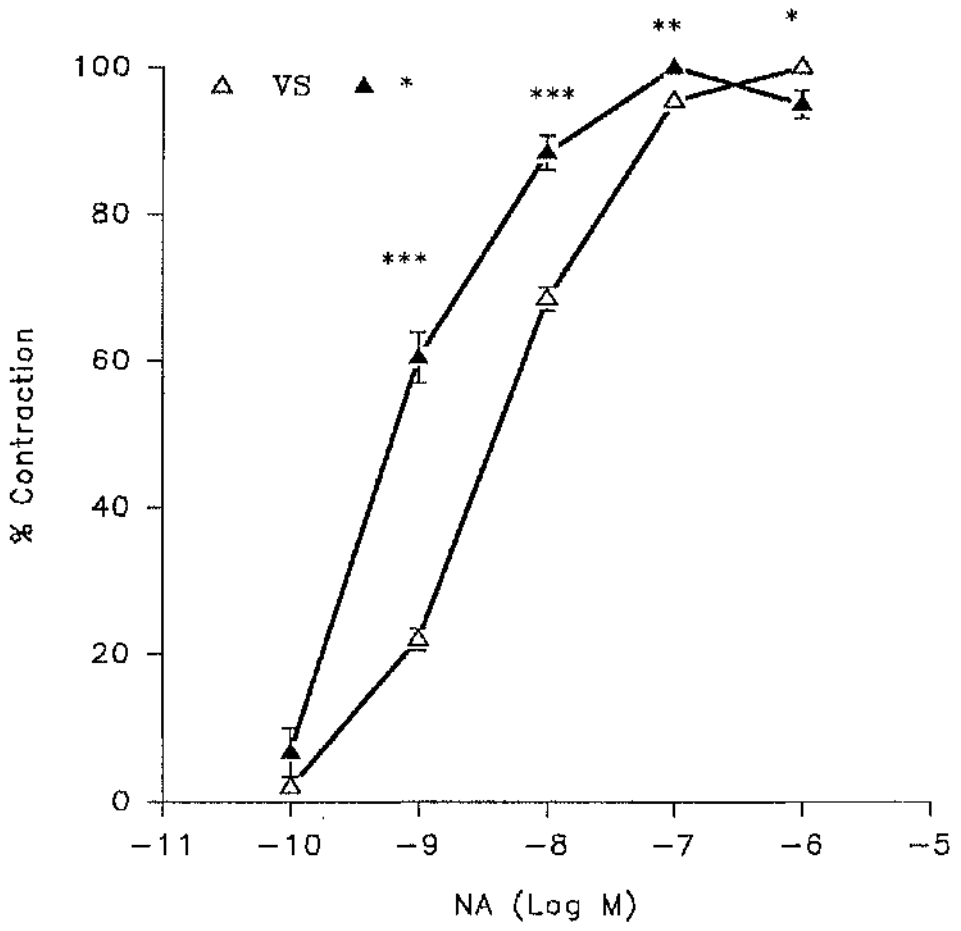

Fig. 3. 13

Comparison between concentration response curves to noradrenaline (NA, 10^{-10} to 10^{-6}M) in MPA (open symbol) and or BPA (closed symbol) rings of Wistar rats. Tension was recorded (mg mg^{-1} Tissue) and NA induced concentration-dependent contractions in these rings. The results are expressed as means of n=7 rings from 7 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (unpaired t test).

Pulmonary Artery	The E_{max} (mg mg^{-1} tissue)	P values
MPA (control) V MPA (treated)	298.89±6.03 V 304.92±6.10	P > 0.05
BPA (control) V BPA (treated)	282.50±18.45 V 288.13±20.32	P > 0.05
MPA (control) V BPA (control)	298.89±6.03 V 282.50±18.45	P > 0.05
MPA (treated) V BPA (treated)	304.92±6.10 V 288.13±20.32	P > 0.05

Table 3.5
Comparison between the maximum responses (E_{max}) to NA (10^{-6}M) in MPA and BPA rings and/or in MPA rings versus (V) BPA rings and in the absence (control) and presence (treated) of cocaine (10^{-6}M). The results are expressed as means of n=7 rings from 7 animals.

Pulmonary Artery	The EC_{50} (Molar)	P values
MPA (control) V MPA (treated)	6.4×10^{-9}M V 3.51×10^{-9}M	*** P < 0.001
BPA (control) V BPA (treated)	8×10^{-10}M V 7.1×10^{-10}M	P > 0.05
MPA (control) V BPA (control)	6.4×10^{-9}M V 8×10^{-10}M	*** P < 0.001
MPA (treated) V BPA (treated)	3.51×10^{-9}M V 7.1×10^{-10}M	*** P < 0.001

Table 3.6
Comparison between the EC_{50} values induced by NA in MPA and BPA rings and/or in MPA rings versus (V) BPA rings and in the absence (control) and presence (treated) of cocaine (10^{-6}M). The results expressed as means of n=7 rings from 7 animals. Asterisks indicate significant differences between means. *p values*: *** P < 0.001 (Student's t test).

3.1.4 *Contractile responses to angiotensin II (Ang II)*

Angiotensin II (Ang II, 10^{-10} to 10^{-7}), administered cumulatively, induced concentration-dependent contractions of MPA and BPA rings (Fig. 3.14). BPA rings were more responsive than the MPA rings to Ang II (10^{-9}M). The EC_{50} values for Ang II in intact MPA and BPA rings were (respectively, 1.7×10^{-9}M; n=9 and 7×10^{-10}M). Comparison of the EC_{50} values for Ang II, indicates that BPA rings were more responsive than MPA rings (2.42 fold; $P < 0.001$). Maximal responses to Ang II, expressed as percentages of the initial maximal contraction to KCl (3×10^{-2}M) in these rings were (MPA: 130.13±3.84% and BPA: 131.07±5.56%; $P > 0.05$; Fig. 3.14). Comparison of the maximal responses to Ang II obtained in MPA rings and BPA rings, indicated no significant differences.

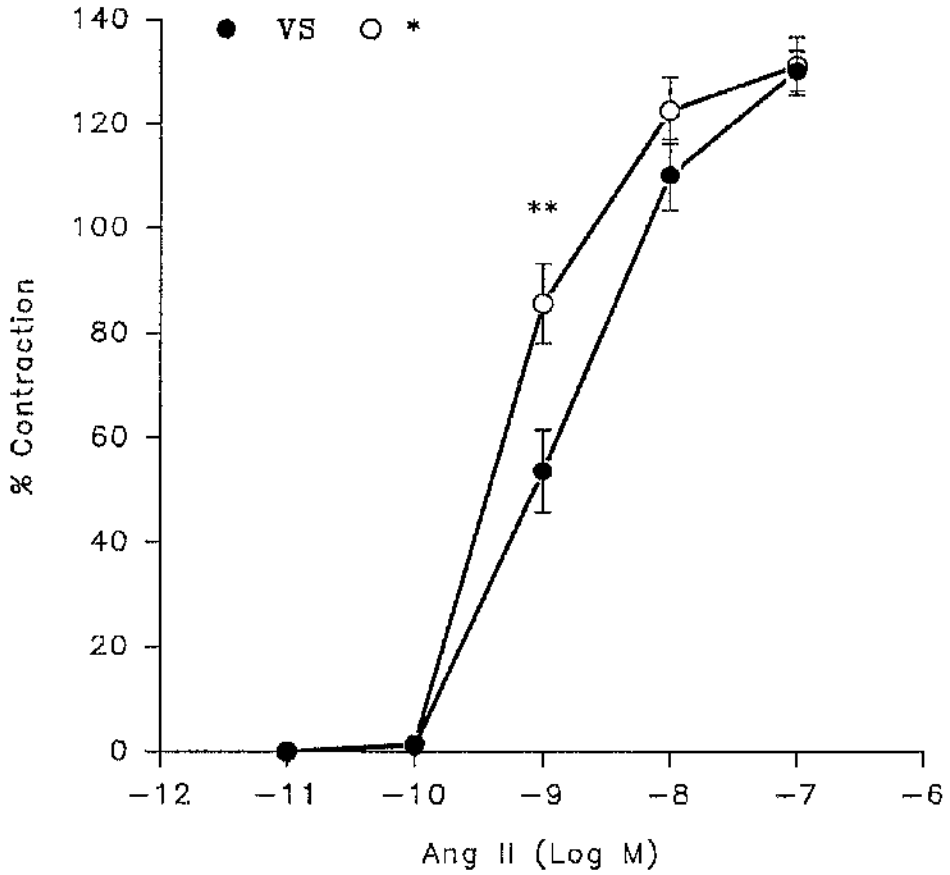

Fig. 3. 14

Concentration-response curves showing contraction to angiotensin II (Ang II, 10^{-11} to 10^{-7}M) in MPA (closed symbol) and BPA (open symbol) rings of Wistar rats. Tension was recorded as a percentage of the maximum contraction to KCl (3×10^{-2}M). The results are expressed as means of n=9-10 from 9-10 animals. The vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p values*: ** $P < 0.01$ (unpaired t test).

3.1.5 *Contractile responses to the thromboxane A_2 mimetic, U46619*

The thromboxane A_2 (TXA_2) mimetic, U46619 (10^{-9} to 10^{-6}M), administered cumulatively, induced large concentration-dependent contractions in isolated MPA (EC_{50}; 8.5×10^{-9}M) and BPA (EC_{50}; 7.2×10^{-9}M; $P > 0.05$) rings of Wistar rats (Fig. 3.15). The maximum contractions to U46619 (10^{-6}M) expressed as percentages of the initial maximum contraction to KCl (3×10^{-2}M) in MPA rings and BPA rings were (respectively, 165.16±5.66% and 178.62±31.03%; $P > 0.05$). There were no differences between the responses to U46619 in the MPA and BPA rings.

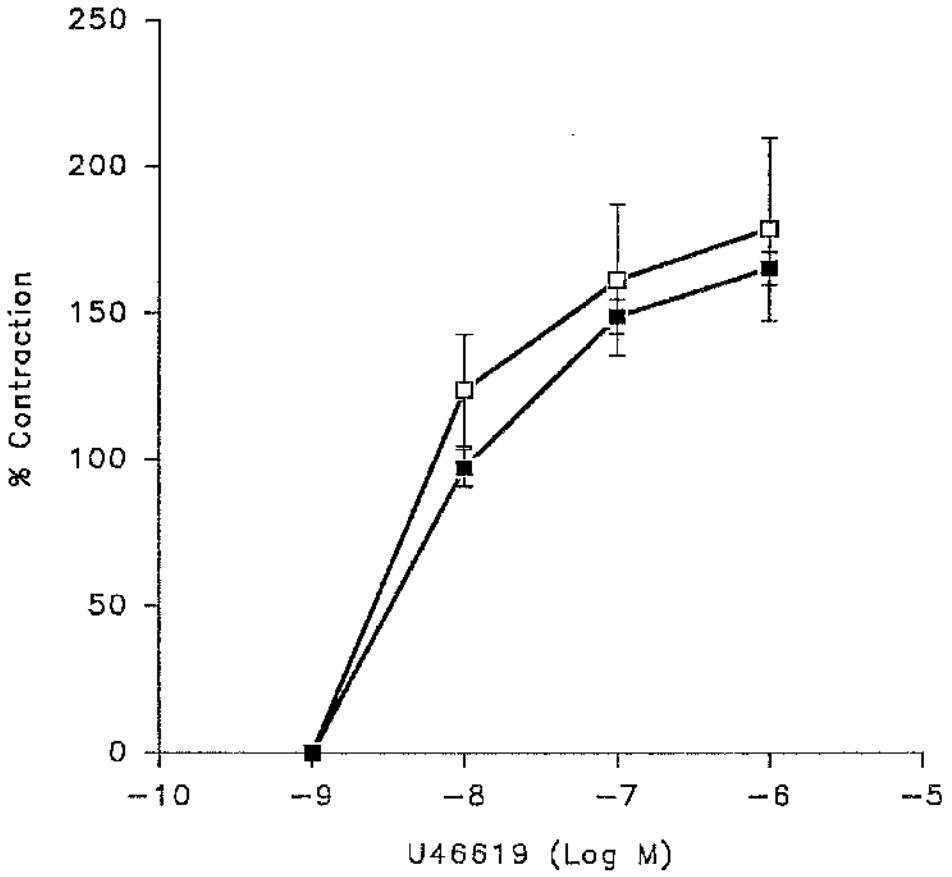

Fig. 3. 15
Concentration-response curves showing contraction to U46619 (10^{-9} to 10^{-6}M) in MPA (closed symbol) and BPA (open symbol) rings of Wistar rats. Tension was recorded as a percentage of the maximum contraction to KCl (3×10^{-2}M). The results are expressed as means of n=7-9 from 7-9 animals. The vertical bars show ± S.E.M.

3.2 Investigation of Agonist-Induced Desensitisation of Isolated Pulmonary Artery Rings of Wistar Rats

3.2.1 *5-Hydroxytryptamine (5-HT)-induced desensitisation*

Prolonged exposure of a variety of cells to drugs or hormones may result in loss of responsiveness to agonists with time. This phenomenon, which has been termed desensitisation, leads to a loss in agonist potency and/or ability to induce a maximal biological response in the tissue. Prolonged exposure of MPA rings and BPA rings with or without endothelium, in the absence and presence of L-NAME (5×10^{-4}M, for 20min) to 5-HT (10^{-4}M, for 30min or 1hr) produced marked desensitisation to 5-HT (Fig. 3.19A). Standard responses to 5-HT before tachyphylaxis, in intact MPA and BPA rings were enhanced by L-NAME, but not in endothelium-denuded rings. Also, L-NAME enhanced the standard responses to 5-HT after tachyphylaxis, in intact MPA and BPA rings, but not in endothelium-denuded rings (Table 3.7 to 3.8; Figs. 3.16 to 3.17 and 3.19B). The mechanical removal of the endothelium by rubbing, enhanced standard responses to 5-HT in MPA rings, but not in BPA rings (Figs. 3.16 to 3.17 and 3.19A). Comparing the maximal responses to 5-HT, before tachyphylaxis, there were no significant differences between MPA and BPA rings, in the absence and presence of L-NAME, whether the endothelium was present or not (Table 3.7). In comparisons between the maximal responses to 5-HT, after tachyphylaxis, there were significant differences between intact MPA and BPA rings, in the absence and presence of L-NAME. There were also differences between denuded MPA and BPA rings in the presence of L-NAME, but not in its absence (Table 3.8). L-NAME depressed the percentage of tachyphylaxis to 5-HT in MPA rings, whether the endothelium was present or not, and in BPA rings when the endothelium was intact (Table 3.7, Fig. 3.18). Prior treatment of MPA and BPA rings with L-NAME (5×10^{-4}M, for 20min)

reduced desensitisation to 5-HT (10^{-4}M). The desensitisation to 5-HT (10^{-4}M) in the absence and presence of L-NAME was more marked in MPA rings than in BPA rings, whether the endothelium was present or not (Table 3.7). The addition of oxidised haemoglobin (HbO, 1mg/ml) or L-NAME (5×10^{-4}M) to desensitised rings with an intact endothelium during prolonged exposure to 5-HT, completely reversed this desensitisation. This effect of L-NAME was inhibited by L-arginine (L-Arg, 2×10^{-4}M), but not in desensitised rings, in which the endothelium had been removed or in rings pretreated with L-NAME. In contrast, prior treatment with L-NAME (5×10^{-4}M, for 20min) or the mechanical removal of the endothelium by rubbing, increased standard contractile responses to 5-HT (10^{-4}M) and reduced but did not prevent the development of desensitisation to 5-HT (Fig. 3.19C). In some experiments, during prolonged exposure to 5-HT (10^{-4}M) or PHE (10^{-5}M) and before the desensitisation had fully developed, the addition of acetylcholine (10^{-6}M) produced relaxations that were reversed by L-NAME (5×10^{-4}M). This action of L-NAME was unaffected by L-arginine (L-Arg, 2×10^{-4}M; Fig. 3.19D). In one series of the experiments, cumulative concentration response curves to 5-HT (10^{-7} to 10^{-4}M) and PHE (10^{-9} to 10^{-5}M), were obtained after tachyphylaxis induced by 5-HT (10^{-4}M) and were compared with those that were obtained before tachyphylaxis. Whereas responses to all of concentrations to 5-HT were inhibited after prolonged exposure to 5-HT, only responses to low concentrations of PHE (10^{-9} to 10^{-8}M) were depressed after tachyphylaxis to 5-HT (Fig. 3.19C; data not shown). Prolonged exposure of MPA rings to PHE (10^{-5}M, for 2 hrs) also produced a desensitisation to PHE. The desensitisation to the α_1-adrenoceptor agonist, PHE, was most marked in MPA rings with an intact endothelium (data not shown). Cumulative response curves to 5-HT before and after tachyphylaxis to PHE were obtained. After tachyphylaxis to PHE only responses to high concentrations of 5-HT (10^{-4}M) were reduced. In comparisons between the percentage of tachyphylaxis to PHE (10^{-5}M) or

5-HT (10^{-4}M), tachyphylaxis to PHE was less than to 5-HT in MPA rings whether in the absence (PHE: 49.71±3.53%, 5-HT: 85.37±5.09%; $P < 0.001$) or presence of L-NAME (PHE: 26.30±2.65%; 5-HT: 65.85±4.63%; $P < 0.001$) (Fig. 3.19). Prior treatment MPA rings with L-NAME (5×10^{-4}M, for 20min) or mechanical removal of the endothelium by rubbing, increased maximal contractile responses to PHE (data not shown) and reduced the percentage tachyphylaxis to PHE (from 49.71±3.53% to 26.30±2.65%; $P < 0.001$; Fig, 3.19). The addition of L-NAME (5×10^{-4}M) to desensitised rings during the prolonged exposure to PHE, produced a contraction which was partially inhibited by L-Arg (2×10^{-4}M).

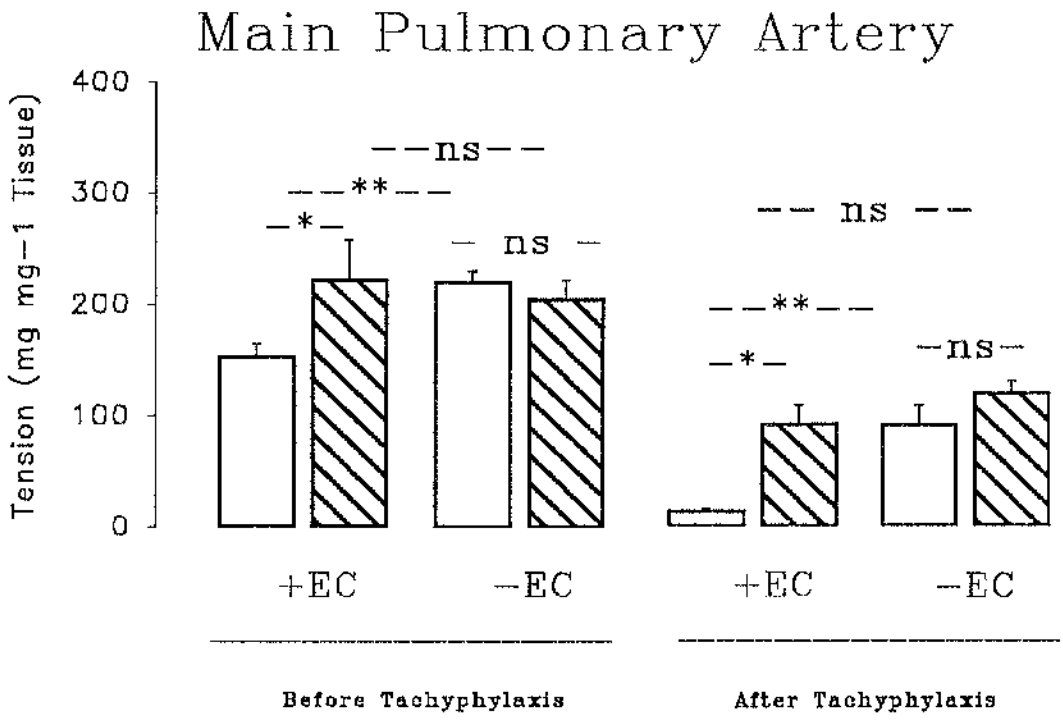

Fig. 3. 16

Histograms showing standard responses of MPA rings to 5-HT before and after prolonged exposure to 5-HT (10^{-4}M, for 30min or 1hr) in intact control rings (+EC) and in rings denuded of their endothelium (-EC) in the absence (open columns) and presence (hatched columns) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. The vertical bars show $\pm$ S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; ns = non-significant (Student's t test).

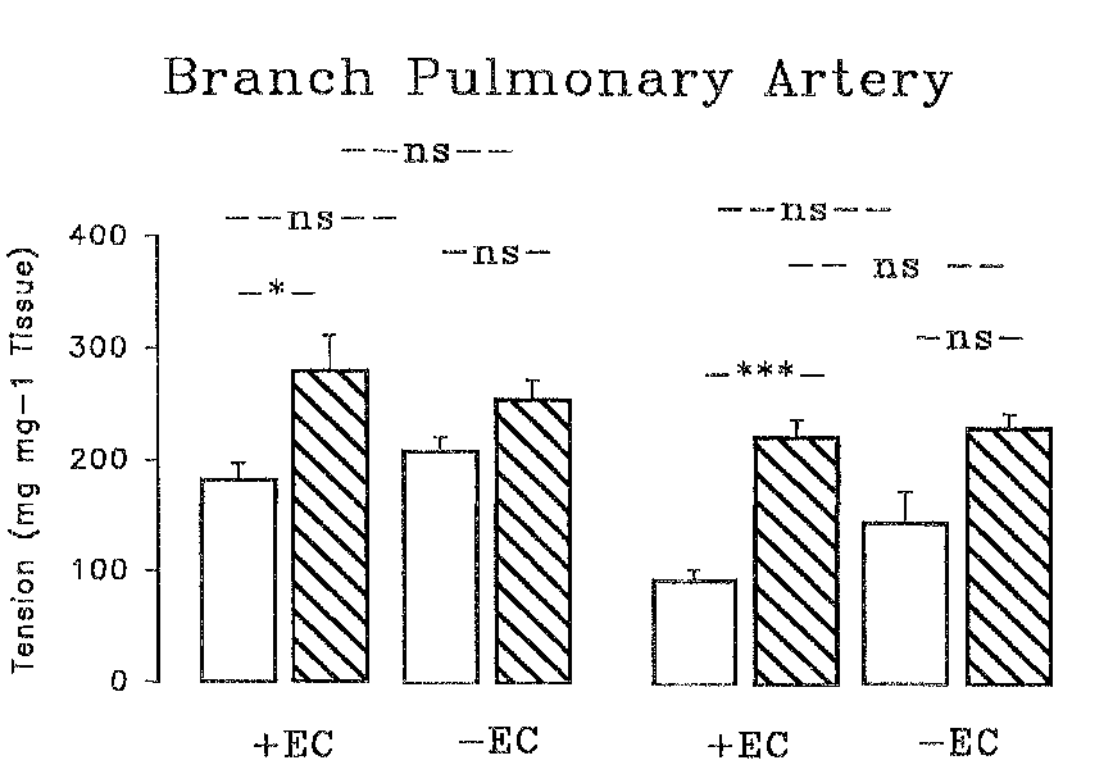

Fig. 3. 17
Histograms showing standard responses of BPA rings to 5-HT before and after prolonged exposure to 5-HT (10^{-4}M, for 30min or 1hr) in intact control rings (+EC) and in rings denuded of their endothelium (-EC) in the absence (open columns) and presence (hatched columns) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; *** $P < 0.001$; ns = non-significant (Student's t test).

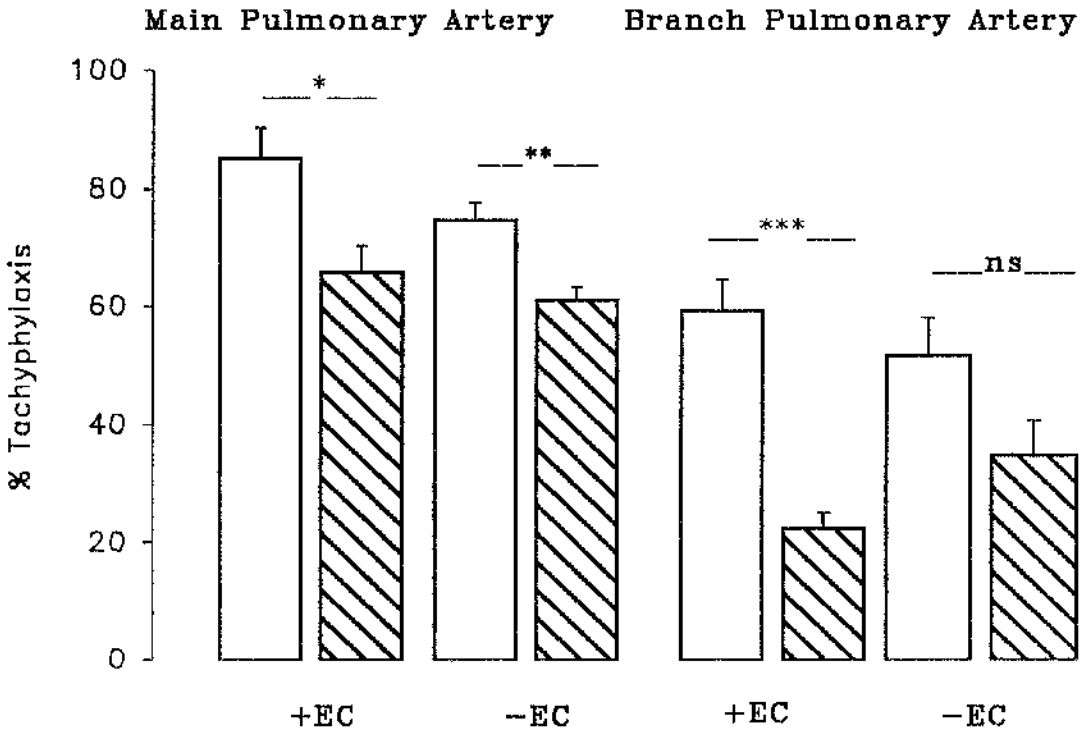

Fig. 3. 18

Histograms showing extent of the tachyphylaxis that occurred in MPA and BPA rings, with and without the endothelium (+EC and -EC) and in the absence (open columns) and presence (hatched columns) of L-NAME (5×10^{-4}M) after prolonged exposure to 5-HT (10^{-4}M, for 30min or 1hr). The results are expressed as means of n=6 rings from 6 animals. The vertical bars show $\pm$ S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns= non-significant (paired Student's t test).

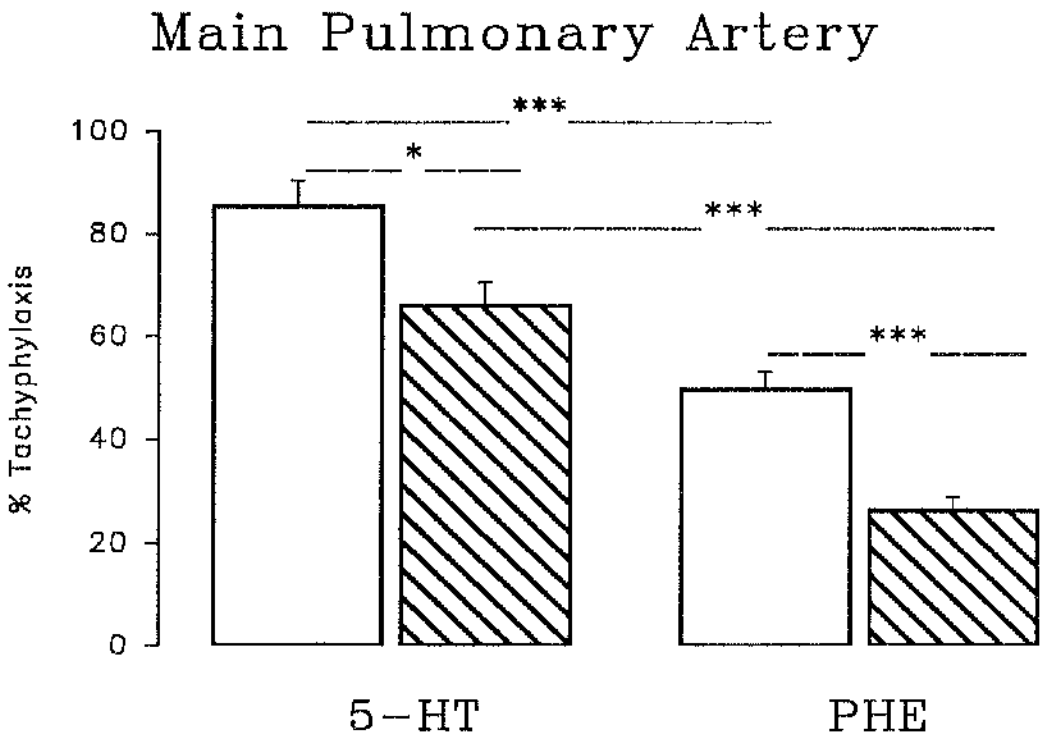

Fig. 3. 19
Histograms showing greater tachyphylaxis to 5-hydroxytryptamine (5-HT, 10^{-4}M) than to phenylephrine (PHE, 10^{-5}M) in MPA rings after prolonged exposure to 5-HT (10^{-4}M, for 30min or 1hr) in the absence (open columns) and presence (hatched columns) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. The vertical bars show $\pm$ S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; *** $P < 0.001$ (Student's t test).

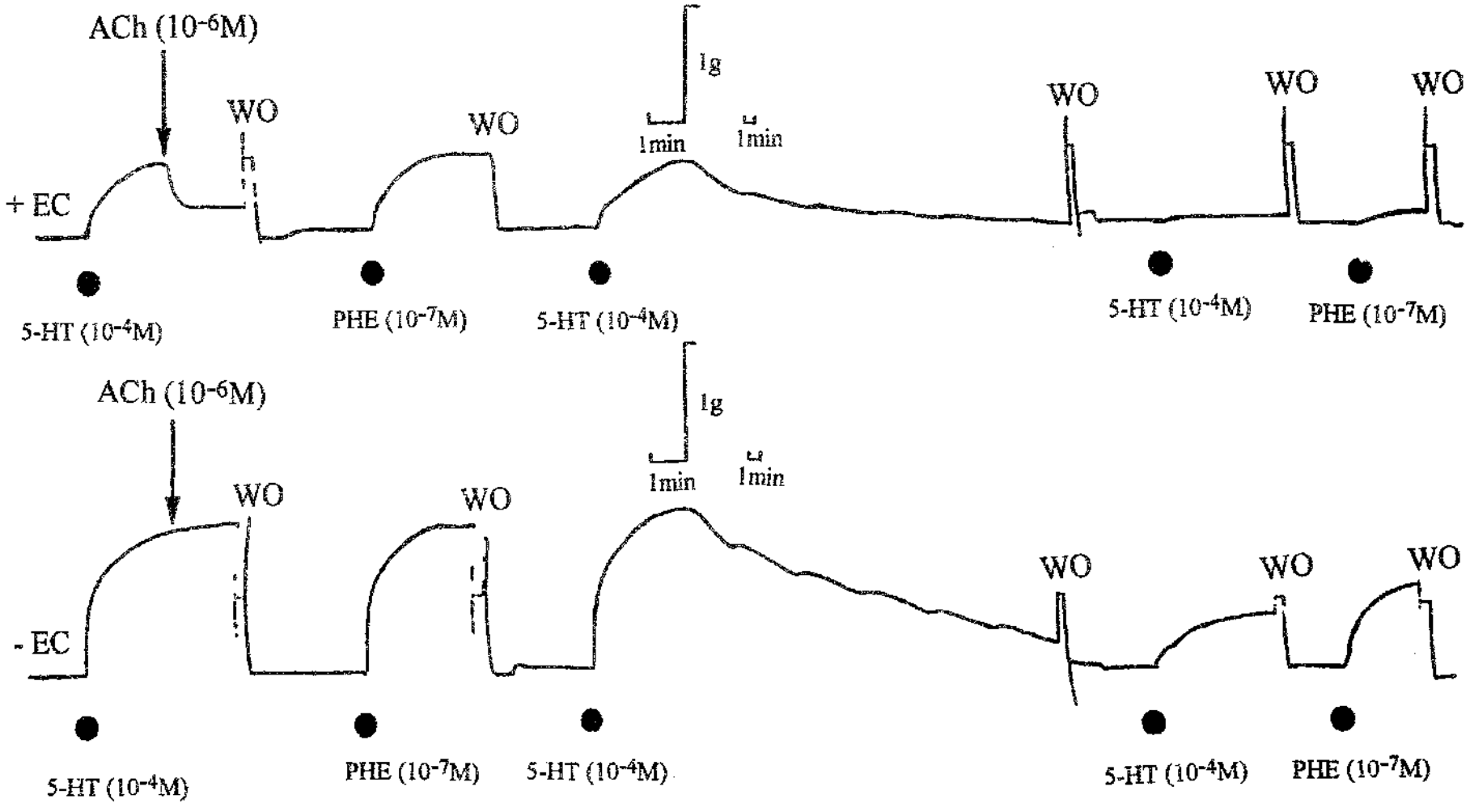

Fig. 3. 19A

Individual traces showing standard responses of main pulmonary artery rings with intact endothelium (+EC) or without endothelium (-EC) to 5-hydroxytryptamine (5-HT, 10^{-4}M) and phenylephrine (PHE, 10^{-7}M) before and after prolonged exposure to 5-HT (10^{-4}M). Symbol (•) indicates drug addition and (WO) indicates wash off.

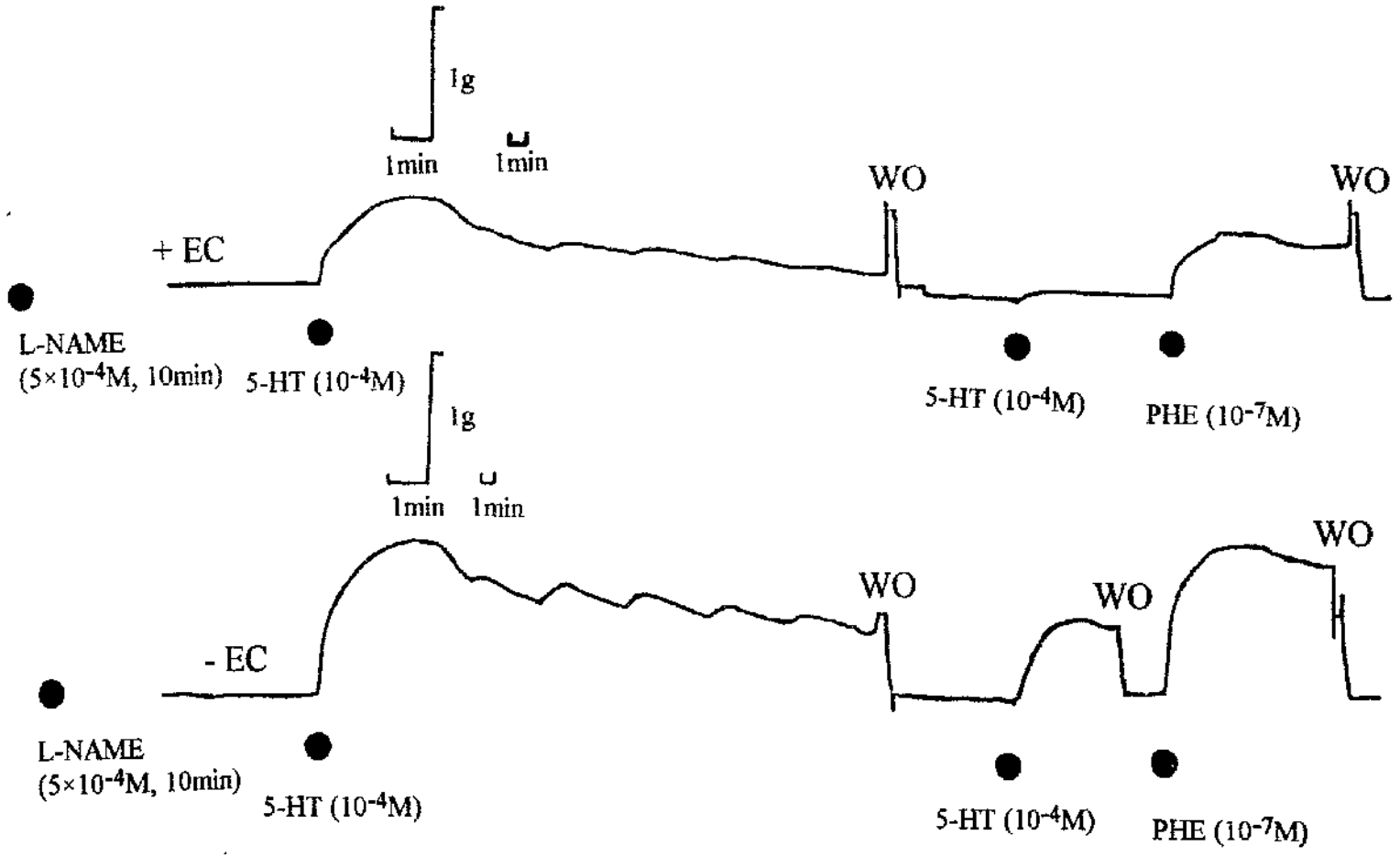

Fig. 3. 19B

Individual traces showing standard responses of main pulmonary artery rings with intact endothelium (+EC) or without endothelium (-EC) to 5-hydroxytryptamine (5-HT, 10^{-4}M) and in the presence of L-NAME (5×10^{-4}M) before and after prolonged exposure to 5-HT (10^{-4}M). Symbol (•) indicates drug addition and (WO) indicates wash off.

