A STUDY OF SMOOTH MUSCLE RELAXATION IN RAT VASCULATURE

A thesis presented for the degree of Doctor of Philosophy in the University of Glasgow

by

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Abbreviations

Commonly used abbreviations used throughout this thesis are listed below:

ADP	-	adenosine 5' diphosphate
ATP	-	adenosine 5' triphosphate
cyclic AMP	-	cyclic adenosine 3'5' monophosphate
cyclic GMP	-	cyclic guanosine 3'5' monophosphate
$[Ca^{2+}]_i$	-	cytosolic free calcium concentration
DAG	-	1,2-diacylglycerol
DMSO	-	dimethylsulphoxide
G protein	-	guanine nucleotide-binding regulatory protein
GTP	-	guanosine 5' triphosphate
$Ins(1,4,5)P_3$	-	inositol 1,4,5-trisphosphate
KCl	-	potassium chloride
M_r	-	relative molecular mass
P_i	-	orthophosphate
Ptd OH	-	phosphatidic acid
Ptd Ins	-	phosphatidylinositol
PIP	-	phosphatidylinositol 4-phosphate
PIP_2	-	phosphatidylinositol 4,5-bisphosphate
4α-PDD	-	4 alpha phorbol 12, 13-didecanoate
PMA	-	phorbol 12-myristate 13-acetate
TCA	-	trichloroacetic acid

SUMMARY

This study examined vascular smooth muscle relaxation in rat isolated aortic rings and related mechanical responses of tissues to the causative intracellular events.
The main areas of research investigated were:
(i) vascular relaxation induced by the bovine inhibitory factor;
(ii) the effects of the contractile agents used to raise muscle tone on the degree and nature of vascular relaxation; and
(iii) vascular relaxation in aortic rings from spontaneously hypertensive rats (SHR).

The principal results obtained in this study are summarised below:
1) The bovine inhibitory factor induced weak endothelium-independent relaxations of rat aortic rings precontracted with noradrenaline (1.6×10^{-8}M).

2) The inhibitory factor (100µl) completely inhibited muscle tone raised with noradrenaline (1.6×10^{-8}M) in rabbit aortic strips.
The relaxant efficacy of the inhibitory factor may therefore be species-dependent.

3) Acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) induced approximately the same degree of relaxation of aortic rings precontracted with noradrenaline (1.6×10^{-8}M).

4) Sodium nitroprusside (10^{-6}M) induced levels of cyclic GMP that were 10-fold higher than those induced by acetylcholine (10^{-5}M).

5) The inhibitory factor (200µl) did not significantly increase levels of cyclic GMP associated with weak vascular relaxation.

6) Acetylcholine and sodium nitroprusside, at concentrations that induced the same degree of relaxation as the inhibitory factor (200µl), did not significantly increase levels of cyclic GMP.

7) Sodium nitroprusside induced almost complete inhibition of muscle tone raised with noradrenaline (1.6×10^{-8}M) without increasing levels of cyclic GMP.
These results suggest that the absolute levels of cyclic GMP measured during vascular relaxation may be of less importance than some other intracellular parameter.

8) Acetylcholine and sodium nitroprusside induced weaker relaxations of aortic rings precontracted with KCl (20mM) than of tissues precontracted with noradrenaline (1.6×10^{-8}M).

9) Acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) induced smaller increases in the levels of cyclic GMP in tissues precontracted with KCl (20mM) than in tissues precontracted with noradrenaline (1.6×10^{-8}M).

10) Acetylcholine and sodium nitroprusside induced weaker relaxations of aortic rings precontracted with phorbol 12-myristate 13-acetate (PMA) (5×10^{-7}M) than of aortic rings precontracted with noradrenaline (2×10^{-7}M).

11) The levels of cyclic GMP induced by acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) in tissues precontracted with PMA (5×10^{-7}M) were significantly smaller than in tissues precontracted with noradrenaline (2×10^{-7}M).
These results contradict findings 3)-7) and show that the absolute levels of cyclic GMP measured during vascular relaxation are related to the degree of smooth muscle relaxation.

12) Noradrenaline induced concentration-dependent increases in the rate of hydrolysis of Ptd Ins, monitored by measuring the levels of Ptd OH.

13) KCl (30mM) and PMA (5×10^{-7}M) did not significantly increase the rate of Ptd Ins hydrolysis.

14) Acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) respectively, significantly decreased the rate of Ptd Ins hydrolysis induced by noradrenaline (2×10^{-7}M).

15) Acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) did not significantly alter the rate of Ptd Ins hydrolysis measured in response to KCl (30mM) or PMA (5×10^{-7}M).
These results suggest that hydrolysis of Ptd Ins may first

be necessary before potent vasorelaxations of aortic rings associated with increased levels of cyclic GMP can be demonstrated.

16) Throughout this study, sodium nitroprusside induced significantly greater relaxations than acetylcholine of precontracted aortic rings. This phenomenon was particularly marked in aortic rings precontracted with PMA (5×10^{-7}M).

17) Nitric oxide, which is the terminal mediator of vascular relaxations induced by both acetylcholine and sodium nitroprusside, induced powerful, concentration-dependent, endothelium-independent and transient relaxations of aortic rings precontracted with noradrenaline (2×10^{-7}M).

18) Nitric oxide-induced relaxations of aortic rings precontracted with PMA (5×10^{-7}M) were diminished to the same extent as those induced by acetylcholine.

19) The level of cyclic GMP induced by nitric oxide (3×10^{-6}M) was significantly smaller in tissues precontracted with PMA (5×10^{-7}M) than in tissues precontracted with noradrenaline (2×10^{-7}M).
These results suggest that PMA, probably via protein kinase C, may have inhibited the relaxant mechanisms activated by acetylcholine and nitric oxide in vascular smooth muscle cells. Therefore, the greater relaxant effect of sodium nitroprusside in tissues precontracted with PMA may be attributed to an additional mechanism of action.

20) Noradrenaline and KCl induced concentration-dependent contractions of aortic rings from SHR that were significantly smaller than those induced in aortic rings from normotensive WKY rats. This is consistent with the higher resting tension recorded in SHR aortic rings than in WKY aortic rings and suggests that the aortic rings were not capable of developing further substantial tension.

21) The basal rate of Ptd Ins hydrolysis measured in SHR aortic rings was significantly higher than that measured in WKY aortic rings. Noradrenaline (2×10^{-7}M) did not further increase the rate of Ptd Ins hydrolysis.

22) At high concentrations (10^{-6}M), sodium nitroprusside induced significantly greater relaxations of SHR aortic rings than of WKY aortic rings. This effect was reflected in significantly higher levels of cyclic GMP.

INTRODUCTION

Individual cells are the basic units of both the structure and the function of living organisms. In the course of evolution, cells have developed characteristics which arise during cellular differentiation, and have resulted in the adaptation of certain cells for particular roles. Four major categories of differentiated cell types are recognised, based on the type of function they perform or on their embryonic origins: (i) nerve cells; (ii) muscle cells; (iii) epithelial cells; and (iv) connective tissue cells. Association of differentiated cells of similar type results in the formation of multicellular tissues which correspond to the four categories of differentiated cell types. All other tissues and organs are composed of these main tissue types.

Muscle tissue is specialised to contract and this property may be expressed as movement or force. Movement is a characteristic of all living cells and is the result of forces generated by the conversion of chemical energy into mechanical energy. This cellular force-generating apparatus was present at an early stage in biological evolution, and the specialised nature of muscle cells is merely an extension and modification of this basic mechanism common to all cells. The function of muscle is as a source of movement, either for locomotion, by its actions on the bones of the skeleton, or for internal motility, by its control over the diameter of blood vessels and hollow organs. Three types of muscle tissues can be distinguished on the basis of structure and contractile properties: (i) skeletal or striated muscle attached principally to the bones of the

skeleton; (ii) cardiac muscle which is present exclusively in the walls of the heart; and (iii) visceral or smooth muscle which is found predominantly in the viscera or internal organs. Smooth muscle may also be present in the walls of blood vessels, with the exception of capillaries, and in the spleen.