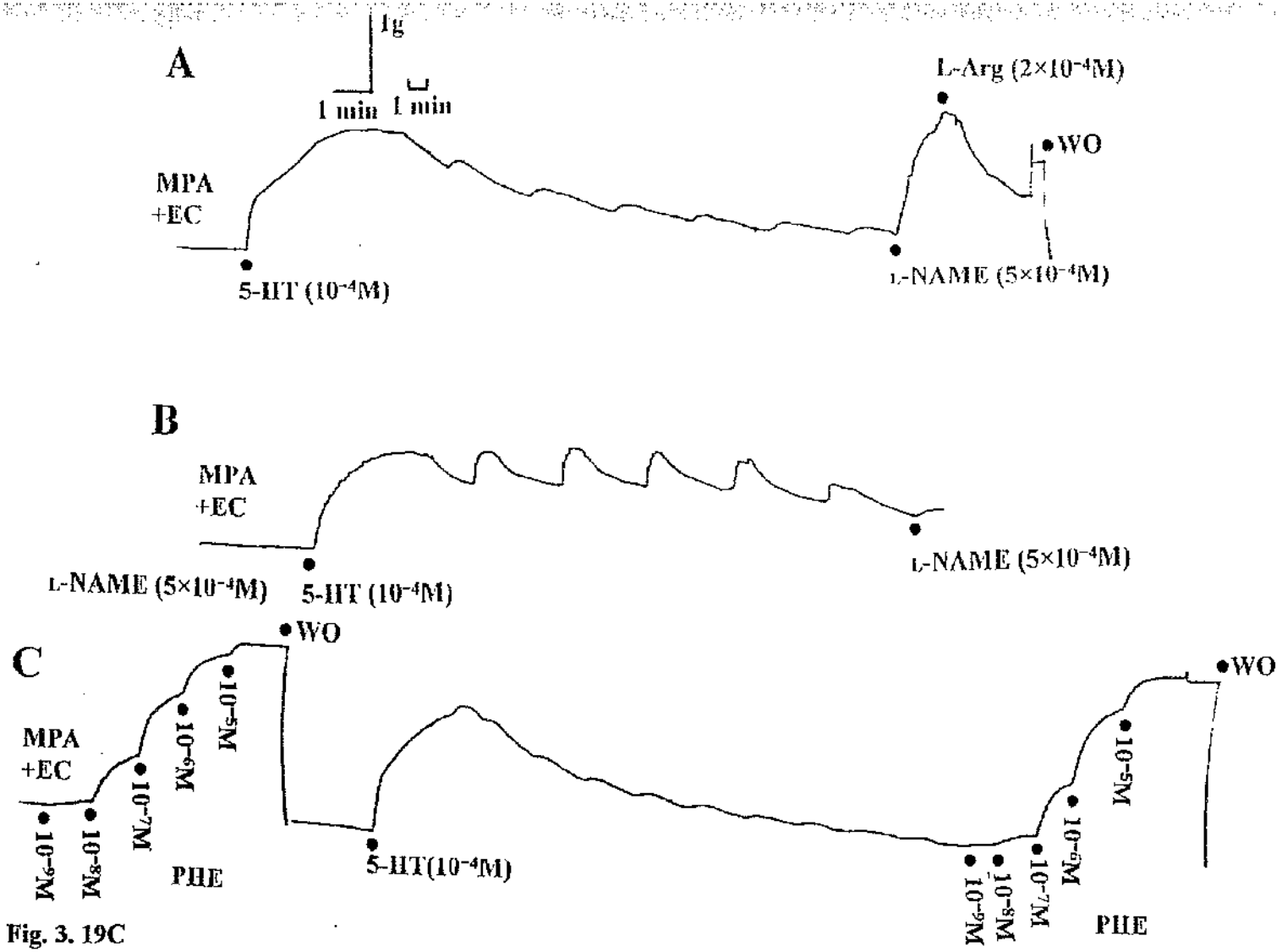

Fig. 3. 19C

Individual traces (A and B) respectively showing the responses of the intact main pulmonary artery (+EC, MPA) rings to prolonged exposure to 5-HT (10^{-4}M). L-NAME (5×10^{-4}M) reversed the decline in the response when artery rings had not been pretreated with L-NAME, and this effect was reversed by L-Arg (2×10^{-4}M). Also trace C showing cumulative dependent-concentration responses to PHE in intact MPA ring before and after prolonged exposure to 5-HT (10^{-4}M). Symbol (•) indicates drug addition and (WO) indicates wash off.

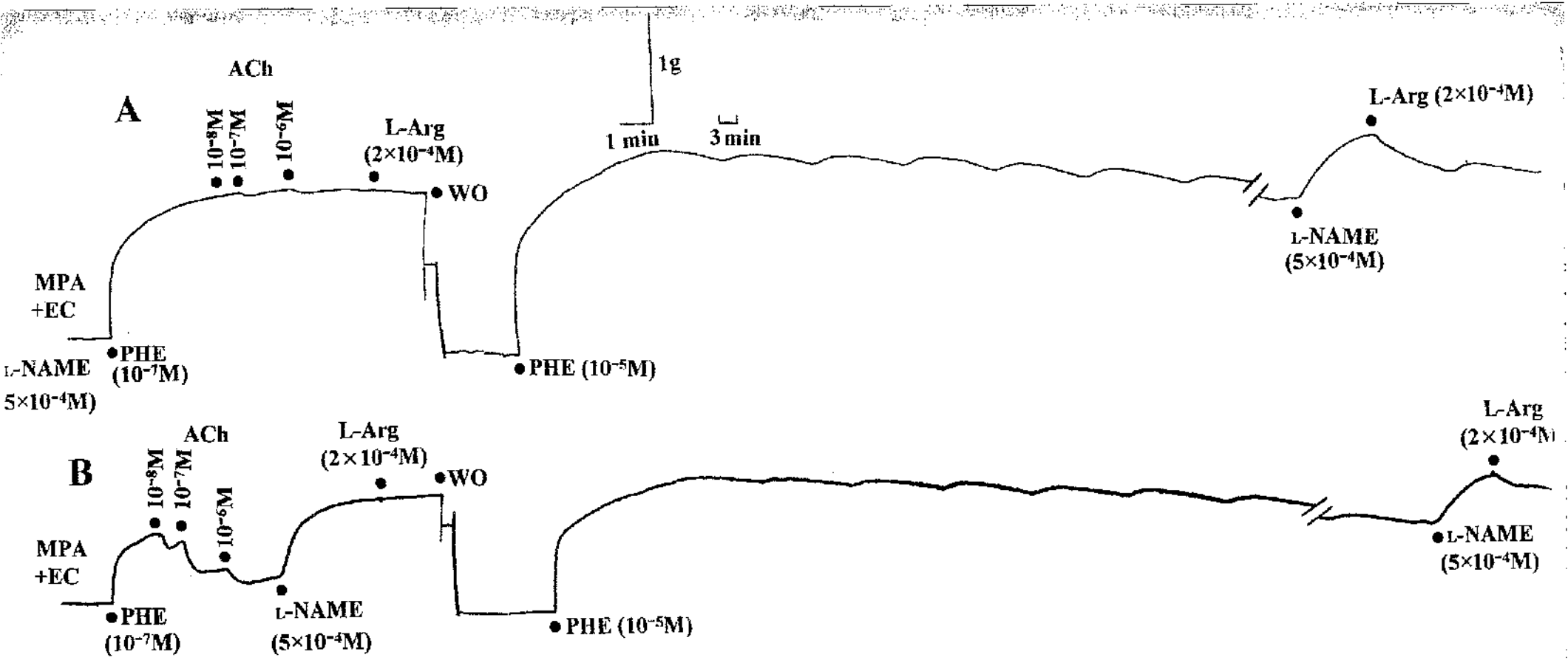

Fig. 3. 19D

Individual traces (A and B) respectively showing responses of intact main pulmonary artery (+EC, MPA) rings to PHE (10^{-7}M) and ACh (10^{-8} to 10^{-6}M) in the presence and absence of L-NAME (5×10^{-4}M) and the effects of L-NAME and L-Arg (2×10^{-4}M) on these induced-responses. Symbol (•) indicates drug addition and (WO) indicates wash off.

Pulmonary Artery	The E_{max} (mg mg^{-1} tissue) (Before tachyphylaxis)	P values
(+EC) MPA (control) V (+EC) MPA (treated)	153.41±11.59 V 238.79±30.38	0.05 > * P > 0.01
(+EC) BPA (control) V (+EC) BPA (treated)	182.50±14.93 V 279.44±32.38	0.05 > * P > 0.01
(+EC) MPA (control) V (+EC) BPA (control)	153.41±11.59 V 182.50±14.93	P > 0.05
(+EC) MPA (treated) V (+EC) BPA (treated)	238.79±30.38 V 279.44±32.38	P > 0.05
(+EC) MPA (control) V (-EC) MPA (control)	153.41±11.59 V 220.54±10.36	0.01 > ** P > 0.001
(+EC) BPA (control) V (-EC) BPA (control)	182.50±14.93 V 208.89±13.27	P > 0.05
(-EC) MPA (control) V (-EC) BPA (control)	220.54±10.36 V 208.89±13.27	P > 0.05
(-EC) MPA (treated) V (-EC) BPA (treated)	204.12±18.20 V 254.44±17.86	P > 0.05

Table 3.7
Comparison between the maximum responses induced by 5-HT (10^{-4}M) before tachyphylaxis in MPA and BPA rings and/or in MPA rings versus (V) BPA rings with endothelium (+EC) or without endothelium (-EC) and in the absence (control) and presence (treated) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$ (Student's t test).

Pulmonary Artery	The E_{max} (mg mg^{-1} tissue) (After tachyphylaxis)	P values
(+EC) MPA (control) V (+EC) MPA (treated)	12.75±2.49 V 91.08±17.76	0.05 > * P > 0.01
(+EC) BPA (control) V (+EC) BPA (treated)	70.00±7.58 V 166.67±11.79	*** P < 0.001
(+EC) MPA (treated) V (-EC) MPA (treated)	91.08±17.76 V 118.96±11.51	P > 0.05
(+EC) BPA (treated) V (-EC) BPA (treated)	166.67±11.79 V 170.83±10.49	P > 0.05
(+EC) MPA (control) V (+EC) BPA (control)	12.75±2.49 V 70.00±7.58	0.01 > ** P > 0.001
(+EC) MPA (treated) V (+EC) BPA (treated)	91.08±17.76 V 166.67±11.79	0.05 > * P > 0.01
(-EC) MPA (control) V (-EC) BPA (control)	90.75±17.80 V 108.33±20.97	P > 0.05
(-EC) MPA (treated) V (-EC) BPA (treated)	118.96±11.51 V 170.83±10.49	0.05 > * P > 0.01

Table 3.8
Comparison between the maximum responses induced by 5-HT (10^{-4}M) after tachyphylaxis in MPA and BPA rings and/or in MPA rings versus (V) BPA rings with endothelium (+EC) or without endothelium (-EC) and in the absence (control) and presence (treated) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's t test).

Pulmonary Artery	% Tachyphylaxis to 5-HT	P values
(+EC) MPA (control) V (+EC) MPA (treated)	85.37±5.09 V 65.85±4.63	0.05 > * P > 0.01
(+EC) BPA (control) V (+EC) BPA (treated)	59.27±5.42 V 22.33±2.77	*** P < 0.001
(+EC) MPA (control) V (+EC) BPA (control)	85.37±5.09 V 59.27±5.42	0.01 < ** P < 0.001
(+EC) MPA (treated) V (+EC) BPA (treated)	65.85±4.63 V 22.37±2.77	*** P < 0.001
(+EC) MPA (control) V (-EC) MPA (control)	85.37±5.09 V 74.78±3.00	P > 0.05
(+EC) BPA (control) V (-EC) BPA (control)	59.27±5.24 V 51.56±6.53	P > 0.05
(+EC) MPA (treated) V (-EC) MPA (treated)	65.85±4.63 V 61.09±2.30	P > 0.05
(+EC) BPA (treated) V (-EC) BPA (treated)	22.33±2.77 V 34.83±5.83	P > 0.05

Table 3.9
Comparison between the percentage of tachyphylaxis to 5-HT (10^{-4}M) in MPA and BPA rings and/or in MPA rings versus (V) BPA rings with endothelium (+EC) or without endothelium (-EC) and in the absence (control) and presence (treated) of L-NAME (5×10^{-4}M). The results are expressed as means of n=6 rings from 6 animals. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's t test).

3.2.2 *Rapid desensitisation to Angiotensin II (Ang II)*

Exposure to the maximum concentration (10^{-7}M) of Ang II produced rapid desensitisation in MPA and BPA rings. The control maximum contractions induced by Ang II in MPA rings and BPA rings were respectively, 257.67±20.94 and 250±22.66; mg mg^{-1} tissue ($P > 0.05$) and after desensitisation produced by Ang II, were 141.67±22.28 (MPA) and 140±23.14 (BPA) mg mg^{-1} tissue ($P > 0.05$). There were significant differences between the standard responses to Ang II (10^{-7}M), in control MPA rings and in desensitised MPA rings ($P < 0.001$; Fig. 3.20) and also between control BPA rings and desensitisised rings of BPA ($P < 0.001$; Fig. 3.20). Following pretreatment of desensitisised rings with a subthreshold concentration of KCl (10^{-2}M, for 5min), responses to Ang II were enhanced. The maximum contractions to Ang II (10^{-7}M) in MPA, and BPA rings, pretreated with KCl after desensitisation, were 226.67±20.70 (MPA) and 242.92±24.77 (BPA) mg mg^{-1} tissue. In comparisons with control (before Ang II-induced tachyphylaxis), there were significant differences between responses to Ang II in MPA rings ($0.01 > P > 0.001$) but not in BPA rings. In other experiments, pretreatment of rings with L-NAME (2×10^{-4}M), or the mechanical removal of the endothelium by rubbing, did not prevent tachyphylaxis to Ang II. In other experiments, in which MPA or BPA rings had been desensitised by repeated exposure to 5-HT (10^{-4}M), subthreshold concentration of KCl (10^{-2}M, for 5min) also enhanced responses to 5-HT (10^{-4}M) (data not shown).

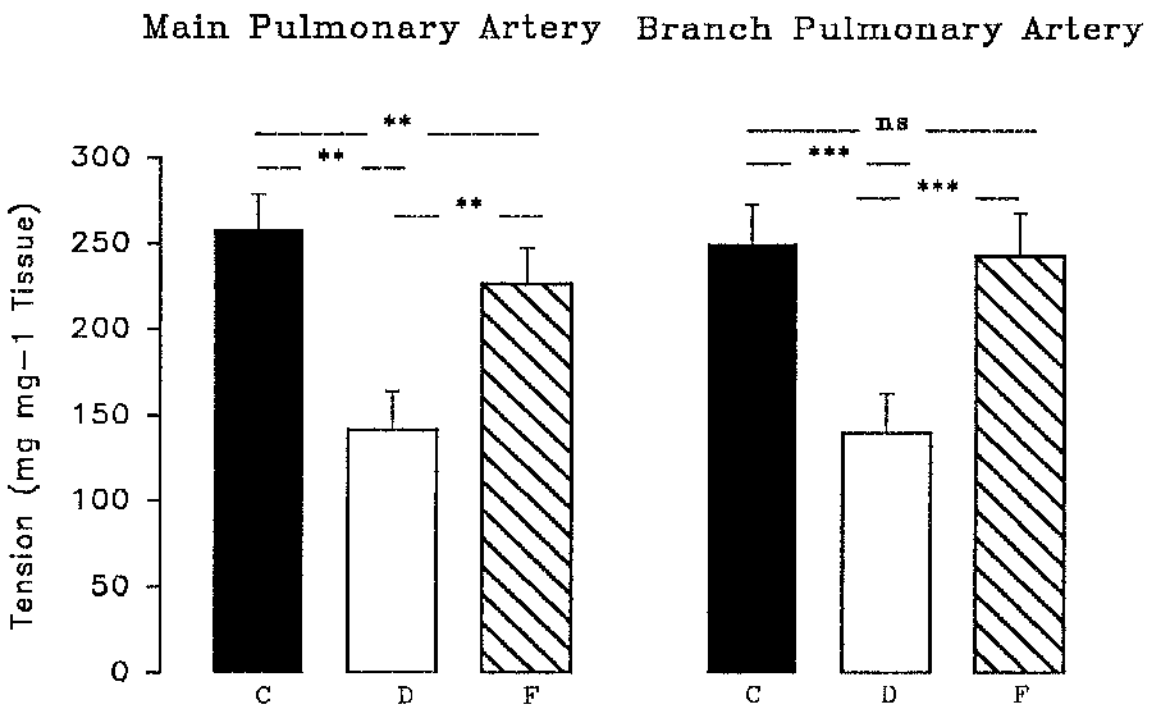

Fig. 3. 20

Histograms showing extent of the maximum response to angiotensin II (Ang II, 10^{-7}M) before (C) and after the desensitisation (D) and the facilitation (F) (pretreated with KCl, 10^{-2}M, for 10-15min)] in MPA and BPA rings of Wistar rats. Tension was recorded (mg mg^{-1} Tissue). The results are expressed as means of n=9-10 from 9-10 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: ** $P < 0.01$; *** $P < 0.001$; ns= non-significant (paired Student's t test).

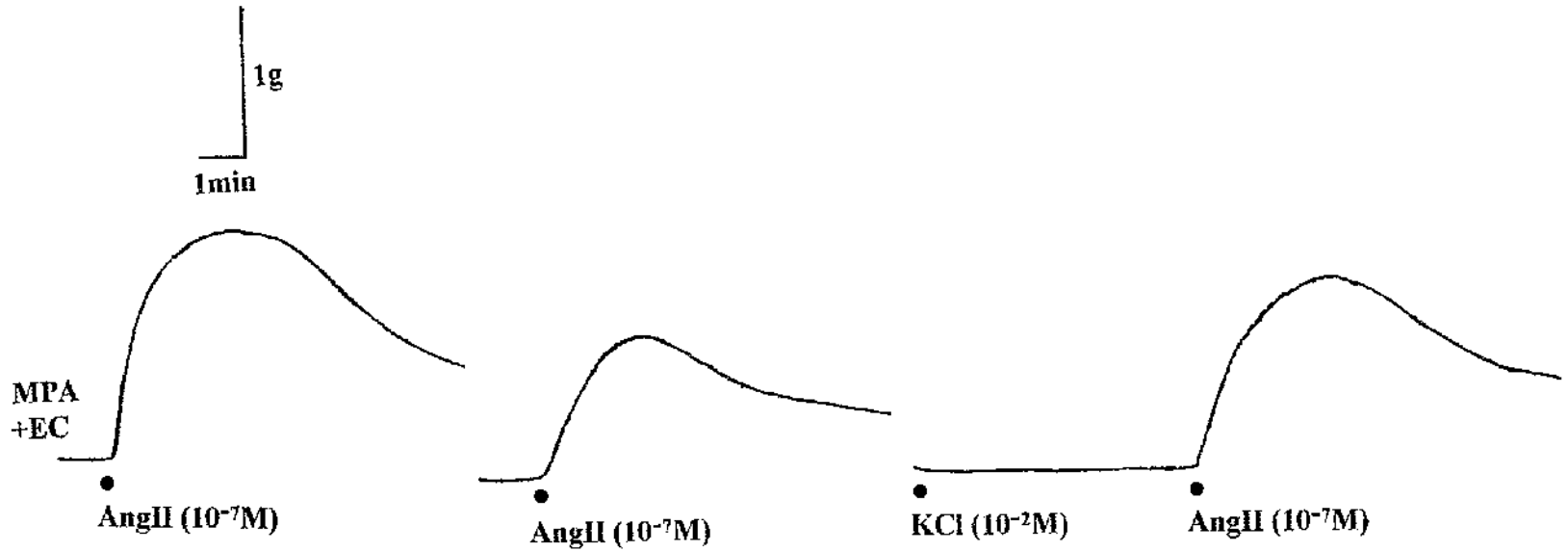

Fig. 3.20A
Individual figures showing extent of the maximum response to angiotensin II (Ang II, 10^{-7}M) before and after rapid desensitisation and the facilitation by KCl (10^{-2}M) in endothelium-intact main pulmonary artery (+EC, MAP) rings of Wistar rats. Symbol (•) indicates drug addition.

3.3 Investigation of vasorelaxant responses to agonists in main pulmonary artery (MPA) and branch pulmonary artery (BPA) rings of Wistar rats

3.3.1 *Vasorelaxant responses to acetylcholine (ACh), carbachol (CARB) and sodium nitroprusside (SNP)*

The endothelium-dependent vasorelaxant acetylcholine (ACh, 10^{-9} to 3×10^{-6}M) induced concentration-dependent relaxations in MPA and in BPA rings precontracted with PHE (EC_{70}, 1.2×10^{-7}M). ACh-induced relaxations in BPA rings were significantly larger than those obtained in MPA rings (Fig. 3.21). The maximum relaxations in BPA and MPA rings were (respectively, 81.79±3.74% and 64.26±3.66%; $0.01 > P > 0.001$). In another series of experiments, the endothelium-dependent relaxant, carbachol, (CARB, 10^{-8} to 10^{-4}M) induced concentration-dependent relaxations in PHE-precontracted (10^{-7}M) MPA and BPA rings (Fig, 3.22). The maximum relaxations in MPA rings and BPA rings were (respectively, 44.33±3.86% and 75.50±3.35%; $P < 0.001$). The BPA rings were more responsive than MPA rings. Pretreatment of the rings with L-NAME (5×10^{-4}M, for 10min) abolished CARB-induced relaxations to low concentrations (10^{-8} to 10^{-7}M), but high concentrations (10^{-6} to 10^{-5}M) of CARB produced concentration-dependent contractions (data not shown). Similarly, the endothelium-independent relaxant sodium nitroprusside (SNP, 10^{-12} to 10^{-6}M) induced concentration-dependent relaxations in PHE-precontracted MPA rings and BPA rings In comparisons between concentration-response curves, MPA rings were more responsive than BPA to SNP (Fig. 3.23). The maximum relaxations in these rings were (MPA: 99.40±0.60% and BPA: 94.61±2%; $P < 0.05$).

In other experiments, concentration response curves to SNP (10^{-11} to 10^{-5}M) were obtained in control rings and in rings pretreatead with L-NAME (5×10^{-4}M). L-NAME had no significant effect on concentration-dependent relaxations to SNP (Fig. 3.24 to 3.25).

In another series of experiments, concentration-response curves to SNP (10^{-12} to 10^{-7}M) were obtained in MPA and BPA rings precontracted with 5-HT (EC_{50}, 10^{-5}M; Fig. 3.26). In comparisons between concentration-response curves, MPA rings were only more responsive than BPA rings to SNP (10^{-7}M) ($0.05 > P > 0.01$; Fig. 3. 26). The maximum responses to SNP in these rings were (MPA: 97.62±2.38% and BPA: 86.43±3.64%; $0.05 > P > 0.01$). Pretreatment of the rings with L-NAME (5×10^{-4}M, for 20min) had no significant effect on concentration-dependent relaxations to SNP (Fig. 3.27 to 3.28).

In comparisons between concentration response curves to the vasorelaxant agonists, the endothelium-independent agonist SNP, was more potent than endothelium-dependent agonist ACh in both MPA rings and BPA rings, precontracted with PHE (EC_{70}; 1.2×10^{-7}M; Fig. 3.29 to 3.30).

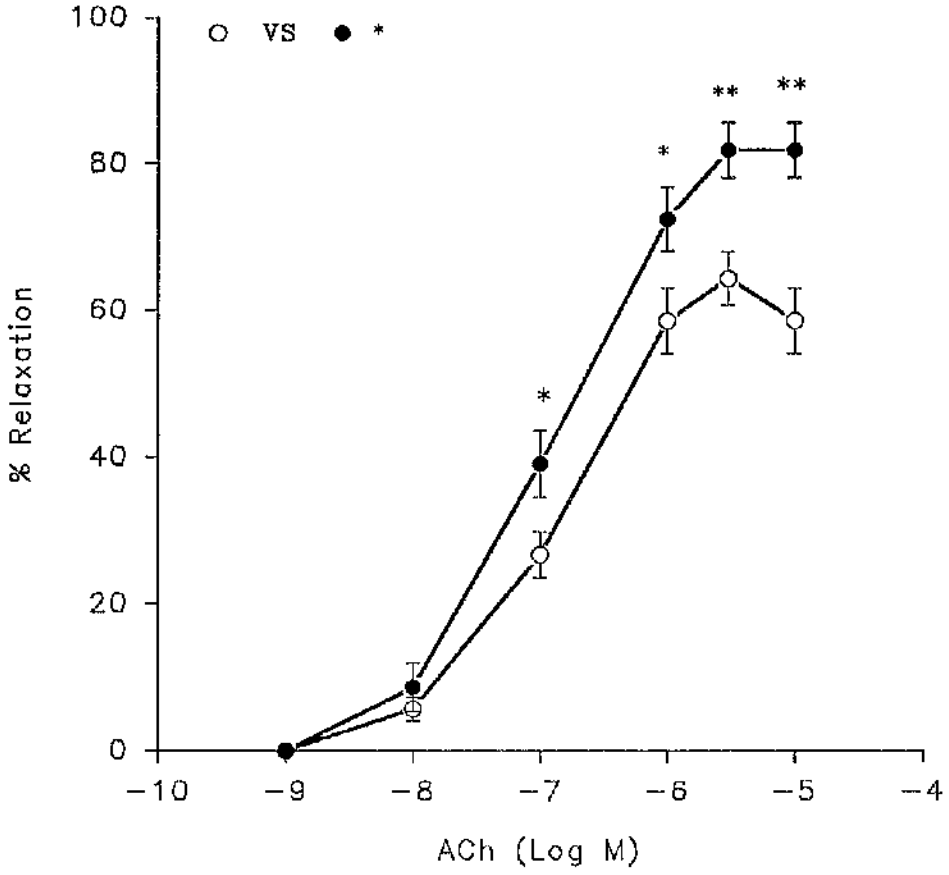

Fig. 3. 21
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 3×10^{-6}M) in MPA (open circle) and BPA (closed circle) rings precontracted with phenylephrine (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$ (unpaired t test).

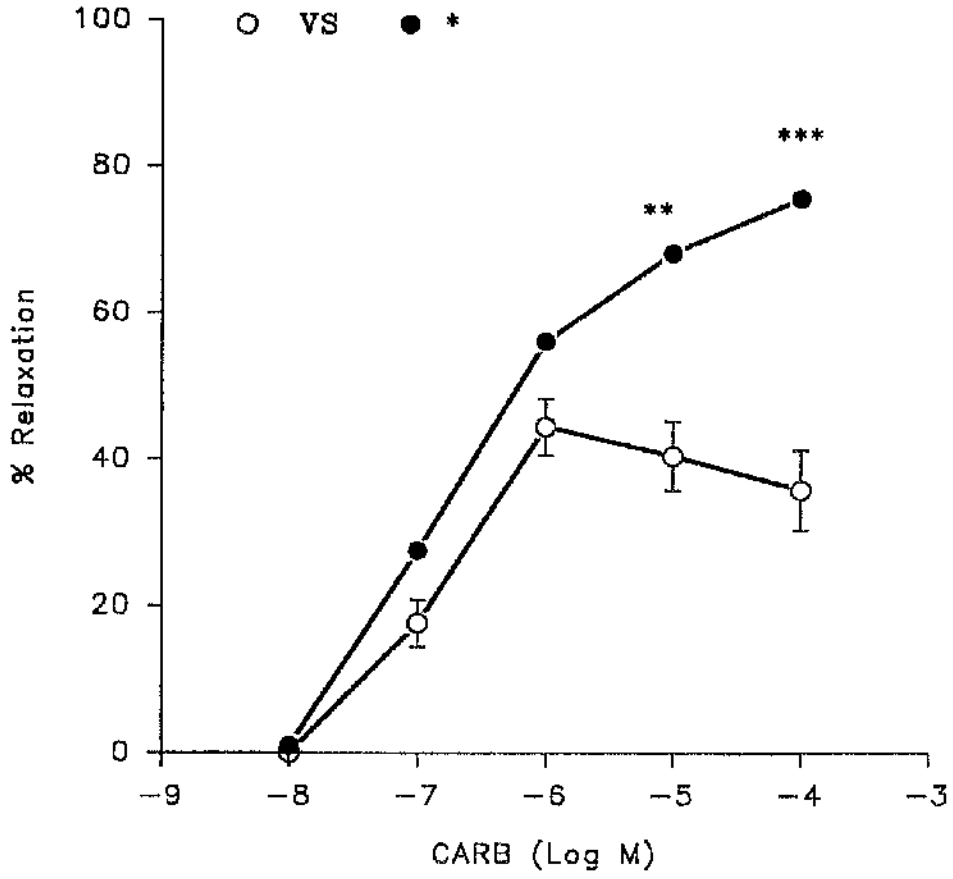

Fig. 3. 22
Comparison between concentration-response curves showing relaxation to carbachol (CARB, 10^{-8} to10^{-4}M) in MPA rings (open circle) and in BPA rings (closed circle) precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p values*: ** $P < 0.01$; *** $P < 0.001$ (unpaired t test).

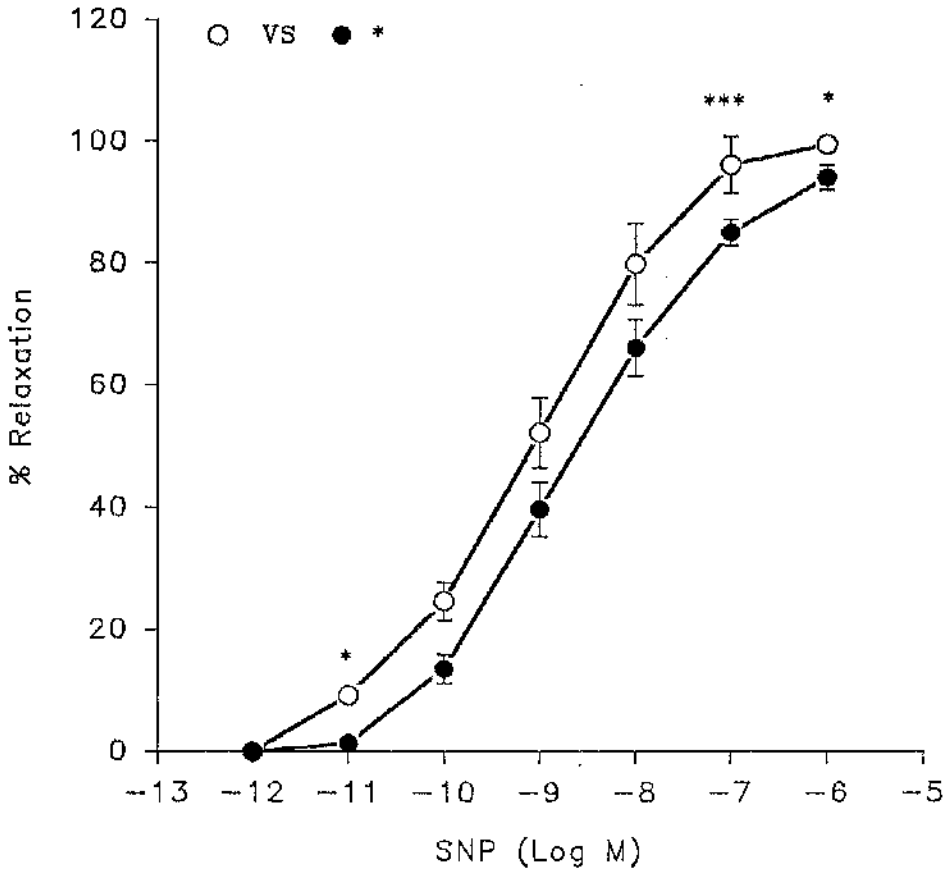

Fig. 3. 23

Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-12} to 10^{-6}M) in MPA (open symbol) and BPA (closed symbol) rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 from 6 animals, vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p value*: * $P < 0.05$; *** $P < 0.001$(unpaired t test).

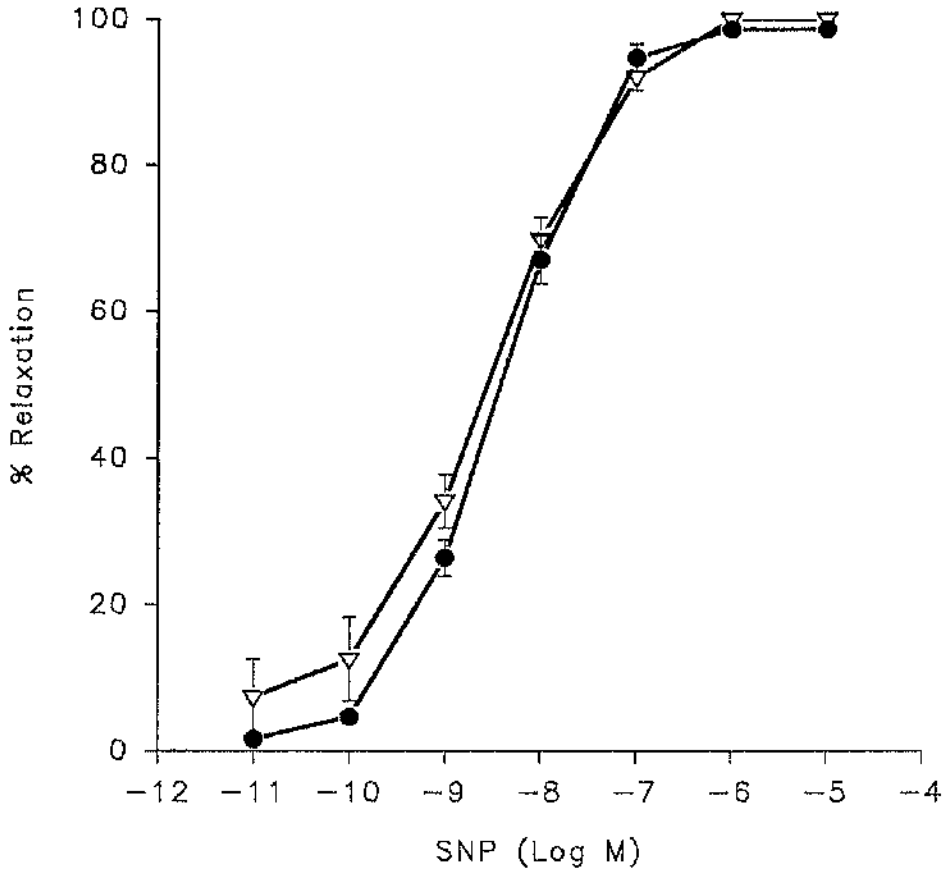

Fig. 3. 24
Concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-5}M) in the absence (closed symbol) and presence (open symbol) of L-NAME (5×10^{-4}M) in MPA rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

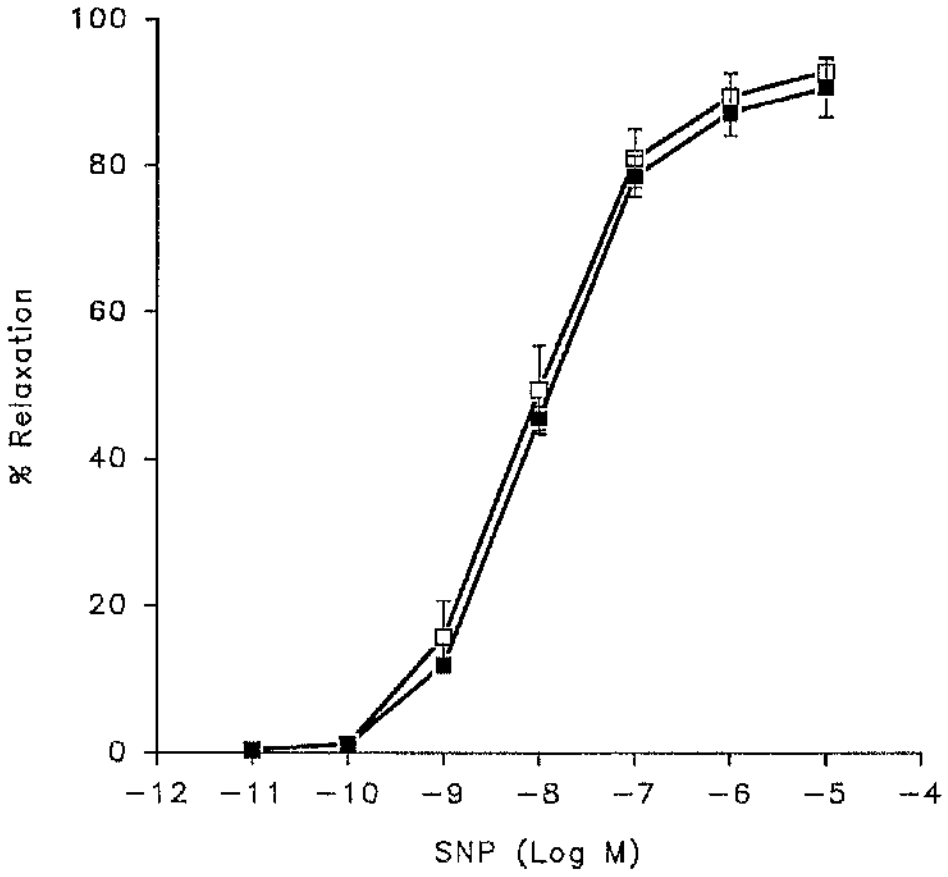

Fig. 3. 25
Concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-5}M) in the absence (closed symbol) and presence (open symbol) of L-NAME (5×10^{-4}M) in BPA rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

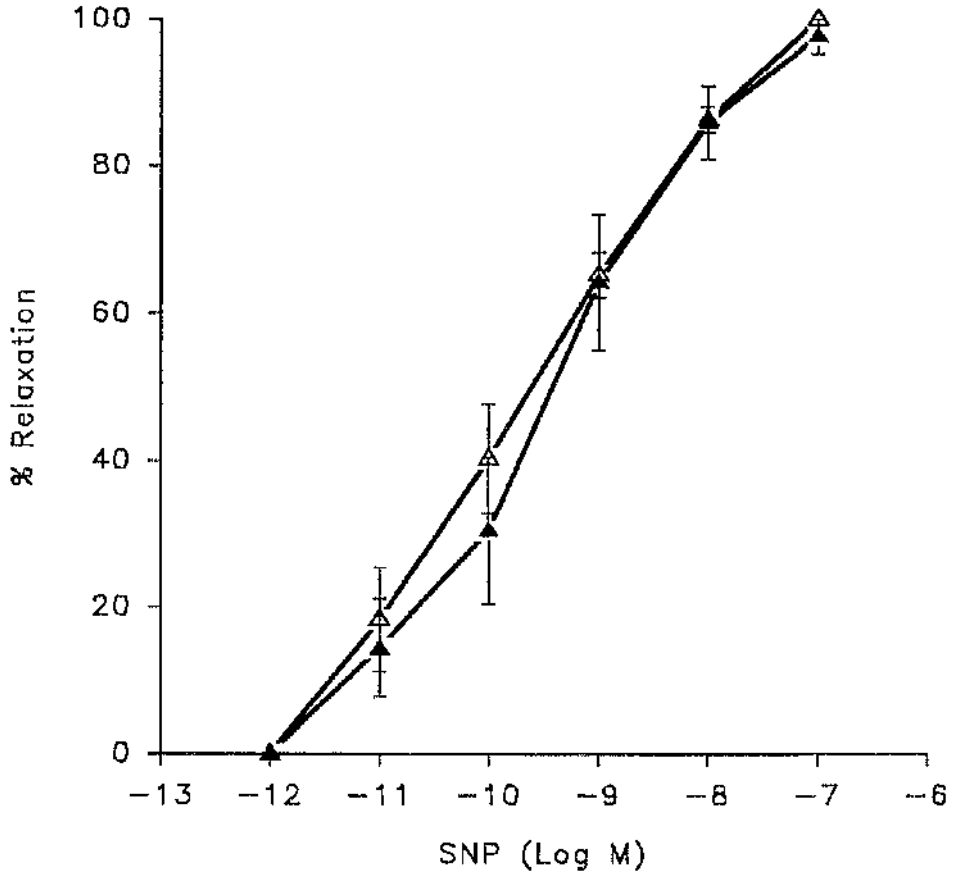

Fig. 3. 26

Concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-12} to 10^{-7}M) in the absence (open symbol) and presence (closed symbol) of L-NAME (5×10^{-4}M) in MPA rings precontracted with 5-HT (EC_{50}; 10^{-5}M). The results are expressed as means of percentage relaxation of 5-HT-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

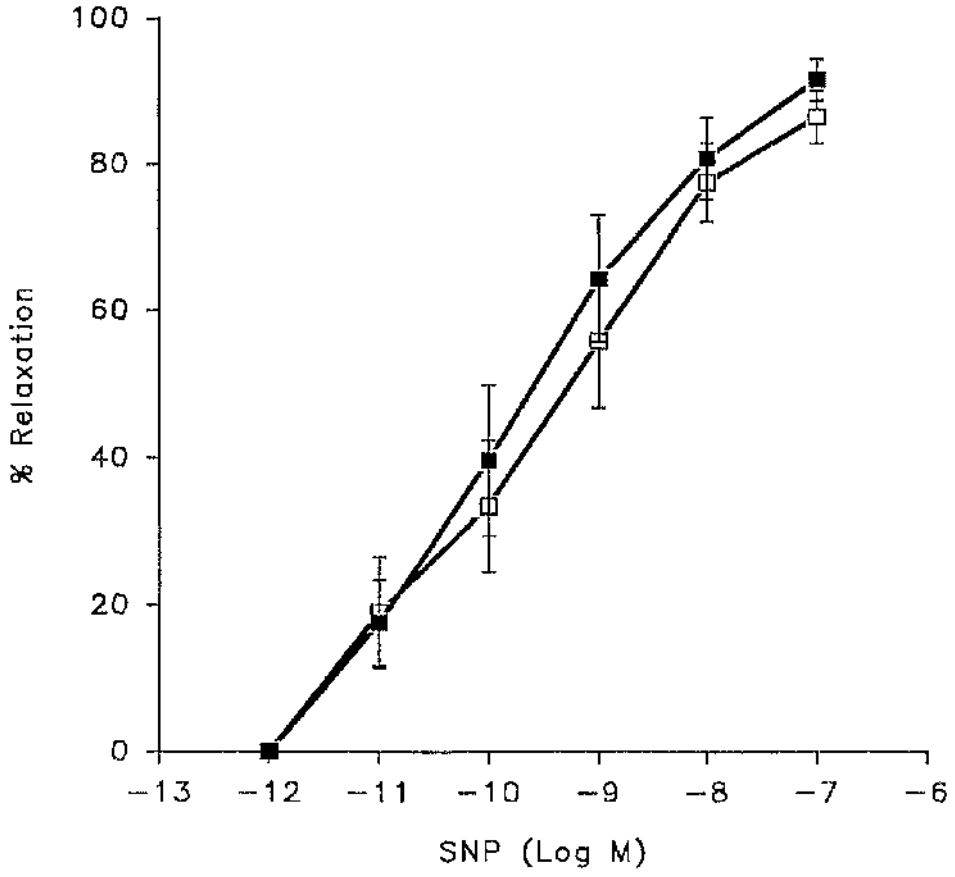

Fig. 3. 27
Concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-12} to 10^{-7}M) in the absence (open symbol) and presence (closed symbol) of L-NAME (5×10^{-4}M) in BPA rings precontracted with 5-HT (EC_{50}; 10^{-5}M). The results are expressed as means of percentage relaxation of 5-HT-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

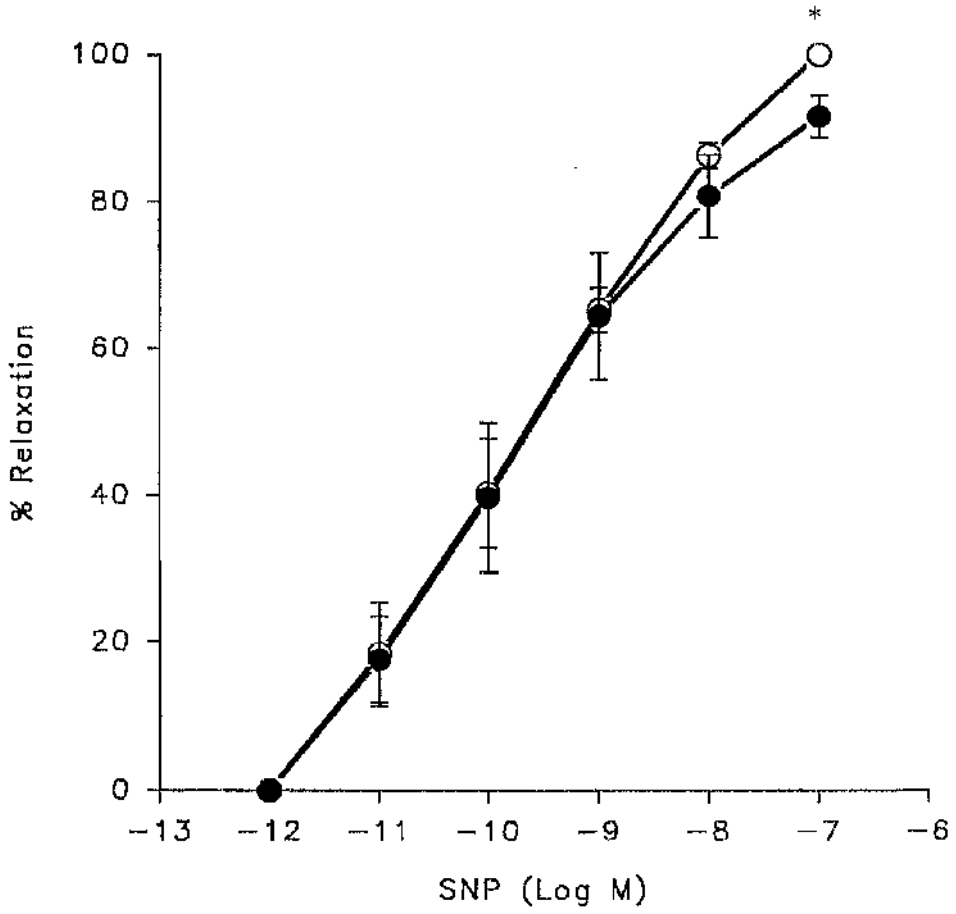

Fig. 3. 28

Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-12} to 10^{-7}M) in MPA .(open symbol) and BPA (closed symbol) rings precontracted with 5-HT (EC_{50}; 10^{-5}M). The results are expressed as means of percentage relaxation of 5-HT-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M. Significant difference between individual means is indicated by *p value*: * $P < 0.05$ (unpaired t test)

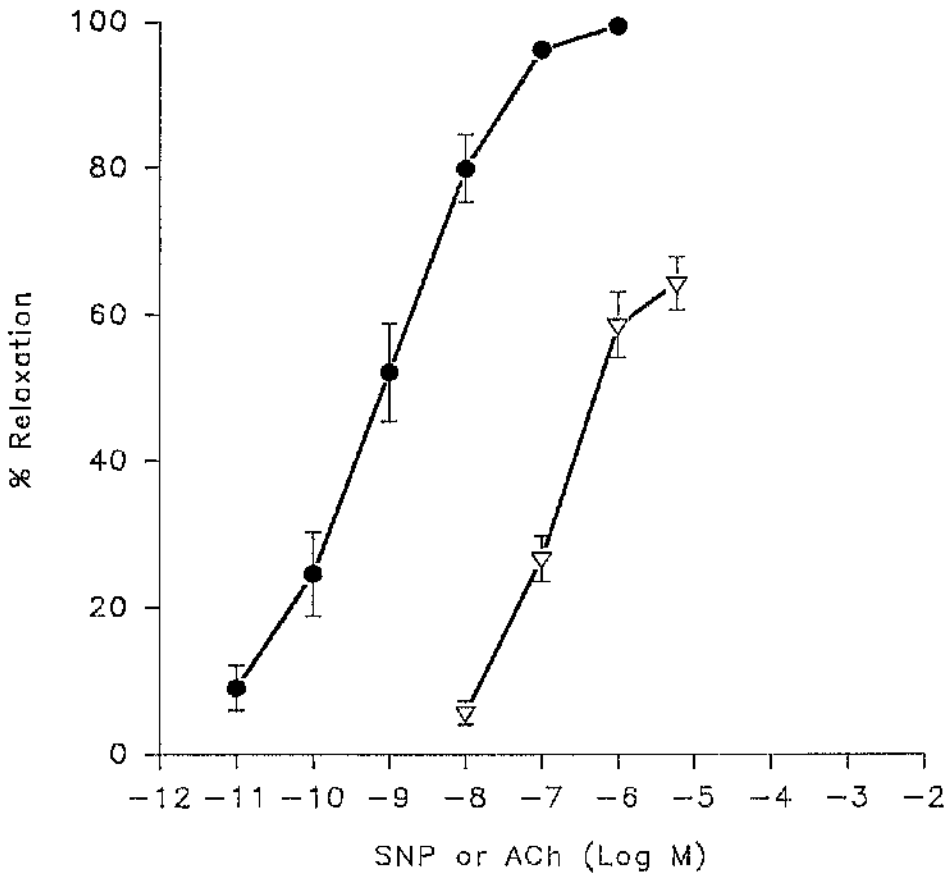

Fig. 3. 29
Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-6}M; closed symbol) and acetylcholine (ACh, 10^{-8} to 3×10^{-6}M; open symbol) in MPA rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-10 rings from 6-10 animals, vertical bars show ± S.E.M.

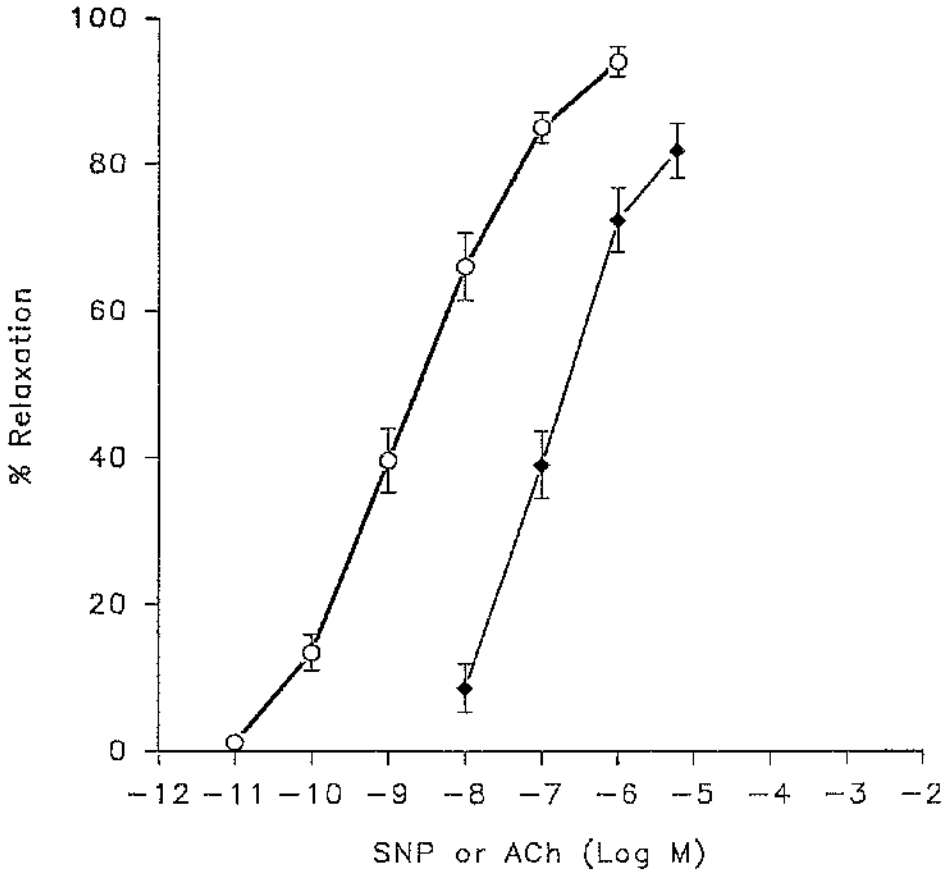

Fig. 3. 30
Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-6}M; open symbol) and acetylcholine (ACh, 10^{-8} to 3×10^{-6}M; closed symbol) in BPA rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-10 rings from 6-10 animals, vertical bars show ± S.E.M.

3.3.2 *Vasorelaxant responses to the purine adenine nucleotides, adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) and adenosine (AD)*

Adenosine 5'-triphosphate (ATP, 10^{-8} to 10^{-5}M) induced concentration-dependent relaxations in MPA and BPA rings precontracted with PHE (10^{-7}M) The maximum relaxant responses to ATP (10^{-5}M) in rings which had been precontracted with PHE were MPA: 80.48±1.93% and BPA: 74.76±3.48% ($P > 0.05$; Fig. 3.31 to 3.32). The mechanical removal of the endothelium by rubbing, reduced relaxant responses to 10^{-6}M of ATP in MPA rings ($P < 0.001$) and to 10^{-7} to 10^{-5}M of ATP in BPA rings ($P < 0.001$). Pretreatment of artery rings with L-NAME (2×10^{-4}M, for 10-15min) reduced responses to ATP (10^{-7} to 10^{-5}M) in both MPA and BPA rings ($P < 0.001$). There were no significant differences between responses to ATP (10^{-8} to 10^{-5}M) in intact MPA and BPA rings ($P > 0.05$; Fig. 3.33). In other experiments, in which the artery rings were pretreated with the blocker of ATP-sensitive potassium channels, glibenclamide (10^{-6}M, for 10min) or with the PDE inhibitor, theophylline (10^{-6}M, for 10min) the relaxant responses to ATP were unaffected (data not shown).

Concentration-dependent relaxations to ADP (10^{-8} to 10^{-5}M) were obtained in MPA and BPA rings precontracted with PHE (10^{-7}M). The maximum relaxant responses to ADP (10^{-5}M) in intact rings precontracted with PHE were MPA: 78.39±2.68% and BPA: 68.45±2.29% ($0.05 > P > 0.01$) (Fig. 3.34 to 3.35). The mechanical removal of the endothelium by rubbing, or pretreatment of the artery rings with L-NAME (2×10^{-4}M, for 10min) reduced responses to all concentrations of ADP (10^{-8} to 10^{-5}M) in both MPA and BPA rings. There were significant differences between responses to high concentrations of ADP (10^{-6} to 10^{-5}M) in intact MPA and BPA rings, with MPA rings being more responsive to ADP than BPA rings ($0.05 > P > 0.01$) (Fig. 3.36).

The β-adrenoceptor antagonist, propranolol (10^{-6}M, for 10min) had no affect on relaxant responses to ADP.

Concentration-dependent relaxations to adenosine (AD, 3×10^{-6} to 10^{-4}M) were obtained in MPA and BPA rings which had been precontracted with PHE (10^{-7}M). The maximum relaxations produced by AD (10^{-4}M) in the artery rings which had been precontracted with PHE were MPA: 72.22±3.0% and BPA: 62.53±3.71% ($P > 0.05$) (Fig. 3.37 to 3.38). Mechanical removal of the endothelium by rubbing or pretreatment of the artery rings with L-NAME (2×10^{-4}M, for 10min) did not affect relaxant responses to AD (Fig. 3.37 to 3.38). There were no significant differences between concentration response curves to AD in MPA and BPA rings (Fig. 3.39). In some experiments, pretreatment of MPA and BPA rings with the PDE inhibitor, theophylline (10^{-6}M, for 10min), completely abolished relaxant responses to AD (3×10^{-6} to 10^{-5}M) (data not shown).

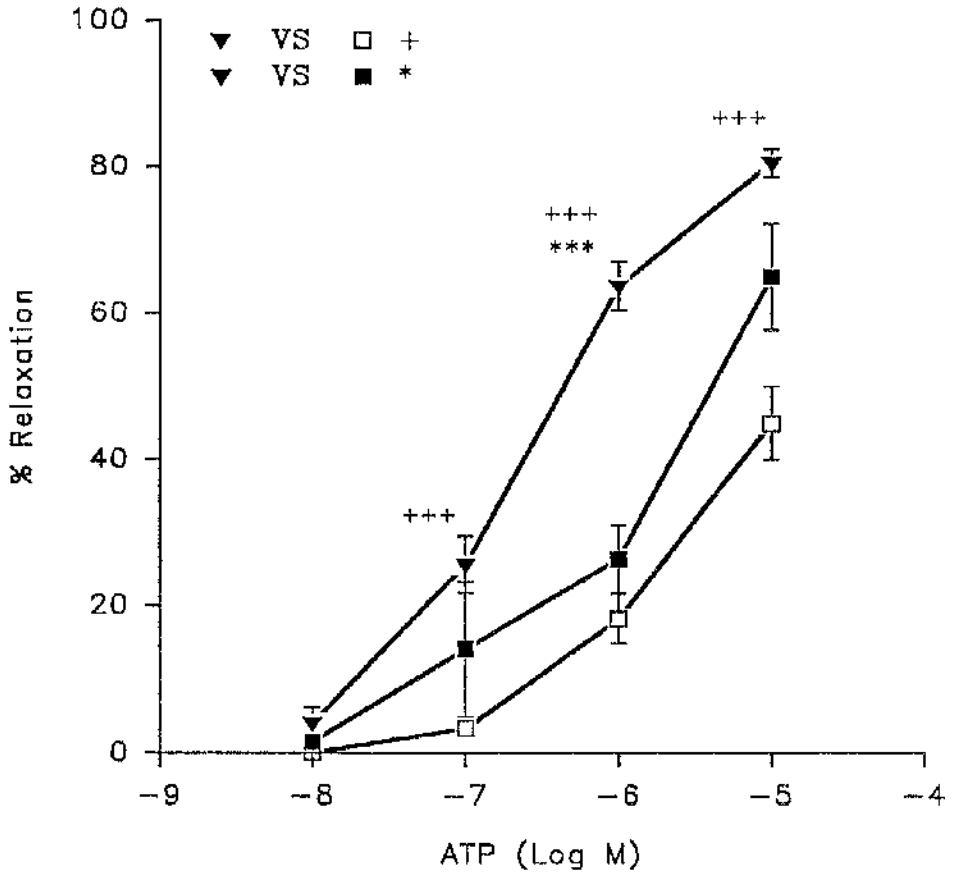

Fig. 3. 31

Comparison between concentration-response curves showing relaxation to adenosine 5'-triphosphate (ATP, 10^{-8} to 10^{-5}M) in MPA rings with endothelium intact in the absence (closed triangle) and presence (open square) of L-NAME (2×10^{-4}M) and in endothelium denuded (closed square) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=5-9 rings from 5-9 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: $^{+++}$ P or *** P < 0.001 (unpaired t test).

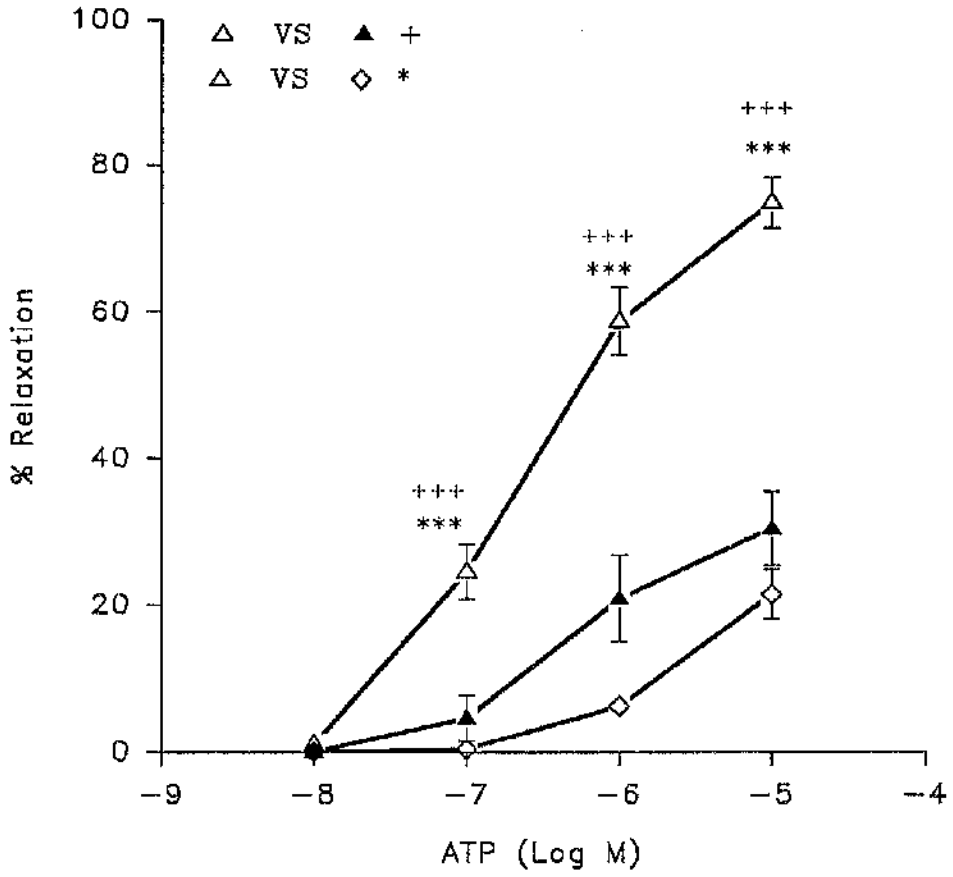

Fig. 3. 32

Comparison between concentration-response curves showing relaxation to adenosine 5'-triphosphate (ATP, 10^{-8} to 10^{-5}M) in BPA rings with endothelium intact, in the absence (open triangle) and presence (closed triangle) of L-NAME (2×10^{-4}M) and in endothelium denuded (open diamond) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=5-8 rings from 5 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: $^{+++}$ $P <$ or *** $P < 0.001$ (unpaired t test).

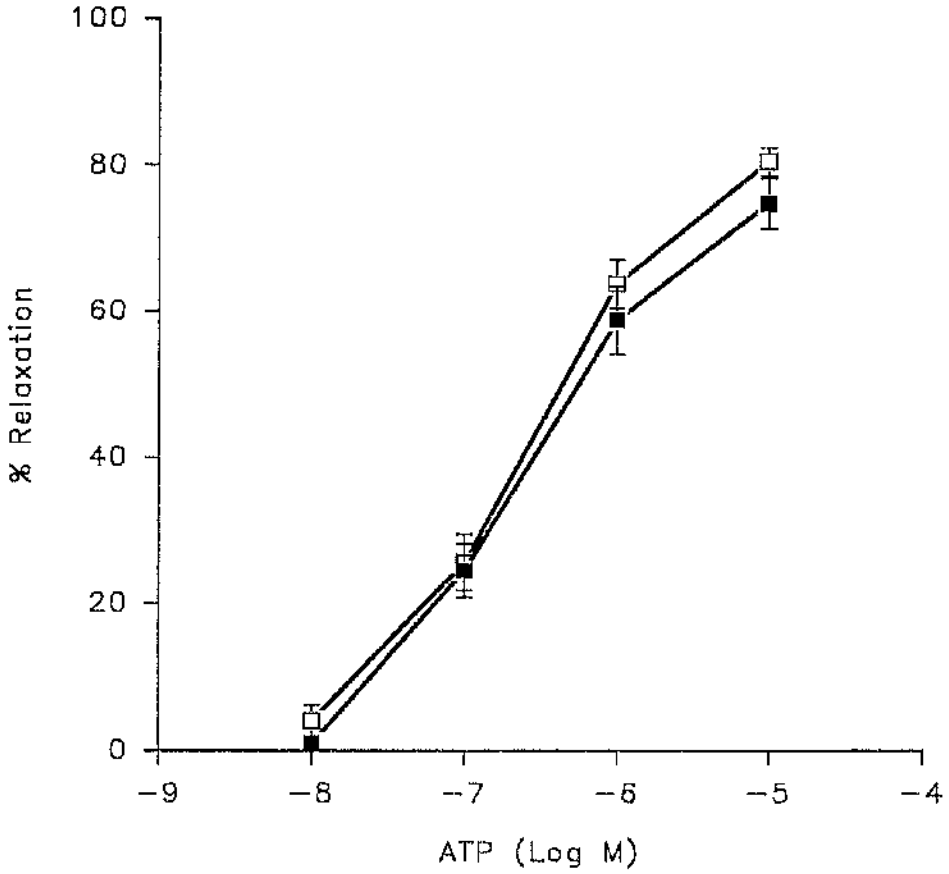

Fig. 3. 33
Comparison between concentration-response curves showing relaxation to adenosine 5'-triphosphate (ATP, 10^{-8} to 10^{-5}M) in MPA (open symbol) and BPA (closed symbol) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=8-9 rings from 9 animals, vertical bars show ± S.E.M.

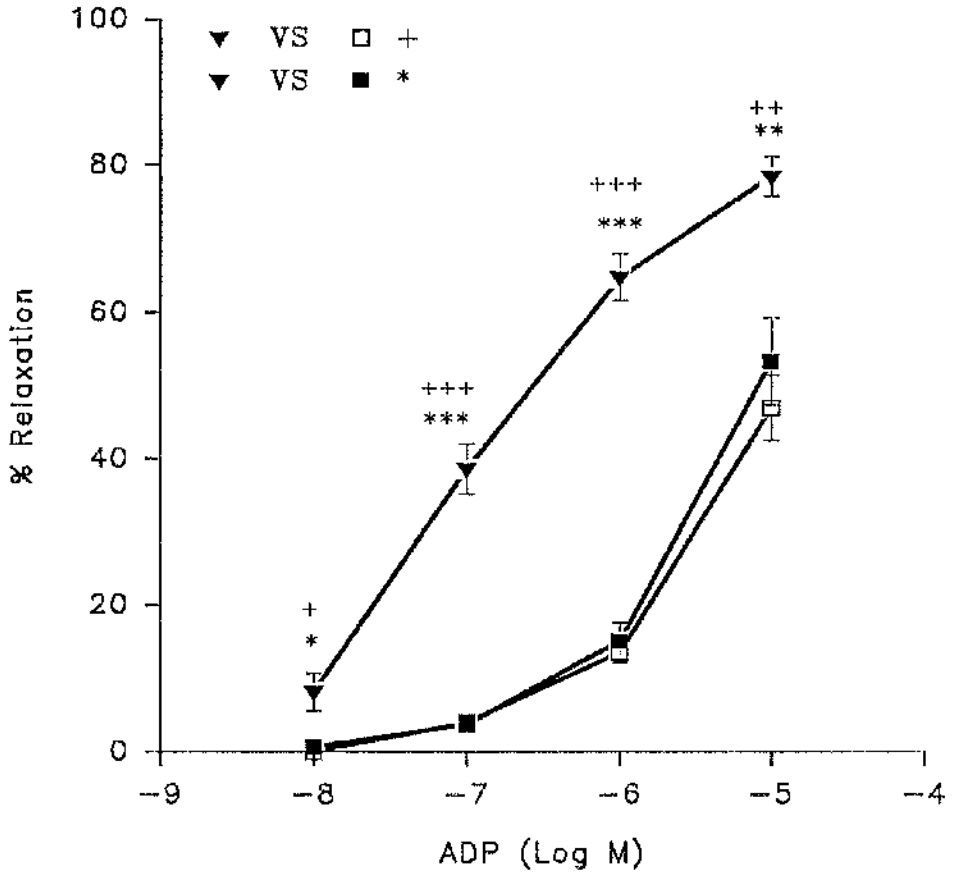

Fig. 3. 34

Comparison between concentration-response curves showing relaxation to adenosine 5'-diphosphate (ADP, 10^{-8} to 10^{-5}M) in MPA rings with endothelium intact, in the absence (closed triangle) and presence (open square) of L-NAME (2×10^{-4}M) and in endothelium denuded (closed square) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-10 rings from 6-10 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: $^{+}$ P < or * P < 0.05; $^{++}$ P < or ** P < 0.01; $^{+++}$ P < or *** P < 0.001 (unpaired t test).

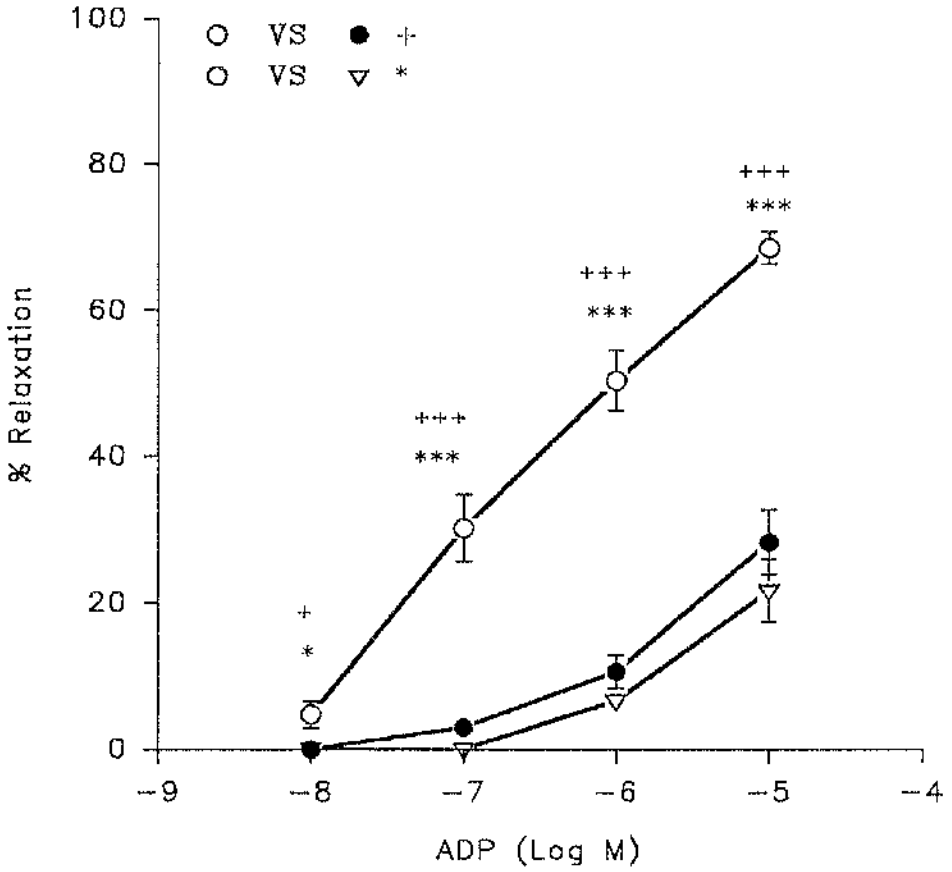

Fig. 3. 35

Comparison between concentration-response curves showing relaxation to adenosine 5'-diphosphate (ADP, 10^{-8} to 10^{-5}M) in BPA rings with endothelium intact, in the absence (open circle) and presence (closed circle) of L-NAME (2×10^{-4}M) and in endothelium denuded (open triangle) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=5-10 rings from 5-10 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: $^{+}$ P < or * **P** < 0.05; $^{+++}$ P < or *** P < 0.001(unpaired t test).

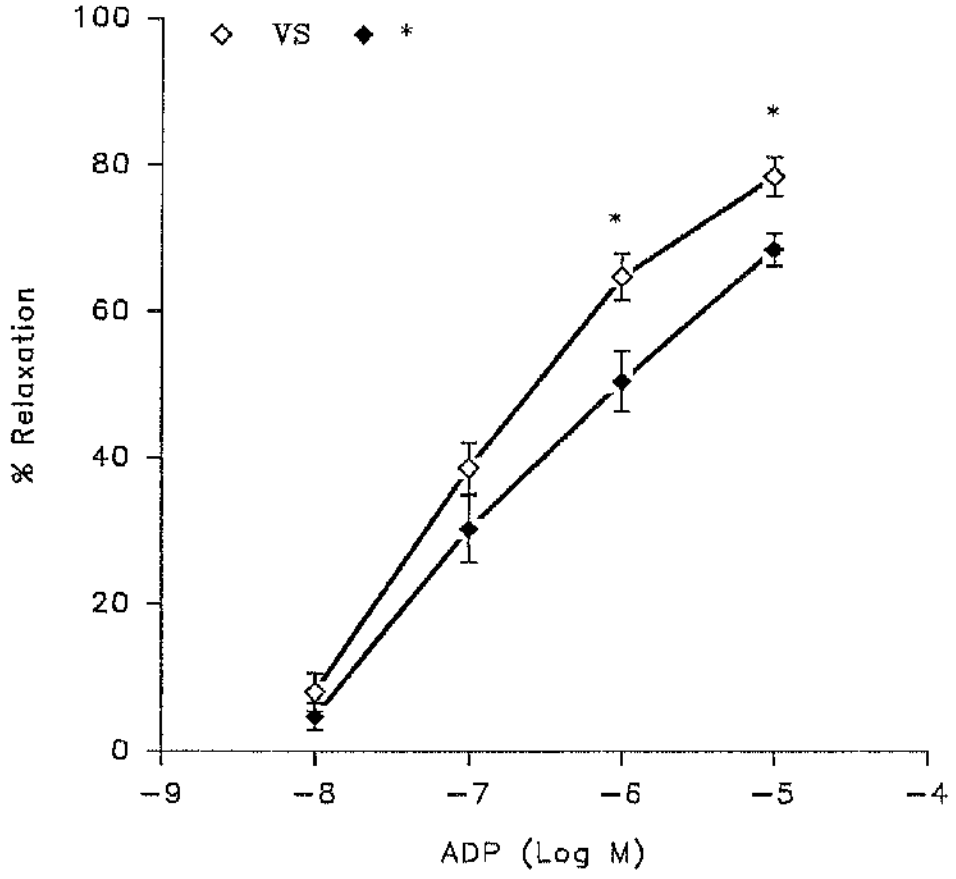

Fig. 3. 36
Comparison between concentration-response curves showing relaxation to adenosine 5'-diphosphate (ADP, 10^{-8} to 10^{-5}M) in MPA (open symbol) and BPA (closed symbol) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-7 rings from 6 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: * $P < 0.05$ (unpaired t test).