Vascular smooth muscle

The basic structure of an artery is shown in figure 1. All blood vessels comprise this fundamental arrangement, a particular blood vessel being characterised by the proportion of each constituent. The morphology and physiology of vascular smooth muscle have been extensively researched and comprehensively reviewed by, among others, Furchgott (1955), Bohr (1964), Somlyo and Somlyo (1968), and Gabella (1981). The role of vascular smooth muscle in the regulation of blood pressure and flow has been recognised for many years. The existence of the blood circulation was first reported by William Harvey in 1621 (Harvey, 1621; cited by Bowman and Rand, 1980), and soon after, its function in the supply of oxygen and nutrients to the tissues, and the removal of carbon dioxide and metabolites to the organs of excretion, was described (Bowman and Rand, 1980). The maintenance of the blood circulation, and hence optimal conditions for cellular function, is dependent on both the rhythmic beating of the heart and the resistance to blood flow exerted by the blood vessels. These two factors together, regulate the arterial blood pressure. The total peripheral resistance to the flow of blood is dependent on the level of vascular tone exerted by the resistance vessels. Vascular smooth muscle is specialised to maintain

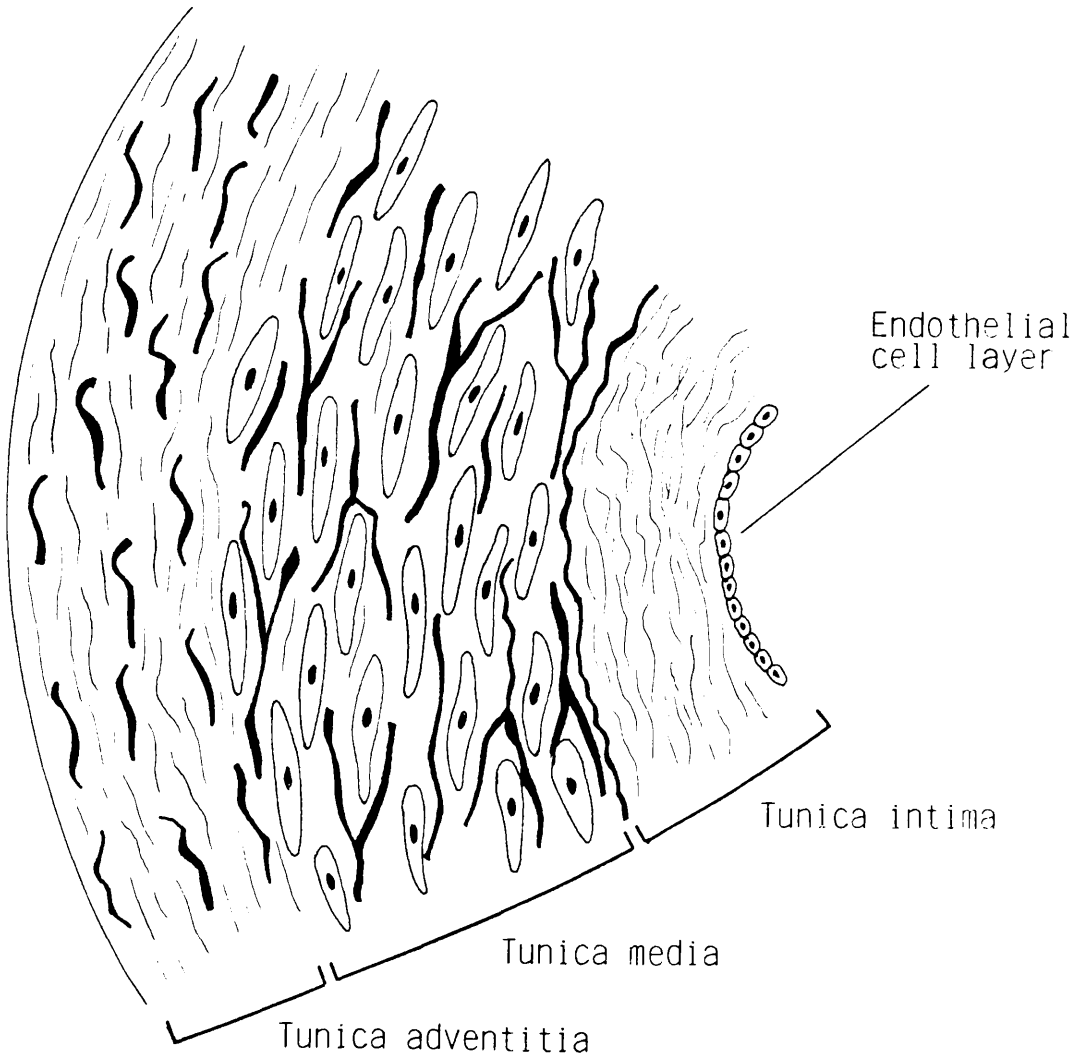


Figure 1.
Diagramatic representation of the constituent layers of a typical artery. The aorta contains concentrically arranged smooth muscle cells and bundles of elastin fibres in the tunica media, and an extensive elastic lamina between the media and the adventitia.

tone, and in contrast with skeletal muscle, it is well suited for maintaining tension for long periods of time with only minimal energy expenditure. Vascular tone may be defined as the degree of muscle contraction maintained by the blood vessel (Morgan, 1987) and is influenced by a multiplicity of factors (Vanhoutte, 1978). Depending on the nature of these stimuli, vascular tone may either be extrinsic or intrinsic in nature. Extrinsic tone is caused by external factors such as neurotransmitters, vasoactive substances and pharmacological agents. Intrinsic tone however, is produced by factors intrinsic to the cell itself eg. structure of vessel wall, cell permeability, and can be observed in isolated smooth muscle preparations even in the presence of neurotransmitter antagonists (Morgan, 1987). The ultimate determinant of vascular smooth muscle tone however, is the rate or magnitude of calcium ion (Ca^{2+}) transport across the smooth muscle cell membrane (Bohr, 1963). The requirement of Ca^{2+} for cell function has long been recognised (Ringer, 1883) and since contraction of vascular smooth muscle is secondary to a rise in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Bolton, 1979; Jones, 1981), the vascular effects of all vasoconstrictor and vasodilator stimuli may be mediated through alterations in the $[Ca^{2+}]_i$ available to the contractile proteins (Bohr, 1963). Indeed, in cardiovascular disorders such as hypertension and myocardial ischaemia, increased vascular tone is associated with an elevated $[Ca^{2+}]_i$ (Cohn, 1983). The common dependence on $[Ca^{2+}]_i$ for muscle contraction is qualitatively applicable to all vascular smooth muscle, although quantitative differences do exist which may account

for the heterogeneity of vascular reactivity. The individuality of the responsiveness and pharmacology of vascular smooth muscle varies with the species, that part of the circulatory system chosen for study, and in the isolated preparation, the experimental conditions used. In general, those factors that affect vascular tone may either be physical or chemical in nature. Those physical considerations include the structural and anatomical arrangement of the blood vessel wall, length-tension relationship of smooth muscle, temperature, and osmolarity of the bathing medium. Chemical factors are more numerous and can be neuronal in origin or contained in the circulating blood. The vascular endothelium plays an important role in the modulation of vascular smooth muscle reactivity and may exhibit properties from both categories.

Factors that affect vascular smooth muscle tone

Physical factors

The principal physical influences on vascular tone are the compositional arrangement of the vascular wall and the length-tension relationship of the vascular smooth muscle.

(i) Structure and anatomical arrangement of blood vessel wall

The structure and tissue composition of a blood vessel wall are related to its function. For example, the aorta and large arteries are thick-walled vessels containing a high proportion of elastin which allows these vessels to act as pressure reservoirs for maintaining blood flow distribution. In contrast, thin-walled arterioles which regulate the flow of blood from the arterial system into the capillaries, are principally composed of circularly-disposed and strongly-

developed smooth muscle fibres. The degree of vascular tone exerted by a blood vessel is dependent on the relative proportion of smooth muscle cells to connective tissue, with respect to the thickness of the vessel wall. This arrangement influences the access of neurotransmitters and vasoactive agents to receptor sites and to the mechanisms of deactivation (Vanhoutte, 1978). In 1949, Goldstein first proposed that the binding of drugs to non-receptor sites may effectively reduce the drug concentration in the vicinity of the receptor. Furthermore, Gillespie (1966), suggested the existence of "silent receptors" for noradrenaline, within the ground plexus of connective tissue. These receptors do not give rise to a tissue response but may reduce the response of the effector cell. In the rabbit ear artery and rat tail, preparations with a high content of collagen and elastic tissue, oxytetracycline has been shown to inhibit the binding of catecholamines to collagen and elastin, and potentiate the response to noradrenaline and adrenergic nerve stimulation (Powis, 1973). However a tissue with a low content of collagen and elastin, the rat anococcygeus, was less susceptible to the potentiating effects of oxytetracycline. Inhibition of the enzyme catechol-O-methyl transferase (COMT) has been demonstrated to cause marked supersensitivity to, and increased maximum relaxation induced by, isoprenaline in the dog saphenous vein (Guimarães et al., 1975). This effect was directly proportional to the mass of smooth muscle cells.

(ii) Length-tension relationship

In 1902, Bayliss first reported that increased stretch of vascular smooth muscle may lead to increased vascular tone.