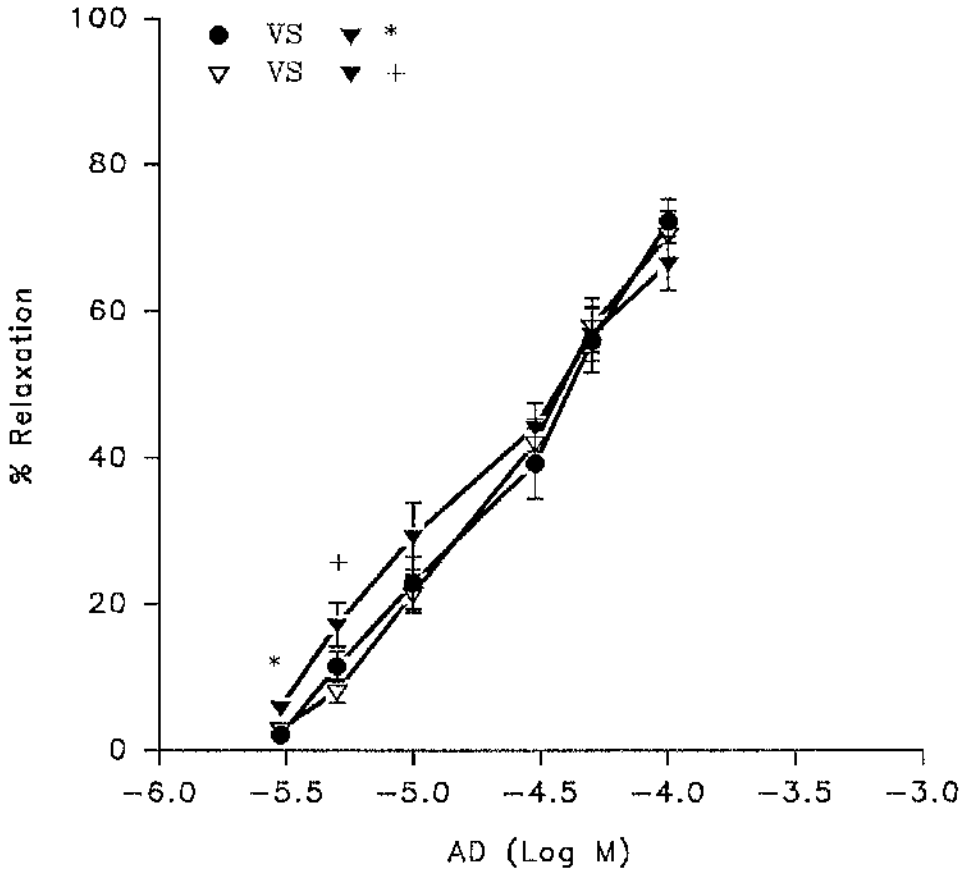

Fig. 3. 37

Comparison between concentration-response curves showing relaxation to adenosine (AD, 3×10^{-6} to 10^{-4}M) in MPA rings with endothelium intact, in the absence (closed circle) and presence (open triangle) of L-NAME (2×10^{-4}M) and in endothelium denuded (closed triangle) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-8 rings, vertical bars show ± S.E.M. Significant difference between means is indicated by: *p values*: $^{+}$ P < or * P < 0.05 (unpaired t test).

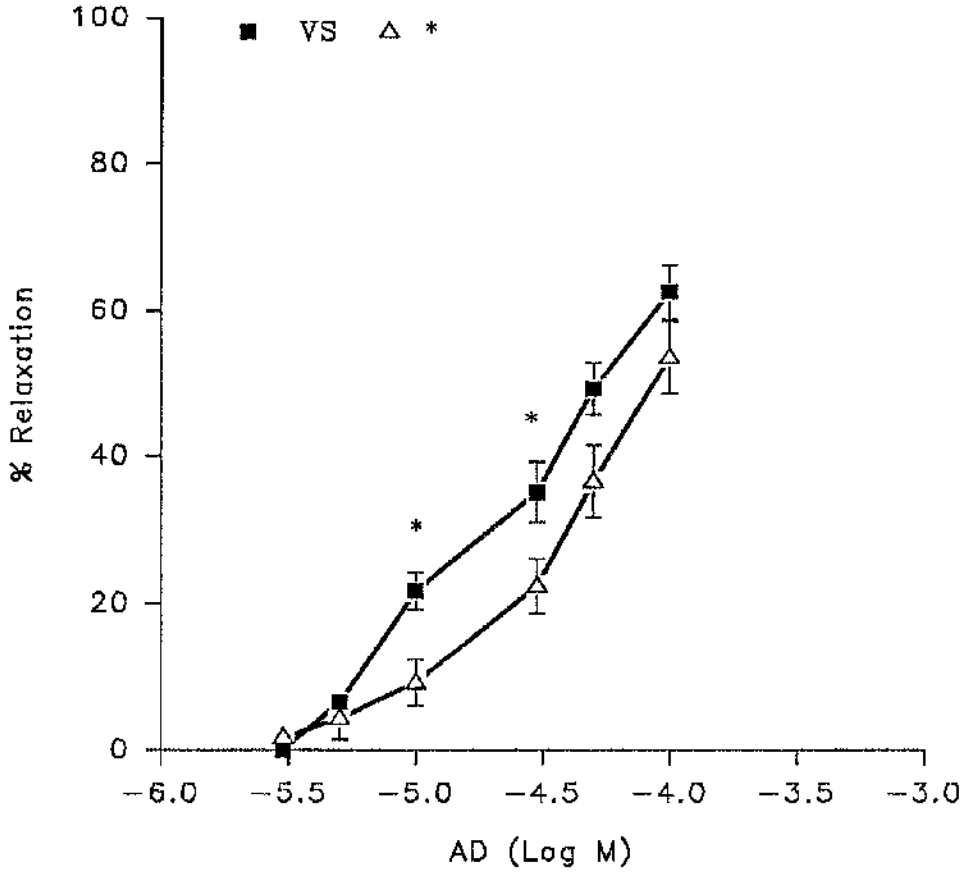

Fig. 3. 38

Comparison between concentration-response curves showing relaxation to adenosine (AD, 3×10^{-6} to 10^{-4}M) in the absence (open symbol) and presence (closed symbol) of L-NAME (2×10^{-4}M) in BPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-7 rings from 4animals, vertical bars show ± S.E.M. Significant difference between means is indicated by: *p values*: * $P < 0.05$ (unpaired t test).

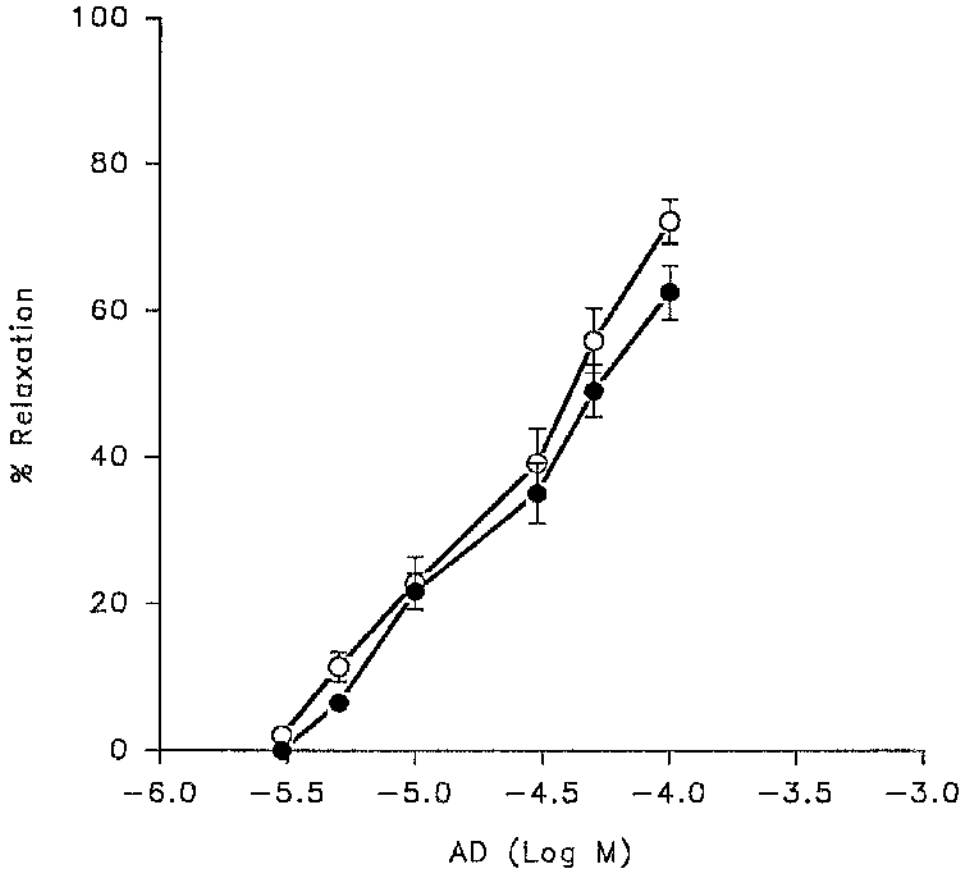

Fig. 3. 39
Comparison between concentration-response curves showing relaxation to adenosine (AD, 3×10^{-6} to 10^{-4}M) in MPA (open symbol) and BPA (closed symbol) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=7-8 rings from 7 animals, vertical bars show ± S.E.M.

3.3.3 *Vasorelaxant and contractile responses to histamine (HIST)*

Histamine (HIST, 10^{-6} to 10^{-3}M) induced concentration-dependent relaxations in MPA and BPA rings precontracted with PHE (Figs. 3.40 and 3.42). The maximum relaxations to HIST (10^{-3}M) in the rings were MPA: 69.52±4.02% and BPA: 78.32±2.92% ($P > 0.05$). The BPA rings were more responsive than MPA rings to HIST (10^{-5} to 10^{-4}M) ($P < 0.05$; Fig. 3.44). Pretreatment of the rings with H_1-receptor antagonist, chlorpheniramine (CPRA, 10^{-6}M, for 10min) reduced relaxant responses to all concentrations of HIST (Figs. 3.40 and 3.42). L-NAME (2×10^{-4}M, for 10min) also, reduced relaxant responses in these rings to all concentrations of HIST (Figs. 3.41 and 3.43). The inhibitory responses to HIST in these tissues was reversed by L-NAME (2×10^{-4}M) and this effect was not inhibited by L-Arg (10^{-3}M). In some experiments, the effects of HIST (10^{-7} to 10^{-3}M) were examined in the absence of PHE-induced tone in resting MPA and BPA rings, which produced small contractile responses to HIST (10^{-4} to 10^{-3}M) in these circumstances (data not shown).

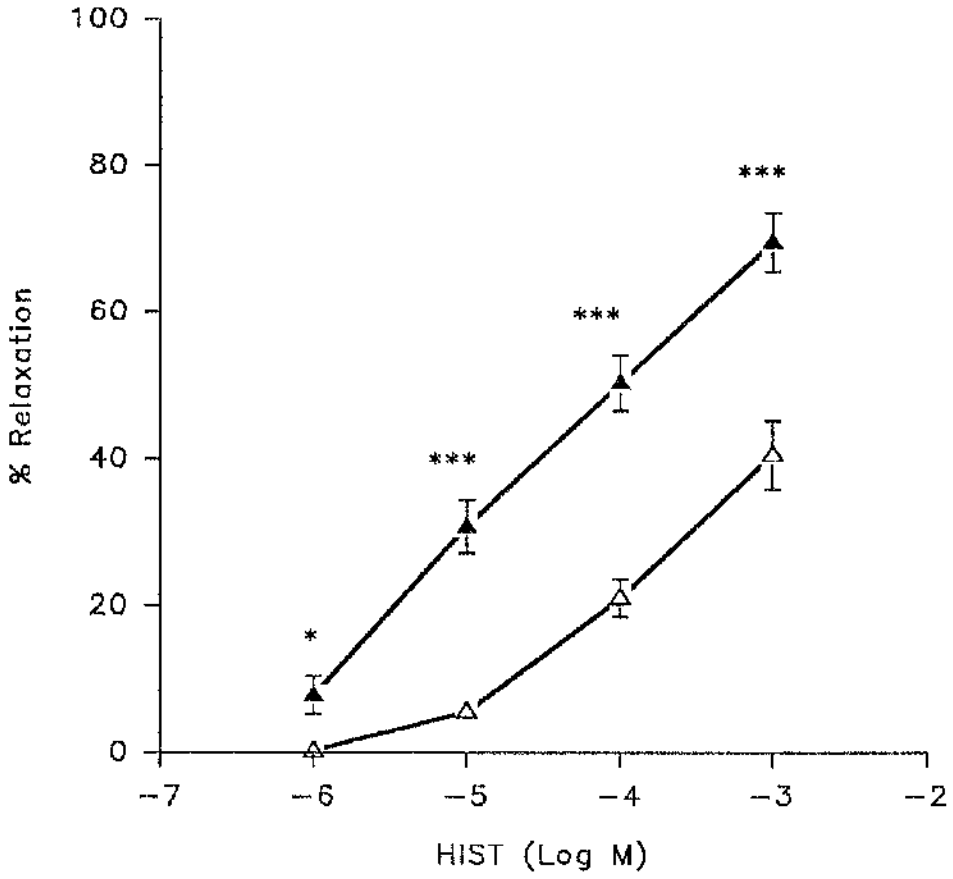

Fig. 3. 40

Comparison between concentration-response curves showing relaxation to histamine (HIST, 10^{-6} to 10^{-3}M) in the absence (closed symble) and presence (open symble) of chlorpheiramine (10^{-6}M, for 10min) in MPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p values*: * $P < 0.05$; *** $P < 0.001$(unpaired t test).

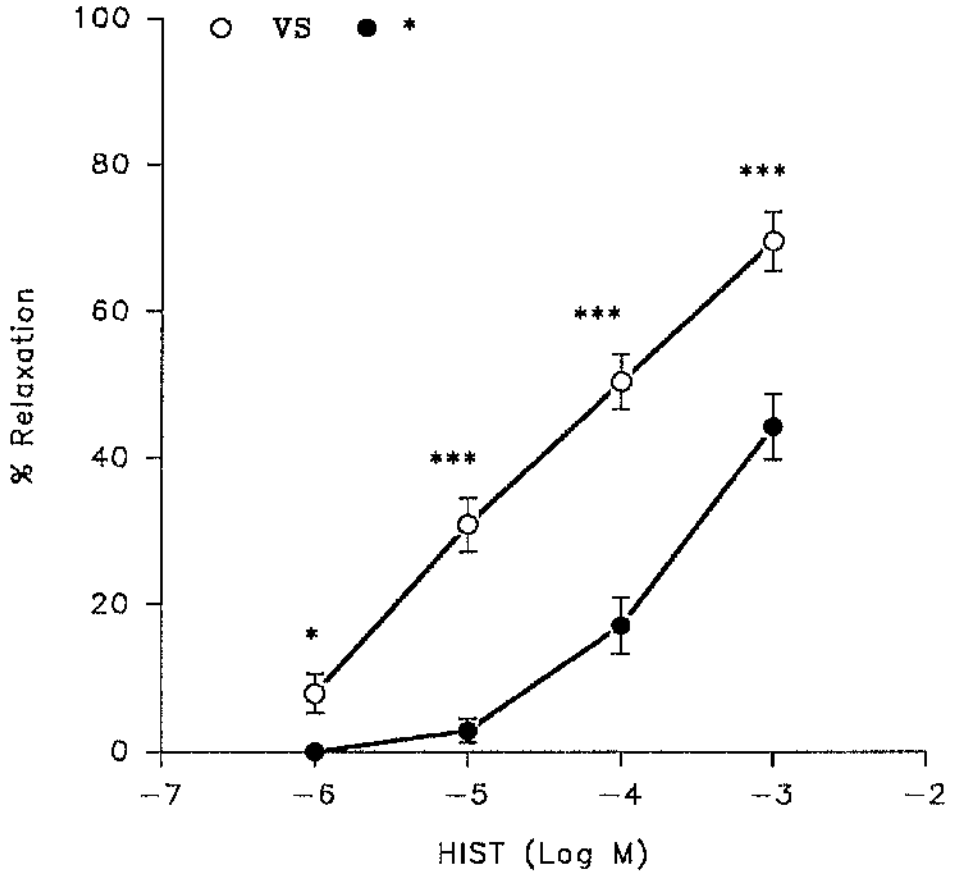

Fig. 3. 41
Comparison between concentration-response curves showing relaxation to histamine (HIST, 10^{-6} to 10^{-3}M) in the absence (open symble) and presence (closed symble) of L-NAME (2×10^{-4}M, for 10min) in MPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=4-10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; *** $P < 0.001$(unpaired t test).

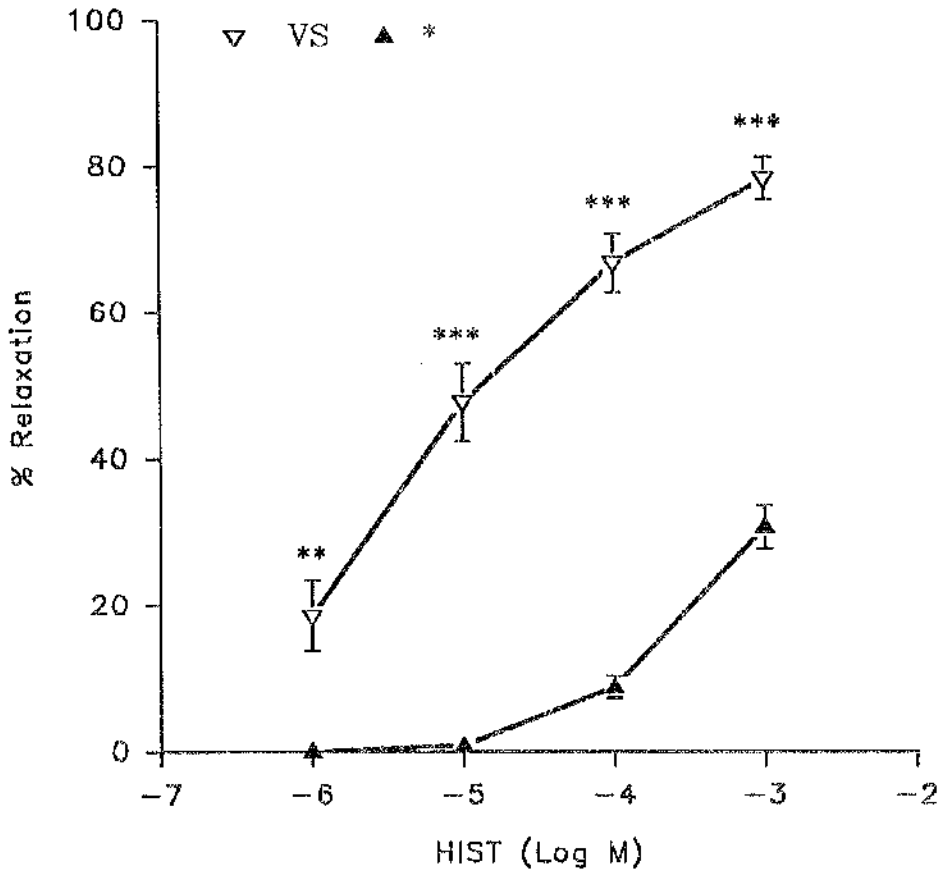

Fig. 3. 42
Comparison between concentration-response curves showing relaxation to histamine (HIST, 10^{-6} to 10^{-3}M) in the absence (open symbol) and presence (closed symbol) of chlorpheniramine (10^{-6}M, for 10min) in BPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: ** $P < 0.01$; *** $P < 0.001$(unpaired t test).

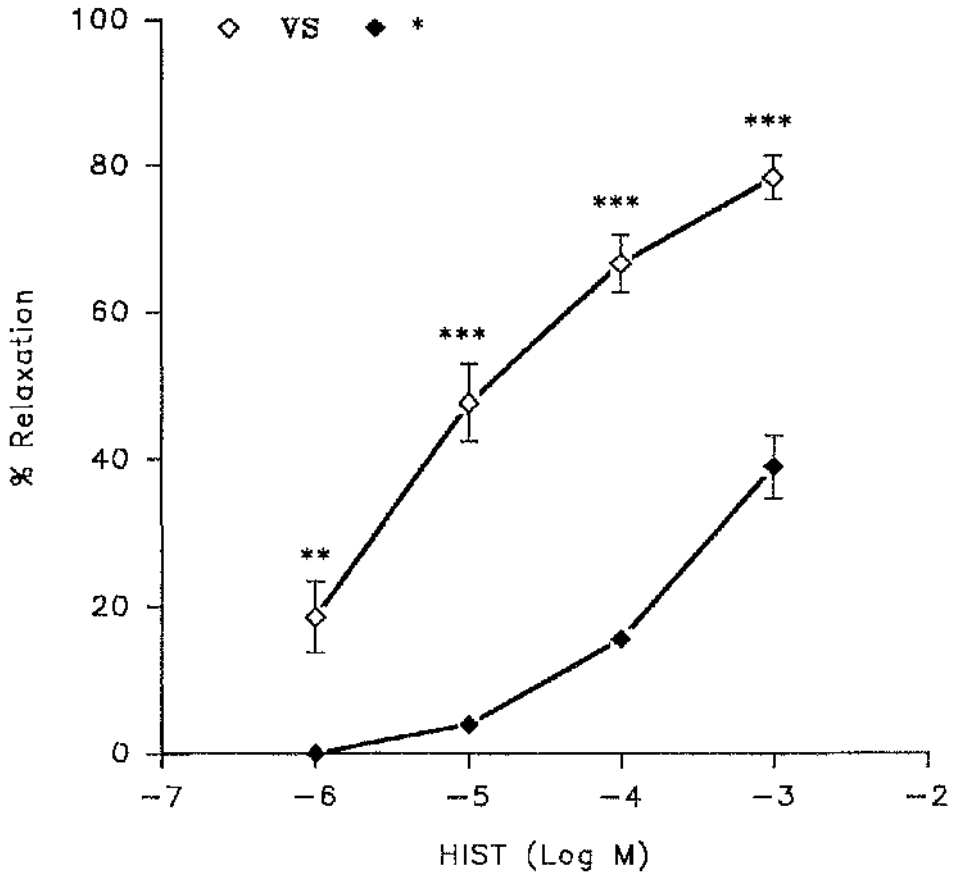

Fig. 3. 43
Comparison between concentration-response curves showing relaxation to histamine (HIST, 10^{-6} to 10^{-3}M) in the absence (open symbol) and presence (closed symbol) of L-NAME (2×10^{-4}M, for 10min) in BPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=4-10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: ** $P < 0.01$; *** $P < 0.001$(unpaired t test).

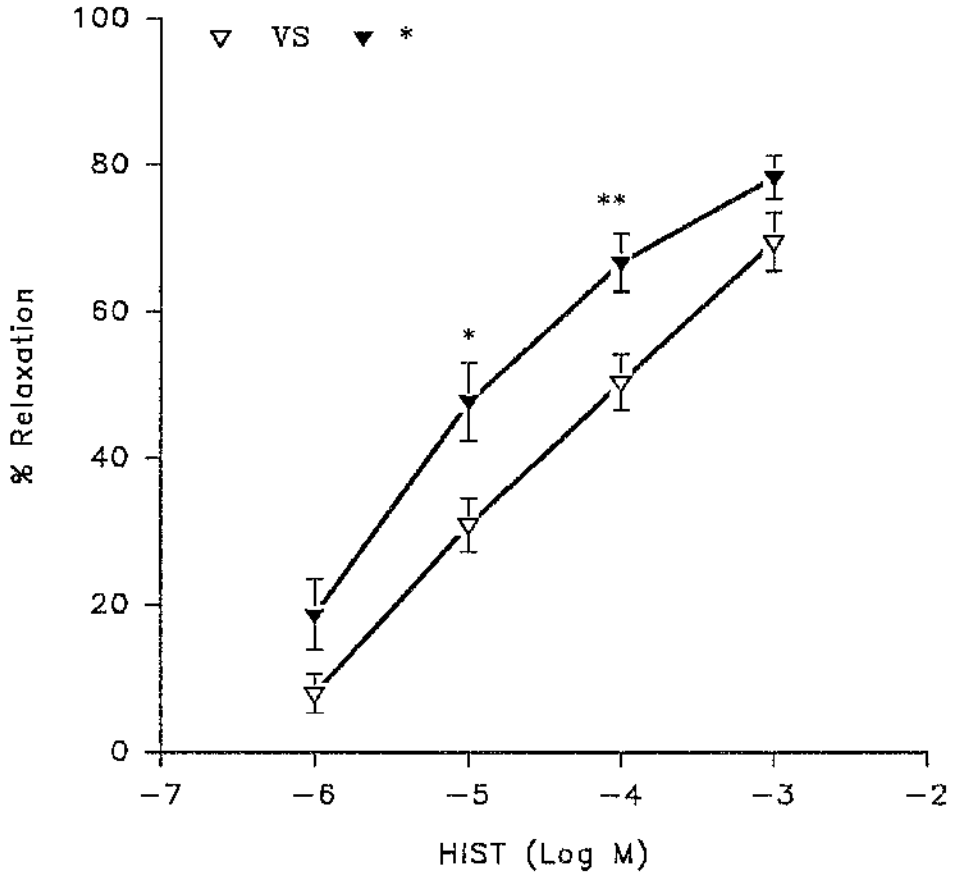

Fig. 3. 44

Comparison between concentration-response curves showing relaxation to histamine (HIST, 10^{-6} to 10^{-3}M) in MPA (open symbol) rings and in BPA (closed symbol) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$(unpaired t test).

3.3.4 *Vasorelaxant responses to the β-adrenoceptor agonist, isoproterenol (ISO)*

The β-adrenoceptor agonist, isoprenaline (ISO, 10^{-10} to 10^{-5}M) induced concentration-dependent relaxations in MPA and BPA rings precontracted with PHE (10^{-7}M; Fig. 3.45 to 3.46). The maximum relaxant responses to ISO (10^{-5}M) in the preparations were MPA: 93.04±1.25% and BPA: 77.21±2.80% ($P < 0.001$). The MPA rings were more responsive than BPA rings to all concentrations of ISO (Fig. 3.47). Mechanical removal of the endothelium by rubbing, or pretreatment of the rings with L-NAME (2×10^{-4}M, for 10min) depressed responses to low concentrations of ISO in these rings (MPA: 10^{-10} to 10^{-7}M and BPA: 10^{-9} to 10^{-8}M) (Fig. 3.45 to 3.46). The β-adrenoceptor antagonist, propranolol (PROP, 10^{-5}M) abolished responses to ISO (data not shown). L-NAME (2×10^{-4}M, for 10min) partially reversed the inhibitory effects produced by ISO (10^{-10} to 10^{-5}M) in MPA and BPA rings. This effect of L-NAME was slightly inhibited by L-Arg (10^{-3}M).

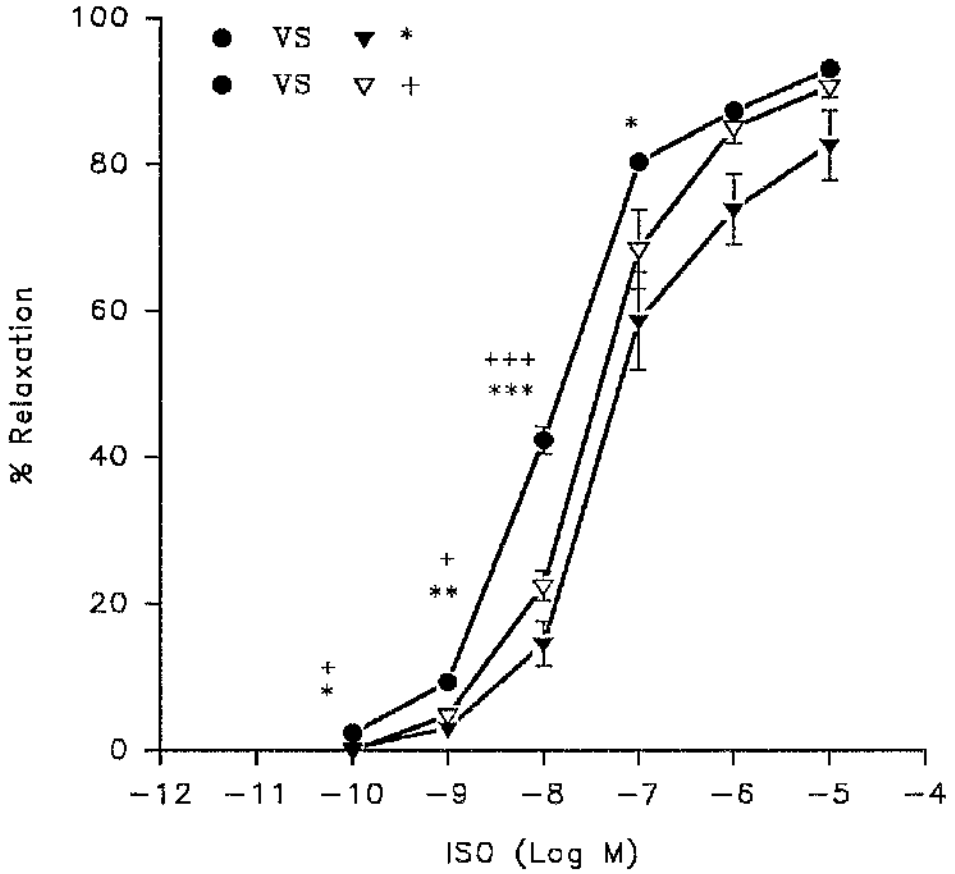

Fig. 3. 45

Comparison between concentration-response curves showing relaxation to isoproterenol (ISO, 10^{-10} to 10^{-5}M) in MPA rings with endothelium intact, in the absence (closed circle) and presence (open triangle) of L-NAME (2×10^{-4}M, for 10min) and in endothelium denuded (closed triangle) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6-8 rings from 6 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: $^{+}$ P < or * P < 0.05; ** P < 0.01; $^{+++}$ P < or *** P < 0.001 (unpaired t test).

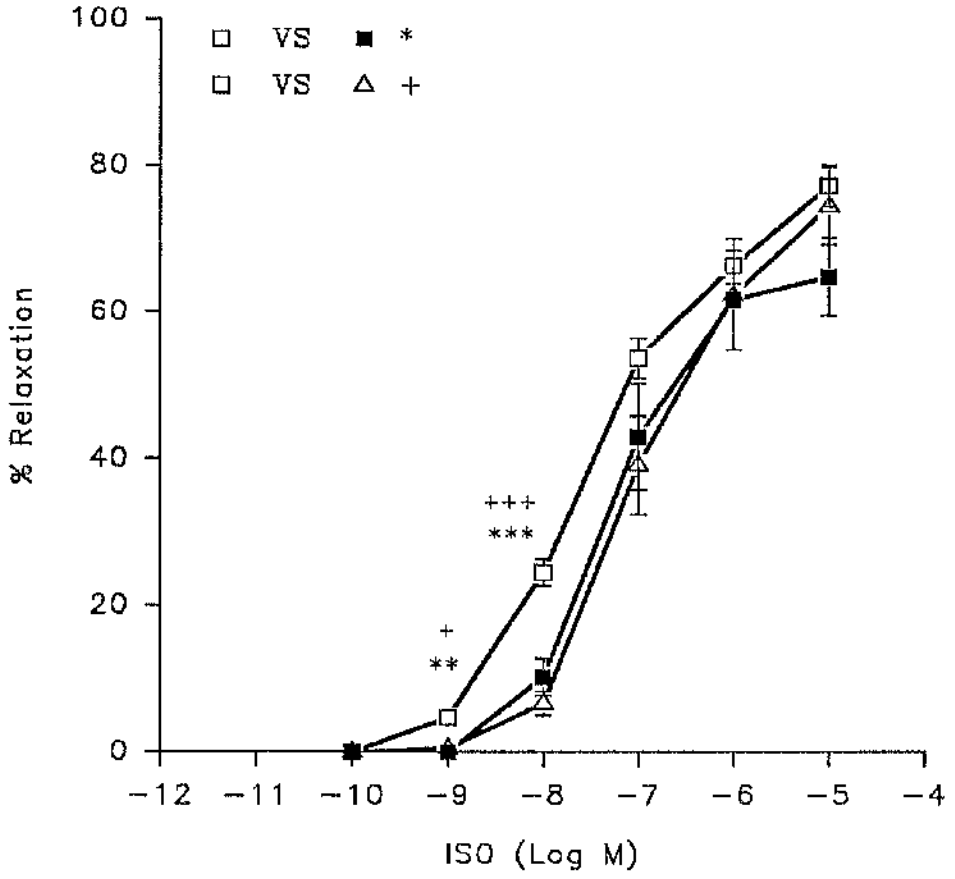

Fig. 3. 46

Comparison between concentration-response curves showing relaxation to isoproterenol (ISO, 10^{-10} to 10^{-5}M) in BPA rings with endothelium intact, in the absence (open square) and presence (closed square) of L-NAME (2×10^{-4}M, for 10min) and in endothelium denuded (open triangle) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=7-8 rings from 4 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values:* $^{+}$ $P < 0.05$; ** $P < 0.01$; $^{+++}$ $P <$ or *** $P < 0.001$ (unpaired t test).

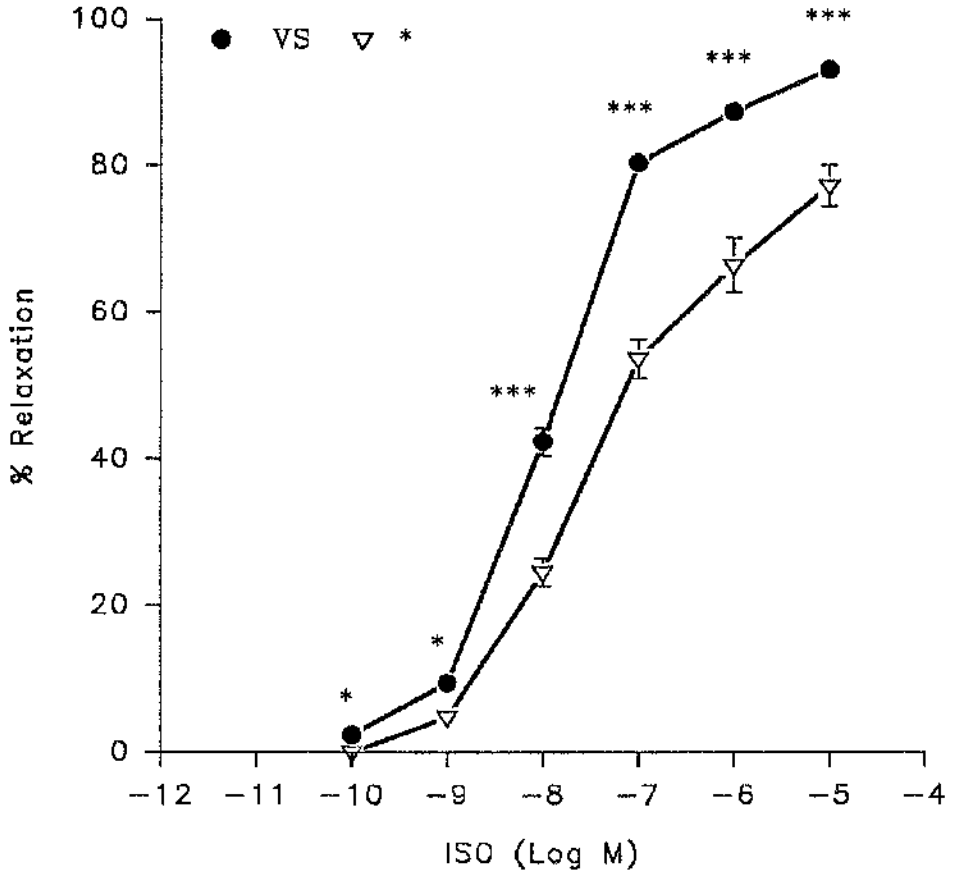

Fig. 3. 47

Comparison between concentration-response curves showing relaxation to isoproterenol (ISO, 10^{-10} to 10^{-5}M) in MPA (closed symbol) rings and in BPA (open symbol) rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=7-8 rings from 8 animals, vertical bars show ± S.E.M. Significant difference between means is indicated by *p values*: * $P < 0.05$; *** $P < 0.001$ (unpaired t test).

3.3.5 *Vasorelaxant responses to 5-hydroxytryptamine (5-HT)*

5-HT (10^{-4} to 5×10^{-4}M) produced concentration-dependent relaxations in isolated MPA and BPA rings precontracted with PHE (EC_{70}, 1.2×10^{-7}M) (Fig. 3.48). The maximum relaxations were 84.37±5.24% (MPA) and 74.12±7.92% (BPA) ($P > 0.05$). Pretreatment of the rings with L-NAME (5×10^{-4}M, for 15min) or with propranolol (10^{-6}M, for 15min) did not abolish 5-HT-induced relaxations in these rings.

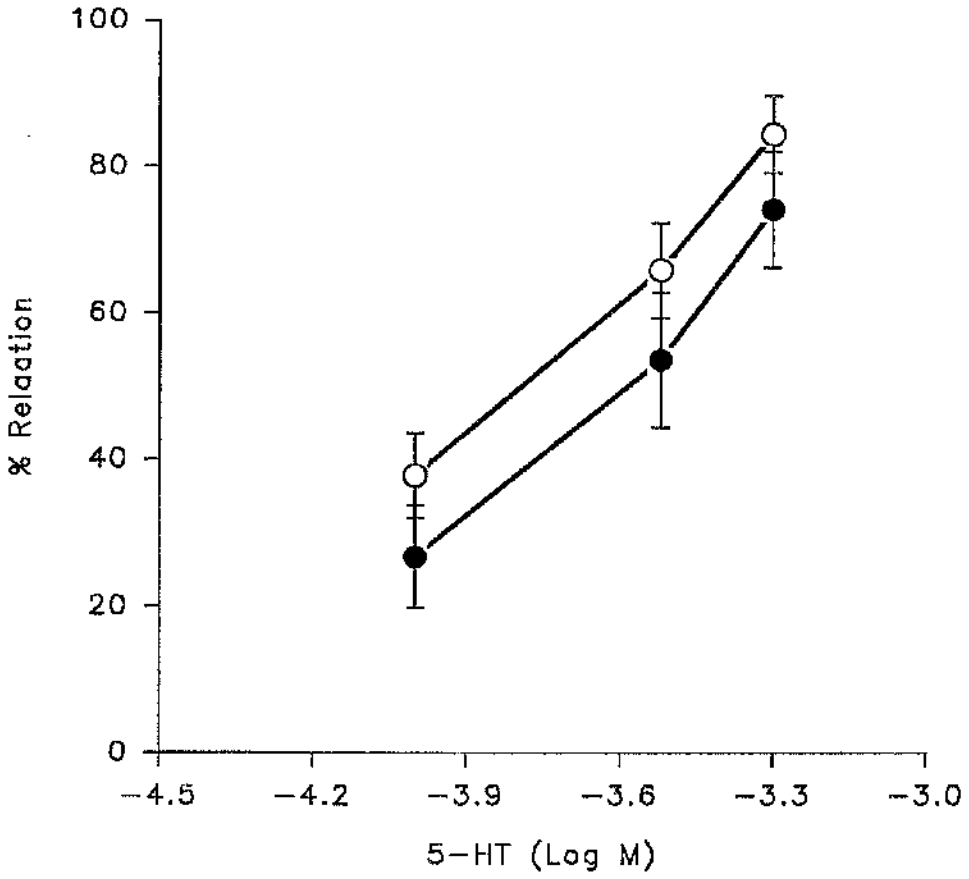

Fig. 3. 48
Comparison between concentration-response curves showing relaxation to 5-HT (10^{-4} to 5×10^{-4}M) in MPA rings (open symbol) and in BPA (closed symbol) rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

3.3.6 *Vasorelaxant effects of atropine*

Atropine (AT, 10^{-8}M to 5×10^{-6}M) induced concentration-dependent relaxations in MPA and BPA rings precontracted with either PHE (10^{-7}M; 3.49) or 5-HT (10^{-5}M), but did not produce relaxations in artery rings precontracted with potassium chloride (KCl, 2×10^{-2}M; Fig. 3.49A). The maximum inhibitions produced by AT in MPA rings and BPA rings, precontracted with PHE, were MPA: 65.87±3.72% and BPA: 51.11±3.33% ($0.05 > P > 0.001$) and when MPA and BPA rings were precontracted with 5-HT, the AT-induced inhibitions were MPA: 62.70±6.2% and BPA: 63.83±6.17% ($P > 0.05$). There were no significant differences between responses to AT in MPA and BPA rings precontracted either with PHE or 5-HT. Pretreatment of these tissues with the nitric oxide synthase inhibitors, L-NAME (5×10^{-4}M, for 10min), or L-NOARG (10^{-4}M, for 10min) and also L-NAME (5×10^{-4}M) in the presence of HbO (1mg/ml) did not abolish AT-induced relaxations (Fig. 3.49B to 3.49C). In one series of experiments the inhibitory effects of AT on contractile responses to PHE in MPA rings with intact endothelium, were completely inhibited by L-NAME (5×10^{-4}M) or HbO (1mg/ml). However, this did not occur in intact rings, which had been pretreated with L-NAME (5×10^{-4}M, for 10min) or in endothelium denuded rings (Fig. 3.49B). This effect of L-NAME was partially reversed by L-arginine (L-Arg, 2×10^{-4}M). The ability of atropine to inhibit PHE-induced tone in MPA rings, with or without the endothelium, was unaffected by β-adrenoceptor antagonist, propranolol (PROP, 10^{-6}M, for 10min; Fig. 3.49C) or with the phosphodiesterase (PDE) inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 10^{-8}M, for 3min). The inhibitory effects of AT on contractile responses to PHE persisted in MPA rings in which the endothelium had been removed, and in the presence of ACh (10^{-6}M). The ACh-induced relaxation (10^{-6}M) was obtained in intact MPA rings which had been precontracted with PHE and these

relaxations were antagonised by AT (10^{-8}M). Also, ACh (10^{-3}M) reversed AT-induced relaxation (5×10^{-6}M; Fig. 3.49D). Comparisons between the concentration response curves to AT in MPA rings and BPA rings which had been precontracted with PHE, revealed that the MPA rings were more responsive than BPA rings to AT (10^{-6} to 3×10^{-6}M).

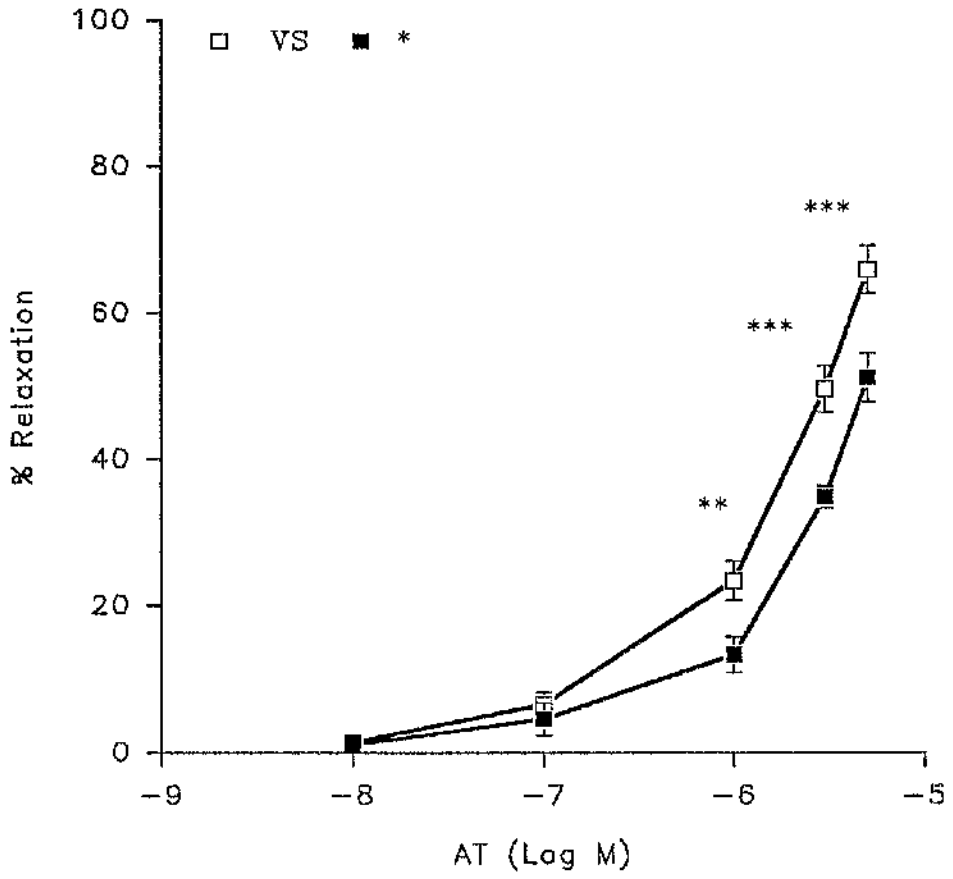

Fig. 3. 49

Comparison between concentration-response curves showing relaxation to atropine (AT, 10^{-8} to 5×10^{-6}M) in MPA (open symbol) and in BPA (closed symbol) rings precontracted with PHE (EC_{70}; 1.2×10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=10 rings from 10 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: ** $P < 0.01$; *** $P < 0.001$ (unpaired t test).

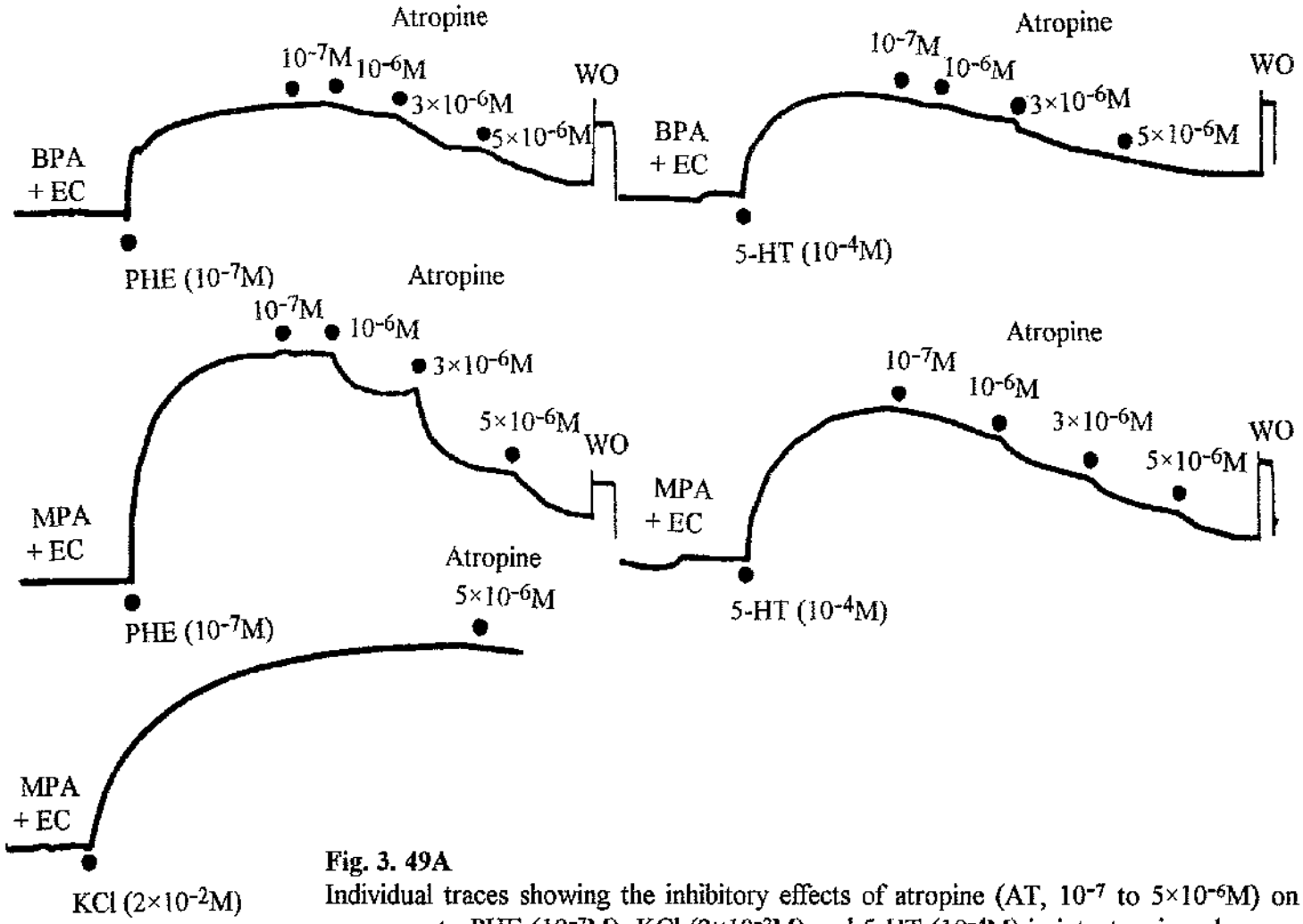

Fig. 3. 49A

Individual traces showing the inhibitory effects of atropine (AT, 10^{-7} to 5×10^{-6}M) on contractile responses to PHE (10^{-7}M), KCl (2×10^{-2}M) and 5-HT (10^{-4}M) in intact main pulmonary artery and branch pulmonary artery (+EC, MPA and BPA) rings. Symbol (•) indicates drug addition and (WO) indicates wash off.

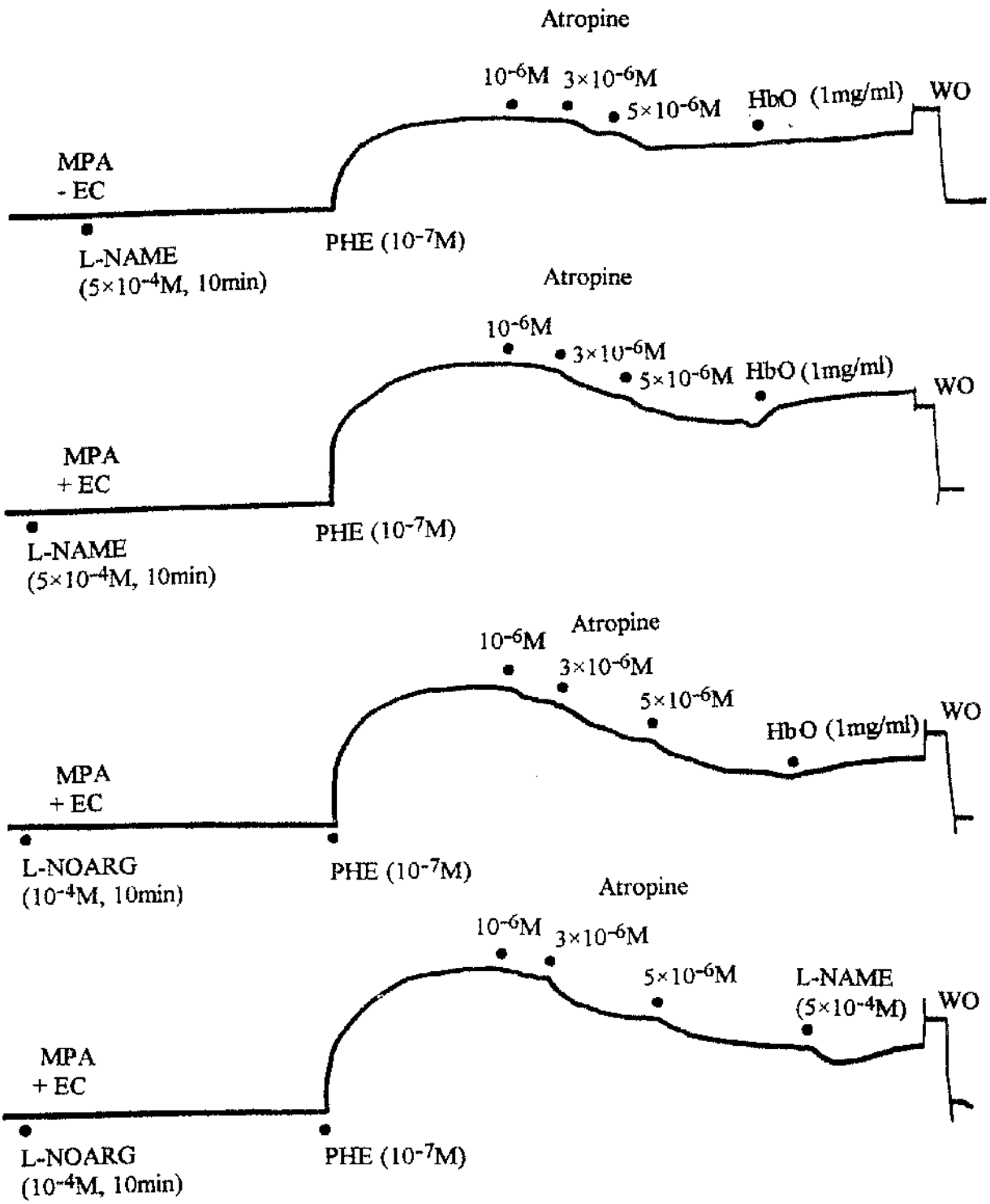

Fig. 3. 49B

Individual traces showing the inhibitory effects of atropine (AT, 10^{-6} to 5×10^{-6}M) in main pulmonary artery (MPA) rings, with (+EC) and without (-EC) the endothelium, in the presence of L-NAME (5×10^{-4}M) or L-NOARG (10^{-4}M) and in the absence and presence of oxy-haemoglobin (HbO, 1mg/ml). Symbol (•) indicates drug addition and (WO) indicates wash off.

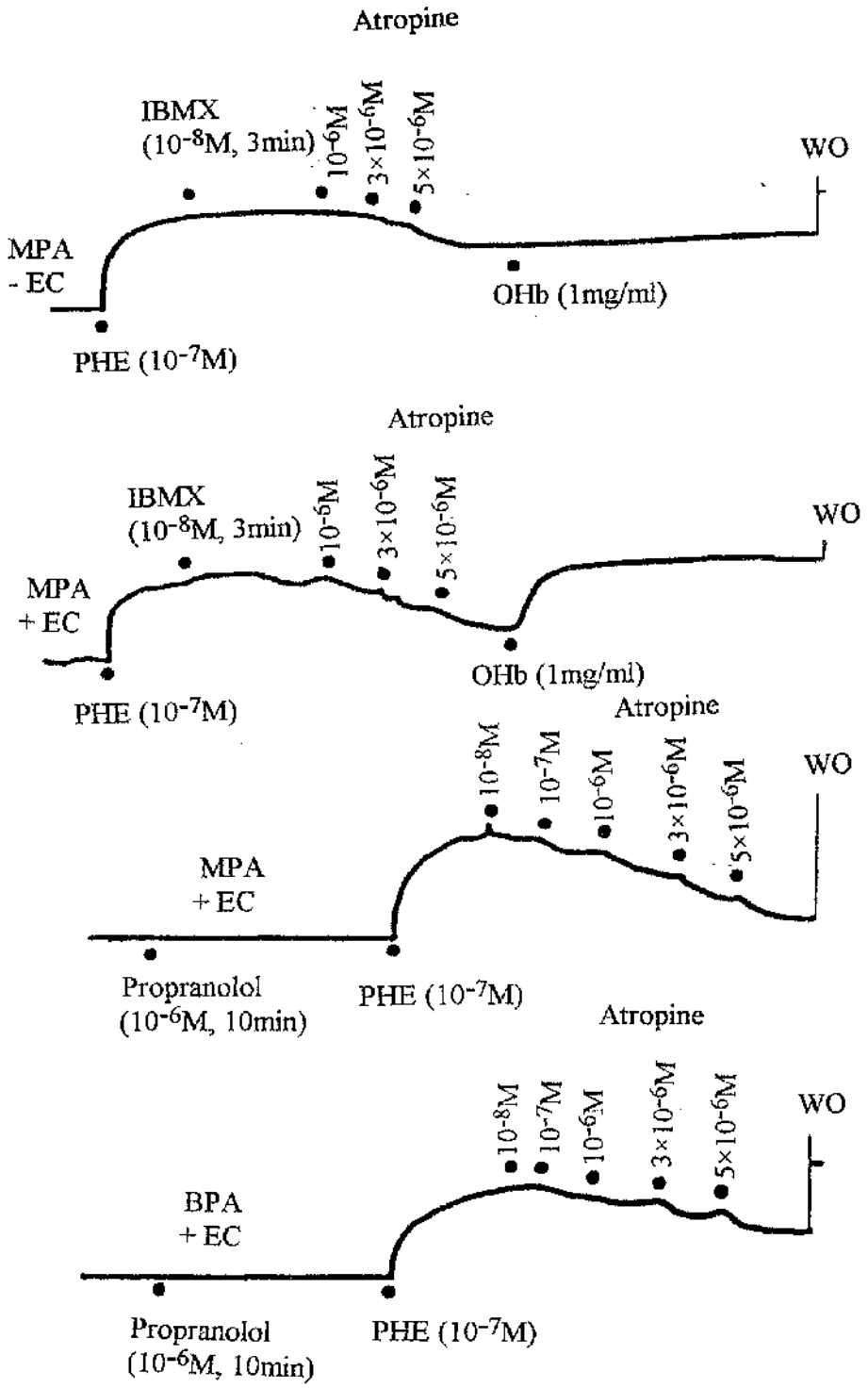

Fig. 3. 49C

Individual traces showing the inhibitory effects of atropine (AT, 10^{-6} to 5×10^{-6}M) in main pulmonary artery (MPA) and branch pulmonary artery (BPA) rings, with (+EC) and without (-EC) the endothelium, in the presence of IBMX (10^{-8}M) or propranolol (10^{-6}M) and in the absence or presence of oxy-haemoglobin (HbO, 1mg/ml). Symbol (•) indicates drug addition and (WO) indicates wash off.

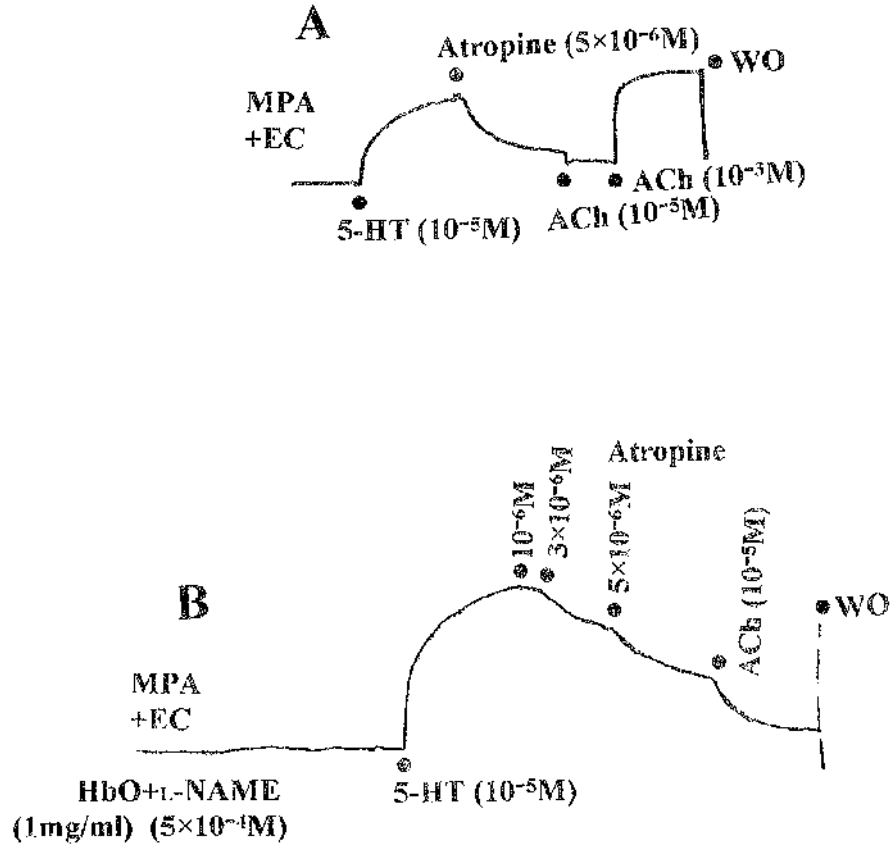

Fig. 3. 49D

Individual traces (A and B) showing responses of intact main pulmonary artery (+EC, MPA) rings, precontracted with 5-HT (10^{-5}M) to atropine (AT, 5×10^{-6}M) in the absence and presence of HbO (1mg/ml)+L-NAME (5×10^{-4}M) and the effects of acetylcholine (ACh, 10^{-5} and 10^{-3}M) on AT-induced responses. Symbol (•) indicates drug addition and (WO) indicates wash off.

3.4 *Frequency-response studies to electrical field stimulation (EFS) in MPA rings of Wistar rats*

Electrical field stimulation (EFS; 70V, pulse width of 0.1 ms, 4, 8, 16 and 32 Hz, 10 seconds) induced a frequency-dependent contraction of the Wistar rat pulmonary artery rings at resting tension. These contractions were abolished by pretreatment of rings with tetrodotoxin (10^{-7}M) and antagonized by α-adrenoceptor antagonist, prazosin (PRAZ, 10^{-9}M), suggesting that this response was due to the activation of α_1-adrenoceptors by neurally-released noradrenaline (NA) from perivascular sympathetic nerves. The EFS-elicited contractions are expressed as means and as a percentage of the maximum response were respectively, 4 Hz : 48.56±5.54, 8 Hz : 70.63±3.48, 16 Hz : 100 and 32 Hz : 94.07 (n=10; Fig. 3.50). Nω-nitro-L-arginine methyl ester (L-NAME, 2×10^{-4}M, for 15min) increased the EFS responses, but this effect was only significant at the frequency which produced the maximal response (16 Hz; $0.05 > P > 0.01$, compared with control, n=8, Fig. 3.50A). Pretreatment of rings with cocaine (10^{-6}M, for 10-15min) had no significant effect on the EFS-induced contractions (Fig. 3.50B).

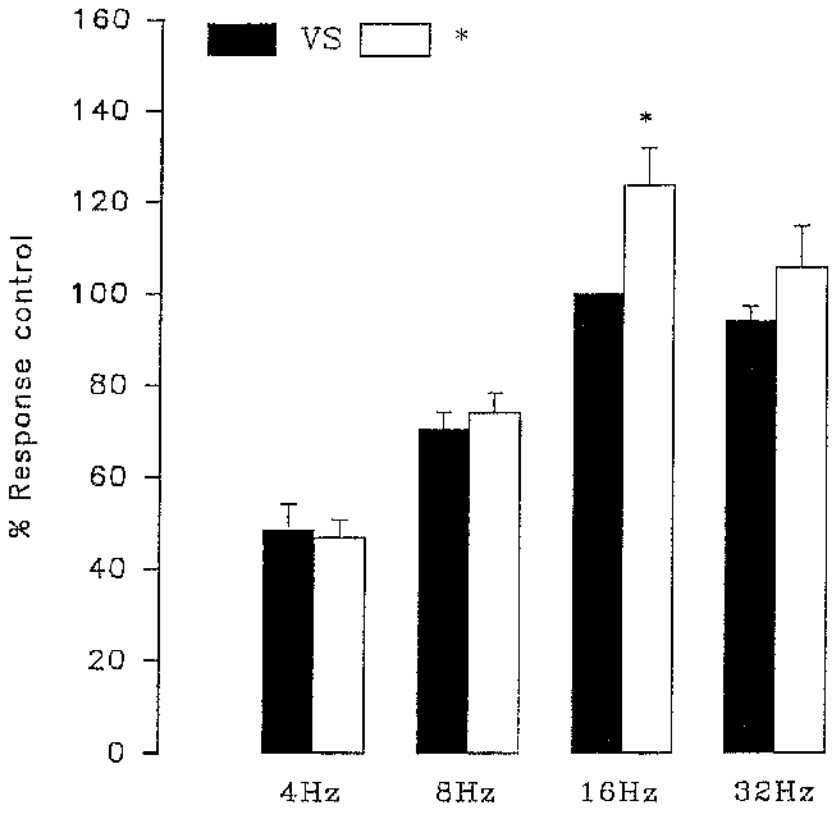

Fig. 3. 50

Histograms showing frequency-dependent responses to electrical field stimulation in the absence (solid column) and presence (open column) of L-NAME (2×10^{-4}M, for 15min) in MPA rings from Wistar rats. The results are expressed as means and as a percentage of the maximum response to EFS of n=8-10 rings from 8-10 animals. The vertical bars show ± S.E.M. Asterisk indicates significant difference between means. *p value*: * $P < 0.05$ (paired t test).

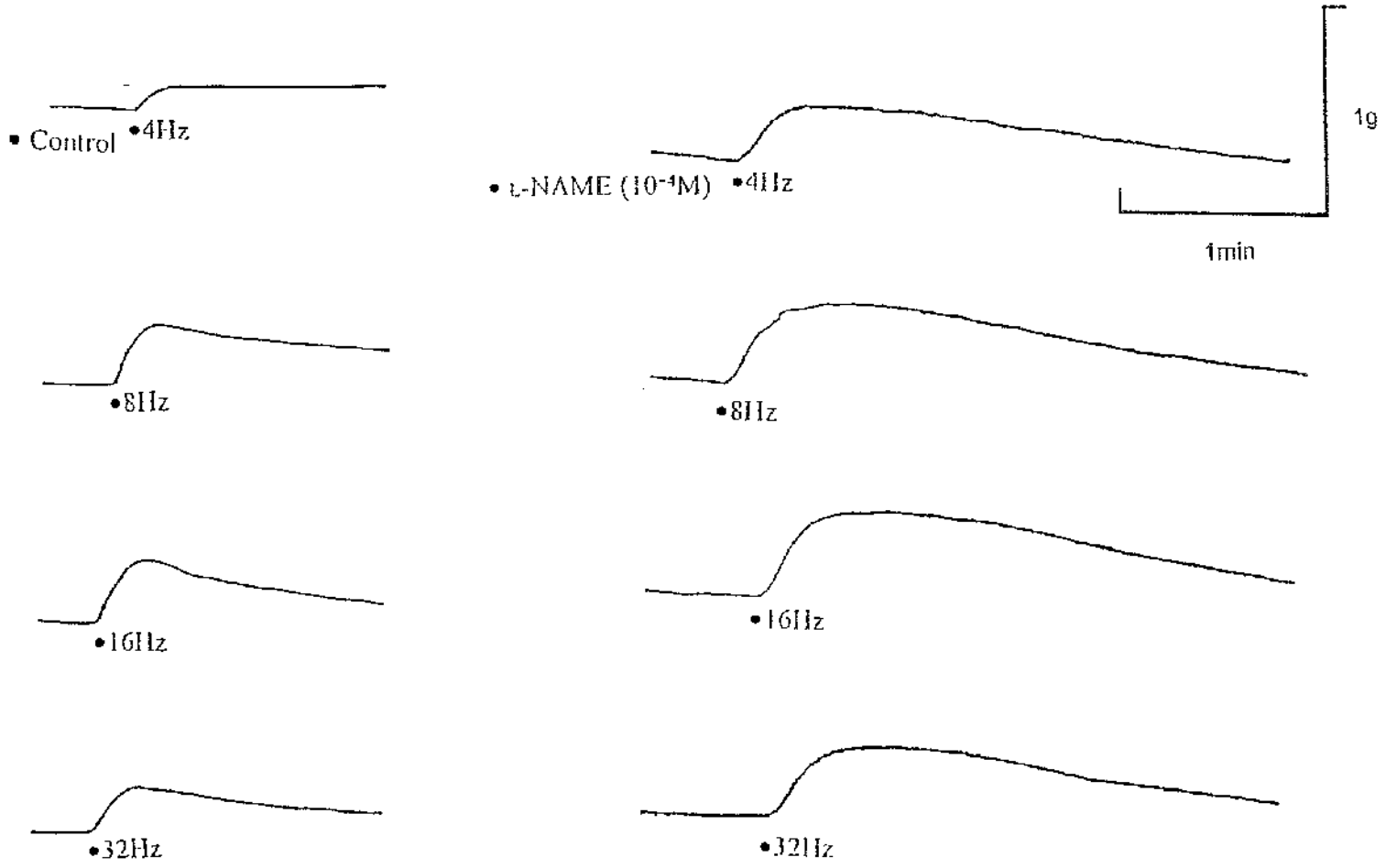

Fig. 3. 50A

Individual traces showing frequency-dependent responses of electrical field stimulation (4 Hz to 16 Hz, for 10sec) in the absence and presence of L-NAME (10^{-4}M) in pulmonary artery (MPA) rings. Symbol (•) indicates drug addition and the onset of electrical field stimulation.

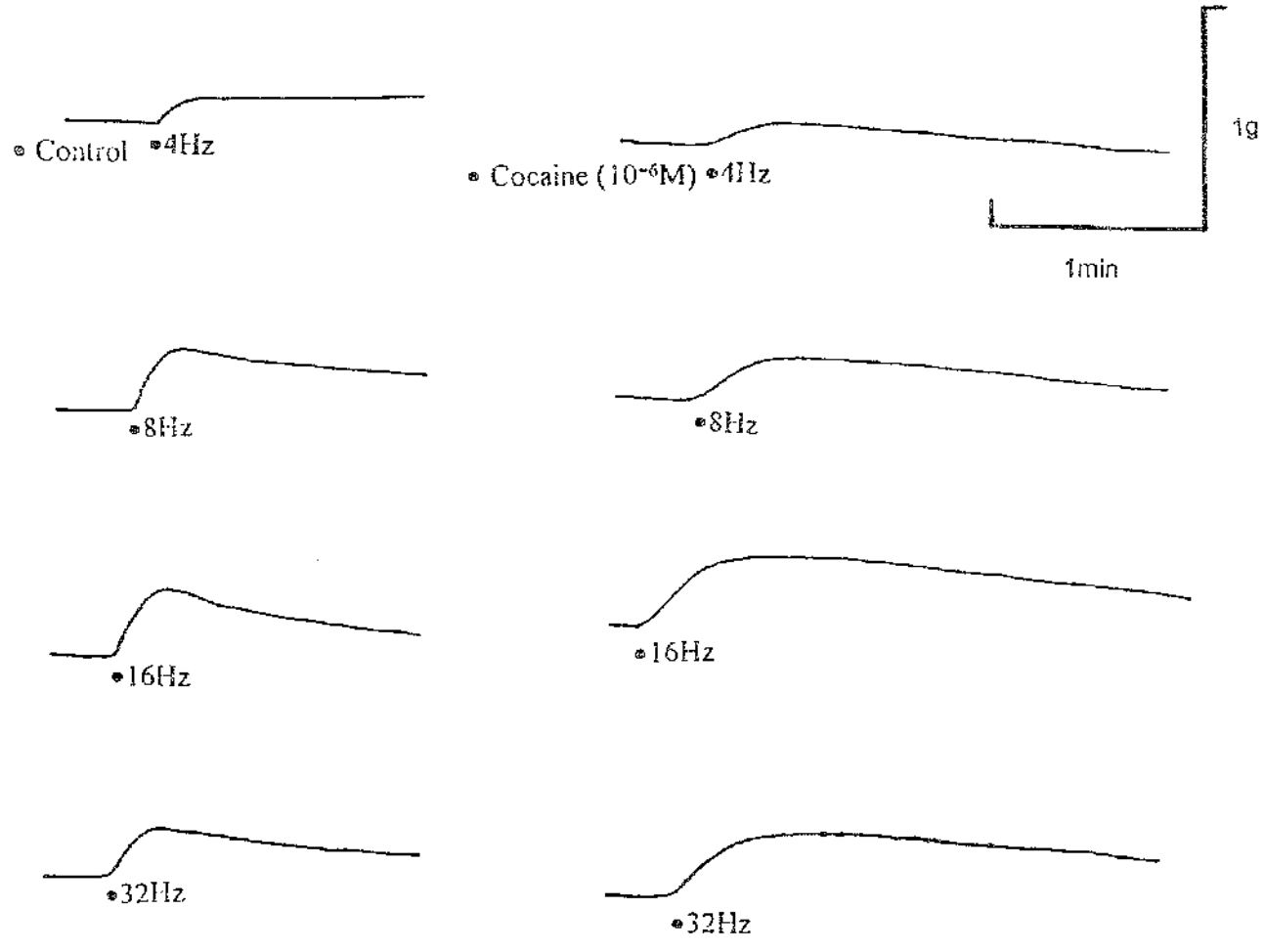

Fig. 3. 50B

Individual traces showing frequency-dependent responses of electrical field stimulation (4 Hz to 16 Hz, for 10sec) in the absence and presence of cocaine (10^{-6}M) in pulmonary artery (MPA) rings. Symbol (•) indicates drug addition and the onset of electrical field stimulation.

3.5 Investigation of the effects cigarette smoke extract (CSE)) on the responsiveness of the pulmonary artery (MPA and BPA) rings of Wistar rats

3.5.1 *Contractile responses*

The cumulative concentration response curves to phenylephrine (PHE, 10^{-9} to 10^{-5}M) were obtained in MPA and BPA rings pretreated with cigarette smoke extract (CSE) and compared with the control responses to PHE in MPA and BPA rings (Fig. 3.51 to 3.52). Pretreatment of rings with CSE (0.1 or 1ml; for 10min) had no effect on contractile responses to PHE in MPA and BPA rings. Whereas, pretreatment of artery rings with 2ml of CSE depressed contractile responses to PHE (10^{-9} to 10^{-7}M) either in MPA or in BPA rings. The cumulative effects of CSE (0.1, 0.2, 0.5, 1 and 2ml) in MPA and BPA rings, which had been precontracted with PHE were performed. The CSE at high concentration (2ml) induced a slight relaxation in some of MPA and BPA rings which precontracted with PHE (10^{-7}M) but not in all of the rings (data not shown). Also, the CSE (2ml) produced a small, time-dependent contraction in some of resting rings.

3.5.2 *Relaxant responses*

The cumulative response curves to acetylcholine (ACh) were obtained in MPA and BPA rings pretreated with cigarette smoke extract (CSE; 1-2ml, for 10min), which had been precontracted with PHE (10^{-7}M) and compared with the control responses to ACh in MPA and BPA rings (Fig. 3.53 to 3.54). The CSE (2ml) reduced relaxant responses to ACh at concentrations (10^{-8} to 10^{-7}M) in MPA rings and at concentrations (10^{-7} to 3×10^{-6}M) in BPA rings (Fig. 3.53 to 3.54), but was unaffected by 1ml of the CSE. The CSE had no

effect on relaxant responses to sodium nitroprusside (SNP, 10^{-11} to 10^{-9}M) in MPA and BPA rings, which had been precontracted with PHE (10^{-7}M). (Fig. 3.55 to 3.56).