Indeed, stretching of both arterial and venous smooth muscle results in an increase in muscle length which is accompanied by an increase in muscle tension. This passive relationship is due to the various constituent viscous elastic properties of the vascular wall, such as elastin, collagen, and smooth muscle (Altura and Altura, 1978) and has been demonstrated in numerous mammalian isolated blood vessels (Speden, 1960; Herlihy and Murphy, 1973; Peiper et al., 1973). An active length-tension relationship has also been demonstrated in the rabbit ear artery (Uchida et al., 1967), isolated arterioles and microvessels of the rat mesentery (Baez, 1969) and in the frog mesenteric microcirculation (Gore, 1972). More recently, the role of the endothelium in stretch-induced vascular tone has been studied. Endothelial cells form a continuous layer lining the entire vascular system and are subjected to wall shear stresses that develop when blood moves through the vessels (Lansman, 1988). It has therefore been proposed that the endothelium may act as a transducer of haemodynamic forces to regulate the local release of vasoactive substances involved in the control of blood pressure by changing vascular tone. Indeed, wall shear stress has been shown to affect the functions of endothelial cells (DeForrest and Hollis, 1978; Frangos et al., 1985). The mechanism of this process remains unclear. However, a K^+-selective, shear stress activated transmembrane current has recently been demonstrated in cultured endothelial cells, which may help explain the phenomenon (Olesen et al., 1988).

Chemical factors

A wide variety of chemical agents affect vascular tone, and

are principally neuronal in origin or vasoactive substances contained in the circulating blood or produced by the vascular endothelium. These external chemical stimuli induce extrinsic vascular tone which is superimposed on the basal or intrinsic tone that is generated by the physical properties of the smooth muscle and its environment. Modulation of vascular tone by chemical stimuli, other than those produced by the endothelial cells, is mediated by stimulation of receptors or "recognition sites" located on the cell membranes of endothelial or vascular smooth muscle cells.

(i) Innervation of vascular smooth muscle

The nerves that innervate blood vessels, the vasomotor nerves, belong to the sympathetic (thoracolumbar) division of the autonomic nervous system and were first described by Claude Bernard (Bernard, 1852; cited by Detweiler, 1978). The principal neurotransmitter released on electrical stimulation of these nerves is noradrenaline which induces vasoconstriction. The precapillary arterioles in skeletal muscle in many species, including man, receive a cholinergic sympathetic innervation. Electrical stimulation of these nerves releases acetylcholine which induces vasodilatation. However neurogenic alterations in vascular tone are predominantly produced by changes in the activity of sympathetic adrenergic nerves (Bevan and Su, 1973). Indeed most chemical, physical and pharmacological factors that control vascular tone also modulate activity of the neurotransmitter released at the neuroeffector junction (Rand et al., 1976; Vanhoutte, 1977; 1980). Moreover, functional changes in vascular smooth muscle associated with

hypertension, may be initiated by alterations in this neurogenic control of vascular reactivity (Winquist et al., 1982). Furthermore, the density of adrenergic innervation and degree of penetration of the nerve endings into the smooth muscle layers are important considerations. Nerves do not penetrate beyond the adventitia-media border in most arteries, whereas in veins they innervate the deep medial smooth muscle layers (Bevan and Su, 1973; Ljung et al., 1975). A differential reactivity to noradrenaline has been demonstrated in the carotid artery of the sheep, in which the adrenergic nerve fibres are restricted to the outer layers of smooth muscle (Keatinge and Torrie, 1976). This effect may be caused by differences in electrical characteristics between muscle cells that receive a different sympathetic innervation (Mekata and Keatinge, 1975). However, ultimately it is the concentration of noradrenaline at the receptor sites on vascular smooth muscle cell membranes that determines the influence of the sympathetic innervation on vascular tone. In adrenergically innervated blood vessels, endogenously released, or exogenously added noradrenaline may stimulate adrenoceptors located on the adrenergic nerve terminal (pre-junctional adrenoceptors), and adrenoceptors situated on the vascular smooth muscle cells (post-junctional adrenoceptors). Pre- and post-junctional alpha-adrenoceptors differ in their pharmacology and function. Pre-junctional alpha-adrenoceptors mediate the regulation of transmitter release from adrenergic nerve endings, whereas post-junctional alpha-adrenoceptors mediate contraction of vascular smooth muscle (Somlyo and Somlyo, 1970; Stjärne and Gripe, 1973;

Starke et al., 1975). Pre-junctional alpha-adrenoceptors have been categorised as $alpha_2$-adrenoceptors and the post-junctional alpha-adrenoceptors may be either $alpha_1$- or $alpha_2$-adrenoceptors (Langer, 1974; Starke et al., 1975; Wikberg, 1979). It has been suggested for the rat isolated aorta, that the post-junctional alpha-adrenoceptors mediating vasoconstriction may possess characteristics of both $alpha_1$- and $alpha_2$-adrenoceptors (Ruffolo et al., 1981; 1982). However, contractions of rat aortic strips induced by the selective $alpha_2$-adrenoceptor agonists B-HT 920 and UK 14,304 were shown to be mediated by $alpha_1$-adrenoceptors, suggesting that postsynaptic alpha-adrenoceptors should be classified as a homogeneous population of $alpha_1$-adrenoceptors (Beckeringh et al., 1984).

(ii) Vasoactive agents and the endothelium

The vascular endothelium is the layer of squamous epithelial cells that lines the entire circulatory system. The endothelium performs multiple functions which include regulation of plasma lipids, capillary transport and participation in haemostasis (Robertson Jr. and Rosen, 1978; Vanhoutte et al., 1986). Furthermore, and presently the subject of intense research, the endothelial layer modulates the reactivity of vascular smooth muscle. This regulatory function is accomplished through two main mechanisms (i) the endothelium imposes a physical barrier between those vasoactive agents circulating in the blood, and the smooth muscle cells and; (ii) the endothelial cells are actively involved in the extraction and metabolism of circulating vasoactive substances, as well as in the secretion of excitatory and inhibitory mediators of vascular tone in

response to vasoactive stimuli (Vanhoutte et al., 1986). The current interest in the role of endothelial cells in vascular smooth muscle responsiveness began in 1980 when Furchgott and Zawadzki first reported that acetylcholine could induce vasodilatation of rabbit isolated aortic rings (Furchgott and Zawadzki, 1980a). This effect was shown to be critically dependent on the presence of functional endothelial cells and has subsequently been demonstrated in numerous mammalian arteries (Chand and Altura, 1981; Lee, 1982; Cohen et al., 1983a), small diameter resistance vessels (Owen and Bevan, 1985) and venous preparations (De Mey and Vanhoutte, 1982). Vasodilatation is thought to be mediated via activation of endothelial muscarinic receptors (Eglen and Whiting, 1985; Rubanyi and Vanhoutte, 1985a), although the exact location of these receptors has proved controversial (Stephenson and Summers, 1987). Stimulation of muscarinic receptors results in the generation and/or release of a diffusable substance(s) which exerts an inhibitory action on smooth muscle cells (eg. Furchgott and Zawadzki, 1980a; van de Voorde and Leusen, 1983; Griffith et al., 1984). This unidentified vasodilator substance(s) was termed "the endothelium-derived relaxing factor(s)" or "EDRF" (Furchgott and Zawadzki, 1980a). Once it was established that vascular smooth muscle tone could be regulated by a potent endothelial substance(s), the search for its identity began. Initially much attention focussed on the metabolic products of arachidonic acid, with the observation that quinacrine, an inhibitor of phospholipase A_2, could prevent the release of the EDRF (Griffith et al., 1984) and also the endothelium-dependent vasorelaxations

observed in various blood vessels (eg. Cherry et al., 1982; De Mey et al., 1982; Singer and Peach, 1983). Furthermore, a possible role for vasodilator prostanoids was proposed (Furchgott and Zawadzki, 1980a,b). However, interpretation of results from these experiments was complicated by the non-specific actions of many of the metabolic inhibitors used and therefore proved inconclusive (reviewed by Furchgott, 1983). In 1987, Furchgott first proposed that the EDRF is nitric oxide (NO) (Furchgott, 1987). This has since been supported by other workers (Palmer et al., 1987). However, the possibility still remains that there exist other EDRF's which may be a product of arachidonic acid metabolism through the P450 pathway (Rubanyi and Vanhoutte, 1987). Also, another nitrogen derivative, ammonia, (NH_3), is presently an attractive candidate for the identity of another EDRF (Vanhoutte, 1987).

In the course of the search for the identity of the EDRF it became apparent that it shares many important properties with the putative transmitter of the non-adrenergic, non-cholinergic (NANC) innervation of the bovine retractor penis muscle (BRP) (reviewed by Gillespie, 1987). This so-called "inhibitory factor" was first described by Ambache et al, (1975), who reported the presence of a smooth muscle relaxant in acid extracts of the BRP. The inhibitory factor has since been shown to be a powerful relaxant of numerous isolated blood vessels (Bowman et al., 1981). Furthermore, EDRF-like activity released from bovine aortic endothelial cells is similar to the inhibitory factor in that they both require acid activation (Murray et al., 1986). This, and other similarities led Furchgott (1987) to draw comparisons

between the EDRF and the inhibitory factor and to suggest that the inactive form of the inhibitory factor is nitrite from which the active species, nitric oxide, is generated by acid. Studies on cultured bovine endothelial cells support this hypothesis although the active species is probably stabilised by some other unidentified constituent of the extract (Martin et al., 1988).