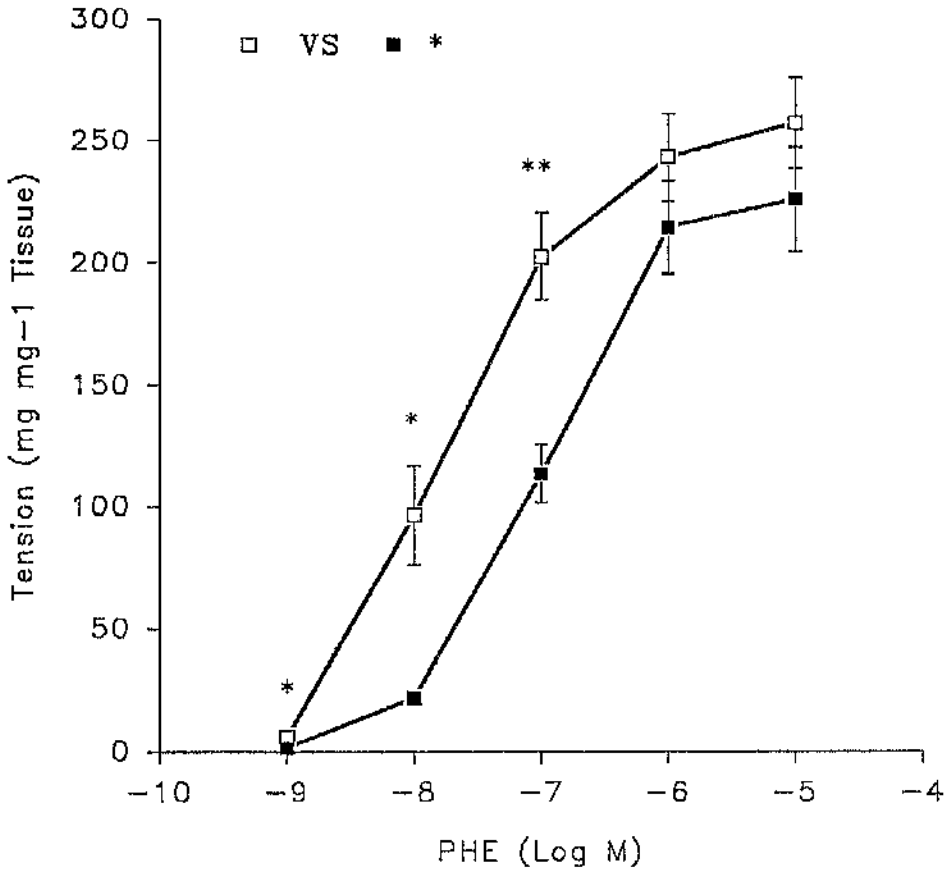

Fig. 3. 51

Cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-5}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in MPA rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and PHE-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$ (paired Student's t test).

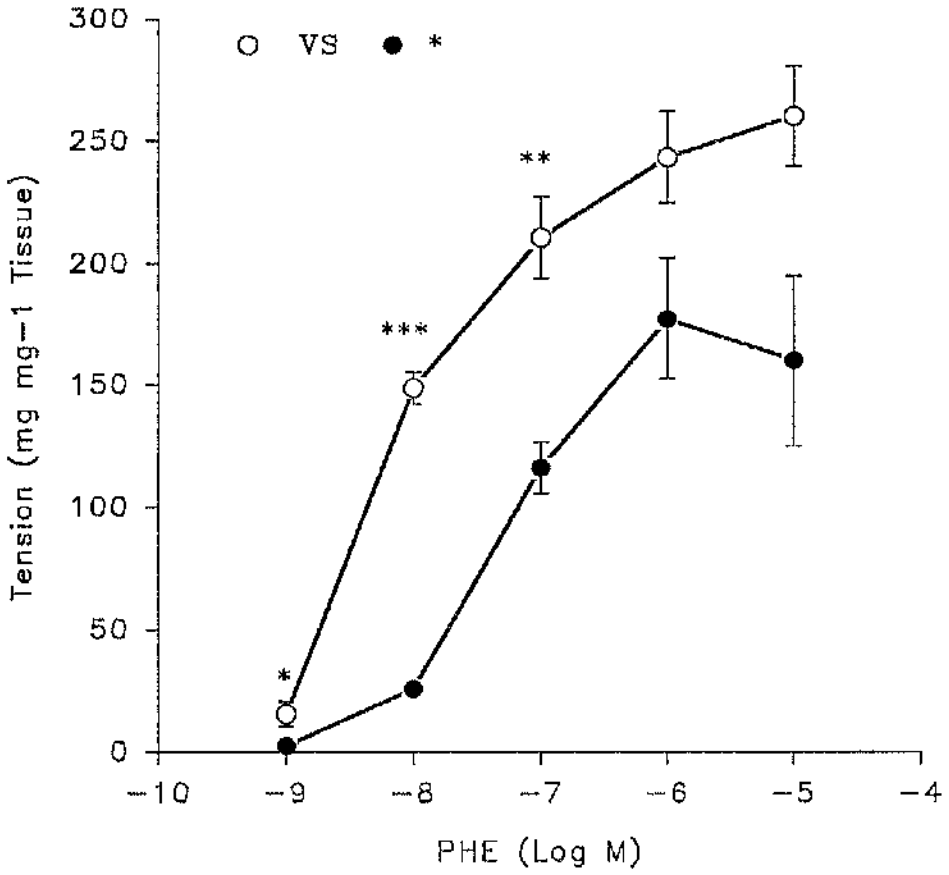

Fig. 3. 52

Cumulative concentration-response curves to phenylephrine (PHE, 10^{-9} to 10^{-5}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in BPA rings of Wistar rats. Tension was recorded (mg mg^{-1} tissue) and PHE-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=6 rings from 6 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (paired Student's t test).

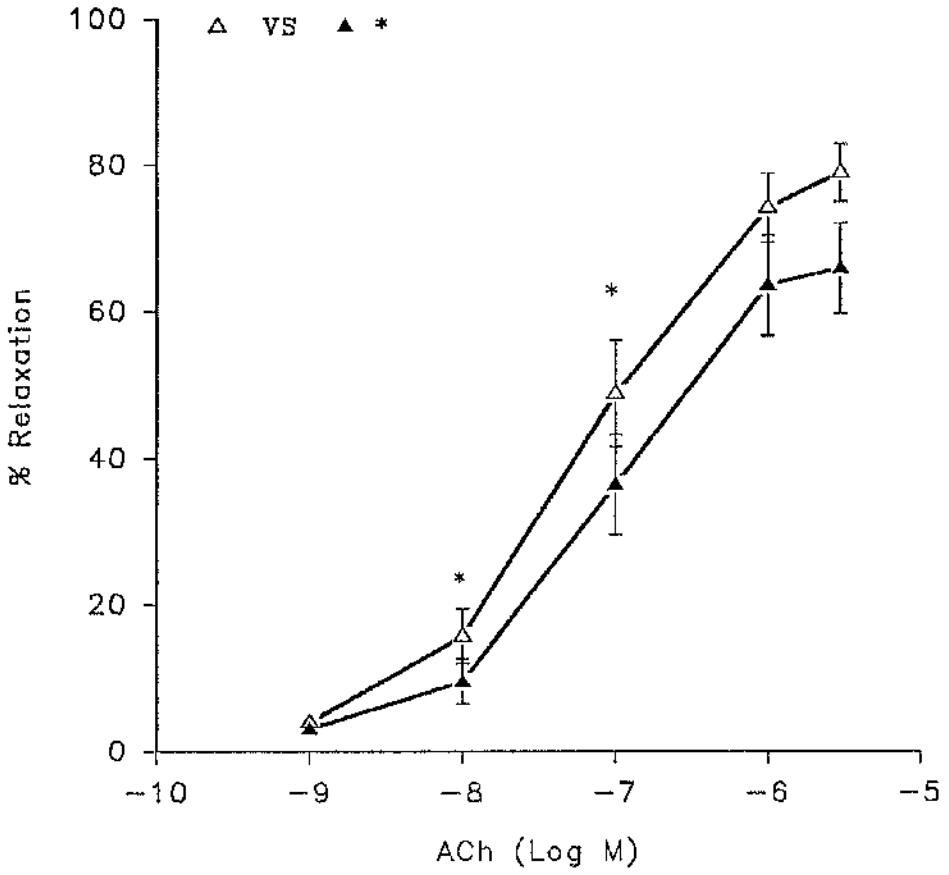

Fig. 3. 53
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 3×10^{-6}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in MPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=7 rings from 7 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$ (paired Student's t test).

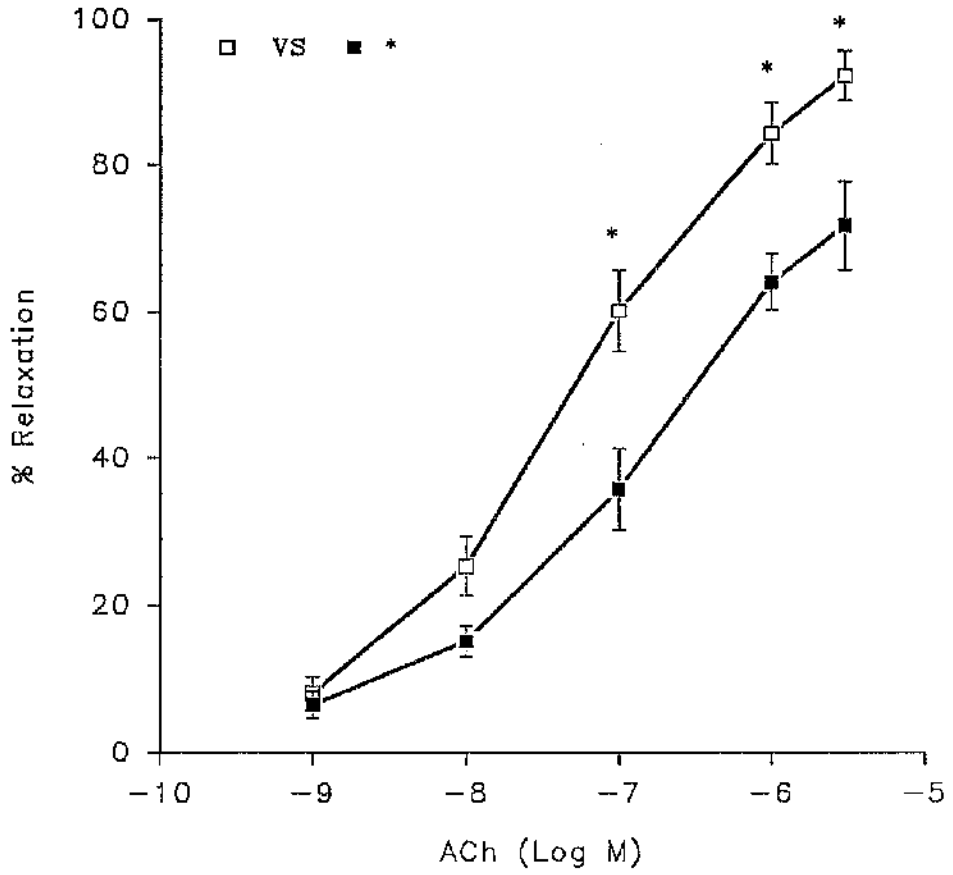

Fig. 3. 54
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 3×10^{-6}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in BPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=5 rings from 5 animals, vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$ (paired Student's t test).

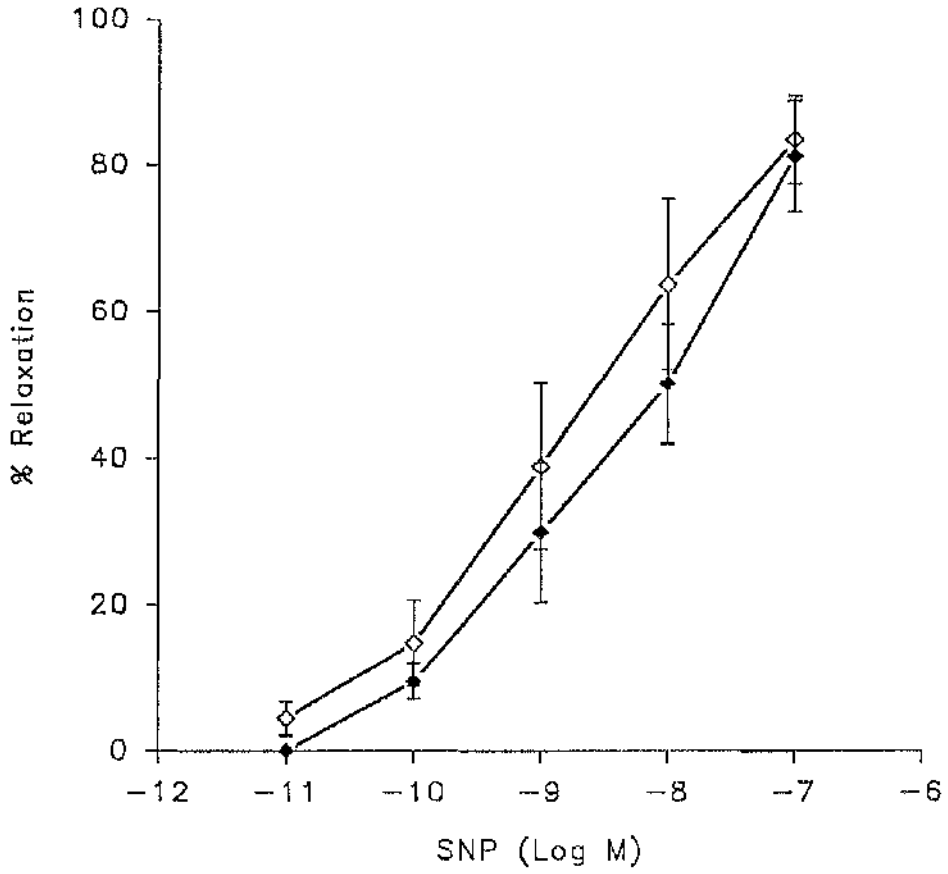

Fig. 3. 55
Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-7}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in MPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=6 rings from 6 animals, vertical bars show ± S.E.M.

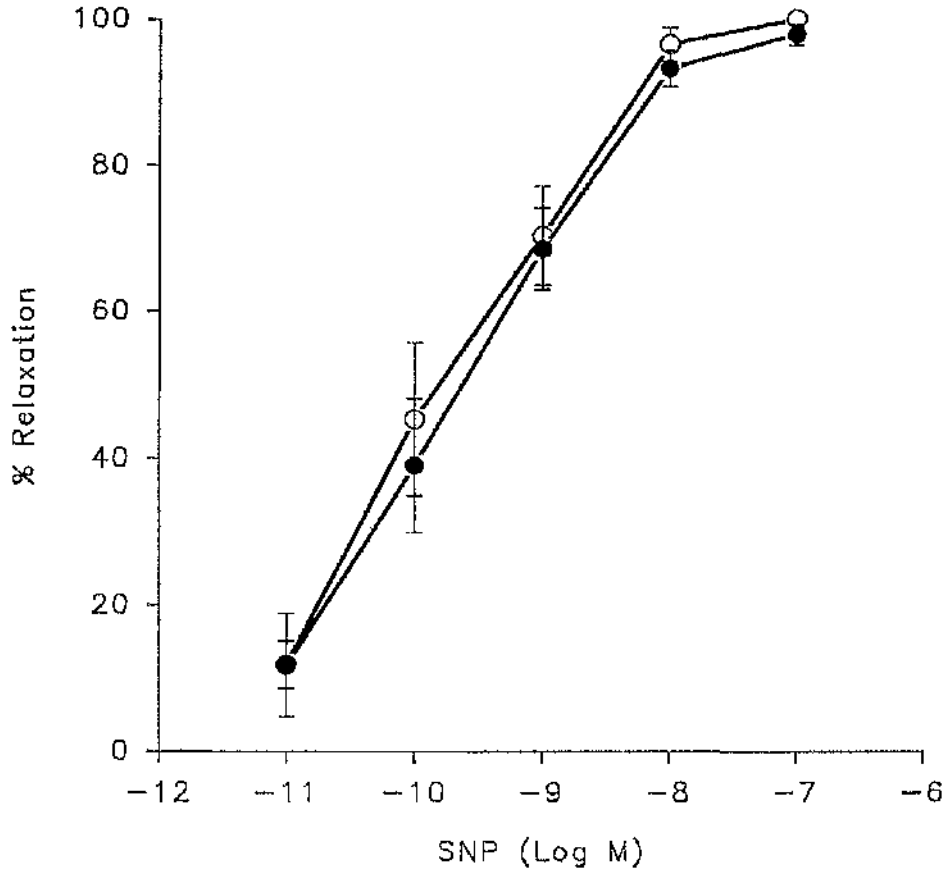

Fig. 3. 56
Comparison between concentration-response curves showing relaxation to sodium nitroprusside (SNP, 10^{-11} to 10^{-7}M) in the absence (open symbol) and presence (closed symbol) of CSE (2ml) in BPA rings precontracted with PHE (10^{-7}M). The results are expressed as means of percentage relaxation of PHE-induced tone. n=7 rings from 7 animals, vertical bars show ± S.E.M.

3.6 Investigation of the effects of chronic heart failure on the responsiveness of the pulmonary vasculature: in main pulmonary artery (MPA) and branch pulmonary artery (BPA) rings of sham and heart failure rabbits

3.6.1 *Effects of contractile responses*

3.6.1.1 *Contractile responsese to 5-hydroxytryptamine (5-HT)*

In this set of experiments, after 1 hour equilibration period, artery rings were contracted with the maximum concentration of KCl (3×10^{-2}M) twice, at 20min intervals, to assess tissue viability and provide a reference contraction for subsequent data analysis. In addition, when experiments with noradrenaline (NA) were performed, the Krebs solution also contained 2.3×10^{-5}M ethylene diaminetetra-acetic acid (EDTA) in order to reduce degradation of the NA.

5-Hydroxytryptamine (5-HT, 10^{-7} to 10^{-4}M) induced concentration-dependent contractions in MPA and BPA rings of 8 weeks sham or of heart failure (HF) rabbits. The maximum contractions to 5-HT (expressed as mg mg^{-1} tissue) in MPA rings were repectively, 54.22±4.54 (n=7 rings from 7 animals) and 58.91±16.53 (n=3 rings from 3 animals) (Fig. 3.57). There were no significant differences between contractile responses to 5-HT in MPA rings of sham and of HF rabbits. Also, 5-HT produced concentration-dependent contractions in BPA rings of sham and of HF rabbits. The maximum contractions (expressed as mg mg^{-1} tissue) were respectively, 195.89±37.27 (n=8 rings from 8 animals) and 135.72±18.62 (n=3 rings from 3 animals), which were not significantly different (Fig. 3.58). BPA rings were more responsive than MPA rings to 5-HT both in sham and HF rabbits ($P < 0.05$; Fig. 3.59 to 3.60).

3.6.1.2 *Contractile responses to noradrenaline (NA)*

Noradrenaline (NA, 10^{-10} to 10^{-5}M) induced concentration-dependent contractions in MPA and BPA rings, both in sham operated and HF rabbits (8 weeks). There were no significant differences between responses to NA both in MPA and in BPA rings of sham operated and of HF rabbits (Fig. 3.61 to 3.62). The maximum contractions (expressed as mg mg^{-1} tissue) in MPA rings of sham operated rabbits were 82.79±6.06 and of HF rabbits were 83.88±2.85, which were not significantly different. The maximum contractions in BPA rings of sham operated rabbits were 158.50±3.03 and of HF rabbits were 195.85±27.13, which were not significantly different. In comparisons between responses to NA in MPA and BPA rings of sham operated rabbits, the latter were more responsive than the former to NA (10^{-8} to 10^{-5}M; Fig. 3.63), also BPA rings of HF rabbits were more responsive than MPA rings to NA (10^{-7} to 10^{-5}M; Fig. 3.64). Cocaine (10^{-6}M) slightly enhanced responses to NA (10^{-7}M) in MPA and to NA (10^{-6}M) in BPA rings of sham rabbits (Fig. 3.65 to 3.66).

3.6.2 *Vasorelaxant responses to acetylcholine (ACh)*

In pulmonary artery rings of sham operated and heart failure (HF) rabbits, which had been precontracted with NA (10^{-6}M), acetylcholine (ACh, 10^{-9} to 10^{-6}M) induced concentration-dependent relaxations. The maximum relaxations to ACh (expressed as the percentage of the PHE-induced tone) in MPA rings were respectively, 73.89±3.89% (in sham) and 69.23±8.24% (in HF), which were not significantly different (Fig. 3.67). Also, the maximum relaxations to ACh in BPA rings were 57.99±8.68 (in sham) and 46.27±4.61 (in HF), which were not significantly different. There was a significant difference between the responses only at lowest concentration of ACh (10^{-9}M)

(Fig. 3.68). Comparisons of concentration response curves to ACh in MPA and BPA rings whether from the heart failure and or from sham rabbits, showed that there were no significant differences (Fig. 3.69 to 3.70).

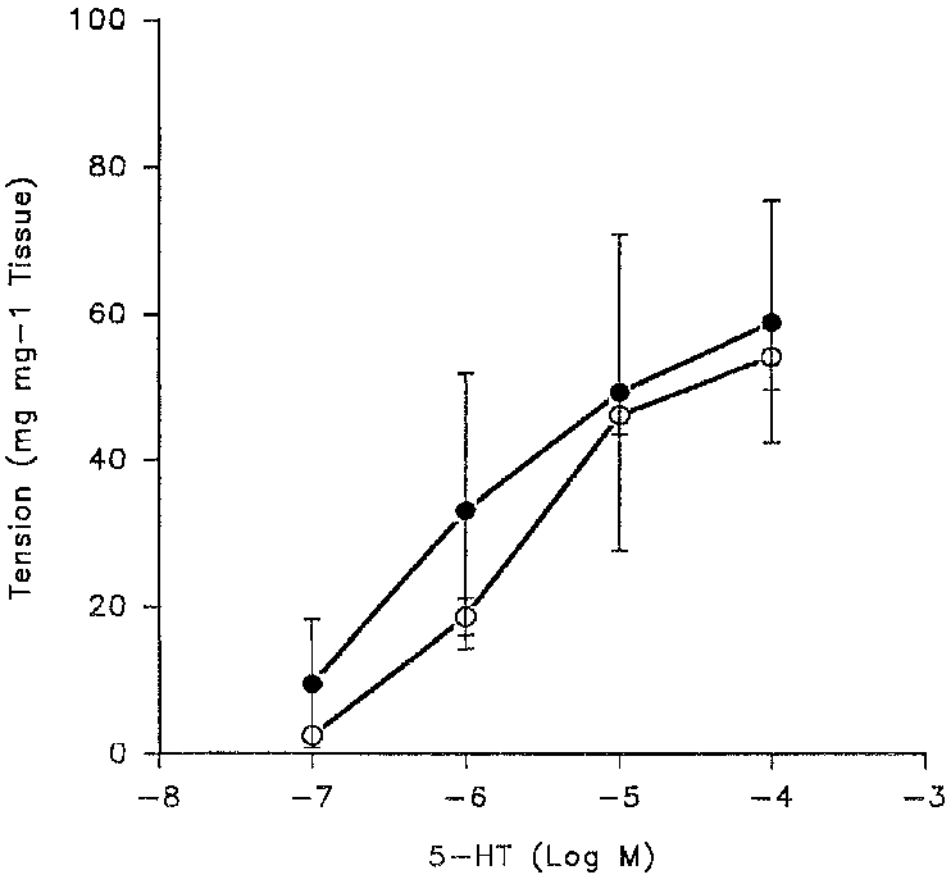

Fig. 3. 57
Cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-4}M) in MPA rings of 8 weeks sham (open symbol) or HF (closed symbol) rabbits. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=3-7 rings from 3-7 animals. The vertical bars show ± S.E.M.

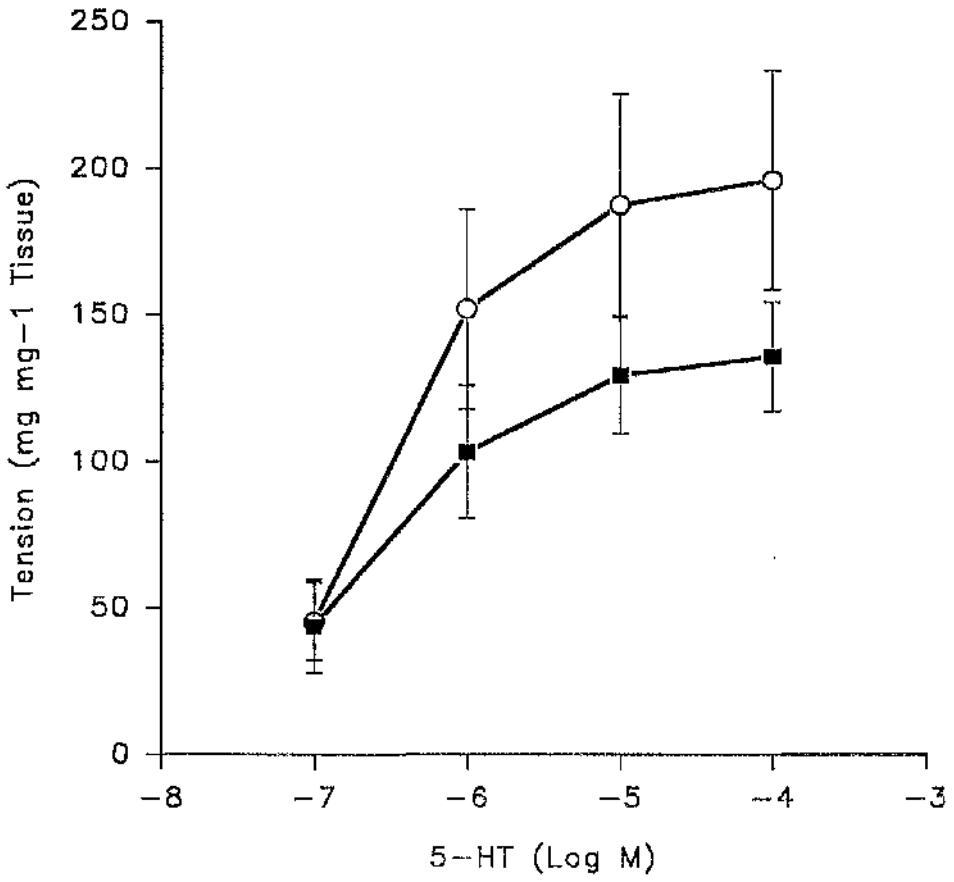

Fig. 3. 58
Cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-4}M) in BPA rings of 8 weeks sham (open symbol) or HF (closed symbol) rabbits. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=3-8 rings from 3-8 animals. The vertical bars show ± S.E.M.

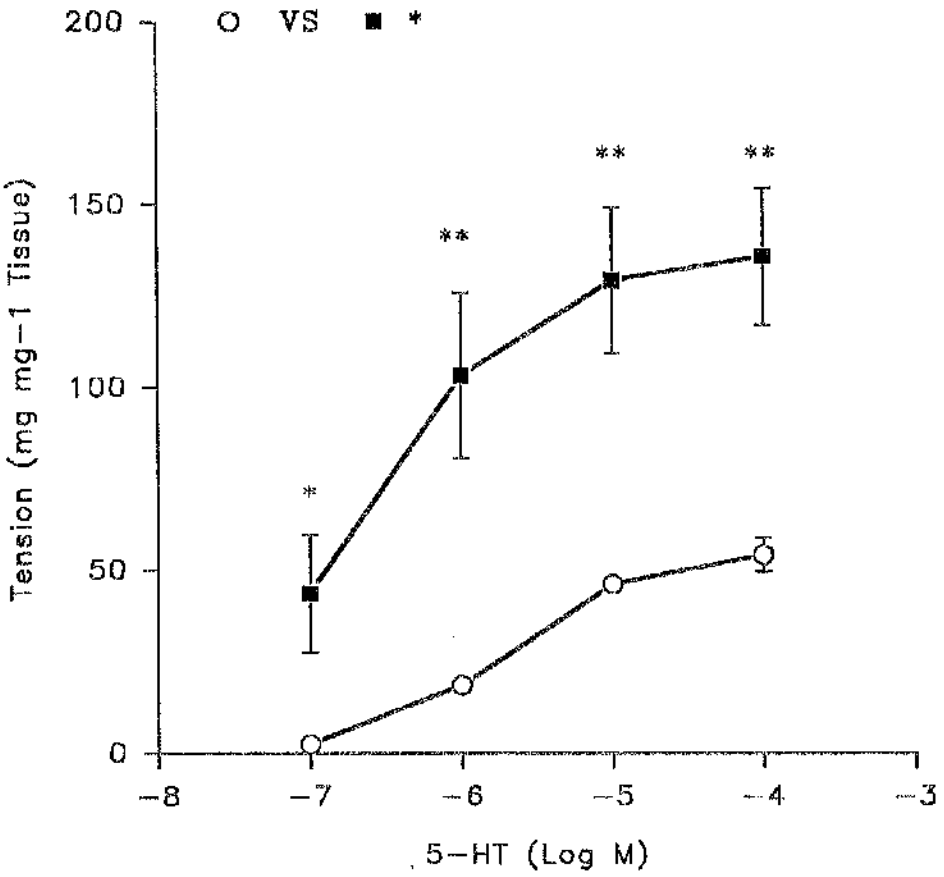

Fig. 3. 59
Comparison between cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-4}M) in MPA (open symbol) and BPA (closed symbol) rings of 8 weeks HF rabbits. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=3 rings from 3 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$ (unpaired t test).

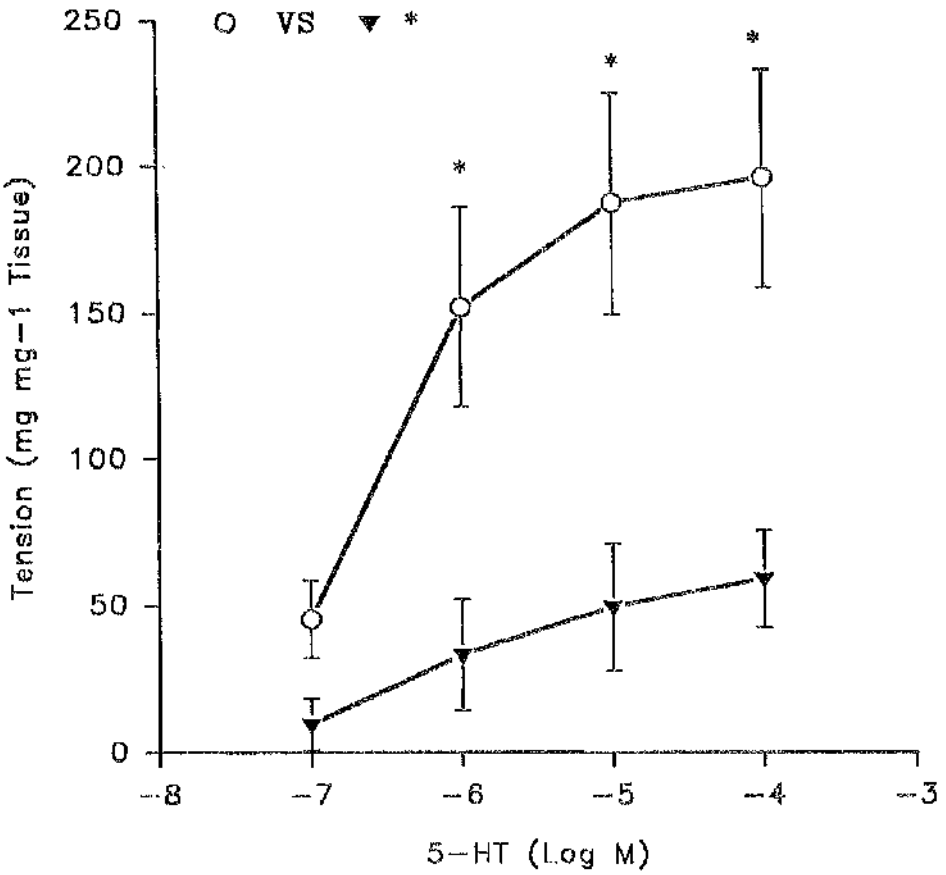

Fig. 3. 60
Comparison between cumulative concentration-response curves to 5-hydroxytryptamine (5-HT, 10^{-7} to 10^{-4}M) in MPA (closed symbol) and BPA (open symbol) rings of 8 weeks sham rabbits. Tension was recorded (mg mg^{-1} tissue) and 5-HT-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=7-8 rings from 7-8 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$ (unpaired t test).

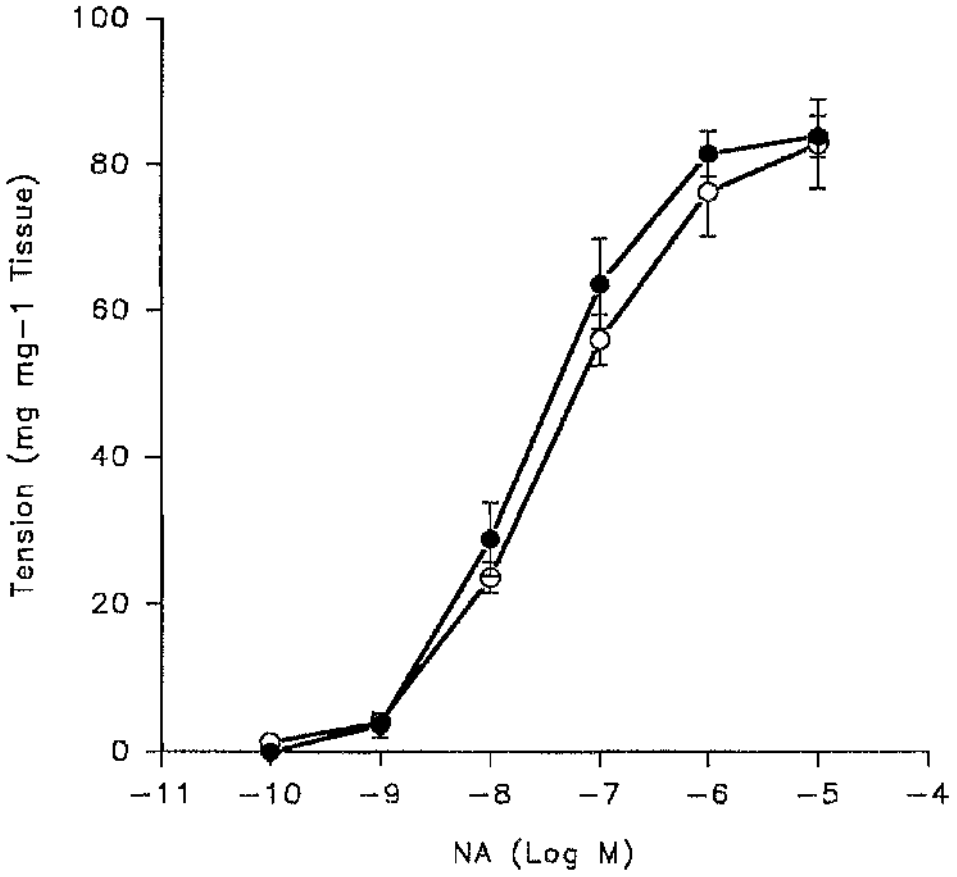

Fig. 3. 61
Comparison between cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in MPA rings of 8 weeks sham (open symbol) or HF (closed symbol) rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=7-9 rings from 7-9 animals. The vertical bars show ± S.E.M.

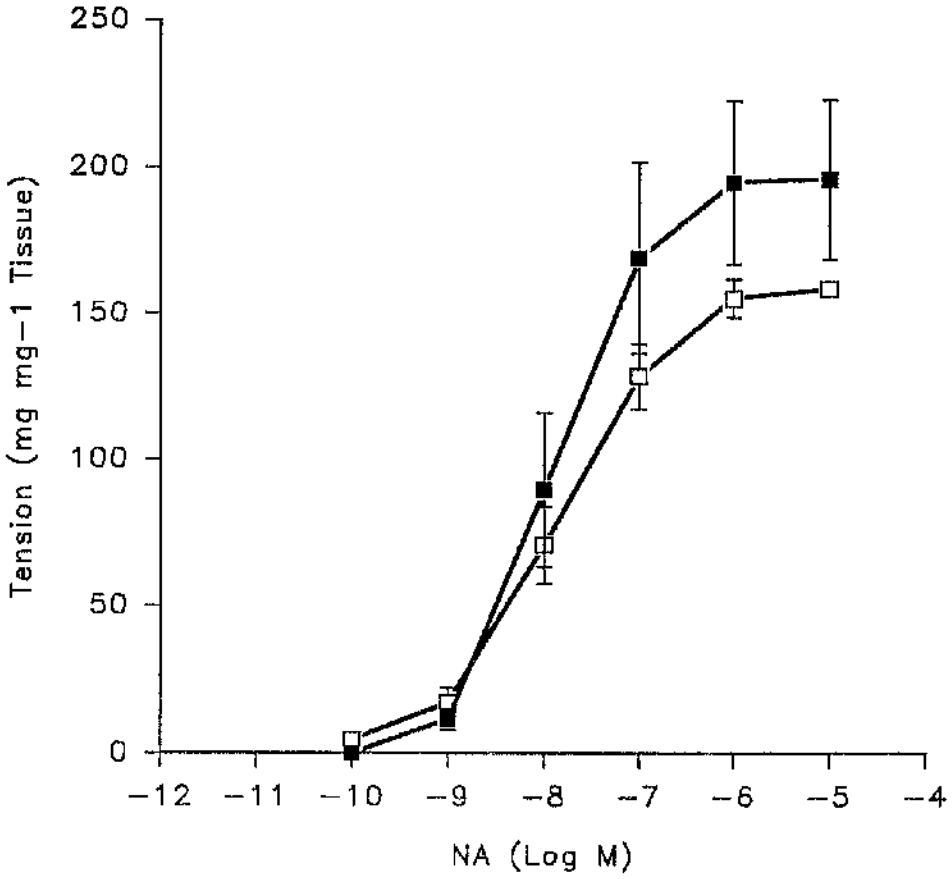

Fig. 3. 62
Comparison between cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in BPA rings of 8 weeks sham (open symbol) or HF (closed symbol) rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=3 rings from 3 animals. The vertical bars show ± S.E.M.