The mediators of haemostasis may modulate vascular reactivity eg. thrombin (De Mey et al., 1982; De Mey and Vanhoutte, 1982; Ku, 1982; Rapoport et al., 1984); aggregating platelets, if tone is first raised with prostaglandin $F_{2\alpha}$, (Cohen et al., 1983a,b; Houston et al., 1985; Houston and Vanhoutte, 1985); serotonin via stimulation of S_1-serotonergic receptors (Cocks and Angus, 1983; Cohen et al., 1983a,b; Houston et al., 1985); adenosine 5' diphosphate (ADP) and adenosine 5' triphosphate (ATP) (De Mey et al., 1982; De Mey and Vanhoutte, 1982; Gordon and Martin, 1983a; Houston et al., 1985); and platelet activating factor (PAF), probably via a non-specific action (Kamitani et al., 1984; Vanhoutte and Houston, 1985).

Various hormones and autocoids circulating in the blood can also regulate vascular reactivity eg. noradrenaline and selective alpha$_2$-adrenoceptor agonists (Cocks and Angus, 1983; Miller and Vanhoutte, 1984); and noradrenaline and isoprenaline via stimulation of beta-adrenoceptors (Rubanyi and Vanhoutte, 1985b). This latter effect however, may not only be attributed to activation of endothelial beta-adrenoceptors but also to the basal release of the EDRF. Therefore the presence of endothelial adrenoceptors may

account for the vasodilatory effects of catecholamines in certain vascular beds and indeed, removal of the endothelium produces vasoconstriction in response to catecholamines via stimulation of alpha-adrenoceptors located on the vascular smooth muscle cells. Thus, in vascular preparations denuded of endothelium, non-selective alpha-adrenoceptor agonists, and in particular noradrenaline and adrenaline, induce enhanced vasoconstriction (Allan et al., 1983; Fortes et al., 1983; Carrier and White, 1985; Godfraind et al., 1985). Endothelium-dependent vasorelaxations have now been demonstrated in blood vessels in response to several other substances present normally or pathologically in blood: angiotensin II (Toda, 1984); bradykinin (Altura and Chand, 1981; Chand and Altura, 1981; Cherry et al., 1982; Regoli et al., 1982; Gordon and Martin, 1983b); histamine via activation of H_1-histaminergic receptors (Rapoport and Murad, 1983a; van de Voorde and Leusen, 1983; Davies and Williams, 1984; Satoh and Inui, 1984); substance P (Angus et al., 1983); vasoactive intestinal peptide (Davies and Williams, 1984); vasopressin and the related peptide oxytocin, via stimulation of V_1 receptors (Katusic et al., 1984a,b); and calcitonin gene-related peptide (Brain et al., 1985).

In addition to mediating vasodilatation, endothelial cells have been implicated in the facilitation of contractile responses of vascular smooth muscle (Hickey et al., 1985). Indeed, recently an endothelium-derived 21-residue vasoconstrictor peptide, endothelin, has been isolated and characterised from cultured endothelial cells (Yanagisawa et al., 1988).

Stimulus response coupling

Regulation of vascular tone by neurotransmitters and vasoactive agents is ultimately effected by alterations in the $[Ca^{2+}]_i$. External chemical stimuli confer their information on vascular smooth muscle cells, in common with other cell types, by interacting with specific cell surface receptors resulting in the generation of a corresponding intracellular signal. It is this signal that mediates the cellular response. The plasma membrane however, forms a barrier to the inward flow of information, but the presence of membrane transduction mechanisms is thought to translate these external stimuli into internal chemical signals in a process called stimulus-response coupling (see figure 2) (Berridge, 1981). These signals are carried by "second messengers" which may either remain in the membrane or diffuse throughout the cell and combine with "acceptors" to modulate the activity of key biochemical reactions that regulate the cellular response. Following binding of its agonist, the cell surface receptor associates with specific transduction proteins; these guanine nucleotide-binding regulatory proteins, "G" proteins, become active upon binding of guanosine triphosphate (GTP) and stimulate an "amplifier" enzyme located on the inner face of the membrane that catalyses the conversion of precursor molecules into second messengers. Those second messengers currently identified as being generated by this mechanism and of importance in vascular smooth muscle reactivity are cyclic adenosine 3'5' monophosphate (cyclic AMP), the products of inositol phospholipid metabolism, 1,2-diacylglycerol (DAG) and inositol 1,4,5,-trisphosphate ($Ins(1,4,5)P_3$) and Ca^{2+}

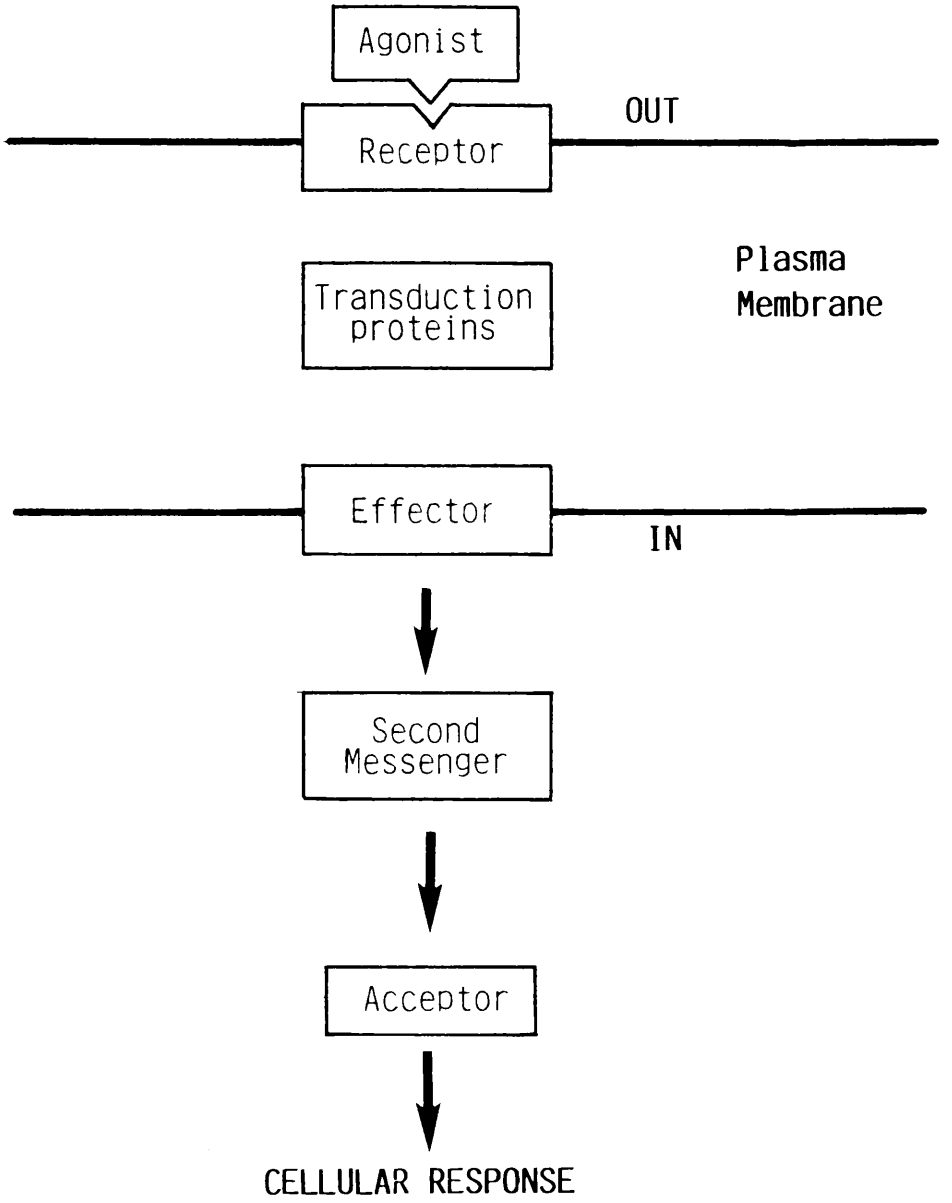


Figure 2.
Mechanism of stimulus-response coupling (see text for details).

(Berridge, 1985). The messenger role of Ca^{2+} is virtually universal in animal cells and is performed in concert with the other intracellular messengers.

Ca^{2+} and Ca^{2+} homeostasis

The Ca^{2+} messenger system operates via two distinct branches: (i) a calmodulin (CaM) branch that is activated by a transient rise in $[Ca^{2+}]_i$ and is responsible for the initial phase of the cellular response; and (ii) a protein kinase C branch that is activated by both the rise in $[Ca^{2+}]_i$ and DAG content of the cell membrane, and is responsible for the sustained phase of cell response.

The extracellular concentration of Ca^{2+} is normally held at approximately 1000μM, whereas in the unstimulated cell, the $[Ca^{2+}]_i$ is maintained between 0.1-0.2 μM (Rasmussen, 1983). The total cellular Ca^{2+} content however, is much higher, the majority of this Ca^{2+} being sequestered in intracellular organelles such as mitochondria, sarcoplasmic reticulum, golgi apparatus and nuclei. Ca^{2+} may also be bound to proteins, membranes or other cellular constituents (Irvine, 1986).

The plasma membrane is principally responsible for maintaining Ca^{2+} homeostasis. However, a role for intracellular membranes is becoming increasingly apparent (Rasmussen and Barrett, 1984).

Role of plasma membrane

The maintenance of cellular Ca^{2+} homeostasis is dependent on the plasma membrane. The low resting Ca^{2+} permeability of this membrane (Borle, 1981) and the presence of a high capacity, high affinity Ca^{2+} pump, a Ca^{2+}-Mg^{2+}-ATPase, which extrudes Ca^{2+} from the cell cytosol into the extracellular

space (Gimble et al., 1982; Niggli et al., 1982; Smallwood et al., 1983), maintain the large Ca^{2+} concentration gradient across the plasma membrane at low metabolic cost. Cell activation is partly mediated by the active, inward movement of Ca^{2+} across the plasma membrane, which permits the use of extracellular Ca^{2+} as an intracellular mediator of cell function (Rasmussen and Barrett, 1984). Influx of Ca^{2+} may occur via two main types of Ca^{2+} channel categorised by the nature of the excitatory stimulus required to open them (Bolton, 1979; Reuter, 1983): (i) the potential operated (voltage-sensitive) (POC) channel is sensitive to changes in the transmembrane potential, whereas (ii) activity of the receptor-operated channel (ROC) appears to be dependent on specific plasma membrane receptors. A third class of non-selective and stretch-sensitve ion channel, which is permeable to Ca^{2+}, has been reported in both skeletal (Guharay and Sachs, 1984) and endothelial cells (Lansman et al., 1987). Ca^{2+} may also enter the cell via the Na^{+} channel, and be actively extruded by a Na^{+}-Ca^{2+} exchange pathway which is driven by the electrochemical potential of the Na^{+} gradient across the cell membrane (Blaustein, 1974).

Role of intracellular membranes

Two other membranes are involved in the regulation of $[Ca^{2+}]_i$: the membrane of the sarcoplasmic reticulum and the inner mitochondrial membrane (Rasmussen and Barrett, 1984). Both of these membranes have pump-leak systems oriented such that there is active extrusion of Ca^{2+} from, and passive leak of Ca^{2+} back into the cytosol. The mitochondrial membrane serves two functions: at low $[Ca^{2+}]_i$ it allows the

stabilisation of $[Ca^{2+}]_i$ at a new higher level, whereas at high $[Ca^{2+}]_i$, mitochondria serve as a reservoir for the storage of Ca^{2+} during periods of excessive cellular Ca^{2+} accumulation. In common with the endoplasmic reticulum in other cell types, the sarcoplasmic reticulum in vascular smooth muscle cells, is a source of Ca^{2+} as an intracellular mediator of cell function during the initial phase of cell activation (Deth and Van Breemen, 1977; Cauvin et al., 1982).

Membrane transduction processes

Both the adenylate cyclase system for synthesis of cyclic AMP and the phospholipase C enzyme system for generation of DAG and $Ins(1,4,5)P_3$ are thought to share the same basic membrane transduction apparatus: a specific plasma membrane receptor, a guanine nucleotide-dependent regulatory protein (G protein) and a catalytic component (Taylor and Merritt, 1986).

Both of these receptor coupling systems are closely associated with regulation of Ca^{2+} metabolism. The nature of both the receptors and the enzymic moiety, differs in the two systems. The similarity between these receptor coupling mechanisms is in the apparent mandatory role of a G protein.

G proteins

The effect of receptor activation on the adenylate cyclase signalling pathway is mediated by two distinct G proteins, G_s and G_i, which regulate the stimulation and inhibition of adenylate cyclase activity respectively (Helmreich and Pfeuffer, 1985). G_s and G_i are structurally similar heterotrimers comprising a larger alpha subunit (41-45,000 M_r), and two smaller subunits: beta (35,000 M_r) and gamma

(10,000 M_r) (Gilman, 1984). It appears that the beta and gamma subunits coexist as a beta-gamma complex and are similar in both G proteins. This suggests that the differences between G_s and G_i are primarily in their alpha subunits. Occupation of the stimulatory receptor, R_s by an agonist, induces a conformational change that is transmitted through the membrane to G_s which becomes more susceptible to binding of guanosine triphosphate (GTP). GTP binding occurs on the alpha subunit, displacing GDP, and constitutes the "on reaction" which results in the dissociation of G_s into its alpha and beta-gamma subunits. The high affinity of receptor R_s for agonists is generally reduced by GTP-G_s binding. The dissociated GTP-bound alpha subunit directly stimulates adenylate cyclase resulting in increased cAMP formation. The subunits remain dissociated for as long as GTP is bound to the alpha subunit. On hydrolysis of GTP by an intrinsic GTP-ase, the subunits reassociate and this constitutes the "off reaction". Inhibition of adenylate cyclase activity via activation of G_i induced by inhibitory agonists, is essentially analogous to that for stimulation of the enzyme. However, an additional mechanism has been proposed whereby liberated beta-gamma subunits of G_i attenuate the activation of G_s (Hildebrandt et al., 1984; Katada et al., 1984), but this mechanism may only operate under resting conditions (Birnbaumer, 1987).

Activation of the enzyme phospholipase C has also been shown to be mediated via a G protein (G_p) (Haslam and Davidson, 1984; Blackmore et al., 1985; Litosch and Fain, 1986). The identity of G_p however remains unknown (Cockcroft, 1987).

Catalytic component

Adenylate cyclase

Adenylate cyclase has been isolated from the myocardium of the rabbit, and identified as a glycoprotein (150,000 M_r) which traverses the plasma membrane (Pfeuffer et al., 1985). This enzyme catalyses the conversion of Mg.ATP into cyclic AMP.

Phospholipase C and inositol phospholipid metabolism

Inositol-containing lipids represent approximately 5% of the total phospholipid content of most mammalian cells (Marcus et al., 1969). Phosphatidylinositol (Ptd Ins) is the major inositol phospholipid (approximately 90% of total) and although the more phosphorylated derivatives phosphatidyl inositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) are present in much smaller quantities, they have a rapid metabolic turnover. These lipids are collectively termed the phosphoinositides. Stimulation of Ca^{2+}-mobilising receptors initially results in the activation of phospholipase C (phosphoinositide phosphodiesterase), which catalyses the hydrolysis of PIP_2 into DAG and Ins(1,4,5)P_3 (Michell et al., 1981). Enhanced rate of metabolism of the phosphoinositides is called the Ptd Ins ("PI") response and has been observed in numerous tissues in response to agonist stimulation (reviewed by Hokin, 1985). The phosphoinositide cycle is shown in figure 3. The cyclical metabolism of the phosphoinositides invoves (i) the sequential dephosphorylation of the inositol phosphates to form free myo-inositol and (ii) the sequential action of DAG kinase and CTP-Ptd OH citidyl transferase to form CDP-DG which combines with myo-inositol to form Ptd