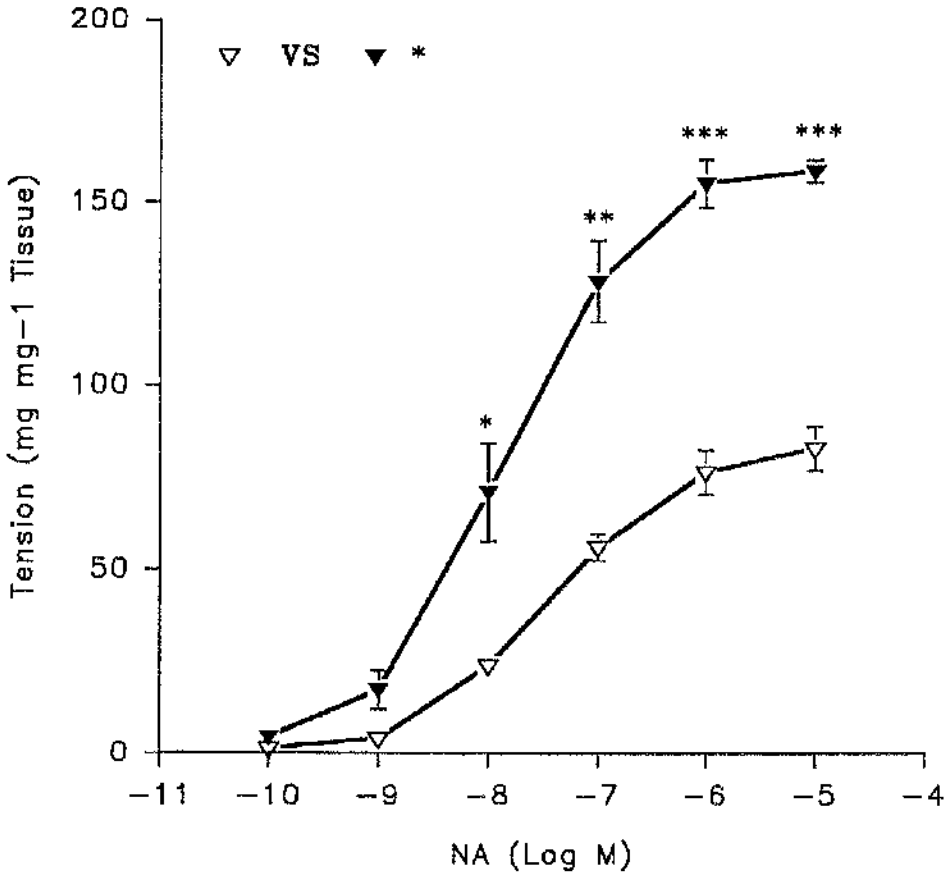

Fig. 3. 63

Comparison between cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in MPA (open symbol) and in BPA (closed symbol) rings of 8 weeks sham rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=7-9 rings from 7-9 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (unpaired t test).

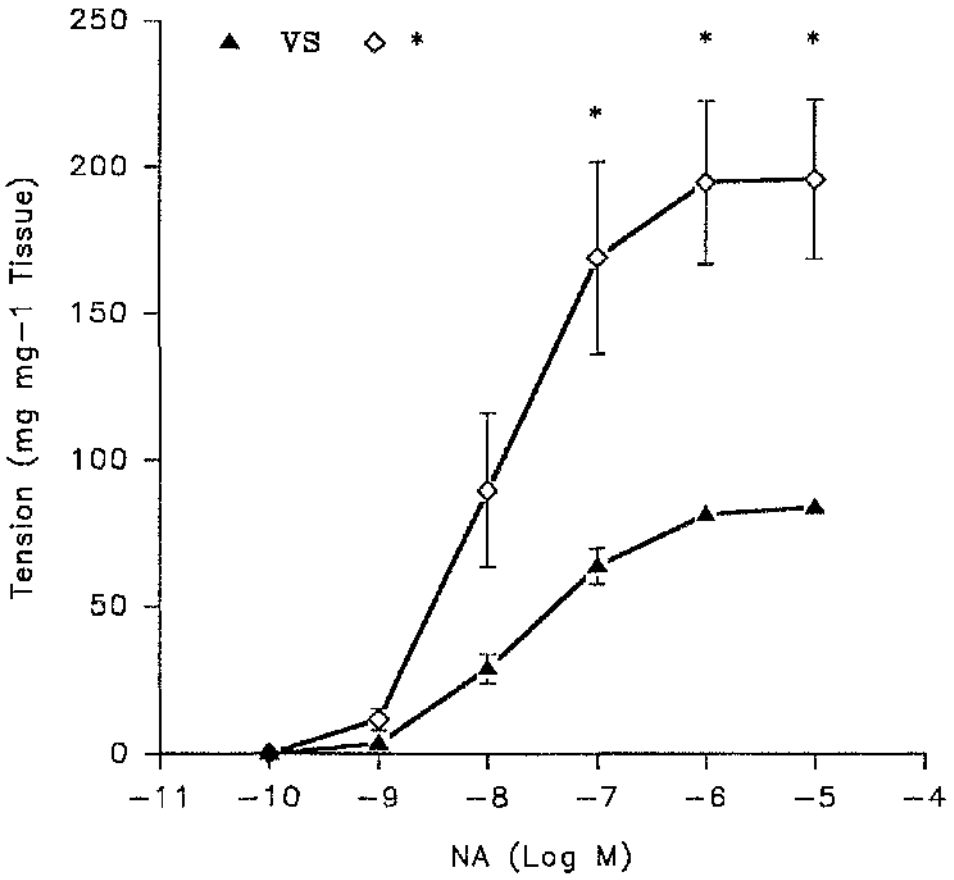

Fig. 3. 64

Comparison between cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in MPA (closed symbol) and in BPA (open symbol) rings of 8 weeks HF rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=3 rings from 3 animals. The vertical bars show ± S.E.M. Asterisks indicate significant differences between means. *p values*: * $P < 0.05$ (unpaired t test).

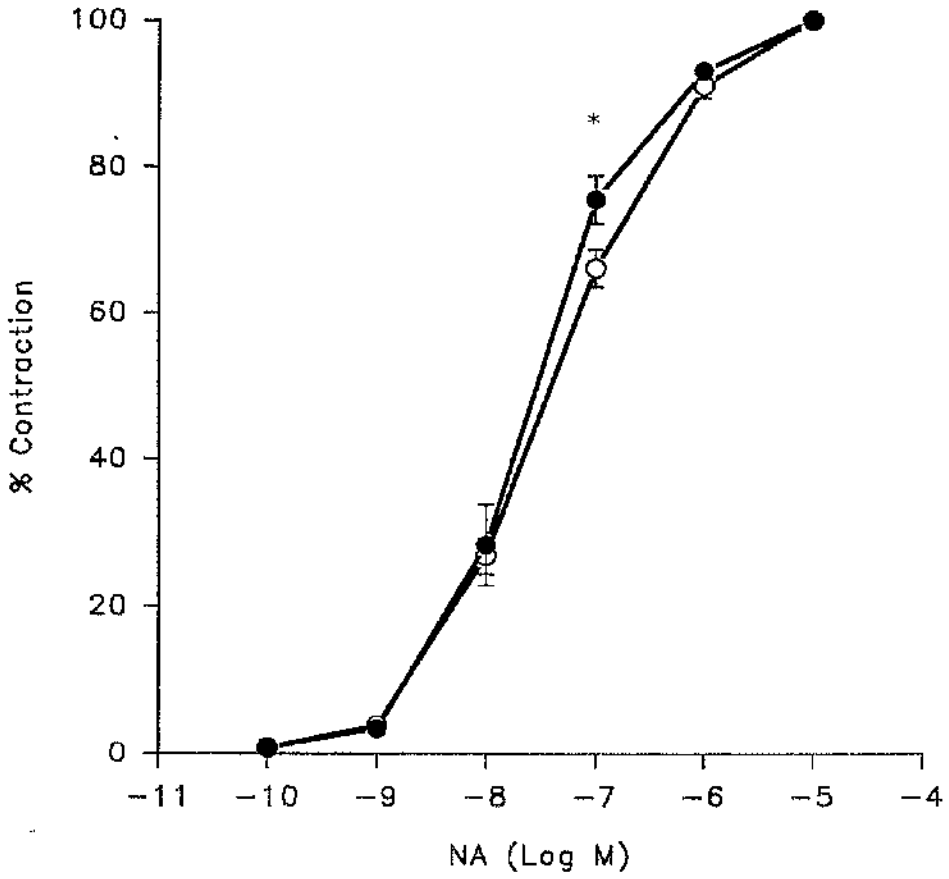

Fig. 3. 65
Cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in the absence (open symbol) and presence (closed symbol) of cocaine (10^{-6}M) in MPA rings of 8 weeks sham rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=9 rings from 9 animals. The vertical bars show ± S.E.M. Asterisk indicates significant difference between means. *p value*: * $P < 0.05$ (paired t test).

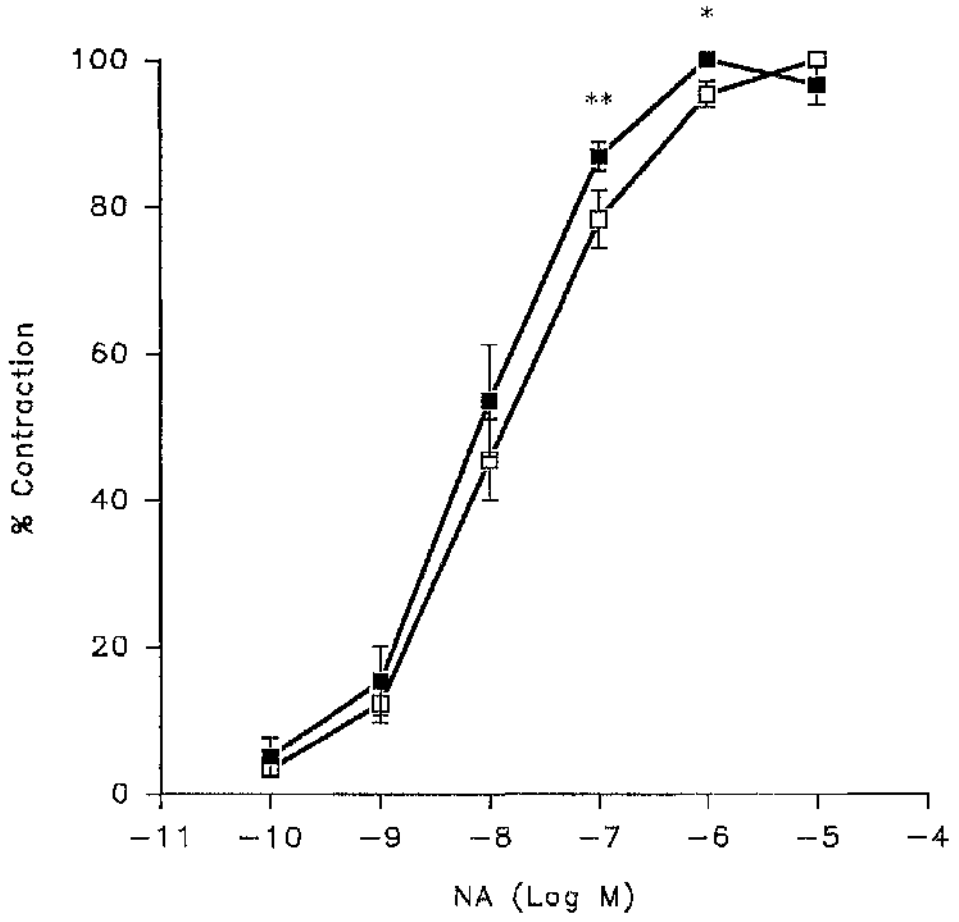

Fig. 3. 66

Cumulative concentration-response curves to noradrenaline (NA, 10^{-10} to 10^{-5}M) in the absence (open symbol) and presence (closed symbol) of cocaine (10^{-6}M) in BPA rings of 8 weeks sham rabbits. Tension was recorded (mg mg^{-1} tissue) and NA-induced concentration-dependent contractions were obtained in these preparations. The results are expressed as means of n=7 rings from 7 animals. The vertical bars show ± S.E.M. Asterisks indicate significant difference between means. *p value*: * < 0.05; ** P < 0.01 (paired t test).

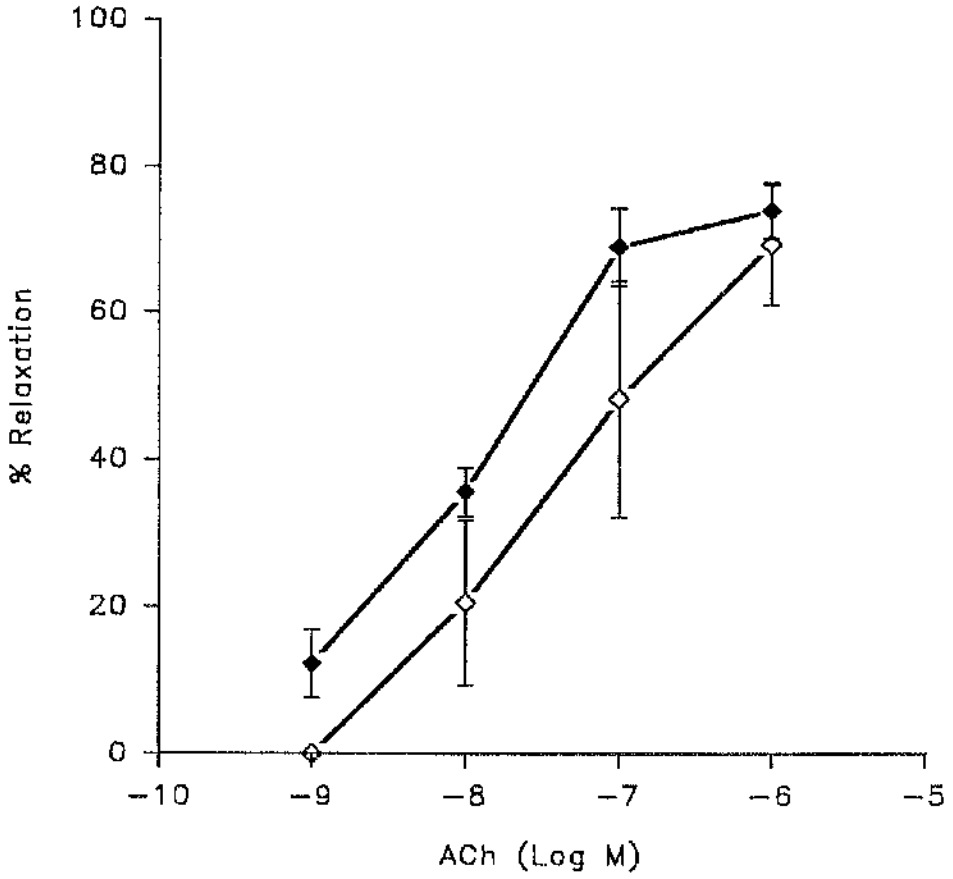

Fig. 3. 67
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 10^{-6}M) in MPA rings precontracted with PHE (10^{-6}M) of 8 weeks sham (closed symbol) or HF (open symbol) rabbits. The results are expressed as means of percentage relaxation of PHE-induced tone. n=3 rings from 3 animals, vertical bars show ± S.E.M.

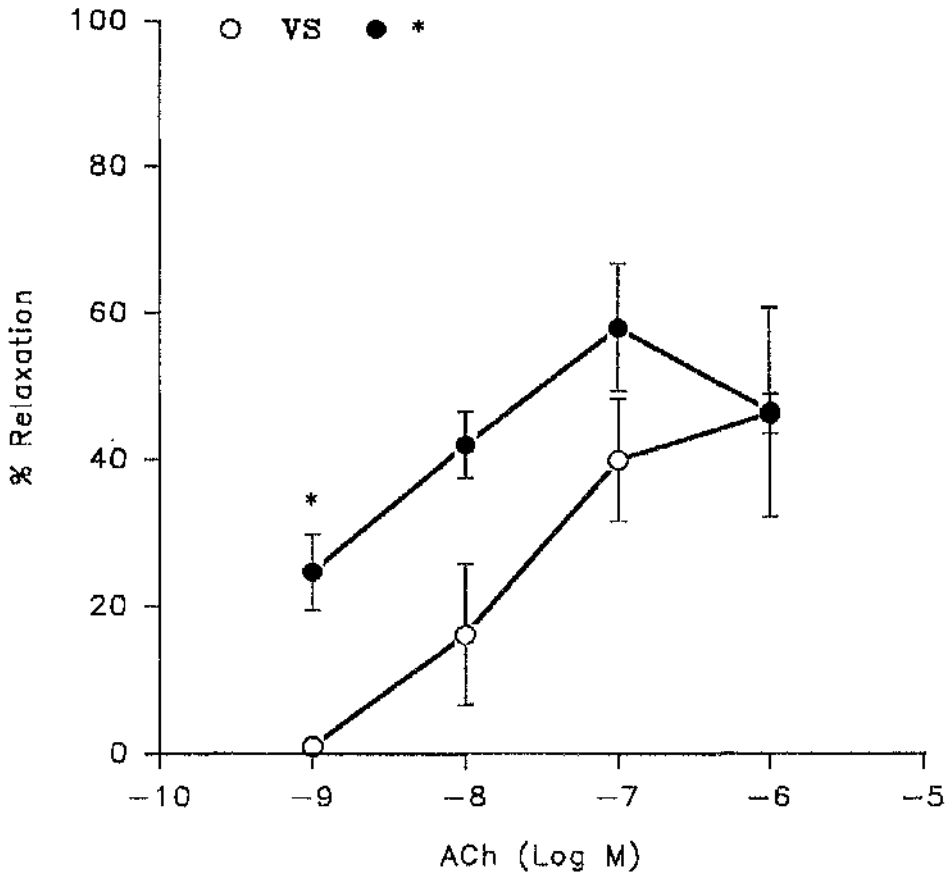

Fig. 3. 68
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 10^{-6}M) in BPA rings precontracted with PHE (10^{-6}M) of 8 weeks sham (closed symbol) or HF (open symbol) rabbits. The results are expressed as means of percentage relaxation of PHE-induced tone. n=3 rings from 3 animals, vertical bars show ± S.E.M. Asterisk indicates significant difference between means. *p value*: * $P < 0.05$ (unpaired t test).

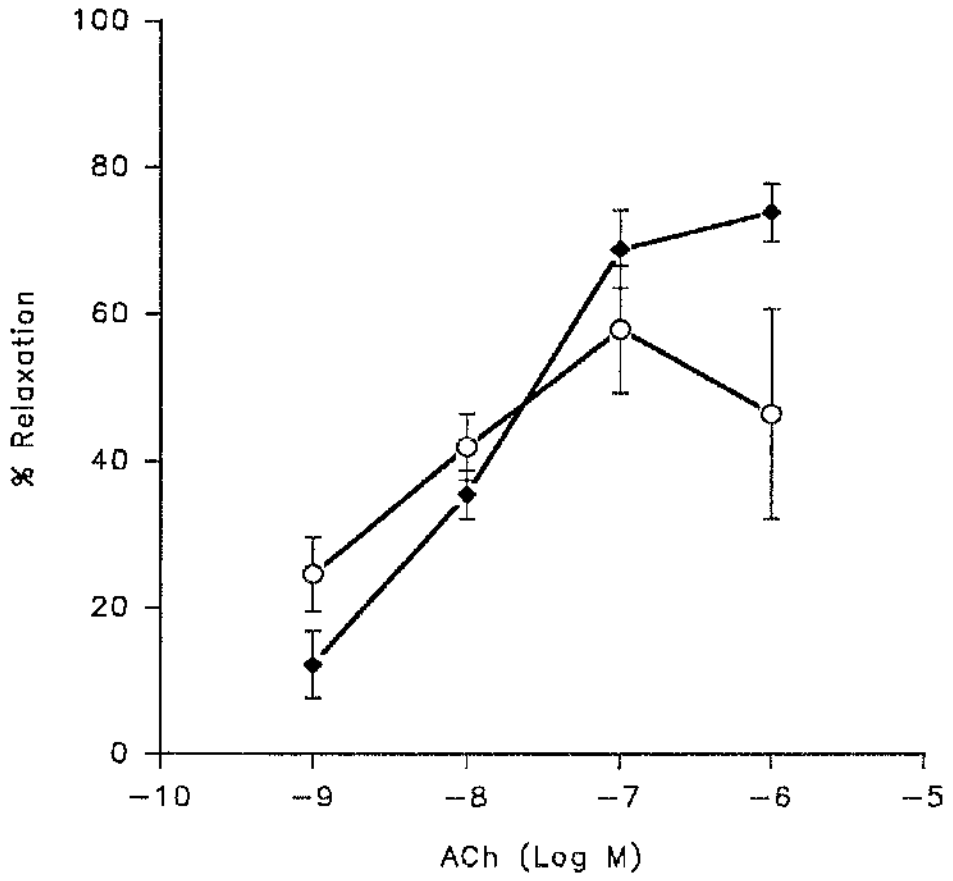

Fig. 3. 69
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 10^{-6}M) in MPA (closed symbol) and in BPA rings (open symbol) precontracted with PHE (10^{-6}M) of 8 weeks sham rabbits. The results are expressed as means of percentage relaxation of PHE-induced tone. n=3 rings from 3 animals, vertical bars show ± S.E.M.

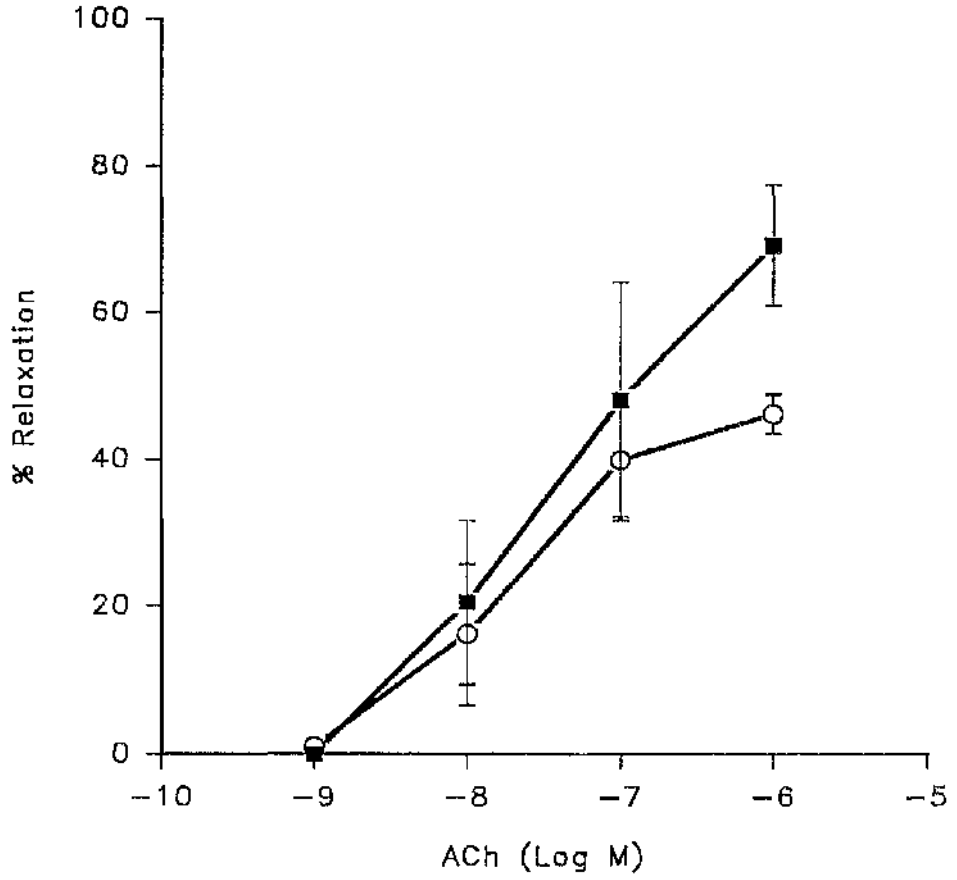

Fig. 3. 70
Comparison between concentration-response curves showing relaxation to acetylcholine (ACh, 10^{-9} to 10^{-6}M) in MPA (closed symbol) and in BPA rings (open symbol) precontracted with PHE (10^{-6}M) of 8 weeks HF rabbits. The results are expressed as means of percentage relaxation of PHE-induced tone. n=3 rings from 3 animals, vertical bars show ± S.E.M.

Chapter 4

DISCUSSION

4 DISCUSSION

The calibre of blood vessels is dependent on vascular smooth muscle (VSM) tone, which is influenced by the adrenergic innervation, and by circulating or locally- produced humoral substances. It is also modulated by the endothelium. The role of endothelium-derived vasoactive substances in the control of vascular tone have been documented widely (Furchgott & Zawadzki, 1980a;1980b; Moncada *et al.*, 1976; Yanagisawa *et al.*, 1988; Palmer *et al.*, 1987; Ignarro *et al.*, 1987b). The vascular endothelium in addition to its other important functions, which include capillary transport, regulation of plasma lipids, and participation in the control of haemostasis, modulates the reactivity of vascular smooth muscle. This regulatory role is accomplished through several mechanisms: I) The endothelial layer interposes a physical barrier between the vascular smooth muscle and hormones and other vasoactive substances circulating in blood; II) it extracts or metabolically degrades vasoactive substances such as noradrenaline (NA), 5-hydroxytryptamine (5-HT), and kinins and thereby prevents or diminishes their activity in vascular smooth muscle; III) it converts precursers (e.g. angiotensin I) into vasoactive products; IV) it may store, synthesis/release inhibitory and excitatory vasoactive substances, including, (PGI_2, EDRF, EDHF, EDCF). The pulmonary artery rings were selected for examination since the pulmonary vascular bed is notable for its large surface area and the important contribution that the endothelial cells make to the metabolic activation and/or degradation of a variety of vasoactive compounds. On the other hand, the pulmonary circulation differs markedly from the systemic circulation in several respects; the different response to acute hypoxia, which causes contraction in pulmonary, but relaxation in systemic vessels (Staub, 1985) is well known; also there are differences in other control mechanisms (Fishman, 1990), the distribution (Hyman *et al.*, 1989) and affinities of adrenoceptors (Shaul *et al.*,

1990), and in the response to some peptides, such as vasoactive intestinal polypeptide (Sata *et al.*, 1986).

4.1 Vascular effects of contractile agonists and the role of the endothelium

5-Hydroxytryptamine (5-HT) exerts complex effects on the cardiovascular system. 5-HT is a potent vasoconstrictor in many vascular tissues. However, the action of 5-HT and related agents vary greatly, depending on the species, the specific vessels under study and the sympathetic tone (Pertz, 1993; Growcott *et al.*, 1992; Corsi *et al.*, 1991). 5-HT can cause either vasoconstriction or vasodilation and these effects have been explained by the wide variety of 5-HT receptors involved and by the different cell types activated by the amine (Feniuk & Humphrey, 1989; Feniuk & Humphrey, 1990). 5-HT inhibits the release of noradrenaline (NA) from sympathetic nerves (Rang & Dale, 1991; Done & Sharp, 1992), and releases nitric oxide (NO) from endothelial cells *via* the 5-HT_1 receptor in the canine coronary artery (Cocks & Angus, 1983; Cohen *et al.*, 1983; Vanhoutte, 1987). The complexity of the effects of 5-HT is indicated by the fact that vasoconstrictor responses to 5-HT usually show desensitisation, which has been attributed to the vasorelaxant effect of NO, released during 5-HT-induced responses (Wakabayashi, 1993). The effects of 5-HT in the pulmonary vessels are further complicated by the fact that 5-HT is taken up by and inactivated in the endothelial cells (Murray, 1986). The main findings of this study of the effects of 5-HT on pulmonary artery rings can be summarized as follows. 1) 5-HT produced concentration-dependent contractions in isolated rings of main pulmonary artery (MPA) and its first branches (BPA), but at high concentration (10^{-3}M), contractile responses to 5-HT in these rings decreased. 2) Branch pulmonary artery (BPA) rings and main pulmonary artery (MPA)

rings were equally responsive to 5-HT. 3) Contractile responses to 5-HT in endothelium-intact rings, were enhanced by the nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 5×10^{-4}M, for 20min) or when the endothelium had been removed by rubbing. The augmentation of the contractile responses to 5-HT in endothelium-denuded rings was very similar to that in endothelium-intact rings incubated with L-NAME. 4) 5-HT produced concentration-dependent relaxations in endothelium-intact artery rings and also in endothelium denuded artery rings, which had been precontracted with phenylephrine (PHE). 5) Prolonged exposure of MPA and BPA rings to 5-HT produced marked desensitisation to 5-HT. The present study demonstrated that 5-HT produced concentration-dependent contractions in MPA and BPA rings. It has been shown that contractile responses to 5-HT in both arteries and veins are usually mediated *via* 5-HT_2 receptors but 5-HT_1-like receptors or a mixture of 5-HT_1-like and 5-HT_2 receptors may also be involved, depending upon tissues and species examined (Roth *et al.*, 1986; Saxena & Villalon, 1990). To characterize the 5-HT-mediated contractile responses, the artery rings (MPA and BPA) were pretreated with the 5-HT_2 receptor antagonist, mianserin (10^{-6}M), which inhibited cumulative contractile responses to 5-HT (10^{-7} to 10^{-5}M). This observation suggested that 5-HT-induced contractile responses in these rings are mediated *via* 5-HT_2 receptors. Surprisingly, the contractile responses to 5-HT in MPA and BPA rings were similar, indicating that contractile responses to 5-HT in these rings did not correlate with artery size in the pulmonary vasculature. Nω-nitro-L-arginine methyl ester (L-NAME, 5×10^{-4}M, which inhibited the maximum responses to endothelium-dependent relaxant, ACh), enhanced responses of intact artery rings to high concentrations of 5-HT, without significantly affecting the EC_{50} values. 5-HT normally has little apparent effect in the pulmonary circulation when it is perfused with Krebs' buffer but in the presence of the nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), 5-HT produced large

pressor responses (Shaw *et al.*, 1992). One explanation of the ability of L-NAME to enhance the pressor effect of 5-HT in the pulmonary circulation is that L-NAME inhibits the synthesis of NO, which acts as a physiological antagonist of 5-HT and normally masks its pressor effects. Another explanation is that L-NAME potentiates the vasoconstrictor effects of 5-HT in the pulmonary circulation by increasing the sensitivity of the 5-HT_2 receptor to the vasoconstrictor action of 5-HT rather than by inhibiting NO synthesis (Lippton *et al*, 1992). Mechanical removal of the endothelium in MPA and BPA by rubbing, caused little reduction of KCl-induced responses. The effectiveness of this procedure was subsequently investigated using ACh, which normally relaxed artery rings but had no effect in rubbed rings. Rubbing also enhanced responses to 5-HT in MPA rings and BPA rings. L-NAME had no effect on 5-HT-induced responses in denuded rings. Interestingly, the concentration-response curves to 5-HT in intact rings, pretreated with L-NAME, was superimposable on the curves to 5-HT in denuded rings, which were not pretreated with L-NAME. These results are consistent with those obtained in the rabbit basilar artery by Trezise and colleagues (1992). Thus, removal of the endothelium or pretreatment artery rings with L-NAME had almost identical effects on the responses to 5-HT. These data collectively suggest that the depressant effect of the endothelium can be fully accounted for by the release of an L-NAME-sensitive endothelium-derived relaxing factor (EDRF). Since L-NAME is an inhibitor of the formation of nitric oxide (NO) from its precursor L-arginine (L-Arg) in vascular endothelial cells (Rees *et al.*, 1990), it seemed probable that the NO/L-Arg pathway was involved. To determine the involvement of the NO/L-Arg pathway, the effect of L-Arg (2×10^{-4} and 10^{-3}M) was investigated in endothelium-intact pulmonary artery rings and in denuded rings. Following incubation in the response of a submaximal concentration of 5-HT, addition of L-NAME (5×10^{-4}M) resulted in an augmentation of tone in intact artery rings, but not in denuded rings and this effect of L-NAME was

completely inhibited by L-Arg (10^{-3}M), whereas L-Arg had no such effect in intact rings precontracted with 5-HT and in the absence of L-NAME. These observations are consistent with those reported in the guinea-pig pulmonary artery by Sakuma and colleagues (1988), and in rat aorta by Frew and colleagues (1993). When the tone was raised in intact pulmonary artery rings with a submaximal concentration of 5-HT (10^{-5}M), ACh (10^{-6}M)-induced relaxations were inhibited by L-NAME (5×10^{-4}M) but these inhibitions were not reversed by L-Arg (10^{-3}M). These observations are consistent with those reported by Randall and Griffith (1991) in rabbit perfused ear arteries. These observations strongly suggest 1) The endothelium-derived nitric oxide formed from L-Arg fully accounts for the depression of 5-HT-induced contractions in these vessels. 2) The depression of contractions to 5-HT was a consequence of the basal release of nitric oxide (NO) from the endothelium, and not of 5-HT-stimulated release. This seems likely since first, L-NAME induced contractions in endothelium-intact artery rings which had been precontracted with 5-HT, but not in endothelium-denuded rings and this effect of L-NAME was reversed by L-Arg; secondly, L-Arg failed to reverse the inhibitory effect of L-NAME in endothelium-intact artery rings, which had been relaxed with ACh. These results therefore support the hypothesis that the vascular endothelium regulated resting pulmonary vascular tone through the synthesis and release of endothelium-derived relaxing factor (EDRF). Similar experiments were carried out using the α_1-adrenoceptor agonist, phenylephrine (PHE), in endothelium-intact rings of MPA and of BPA and in endothelium-denuded rings, in the absence and presence of L-NAME, (i) to investigate responses of MPA and BPA rings to this agonist and (ii) to compare responses induced by 5-HT and PHE in MPA and BPA rings. These experiments demonstrated that phenylephrine (PHE) produced concentration-dependent contractions in MPA rings and BPA rings. MPA rings were less responsive than BPA rings to PHE. Contractile responses to PHE were enhanced in MPA and BPA rings

pretreated by L-NAME (5×10^{-4}M, for 20min) and this effect L-NAME was inhibited by L-Arg. Removal of the endothelium by rubbing in MPA rings, enhanced and potentiated contractile responses to PHE. The augmentation of the contractile responses to PHE were very similar in intact MPA rings pretreated by L-NAME to that seen in endothelium-denuded rings. These results indicate that the endothelium exerts a depressant effect on the contractility of these rings to PHE. Contractile responses to high concentrations of PHE (10^{-4}M) in these preparations were poorly maintained. Pretreatment of the preparations with propranolol (10^{-6}M, for 10min) prevented this reduction in the contractile response which occurred with high concentrations of PHE (10^{-4}M), without having any other effect on the contractile responses to PHE. This suggests that at high concentrations of PHE, a β-adrenoceptor mediated response occurs and that this β-adrenoceptor-mediated relaxation overcomes the α_1-adrenoceptor mediated vasoconstriction at this point. It has been shown that contractile responses to PHE are mediated *via* α_1-adrenoceptors (Langer & Hicks, 1984). In some experiments concentration response curves to PHE were obtained in MPA rings pretreated with the α-adrenoceptor antagonist, prazosin (PRAZ, 10^{-8}M). PRAZ, which inhibited completely the contractile responses to PHE (10^{-9} to 10^{-5}M), indicating that contractile responses to PHE are mediated *via* α_1-adrenoceptors in these artery rings. Comparisons between contractile responses to PHE, in MPA and BPA rings demonstrated that BPA rings were more responsive than MPA rings to PHE, indicating that the contractile response to PHE is correlated with artery size in the pulmonary vasculature, and smaller arteries were more sensitive than bigger arteries. The results obtained are not consistent with the results reported by Leach and colleagues (1992). There may be a biochemical explanation of the greater ability of BPA rings to respond to this vasoconstrictor. I) It is possible that basally-released NO acts as a physiological antagonist of PHE in rat isolated BPA rings and this occurs to a

lesser extent than in MPA rings. II) It is possible that BPA rings were less sensitive to basally-released NO than MPA. This view seems likely since there were significant differences between MPA and BPA rings after treatment of the rings with the nitric oxide inhibitor-synthase, Nω-nitro-L-arginine methylester. III) It is possible that BPA rings have either more α_1-adrenoceptors than MPA rings or that their contractile mechanisms were more responsive to this agonist. Consequently, activation of α_1-adrenoceptors in BPA rings was perhaps more likely to induce more PI-hydrolysis and in turn increase Ca^{2+} from intracellular stores, and presumably, produce larger contractile responses. Comparisons between contractile concentration response curves to 5-HT and PHE in MPA and BPA rings, reveal that the latter agonist was more potent than the former. There are several explanations of the greater ability of PHE to contract these rings. I) It is possible that 5-HT_2-receptor-mediated response was more susceptible than the α_1-adrenoceptor-mediated response to NO and/or there was less NO released by the α_1-agonist than by 5-HT. II) It is possible that the α_1-adrenoceptors were more numerous than the 5-HT_2-receptors and/or more G-proteins participate to promote receptor-phospholipase C coupling in α_1-adrenoceptor-mediated responses than in 5-HT induced responses. To determine which explanation is correct will require the use of techniques which would involve e.g. measurement of cGMP levels, receptor cloning and radioligand studies. In another series of experiments, the effects of other vasoconstrictors (noradrenaline, angiotensin II and the thromboxane A_2 mimetic, U46619) were examined (i) to determine the effects of these agonists on pulmonary artery rings and (ii) to compare their effects in MPA and BPA rings and to determine whether there were any significant differences. All of these agonists produced concentration-dependent contractions in MPA and BPA rings. As with the results obtained in BPA rings with PHE, responses to noradrenaline (NA) in BPA rings were larger than in MPA rings. The explanations given previously for the greater ability of BPA rings to respond to

the α_1-adrenoceptor agonist, PHE may also apply to NA. In addition, BPA rings were more responsive than MPA rings to angiotensin II (AII) but only at a concentration of 10^{-9}M. Whereas, there were no significant differences between MPA and BPA rings in responses to thromboxane A_2 mimetic, U46619. Generally, the results obtained showed that the smaller pulmonary arteries (BPA) were more responsive and sensitive than the larger MPA to vasoconstrictors.