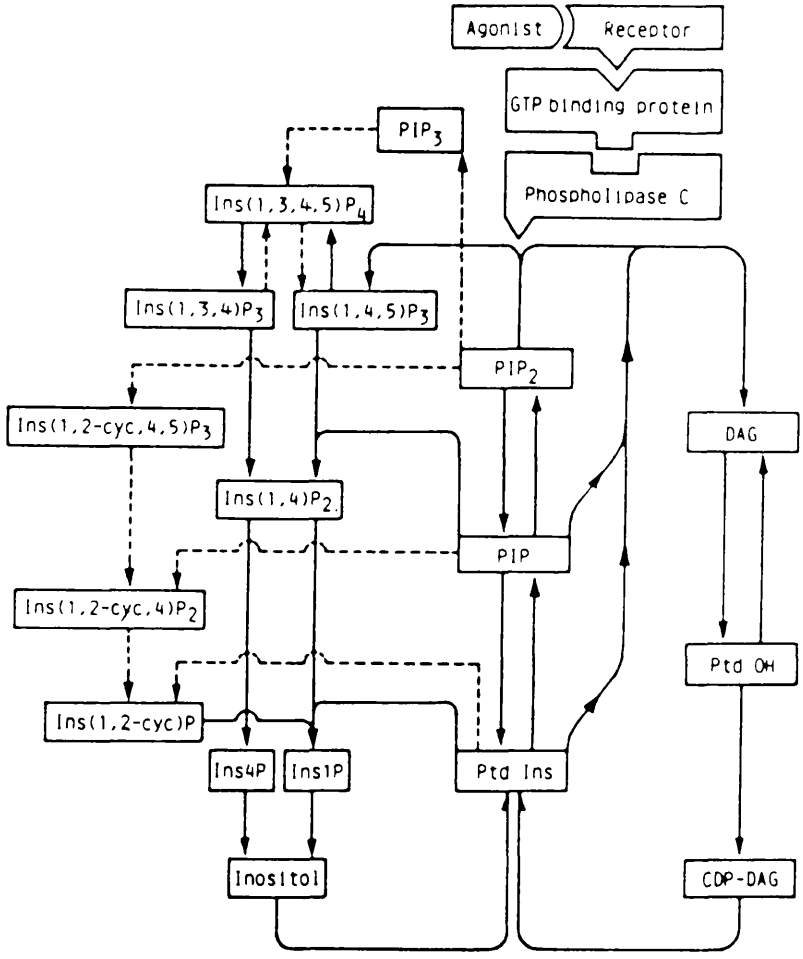


Figure 3.
Phosphoinositide cycle: pathways for the synthesis and degradation of phosphoinositides and generation of phosphoinositide messenger molecules.

Ins. The understanding of phosphoinositide metabolism has been further complicated by the discovery of cyclic, and highly phosphorylated derivatives of the inositol phosphates. These new-found metabolites are generated respectively from the phospholipase C catalysed hydrolysis of phosphoinositides (Wilson et al., 1985a), and the sequential phosphorylation of Ins(1,4,5)P_3 (Batty et al., 1985; Michell, 1986).

Cyclic GMP

Another cyclic nucleotide, cyclic 3'5' guanosine monophosphate (cyclic GMP), has emerged as one of the few fundamental second messengers. The synthesis of cyclic GMP is catalysed by the enzyme guanylate cyclase which is apparently not directly regulated by G proteins. However, in certain cell systems the activity of guanylate cyclase may be influenced by the stimulation of phosphoinositide hydrolysis (Berridge, 1985). Much of the work on guanylate cyclase structure, regulation and function has been carried out in tissues other than vascular smooth muscle, although there are strong similarities in the characteristics of guanylate cyclase between different tissue types (Hardman, 1984). Guanylate cyclase activity has been reported in nearly all cell types studied. There are two forms of the enzyme: (i) a soluble (cytosolic) form; and (ii) a particulate (membrane-associated) form. These isozymes can coexist in the same cell, and indeed from studies on the subcellular distribution of guanylate cyclase, it is clear that there are at least two forms of the enzyme in most cell types (Waldman and Murad, 1987). Particulate guanylate cyclase is distributed in most intracellular organelles in a

variety of tissues and phyla (eg. Kimura and Murad, 1975; Schultz and Klumpp, 1980), although compartmentalisation of the soluble enzyme remains undetermined. Activation of soluble guanylate cyclase is of major importance in the modulation of vascular reactivity, although a family of atrial peptides, atriopeptins, involved in the regulation of electrolyte balance and blood pressure, stimulate particulate guanylate cyclase exclusively, in vascular tissues (Waldman et al., 1984; Winquist et al., 1984). The soluble enzyme is probably a dimeric protein with two identical subunits, and a molecular weight of approximately 150,000 M_r (Braughler et al., 1979; Lewicki et al., 1980). However, there is disagreement on the composition of the enzyme (Garbers, 1979; Zwiller et al., 1981). Particulate guanylate cyclase is of greater molecular weight, 200-300,000 M_r (Waldman et al., 1983) but it is not known if it exists in the membrane as a mono- or dimeric entity. The catalytic conversion of Mg.GTP into cyclic GMP occurs via displacement of the nucleotide pyrophosphate group by the 3'-hydroxyl group on the ribose molecule (Senter et al., 1983).

Cyclic nucleotides are inactivated by phosphodiesterases. The action of the enzyme cyclic 3',5'-nucleotide phosphodiesterase, which catalyses the metabolism of cyclic AMP into 5'-AMP, was first described (Butcher and Sutherland, 1962), and it is now clear that there are multiple forms of phosphodiesterases exhibiting characteristic substrate affinities, kinetic behaviour, molecular size and sensitivities to activators (Strada and Thompson, 1978). Three general groups of cyclic nucleotide

phosphodiesterases are recognised and can be clearly separated by DEAE-cellulose chromatography (Hidaka et al., 1977): (i) cyclic GMP phosphodiesterase (FI) has a higher affinity for cyclic GMP than for cyclic AMP and hydrolyses principally cyclic GMP, even at low substrate levels; (ii) Ca^{2+}-phosphodiesterase (FII) preferentially hydrolyses cyclic GMP and requires Ca^{2+} and calmodulin (CaM) for its activity; and (iii) cyclic AMP phosphodiesterase (FIII) is a specific cyclic AMP phosphodiesterase.

Regulation of guanylate cyclase

Activation of soluble guanylate cyclase was first reported from studies on the kinetic properties of the soluble and particulate forms of the enzyme, in response to the GTP-ase inhibitor, sodium azide (Kimura et al., 1975). Since then, a diverse group of nitrogen-containing organic and inorganic compounds has been found to stimulate this enzyme (reviewed by Ignarro and Kadowitz, 1985). Under the appropriate conditions, all of these compounds are potential sources of nitric oxide, which has been implicated as the proximal activator of soluble guanylate cyclase, and which may represent a common pathway for regulation of enzyme activity by these agents (Murad et al., 1978; Mittal and Murad, 1977a). The generation of nitric oxide may occur enzymatically or non-enzymatically from these precursor compounds (Murad et al., 1978). These nitrogen-containing compounds can also relax vascular and non-vascular smooth muscle and are therefore called "nitrovasodilators" and include hydroxylamine, nitroglycerin, sodium nitroprusside and nitrosamines (Ignarro and Kadowitz, 1985). Furthermore, stimulation of soluble guanylate cyclase activity can occur

in response to endothelium-dependent vasodilators via the EDRF which has now been identified as nitric oxide (Palmer et al., 1987). Observations that oxidising agents inhibited, and reducing agents promoted the activation of guanylate cyclase by nitrovasodilators, suggested that activation of the enzyme probably involves several oxidation-reduction reactions (Kimura et al., 1975; Braughler et al., 1979). Oxidising agents and oxygen could prevent enzyme activation by converting nitric oxide to other less efficacious oxides of nitrogen eg. nitrogen dioxide, whereas reducing agents could promote activation by preventing this oxidation (Katsuki et al., 1977).

Both cyclic AMP and cyclic GMP can be formed by the action of soluble guanylate cyclase in response to nitrovasodilators. Indeed GTP has been shown to inhibit the formation of cyclic AMP, and conversely, ATP inhibit cyclic GMP formation (Mittal and Murad, 1977b). The physiological relevance of this alternative mechanism for cyclic AMP synthesis remains obscure.

The principal second messengers that mediate vascular smooth muscle responsiveness are: cyclic AMP, cyclic GMP, $Ins(1,4,5)P_3$, DAG, and Ca^{2+}. The EDRF and endothelin may also be regarded to perform a "messenger-like" role.

Vascular tone is the resultant of smooth muscle contraction and relaxation, and is determined by the $[Ca^{2+}]_i$. Muscle contraction is mediated by increased $[Ca^{2+}]_i$, whereas relaxation is associated with a lowering of $[Ca^{2+}]_i$, and complex interactions between second messengers, including Ca^{2+}, regulate this $[Ca^{2+}]_i$. In studying the basic intracellular events that lead to vascular relaxation, it is

important to also consider the underlying coupling mechanisms that mediate smooth muscle contraction, because vasorelaxation is usually examined in precontracted tissues and the two processes are closely linked through the common functions of the second messenger system. Moreover, the degree of vascular relaxation may be dependent on the nature of the contractile agent used to raise muscle tone and the intracellular mechanism involved (Furchgott, 1983).

Contraction

Contraction of vascular smooth muscle is predominantly mediated via stimulation of Ca^{2+}-mobilising receptors. This results in an accelerated metabolism of phosphoinositides ("PI" response) and the rapid generation of DAG and Ins(1,4,5)P_3 (Berridge, 1981; Michell et al., 1981), which both satisfy the criteria for a second messenger function (Berridge, 1984). The Ptd Ins response initiates a bifurcating signal pathway, leading to muscle contraction via two main mechanisms: (i) the Ca^{2+}-CaM pathway; and (ii) activation of protein kinase C. Both of these pathways result in the phosphorylation of the contractile proteins.