5-HT also induced concentration-dependent relaxations in the rings, which had been precontracted with PHE (10^{-7}M). 5-HT-induced relaxations were unaffected either by pretreating the preparations with L-NAME (5×10^{-4}M) or by mechanical removal of the endothelium by rubbing. The vasorelaxant effect of 5-HT was therefore unlike that of ACh which also relaxed PHE-induced tone in these vessels. Unlike 5-HT-induced relaxations, ACh-induced relaxations were inhibited by L-NAME (5×10^{-4}M) and this effect was not reversed by L-arginine (10^{-3}M). The results obtained with ACh in the pulmonary artery rings are consistent with those of Randall and Griffith (1991), who suggested that different mechanisms may be involved in basal and agonist-stimulated release of NO. The synthesis of NO involved in both types of release was inhibited by L-NAME, but the effect of L-NAME was only reversed by L-arginine when the NO was basally-released. By this criterion, ACh released NO in pulmonary artery rings but 5-HT did not, since its ability to relax rings which had been precontracted with PHE was reversed by L-NAME and this action of L-NAME was then antagonised by L-Arg. However, this distinction between basally-released and stimulation-release may be spurious since there is evidence that L-NAME can also block muscarinic receptors (Buxton *et al.*, 1993). Thus L-NAME would block the vasorelaxant effect of ACh for two reasons. First, by inhibiting nitric oxide synthase and secondly, by antagonising the action of ACh at its receptor. L-Arginine would be able to reverse the action of L-NAME on the synthesis of NO but would not affect the action of

L-NAME at the muscarinic receptors and so L-arginine would not restore the vasorelaxant response to ACh. Since muscarinic receptors are not involved in basal release of NO, the effect of L-NAME would be readily reversed by L-arginine. Furthermore, since L-NAME does not block the action of 5-HT at its receptor, its ability to reverse any NO-mediated vasorelaxation that occurred in the presence of 5-HT, would be exclusively due to blockade of NO synthesis. Thus contraction produced by L-NAME in the presence of 5-HT would be reversed by L-arginine irrespective of whether the NO was basally-released or released by 5-HT. The ability of L-arginine to reverse L-NAME induced contractile responses, obtained in the presence of 5-HT, merely confirms that NO is present and reduces contractile response to 5-HT and also, it is thought that the NO is basally-released and is not released by 5-HT. Therefore, it is possible that the vasorelaxant action of 5-HT is mediated either by relaxant receptors which are only susceptible to high concentrations of 5-HT or it is a non-specific effect of 5-HT and/or it is that desensitisation occurred.

Homologous desensitisation is a widespread phenomenon in which initial exposure to an agonist results in decreased cellular responsiveness on second exposure to the same agonist. This study also investigated the maximum contractile effects of 5-HT in the absence and presence of L-NAME in endothelium-intact rings of MPA or of BPA and in rings with denuded-endothelium to determine if desensitisation occurred and whether it was specific to 5-HT and if it could be explained by the release of nitric oxide (NO). For comparison, responses to phenylephrine (PHE) were also examined. In some experiments artery rings were exposed to high concentration of 5-HT (10^{-4}M) for 30 minutes or 1 hour, after which cumulatively administered submaximal responses to 5-HT and PHE were examined. The effect of this treatment were also examined in the presence of L-NAME, and in rings in which the endothelium had been removed by rubbing. This study has shown

that L-NAME statistically enhanced the maximum contractile responses to 5-HT in main pulmonary artery rings and in its branches. This result suggests that endogenous nitric oxide (NO) acts as a physiological antagonist of 5-HT in the pulmonary vasculature. Two possible explanations of the ability of L-NAME to potentiate responses to 5-HT in the pulmonary vessels exist. First, L-NAME may have prevented desensitisation to 5-HT occurring. This is more likely to occur with high concentrations of 5-HT and so this is a plausible explanation of the ability of L-NAME to enhance responses to high concentration of 5-HT in pulmonary artery rings. A second possibility is that 5-HT can release NO and its ability to do this is, like its ability to contract the artery rings, concentration-related. The maximum contractile responses to phenylephrine (PHE) were enhanced also by L-NAME in pulmonary artery rings and its first branches. Prolonged exposure of pulmonary artery rings to 5-HT for periods of up to an hour produced desensitisation, which was characterised by abolition of contractile responses to 5-HT and inhibition of responses to low concentrations of PHE. This desensitisation to 5-HT was most marked in pulmonary artery rings in which the endothelium was intact. Investigation of the effects of 5-HT in pulmonary artery rings in which the endothelium had been removed by rubbing and in which ACh had no relaxing effect, showed that the extent of 5-HT-induced desensitisation was reduced but not abolished. Pretreatment with L-NAME also reduced but did not abolish 5-HT-induced desensitisation and even the combination of L-NAME and removing the endothelium did not abolish the phenomenon. It is clear that the desensitisation produced by prolonged exposure to 5-HT is complex and is produced by several mechanisms. One component could be physiological antagonism caused by NO derived from the endothelium. This seems likely since removing the endothelium reduced the extent of the desensitisation. However, the possibility that endothelial PGI_2 was responsible for this component of the desensitisation can be excluded; since indomethacin

pretreatment of artery rings had no effect. The involvement of NO is suggested by the effect of L-NAME , which when administered before exposure of the artery to 5-HT, also reduced but did not abolish the desensitisation. In some prolonged experiments, artery rings were exposed to 5-HT and then, during the desensitisation and in the presence of 5-HT, were exposed to haemoglobin or L-NAME. In these experiments haemoglobin or L-NAME rapidly reversed the desensitisation and this effect of L-NAME was opposed by L-arginine. This observation suggests that during prolonged experiments, in which the artery rings were exposed only to 5-HT, NO was entirely responsible for the desensitisation, which was due to physiological antagonism of the contractile effect of 5-HT by NO, released mainly from the endothelium. In contrast, when the L-NAME was administered prior to exposure to 5-HT, the result obtained was very different. In this situation there was still desensitisation to the contractile response to 5-HT but the administration of haemoglobin or L-NAME during the desensitisation had no effect. This result indicates that in the presence of L-NAME and presumably in the absence of NO, prolonged exposure to 5-HT still causes subsensitivity to 5-HT. This subsensitivity may be due to (i) alterations in the 5-HT receptor or its coupling mechanism; whose integrity may normally be maintained by nitric oxide; however, attenuation of 5-HT_2 receptor-mediated phosphatidylinositol turnover *via* an activation of protein kinase C has been reported (Roth *et al.*, 1986; Aghajanian, 1990); such inhibition may serve (a) to protect cells from over stimulation, (b) as a modulatory substrate by other regulators of cellular activity. (ii) internalisation or sequestration of receptors by which hydrophilic agonists are prevented access to receptors; sequestration of β-adrenoceptors has been extensively studied (Waldo *et al.*, 1983; Tocws *et al.*, 1984; Kassis *et al.*, 1986).

4.2 *Vascular effects of relaxant agonists and the role of the endothelium*

A number of agents have been shown to elicit relaxation of the pulmonary artery either through the release of nitric oxide (NO) or by promoting (NO) synthesis, through stimulation of specific endothelial cell receptors. This study investigated the effects of various vasorelaxants including acetylcholine (ACh), carbachol (CARB), adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), histamine (HIST), isoprenaline (ISO), adenosine (AD), bradykinin (BK), substance P (SP) and sodium nitroprusside (SNP) in MPA and BPA rings with or without endothelium and in the absence and presence of L-NAME (2×10^{-4}M). The purpose of this study was : (i) to examine the effects of these vasorelaxants in MPA and BPA rings (ii) to determine the contribution of nitric oxide on the agonist-induced endothelium-dependent or -independent relaxations and (iii) to investigate agonist-induced relaxant responses in the presence of their specific antagonists. The vascular endothelium regulates the tone of the underlying vascular smooth muscle through the release of two important nonprostanoid agents, these are EDRF, which is likely to be nitric oxide (NO), and EDHF, which has yet to be identified (Palmer *et al.*, 1987; Ignarro *et al.*, 1988; Feletou & Vanhoutte, 1988; Chen *et al.*, 1988; Keef & Bowen, 1989; Brayden, 1990; Nagao & Vanhoutte, 1991; Chen *et al.*, 1991). These are likely to be distinct factors because NO does not alter membrane potential (Huang *et al.*, 1988; Komori *et al.*, 1988) and also because inhibitors of nitric oxide do not alter endothelium-dependent hyperpolarization (Chen & Cheung, 1992; Chen *et al.*, 1991). Acetylcholine and related cholinomimetics cause both vasorelaxation and hyperpolarization in vascular smooth muscle cells by endothelium-dependent mechanisms (Bolton *et al* ., 1984; Chen *et al.*, 1988; Feletou & Vanhoutte, 1988). The mechanism of the vascular smooth muscle relaxation to acetylcholine (ACh) and other muscarinic agents has been studied extensively (Furchgott & Zawadzki, 1980b; Chand & Altura, 1981;

De Mey & Vanhoutte, 1981; Cocks & Angus, 1983) and shown to be dependent upon the presence of the endothelium (Furchgott & Zawadzki, 1980b). Now, it is understood that acetylcholine and other muscarinic agents cause a diffusible factor namely endothelium-derived relaxing factor (EDRF) (Angus & Cocks, 1989) which is released from endothelial cells following the binding of ACh to muscarinic receptors (Hynes *et al.*, 1986; Dauphin & Hamel, 1990). EDRF is thought to be nitric oxide (NO) (Palmer *et al.*, 1987; Ignarro, 1989) which is formed from the conversion of L-arginine to L-citrulline (Palmer *et al*, 1988b) or a derivative, since relaxation to acetylcholine is blocked by compounds including haemoglobin (Angus & Cocks, 1989), or the L-arginine analogues, N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA) and N^G-nitro-L-arginine methyl ester (L-NAME) (Sakuma *et al.*, 1988; Moore *et al.*, 1990; Rees *et al.*, 1990). NO diffuses into the adjacent vascular smooth muscle cells where it causes activation of guanylyl cyclase (GC), the enzyme that catalyzes the production cGMP from guanosine 5'-trisphosphate (GTP) within the smooth muscle (Griffith *et al.*, 1985; Waldman & Murad, 1987; Ignarro, 1989; Moncada *et al.*, 1991). In turn, cGMP probably inhibits contraction of vascular smooth muscle through activation of cGMP-dependent protein kinase (PKG). Both the relaxation and enhanced guanylyl cyclase activity produced by ACh can be mimicked by addition of authentic NO (Gruetter *et al.*, 1981; Ignarro *et al.*, 1987a). The present experiments demonstrated that ACh (10^{-8} to 3×10^{-6}M) induced concentration-dependent relaxations in MPA and BPA rings. The maximum relaxation to ACh in MPA rings and BPA rings which had been precontracted with PHE (EC_{70}, 1.2×10^{-7}M) were respectively, (64.26±3.66% in MPA and 81.79±3.74% in BPA). In comparisons between relaxant response curves to ACh, BPA rings were more responsive than MPA rings. This observation is consistent with that reported in rabbit ear artery resistance vessels and in central ear arteries to ACh by Owen and Bevan (1985). There were significant

differences between MPA and BPA rings to the maximum effect of ACh ($0.01 > P > 0.001$). Moreover, in another series of experiments, similar results were obtained between MPA and BPA rings in relaxant responses to carbachol (CARB, 10^{-8} to 10^{-4}M). Thus, it is possible that the smaller pulmonary arteries produce higher cGMP levels than large arteries in relaxant responses to muscarinic agonists. This view is supported by the results obtained in bovine pulmonary arteries of different sizes by Ignarro and colleagues (1987). Pretreatment of isolated rings with blockers of the NO pathway, L-NAME or L-NOARG (5×10^{-4}M, for 10min) or mechanical removal of the endothelium by rubbing completely abolished relaxant responses to ACh or CARB in pulmonary artery rings. The inhibitory effect of ACh was reversed by L-NAME and this action of L-NAME was not antagonised by L-Arg (10^{-3}M). These results indicate that (i) vascular smooth muscle relaxation induced by ACh or CARB, to be dependent upon endothelium (Furchgott & Zawadski, 1980b), (ii) ACh- or CARB-induced relaxations were mediated by agonist-stimulated endothelial-release EDRF, since the L-Arg failed to reverse the contractile action of L-NAME, whereas the contractile effect of L-NAME was inhibited by L-Arg when the NO was basally-released and (iii) ACh or CARB are unlikely to have produced hyperpolarization in pulmonary artery rings, since, L-NAME completely abolished relaxant responses both to ACh and CARB, thus suggesting that EDRF was the sole inhibitory mediator released from endothelium cells. In blood vessels, the release of EDRF evoked by ACh is mediated *via* the activation of different muscarinic receptors. The M_3 muscarinic receptors mediate endothelium-dependent relaxation in the rat pulmonary artery (McCormack *et al.*, 1988). This study demonstrated that the relaxant effect ACh (10^{-6}M) was antagonized by atropine (10^{-8}M), indicating relaxant responses to ACh mediated *via* muscarinic receptors in pulmonary artery rings. It has also been demonstrated that nitro-vasodilators such as sodium nitroprusside (SNP) induce endothelium-independent relaxation

through formation of cGMP and activation of soluble guanylate cyclase (Rapoport & Murad, 1983). Nitro-vasodilators probably lead to the formation of nitric oxide (NO) which activates guanylate cyclase (Katsuki *et al.*, 1977). In present experiments, concentration-response curves to SNP were obtained in MPA rings and its first branches (BPA), which were precontracted either with PHE (10^{-7}M) or with 5-HT (10^{-5}M). MPA rings were more responsive than BPA rings especially those which had been precontracted with PHE. Pretreatment of the preparations with L-NAME (5×10^{-4}M) or mechanical removal of the endothelium had no significant effect on SNP-induced relaxations, whether in artery rings (MPA and BPA) which were precontracted with PHE or with 5-HT. These results confirm that relaxant responses to SNP are endothelium-independent. In comparing relaxation response curves to ACh or SNP, the latter was able to induce greater relaxation than the former in these tissues.

Extracellular adenosine and adenine nucleotides, adenosine 5'-triphosphate (ATP) and adenosine 5'-diphosphate (ADP) play an important part in the regulation of many biological processes including the control of vascular tone and haemostasis by interaction with specific receptors on the cell surface. The cellular responses to ATP and ADP are mediated by P_2 purinoceptors as opposed to P_1 purinoceptors which interact preferentially with adenosine (Burnstock, 1978; Burnstock & Kennedy, 1985; Gordon, 1986; Olsson & Pearson, 1990). ATP and ADP can be liberated into the extracellular space as a consequence of vessel wall damage and local platelet aggregation (Gordon, 1986), after which they are rapidly sequentially dephosphorylated to adenosine by ectonucleotidases of endothelium and circulating blood cells (Coade & Pearson, 1989). The endothelium-independent effect of ATP can be either vasodilation or vasoconstriction, depending on the subclass of the P_2 purinoceptor in question (Kennedy *et al.*, 1985; Houston *et al.*, 1987). The endothelium-dependent effect of ATP is vasodilation and this is due to its

action at P_{2Y} purinoceptors, releasing the endothelium-derived relaxing factor (EDRF-NO) and/or prostacyclin (PGI_2) (De Mey & Vanhoutte, 1981; Gordon & Martin, 1983; Needham *et al.*, 1987; Boeynaems & Pearson, 1990). The responses of vascular endothelial cells to adenine nucleotides are generally attributed to the stimulation of P_{2Y} receptors. However, the possibility of a heterogeneous population of ATP-sensitive receptors on vascular endothelial cells has recently been reported and two types of ATP receptors (P_{2Y} and P_{2U}) might coexist on endothelial cells (O'Connor *et al.*, 1991; Motte *et al.*, 1993). The role of EDRF in the relaxations to ATP and ADP was demonstrated in canine arteries (De Mey & Vanhoutte, 1981), and in the rat aorta (Olsson & Pearson, 1990; White *et al.*, 1985). It has also been demonstrated that ATP- or ADP-induced relaxations are prevented by inhibitors of nitric oxide synthase (Dominiczack *et al.*, 1991; Koga *et al.*, 1992). It is suggested that vasodilator action of adenosine (AD) is mediated *via* the A_2 subclass of P_1 purinoceptors, leading to stimulation of adenylate cyclase (AC) in smooth muscle (Collis & Brown, 1983; Ramagopal *et al.*, 1988). The location of adenosine receptors has not been well-defined and both endothelium-dependent (Gordon & Martin, 1983; Rubanyi & Vanhoutte, 1985b) and endothelium-independent (Kennedy *et al.*, 1985; White & Angus, 1987) responses have been described. A variety of evidence indicates a key role for ATP-sensitive potassium channels in certain of the cardiovascular effects induced by activation of adenosine A_1 or A_2 receptors. It has been suggested that the xanthines, such as theophylline and caffeine, are P_1 receptor antagonists (Olsson & Pearson, 1990). The present study demonstrated that MPA and BPA rings, which had been precontracted with the α_1-adrenoceptor agonist phenylephrine (PHE) underwent endothelium-dependent relaxations when exposed to either ATP or ADP. Vasorelaxant responses both to ATP and to ADP were prevented by pretreatment artery rings with the nitric oxide synthase inhibitor, L-NAME or by mechanical removal of the endothelium by rubbing. Concentration-

dependent response curves to ATP and ADP were unaffected with pretreatment artery rings by a blocker of intracellular ATP-sensitive K^+ channels, glibenclamide or the PDE inhibitor, theophylline and/or the β-adrenoceptor blocker, propranolol. The results obtained indicated (i) ATP- or ADP-induced relaxations to be endothelium-dependent (ii) ATP-sensitive K^+ channels were not involved in relaxant responses to ATP or ADP in MPA and BPA rings, and it is possible that relaxant responses to ATP or ADP were mediated by P_2 purinoceptors. There were no significant differences between MPA and BPA rings in their responses to ATP, but, there were differences in response to ADP. MPA rings were more responsive than BPA rings to high concentrations of ADP (10^{-6} to 10^{-5}M).

Adenosine (AD) induced slowly-developing and sustained endothelium-independent relaxations in MPA and BPA rings. This effect of adenosine was antagonized by the P_1 receptor antagonist, theophylline, suggesting that adenosine produced this effect *via* P_1 receptors in these artery rings. Pretreatment of MPA and BPA rings with L-NAME or mechanical removal of the endothelium by rubbing did not affect relaxant responses to AD, indicating that relaxant responses to AD were endothelium-independent.

The effects of histamine (HIST) have been studied in different blood vessels and various species and two types of receptors for HIST (H_1 and H_2) are known to control vascular smooth muscle tone (Levie *et al.*, 1982). The contribution of each receptor type varies in different species. HIST decreases coronary blood flow in rat at higher concentrations (Bartlet, 1963), whereas this amine increases coronary blood flow in the dog and cat (Altura & Halevy, 1978). Harvey and Owen (1979) have shown that rat uterine vasculature is sensitive to HIST, which produced H_1- and H_2-mediated vasodilation when given intravenously to anaesthetized rats. Histamine is generally considered to

be a pulmonary vasoconstrictor, but it causes vasodilation when the pulmonary vascular tone is abnormally elevated (Tucker *et al.*, 1975). It has also shown that vasodilatation to HIST in some species may be due to the release of endothelium-derived relaxing factor (EDRF) (Furchgott & Vanhoutte, 1989). The purpose of this study was (i) to determine the effect of HIST on the isolated pulmonary artery (MPA and BPA rings) of the rat (ii) to investigate the role of the endothelium in HIST-induced responses and (iii) to investigate the specificity of HIST-receptors in MPA and BPA rings. The response of pulmonary vessels to HIST has rarely been studied in rats. In this study HIST induced concentration-dependent relaxations in MPA and BPA rings, which had been precontracted with PHE. BPA rings were apparently more responsive than MPA rings to HIST (10^{-5} to 10^{-4}M), but no significant differences were seen in the maximum responses. L-NAME reduced relaxant responses to HIST, indicating that HIST-induced relaxations involved the synthesis of nitric oxide (NO). The present study indicated that HIST-induced vasorelaxant responses in these rings were mediated through H_1-receptors. This hypothesis is further suggested by the fact that HIST concentration-response curves obtained in the presence of the H_1-receptor antagonist, chlorpheniramine, were shifted to the right of the control. This result is consistent with that obtained by Tucker and colleagues (1975), who have shown that the H_1 histamine receptors mediate pulmonary vasodilator responses in the dog. Also, high concentrations of HIST (10^{-4} to 10^{-3}M) produced small concentration dependent-contractile responses in resting MPA and BPA rings. This result is consistent with that obtained by Tucker and colleagues (1975), who have shown that the HIST is a pulmonary vasoconstrictor when the vascular tone is not elevated.

In some experiments, the effects of bradykinin (BK) and substance P (SP) were examined in MPA and BPA rings, which had been precontracted with PHE or 5-HT and/or the thromboxane A_2 mimetic, U46619. Neither BK nor SP had

any effect in MPA and BPA rings, which had been precontracted with one of these agonists.

Physiological studies indicate that β-adrenoceptors induce vasodilatation in pulmonary and systemic vascular beds (Hyman & Kadowitz, 1986). Vascular smooth muscle relaxation mediated *via* β-adrenoceptors involves activation of adenylate cyclase and increases synthesis of cAMP (Scheid *et al.*, 1979). However, recently, conflicting reports have suggested that the endothelium may be involved in β-adrenoceptor-mediated vasorelaxation and have implicated the L-Arg/NO pathway in this response. The results obtained *in vitro*, in canine coronary artery have indicated that isoprenaline-induced relaxation was either endothelium-independent (MacDonald *et al.*, 1987; O'Rourke & Vanhoutte, 1990) or endothelium-dependent (Rubanyi & Vanhoutte, 1985b) and in rat thoracic aorta was completely endothelium-dependent, suggesting that the rise in adenosine 3' : 5'-cyclic monophosphate (cAMP) could lead to the stimulation of NO synthase (Gray & Marshall, 1992). These results were totally at variance with those of Béa and colleagues (1994), who suggested that NO was not directly involved in β-adrenoceptor-mediated relaxation in large epicardial canine coronary arteries, but the presence of a functional endothelium may facilate this relaxation, probably through the basal release of NO and consequently an increase in the basal content of cGMP in vascular smooth muscle. Other investigators have demonstrated that the nitrovasodilator, nitroprusside, and the β-adrenoceptor agonist, isoprenaline, interact synergistically, either to relax or to inhibit the contraction of rat aortic smooth muscle (Maurice *et al.*, 1991). Moreover, nitroprusside markedly increased the accumulation of cAMP caused by additional of isoproterenol to this tissue (Maurice & Haslam, 1990). Recently, evidence has suggested that effects of cAMP in vascular smooth muscle may be mediated, at least in part, by cGMP-dependent protein kinase (Jiang *et al.*,

1992; Lincoln & Cornwell, 1993). The present experiments demonstrated that the β-adrenoceptor agonist, isoprenaline (ISO) induced concentration-dependent relaxations both in endothelium-intact rings and in rings (MPA and BPA) without endothelium ,which had been precontracted with PHE. Removal of endothelium or pretreatment of artery rings with L-NAME reduced submaximal relaxant responses to ISO, particularly to low concentrations, but had no effect on maximum relaxant responses to ISO. However, when L-NAME was added during maximum relaxation to ISO, a partial reversal of the relaxation was observed and this effect of L-NAME was slightly inhibited by L-Arg (10^{-3}M). These results confirm the possibility, suggested by Béa and colleagues (1994) that NO is not required for β-adrenoceptor-mediated relaxation, but the presence of functional endothelium may facilate this relaxation, probably through the basal release of NO and consequently an increase in the basal content of cGMP in vascular smooth muscle. The β-adrenoceptor antagonist, propranolol (10^{-5}M) abolished relaxant responses to ISO (10^{-10} to 10^{-6}M), and reduced the maximum induced-relaxation by ISO (10^{-5}M), indicating that relaxant responses were mediated *via* β-adrenoceptors in these artery rings. MPA rings were more responsive than BPA rings in their response to this agonist, suggesting that β-adrenoceptor-mediated mechanisms may be more active in MPA rings than in BPA rings.

4.3 *Vascular effects of atropine and the role of the endothelium*

Large doses of atropine cause vasodilation of the blood vessels in the skin (Bowman & Rand, 1980). This effect is apparently unconnected with the antimuscarinic activity of atropine and seems to be due to a direct action on the blood vessel. This direct vasodilator action of atropine gives rise to the characteristic "flush" which is caused by dilation of superficial blood vessels of

the skin in the neck and face and is a sign of atropine toxicity (Bowman & Rand, 1980). This study investigated the effects of atropine in MPA and BPA rings with endothelium-intact or with denuded endothelium and in the presence of NO synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 5×10^{-4}M, for 15-20min) when responses to acetylcholine (ACh, 10^{-3}M) were abolished. L-NAME had no effect on AT-induced relaxation. Paradoxically, when L-NAME (5×10^{-4}M) was added during maximum relaxation to atropine (5×10^{-6}M), the relaxant response to AT was completely reversed in endothelium-intact but not in rings with denuded endothelium, and this effect of L-NAME was inhibited by L-arginine (L-Arg, 2×10^{-4}M). The vasorelaxant effect of AT was neither blocked by propranolol (PROP, 10^{-6}M) nor enhanced by isobutylmethylxanthine (IBMX, 10^{-7}M). These results indicate that the ability of atropine to relax pulmonary artery rings (MPA and BPA) may be dependent upon the mechanism of action of the precontracting agonist and suggest that the vasorelaxant effect of atropine is not wholly mediated by the release of nitric oxide (NO).

4.4 *Vascular effects of electrical field stimulation (EFS)*

Extrinsic physiological control of vascular smooth muscle cells is primarily achieved by sympathetic nerve-released noradrenaline (NA). Little is known about the role of the endothelium on the neural control of vascular smooth muscle. The endothelium has been shown to metabolise norepinephrine released from adrenergic nerves in dog pulmonary artery by transmural electrical stimulation (Rorie & Tyce, 1985). The vascular endothelium has an inhibitory effect on adrenergic nerve stimulation in the rabbit carotid artery (Tesfamariam *et al.*, 1987) and rat caudal artery (Hynes *et al.*, 1988). This inhibitory effect of the endothelium on contractile response to adrenergic nerve stimulation is believed to be due to the release of relaxing factors from

endothelial cells (Tesfamariam *et al.*, 1987; Cohen & Weisbrod, 1988). Recently, Liu and colleagues (1991) implicated NO as the factor responsible for the inhibition adrenergic neurogenic vasoconstriction to modulate adrenergic neural responses in guinea-pig pulmonary artery *via* a postjunctional mechanism and basal release of NO from endothelial cells. The present study demonstrated that electrical field stimulation (EFS) induced frequency-dependent contraction responses (FCR) in MPA rings. These FCRs were significantly reduced when stimulation was repeated, suggesting the possibility of exhaustion of NA stores, since there were no significant differences on contractile responses to NA (10^{-7}M) before or after stimulation. Tetrodotoxin (10^{-7}M) abolished the contractile responses to EFS, indicating that the FCRs were neuronally-mediated. Prazosin also inhibited contractile responses to EFS, suggesting that these responses were due to the activation α_1-adrenoceptors by neural-released noradrenaline (data not shown). FCRs were enhanced by L-NAME (10^{-4}M), but this was only significant with maximum responses to EFS (16 Hz), suggesting that the endothelium can modulate neurogenic responses by releasing endothelial-released relaxing factors, especially, endothelium-derived relaxing factor (EDRF/NO). Treatment with cocaine (10^{-6}M), which is an inhibitor of neuronal NA uptake (Iverson, 1967), did not significantly increase contractile responses to EFS at all frequencies, but contractile responses were prolonged in comparison with time controls, suggesting that cocaine inhibited NA uptake.

4.5 *Vascular effects of cigarette smoke extract (CSE)*

The purpose of the present study was to investigate the effect of CSE on the endothelium of pulmonary artery rings. The most important findings of this study were that the CSE (i) depressed contractile responses to PHE and (ii) inhibited the endothelium-dependent-relaxant responses in MPA and BPA

rings but not endothelium-independent relaxant responses. The results suggest that the endothelium may be affected by CSE in MPA and of BPA rings and this is consistent with evidence from other studies, which have demonstrated that components of CS reach the endothelium of pulmonary blood vessels *in vivo*. It has been demonstrated that the components of CS are rapidly absorbed into the bloodstream (Koch *et al.*, 1980). The vasoactive compound, carbon monoxide (CO) reaches a peak concentration in systemic venous blood within a minute of cessation of smoking (Woodman *et al.*, 1986; Zacny *et al.*, 1987), and a similar rapid peak of nicotine concentration has also been demonstrated in the venous blood (Feyerabend *et al.*, 1985). Zacny and colleagues (1987) demonstrated that the constituents of CS are actually present in recirculating blood for several hours after smoking. It has also been demonstrated that in smokers there is an increase in the uptake of small molecular weight compounds (Jones *et al.*, 1980). NO can also be found in high concentration in cigarette smoke (Norman & Keith, 1965). The present study demonstrated that incubation of resting rings with CS caused a slowly-developing contraction in some artery rings tested. This may be due to the effect of CO in CSE. It may also be due to inhibition of basally-release NO. It has been shown that the content of CO is high in cigarette smoke (First, 1984) and also inhalation of CO causes a vasoconstrictor response in the pulmonary circulation of the pig, but the mechanism of action of this remains unclear. Paradoxically, CS-induced bronchial vasodilator effects have been attributed to a combination of CO and NO (Alving *et al.*, 1993). It has been suggested, that this effect may cause direct activation of soluble guanylate cyclase (Marks *et al.*, 1991). It has been shown that cigarette smoke relaxes the pulmonary circulation of the pig isolated lung (Gilman *et al.*, 1981). The present study demonstrated that CSE (1-2ml) induced relaxations in MPA and BPA rings, which had been precontracted with PHE. These relaxations were unaffected by L-NAME (10^{-4} to 5×10^{-4}M) whether the L-NAME was administered before or after

exposure to CSE. It does not seem likely that CSE-induced relaxations were caused by NO synthesis, through stimulation of specific endothelial cell receptors, since L-NAME failed to reverse this CSE-induced relaxant effect. Nor was it due to NO_2, since, the absence of NO_2 in fresh cigarette smoke has been demonstrated (Norman & Keith, 1965; Alving *et al.*, 1993). The relaxant effects of CSE seem in this study is consistent to some extent with the results obtained by Holden and colleagues (1990), who found the CSE produced biphasic responses in isolated porcine intrapulmonary arteries, in which CSE produced relaxant responses at low concentrations (0.001-0.01%) and contractile responses at high concentrations (0.1-1%)., It has also been shown that the vasodilatory effects of NO were similar to those of filtered smoke in the pulmonary circulation (Alving *et al.*, 1992). These workers concluded that the NO content of the gas phase of CS was likely to account for the dilatation of the pulmonary circulation. However, they observed that whole CS did not consistently relax the porcine pulmonary circulation, therefore, the particulate phase, including nicotine, must counteract the vasodilatory effect caused by NO, possibly by reflex mechanisms. This latter view was supported by the fact that vasodilator responses in the pulmonary circulation to whole CS were seen in the isolated porcine lung, where reflexes would be absent (Gilman *et al.*, 1981). It has also been shown that both CS and NO increase the levels of guanosine 3' :5'-cyclic monophosphate in the lung tissue (Arnold *et al.*, 1977). This study demonstrated that the high concentrations of CSE produced by administration of 2ml of extract depressed concentration-dependent contractile responses to PHE. However since, nicotine, a major component of CS, had no such effect, and since aliquots of an aqueous extract of unburned tobacco had no such effect it appears that this depression is not due to natural compound(s) in the cigarette, but may instead be due to some of the compound(s) produced on combustion. It is possible that the NO content of the CSE acts to increase cGMP levels and thus causes depression of the PHE-induced contractile

effects. CSE reduced relaxant responses to ACh but not to SNP. These results suggest that the CSE might impair the vascular endothelium, which confirms the hypothesis that smoking might impair endothelium-derived vascular relaxation because of its toxic effect on endothelial cells in human (Jacobs *et al.*, 1993) and also consistent with the observation that CSE impaired the dilatation of hamster cheek pouch arterioles to ACh, without impairing the response to nitroglycerin (NG) (Rubinstein *et al.*, 1991). These workers also reported that indomethacin prevented the CSE-induced impairment of vasodilatation in response to ACh but did not effect the dilatory response to NG, suggesting that the mechanism of impaired dilatation after CSE exposure may be related to the release of cyclooxygenase products. Also, Bakhle and colleagues (1979) demonstrated that CSE increases the perfusate concentration of PGs in isolated lung of rat, but the vascular effects of CSE were not mediated by PGs. Also, it has been reported that although CSE impairs the production of dilator products produced *via* the cyclooxygenase pathway, it does not appear that this is the primary mechanism by which CSE impairs ACh-induced vasodilation (Rubinstein *et al.*, 1991). They have proposed that the probable mechanism of impaired dilatation is related to the release of a constrictor substance(s) produced *via* the cyclooxygenase pathway. Also, it has been reported that CSE inhibits the synthesis of prostacyclin (PGI_2) in human, rabbit and rat vascular tissue (Jeremy *et al.*, 1985). However, the nature of the constrictor substance(s) is not yet known. Also, it has been demonstrated that both the vasodilator and vasoconstrictor effects of CSE were inhibited by the removal of the endothelium, suggesting that the endothelium may be involved in the vascular response of intrapulmonary arteries during CS exposure (Holden *et al.*, 1990).

In conclusion, the results obtained in this study indicated that CSE impairs endothelium-dependent vasorelaxant responses of the pulmonary arteries. Furthermore, such CSE-induced effects cannot be attributed to nicotine, which

is a major component of CS, nor are they due to a natural product in the cigarette, but rather are the result of combustion.

4.6 *Vascular effects of chronic heart failure due to experimental coronary artery ligation*

Detailed studies on the responsiveness of vessels from chronic heart failure (CHF) patients are notoriously difficult to perform due to the practical and ethical difficulties involved in obtaining suitable tissues from disease and control subjects. Most of the current knowledge comes from animal models. The results obtained in this study were: 5-Hydroxytryptamine induced concentration-dependent contractions in MPA and BPA rings of the eight week either sham operated or heart failure (HF) rabbits. No significant differences were seen between concentration response curves obtained in rings from sham operated or HF rabbits to 5-HT. BPA rings were more responsive than MPA rings to 5-HT in both sham and HF rabbits. Noradrenaline (NA) also induced concentration-dependent contractions in artery rings from sham and from HF rabbits. There were no significant differences between contractile concentration response curves to NA obtained in rings from sham operated and HF rabbits. BPA rings were more responsive than MPA rings from both sham operated and HF rabbits. These results indicated that contractile responses were not different in artery rings from sham operated and HF rabbits.

Acetylcholine (ACh) induced concentration-dependent relaxations in both sham operated rabbits and in HF rabbits. There were significant differences between the responses in rings from sham operated rabbits and in rings from HF rabbits but only at the lowest concentration of ACh (10^{-9}M). Whether or not the smaller inhibiting responses to ACh (10^{-9}M) seem in rings from HF rabbits was biologically significant and indicated that in these animals, the endothelium-dependent vasorelaxation mechanism is impaired, remains to be clarified-perhaps using different models of HF.

In conclusion, this study has shown that in isolated segments of pulmonary artery, there is a basal release of nitric oxide (NO), which may be responsible for the low tone in the pulmonary vasculature. In addition, the basal release of NO is responsible for attenuating contractile responses to vasoconstrictors, such as phenylephrine (PHE), noradrenaline (NA) and angiotensin II (Ang II). This was suggested by the ability of the NO synthase inhibitor, L-NAME, to enhance contractile responses to these agonists. Another major finding was that the smaller, branch pulmonary artery was not only more responsive than the larger, main pulmonary artery to vasoconstrictors such as PHE, NA and Ang II and also more responsive to the endothelium-dependent vasodilators, acetylcholine (ACh), carbachol (CARB), and histamine (HIST) but less responsive to endothelium-independent vasodilators such as sodium nitroprusside (SNP) and isoprenaline (ISO). In addition, this study demonstrated that tachyphylaxis to the contractile responses of the pulmonary artery to 5-hydroxytryptamine (5-HT) occurred and this was partly due to the release by 5-HT of NO from the endothelium. The effects of an aqueous extract of cigarette smoke were also investigated in isolated rings of pulmonary artery. These experiments showed the cigarette smoke extract (CSE) inhibited vasodilator responses to ACh but not to SNP and did not potentiate contractile responses to vasoconstrictors and even inhibited these responses. This is an important observation, since it suggestes that a component of cigarette smoke can inhibit the agonist stimulated release of NO from the endothelium but does not affect either the basal release of NO or the ability of the vascular smooth muscle to relax to NO derived from SNP.

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