DAG

DAG, formed transiently by the action of phospholipase C on the polyphosphoinositides, activates a lipid-dependent protein kinase, protein kinase C, causing the phosphorylation of specific target proteins (Takai et al., 1979; Nishizuka, 1983). Protein kinase C, which is distributed in most tissues, including vascular smooth muscle (Kuo et al., 1980), requires Ca^{2+} and phosphatidylserine as cofactors for activation by DAG. Furthermore, the Ca^{2+} requirement of protein kinase C is

markedly reduced in the presence of DAG, and is therefore active at resting $[Ca^{2+}]_i$. Protein kinase C has been implicated in the activation of smooth muscle contraction since it can phosphorylate a threonin residue of the regulatory light chains (Endo et al., 1982; Naka et al., 1983). Moreover, it has been postulated that activation of protein kinase C is solely responsible for sustained muscle contraction (DeFeo and Morgan, 1985; Park and Rasmussen, 1985; Chatterjee and Tejada, 1986), although this has proved contentious (Khalil and van Breemen, 1988). The effects of endogenous DAG can be mimicked by tumour-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA) (Castagna et al., 1982). These phorbol esters are thought to intercalate into the cell membrane and substitute for DAG, permanently activating protein kinase C (Kikkawa et al., 1983).

Ins(1,4,5)P_3

Michell, 1975, first proposed that agonist-induced hydrolysis of phosphoinositides may be responsible for mobilising Ca^{2+}. Consistent with this hypothesis are the observations, in various tissues, that the activity of phospholipase C does not change significantly over a physiological range of $[Ca^{2+}]_i$, suggesting that agonist stimulated breakdown of inositol lipids precedes any increase in $[Ca^{2+}]_i$ (Jones and Michell, 1975; Fain and Berridge, 1979; Irvine et al., 1984). The first report that Ins(1,4,5)P_3 mobilises intracellular Ca^{2+} came from studies on the release of Ca^{2+} from the endoplasmic reticulum of permeabilised pancreatic cells (Streb et al., 1983). Ins(1,4,5)P_3-induced release of Ca^{2+} from a non-

mitochondrial store has since been demonstrated in vascular smooth muscle (Capponi et al., 1985; Nabika et al., 1985). Moreover, this source of Ca^{2+} has been identified as the sarcoplasmic reticulum (Kowarski et al., 1985). Indeed release of Ca^{2+} induced by $Ins(1,4,5)P_3$ has been correlated with vascular smooth muscle contraction both in vivo and in vitro (Somlyo et al., 1985; Walker et al., 1987). Stimulation of Ca^{2+} release by $Ins(1,4,5)P_3$ from the sarcoplasmic reticulum is evoked by the opening of a receptor-linked Ca^{2+}-channel in the membrane of the sarcoplasmic reticulum (Prentki et al., 1984; Muallen et al., 1985; Bingham Smith et al., 1985). Stable GTP analogues mimic and enhance the effect of $Ins(1,4,5)P_3$, suggesting the presence of a G protein in the receptor coupling mechanism (Dawson et al., 1986). The cyclic derivative of $Ins(1,4,5)P_3$, 1,2 (cyclic) 4,5-trisphosphate, is also thought to be a potent stimulator of Ca^{2+} release from the sarcoplasmic reticulum (Wilson et al., 1985b). In addition to the release of Ca^{2+} from the sarcoplasmic reticulum, there may be release of Ca^{2+} from plasma membrane-bound stores into the cytosol (Buckley and Hawthorne, 1972). It is now established that $Ins(1,4,5)P_3$ stimulates release of Ca^{2+} from the sarcoplasmic reticulum, mediating the initial phase of agonist-induced contraction. However, prolonged muscle contraction requires a maintained elevation of $[Ca^{2+}]_i$ and is effected by an increase in the plasma membrane Ca^{2+} permeability (Berridge and Irvine, 1984). The detailed nature of this change in membrane permeability however, is controversial. Originally it was thought that the product of DAG phosphorylation, phosphatidic acid (Ptd

OH), may act as a Ca^{2+} ionophore (Tyson et al., 1976). However, this effect was probably due to lyso-Ptd OH (Benton et al., 1982). More recently, it has been shown that Ins(1,4,5)P_3 causes influx of Ca^{2+} through a specific receptor-operated Ca^{2+} channel (Kuno and Gardner, 1987). Putney, 1986, has also proposed a role for Ins(1,4,5)P_3 in the inward flux of Ca^{2+}. Furthermore, inositol 1,3,4,5 tetrakisphosphate (Ins(1,3,4,5)P_4) has been suggested to stimulate translocation of extracellular Ca^{2+} into the cell cytosol (Irvine and Moor, 1986). Elevation of $[Ca^{2+}]_i$ is detected by the Ca^{2+}-receptor protein, CaM, which binds Ca^{2+} and then forms an active complex with myosin light chain kinase (MLCK) (Adelstein and Klee, 1981). This complex activates the actomyosin contractile system via phosphorylation of the regulatory myosin light chain, resulting in smooth muscle contraction (Rüegg, 1988). Contraction of smooth muscle has also been reported to be associated with increased tissue levels of cyclic GMP (reviewed by Rapoport and Murad, 1983b). Indeed, in the bovine coronary artery, increased levels of cyclic GMP accompanied contraction in a time- and concentration-dependent manner (Kukovetz et al., 1982). However, from experiments studying the temporal relationship between formation of cyclic GMP and muscle contraction, it appears that contraction precedes elevation of cyclic GMP levels (Fiscus and Dyer, 1981). It has been proposed therefore, that cyclic GMP may act as a feedback inhibitor of contraction (Kukovetz et al., 1982).

Relaxation

Both the $[Ca^{2+}]_i$ and the sensitivity of the contractile

apparatus to Ca^{2+} in smooth muscle, may be controlled by the cyclic nucleotides, cyclic AMP and cyclic GMP (reviewed by Hardman, 1984). It appears that these cyclic nucleotides act via cyclic nucleotide-dependent protein kinases to alter the phosphorylation pattern of a diverse range of target proteins.

Smooth muscle relaxation induced by beta-adrenoceptor agonists and other agents, including cyclic nucleotide phosphodiesterases, may be mediated by cyclic AMP (reviewed by Kramer and Hardman, 1980; Hardman, 1981). The first clear evidence for the involvement of cyclic AMP came from experiments in which cyclic AMP, injected into smooth muscle cells, induced relaxation (Scheid et al., 1979). The mechanism of this relaxation is thought to involve the phosphorylation and inhibition of MLCK, via a cyclic AMP-dependent protein kinase (de Lanerolle et al., 1984). Furthermore, the proteins of the cell membrane, and the protein phospholamban of the sarcoplasmic reticulum, may similarly be phosphorylated, reducing the $[Ca^{2+}]_i$ by the following mechanisms: (i) inhibition of Ca^{2+} influx through Ca^{2+} channels (Meisheri and van Breemen, 1982); (ii) promotion of Ca^{2+} extrusion through the cell membrane (Bülbring and den Hertog, 1980); and (iii) enhancement of the uptake of Ca^{2+} into the sarcoplasmic reticulum (Mueller and van Breemen, 1979).

It is now generally accepted that vascular smooth muscle relaxation induced by nitrovasodilators, endothelium-dependent vasorelaxants and the atriopeptins, is mediated by increased levels of cyclic GMP (Rapoport and Murad, 1983b; Winquist, 1985). Indeed, the effects of these relaxants on

cyclic GMP levels are associated with vascular relaxation in a time- and concentration-dependent manner (Rapoport et al., 1983; Waldman et al., 1984; Winquist et al., 1984). Furthermore, nitrovasodilators and the atriopeptins induce vascular relaxation and increase levels of cyclic GMP even in the absence of endothelium (Winquist, 1985), whereas the effect of endothelium-dependent vasorelaxants on muscle tone and cyclic GMP levels requires functional endothelial cells (Rapoport et al., 1983). The mechanism by which cyclic GMP mediates vascular smooth muscle relaxation is not well understood. There is considerable evidence however, to suggest that cyclic GMP acts via cyclic GMP-dependent protein kinases to (i) directly alter protein phosphorylation and dephosphorylation of MLCK (Rapoport and Murad, 1983b) and (ii) reduce levels of $[Ca^{2+}]_i$ (Morgan and Morgan, 1984). This latter effect may result in reduced phosphorylation of the contractile proteins by the Ca^{2+}-CaM signal pathway. Therefore, cyclic GMP- and cyclic AMP-mediated vasorelaxations appear to be similar in nature, although in the rat aorta, distinct differences between the two systems have been demonstrated (Rapoport et al., 1982; Lincon and Fisher-Simpson, 1983). The details of cyclic GMP-mediated vasodilatation however, remain unclear.

Aims of study

The general aims of this study were to examine the mechanisms leading to smooth muscle relaxation in rat vasculature. The descending thoracic aorta of the rat provided a convenient model for vascular smooth muscle, since this tissue both possesses properties of conducting vessels and may be manipulated experimentally. In this latter respect, the vascular endothelium may easily be removed, and mechanical and biochemical measurements made. Furthermore, genetic control of rat breeding gives rise to standardised preparations of normal and pathological animals and so reproducible results may be obtained.

In particular, this study set out to examine:

(1) the nature of vasodilatation induced by the bovine inhibitory factor and to compare the characteristics of relaxation with those induced by other relaxants. Vasodilatation was correlated with tissue cyclic GMP levels;

(2) the effect of the contractile agent used to raise muscle tone and the underlying mechanism involved, on the degree and nature of vasorelaxation induced by both endothelium-dependent, and endothelium-independent vasodilators. The intracellular changes that mediated the vascular response were measured: (i) the rate of Ptd Ins hydrolysis associated with smooth muscle contraction, and (ii) the levels of cyclic GMP associated with vasodilatation;

(3) the vascular responsiveness to both contractile and relaxant agents under pathophysiological conditions. The disorder chosen for study was hypertension and was examined in spontaneously hypertensive rats. Again the intracellular correlates were measured.

METHODS AND MATERIALS

Experimental Animals

In general, adult male Wistar rats (200-300g) were used throughout this study. However, in certain experiments male rats genetically predisposed to hypertension, spontaneously hypertensive rats (SHR), and normotensive wistar kyoto (WKY) rats were used. Also, in one experiment, a male New Zealand rabbit (3.5kg) was used. All animals were housed in a controlled environment under conditions of a circadian cycle of light and dark (light: 0630-1830hrs.), and allowed tap water and food (Labsure CRM diet) ad libitum. The ambient temperature was maintained between 18-22^{o}C.

Dissection of aorta

The dissection procedure was basically the same for the rat and rabbit. Rats and the rabbit were killed by stunning and exsanguination. The chest wall was cut along the midline and the thoracic cavity exposed. The lungs, inferior vena cava and oesophagus were excised revealing the descending thoracic aorta attached to the distal wall of the thoracic cavity. The aorta was cleared of adhering fat and connective tissue, carefully removed from the thoracic cavity and gently washed in a beaker containing modified Krebs bicarbonate buffer (2^{o}C), similar to that first described by Krebs and Henseleit (1932), with the following composition; (mM): NaCl, 118.5; KCl, 4.75; $CaCl_2$, 2.5; $MgSO_4.7H_2O$, 1.0; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 22.2.

Mechanical responses of tissues in organ bath

(i) Preparation of aortic rings

Two pairs of transverse aortic rings (2-3mm in length) were cut from each aorta, weighed and suspended between stainless

steel wire hooks under a resting tension of 2g in 25ml organ baths (see figure 4). Care was taken at all times to protect the endothelium from damage. The tissues were bathed in Krebs bicarbonate buffer at 37^{oC} and gassed continuously with a mixture of 95% O_2/ 5% CO_2. Isometric tension was recorded using Statham force displacement transducers and displayed on either a Linseis recorder or Grass polygraph (model 7 PCPB). The tissues were allowed to equilibrate for 60 minutes prior to the start of experiments, during which time the resting tension was maintained at 2g and the tissues were washed twice.

(ii) Preparation of aortic spiral strips

Transverse aortic rings were first prepared as detailed above, and positioned over the end of a wooden stick. A continuous cut was made at 45^{o} into the aortic ring while slowly turning the stick in a clockwise direction. After weighing, each aortic strip was secured at one end to a glass hook in a 25ml organ bath and attached, by thread, at the other end to a Statham force displacement transducer. A resting tension of 1g was applied to the tissues which were exposed to the same experimental conditions as the aortic rings.

(iii) Removal of endothelial cells

In certain experiments, the endothelium was removed mechanically from aortic rings by gently rubbing the intimal surface with a wooden stick (Furchgott and Zawadzki, 1980a). Successful removal of, or damage to the endothelial cells was routinely established by the inability of acetylcholine to relax precontracted aortic rings treated in this manner (Furchgott and Zawadzki, 1980a). However, in preliminary

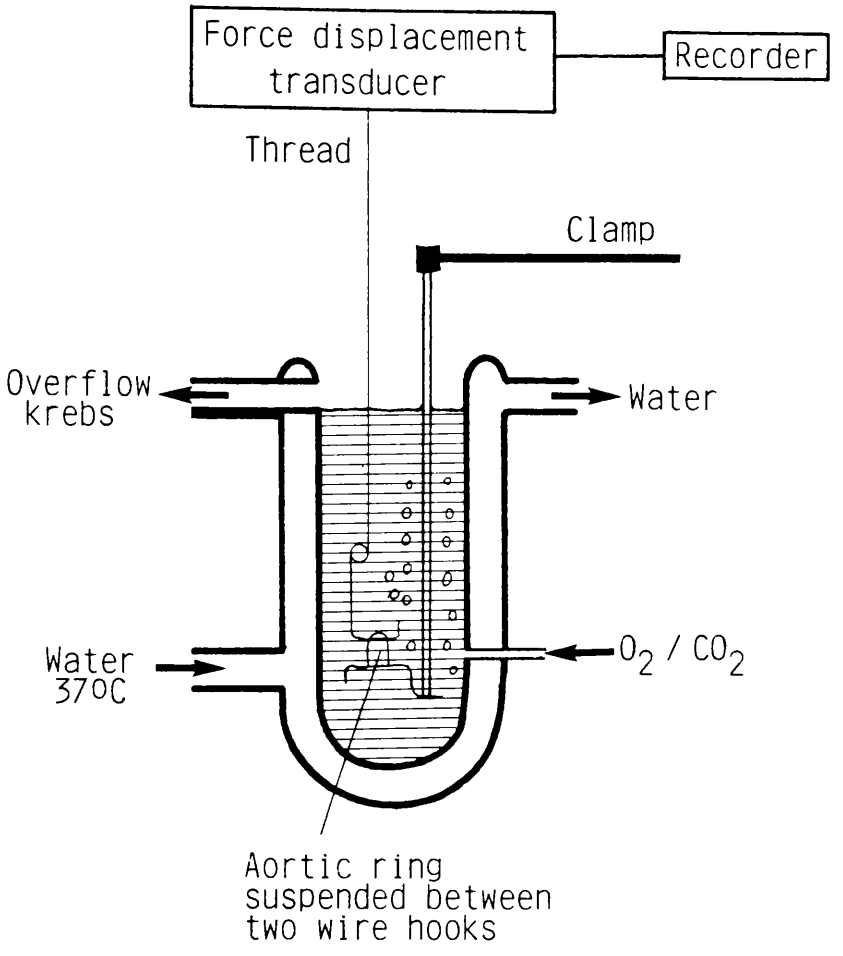


Figure 4.
Apparatus for recording mechanical responses of aortic rings. A similar apparatus was used for recording mechanical responses of aortic strips (see text for explanation).

experiments, the successful removal of endothelial cells was determined by electron microscopy (see photographs 1 and 2).

(iv) Addition of drugs

All drugs were added in volumes not exceeding 0.3ml, and the drug concentration was expressed as the final dilution in the organ bath. Cumulative concentration-response graphs were obtained for each contractile agent and vasorelaxant. The degree of smooth muscle relaxation induced by each vasodilator was expressed as a % of the maximum contraction elicited by the contractile agent (EC_{50} or EC_{70} concentration).

Measurement of cyclic GMP

(i) Experimental

In most experiments, cyclic GMP levels were measured in aortic rings in which mechanical responses had been recorded. However, in certain experiments aortic rings were held by a wire hook, under no applied tension, in incubation flasks. At intervals following the addition of drugs the aortic rings, while still held by wire hooks, were removed from the organ baths or incubation flasks and frozen in liquid nitrogen. This step was performed in less than 2 seconds.

(ii) Extraction of cyclic GMP

The frozen aortic rings were deproteinised by extraction in trichloroacetic acid (T.C.A.) (1.6ml; 5%w/v). After a period of 2hrs. the tissues were detached from the wire hooks, re-immersed in T.C.A. and hand homogenised using Potter-Elvehjem glass-glass homogenisers. The tissue homogenates were then left for a further 18 hrs. at $4^{o}C$. Homogenisation was thought to break down the tissue structure and increase

the surface area across which T.C.A. could permeate and therefore facilitate extraction of cyclic GMP. However, it has been reported that tissue homogenisation does not significantly increase the yield of cyclic GMP during acid extraction (Bowman and Drummond, 1984). In contrast with this finding, the present study found that homogenisation of aortic rings during acid extraction did increase the recovery of cyclic GMP (see figure 5).

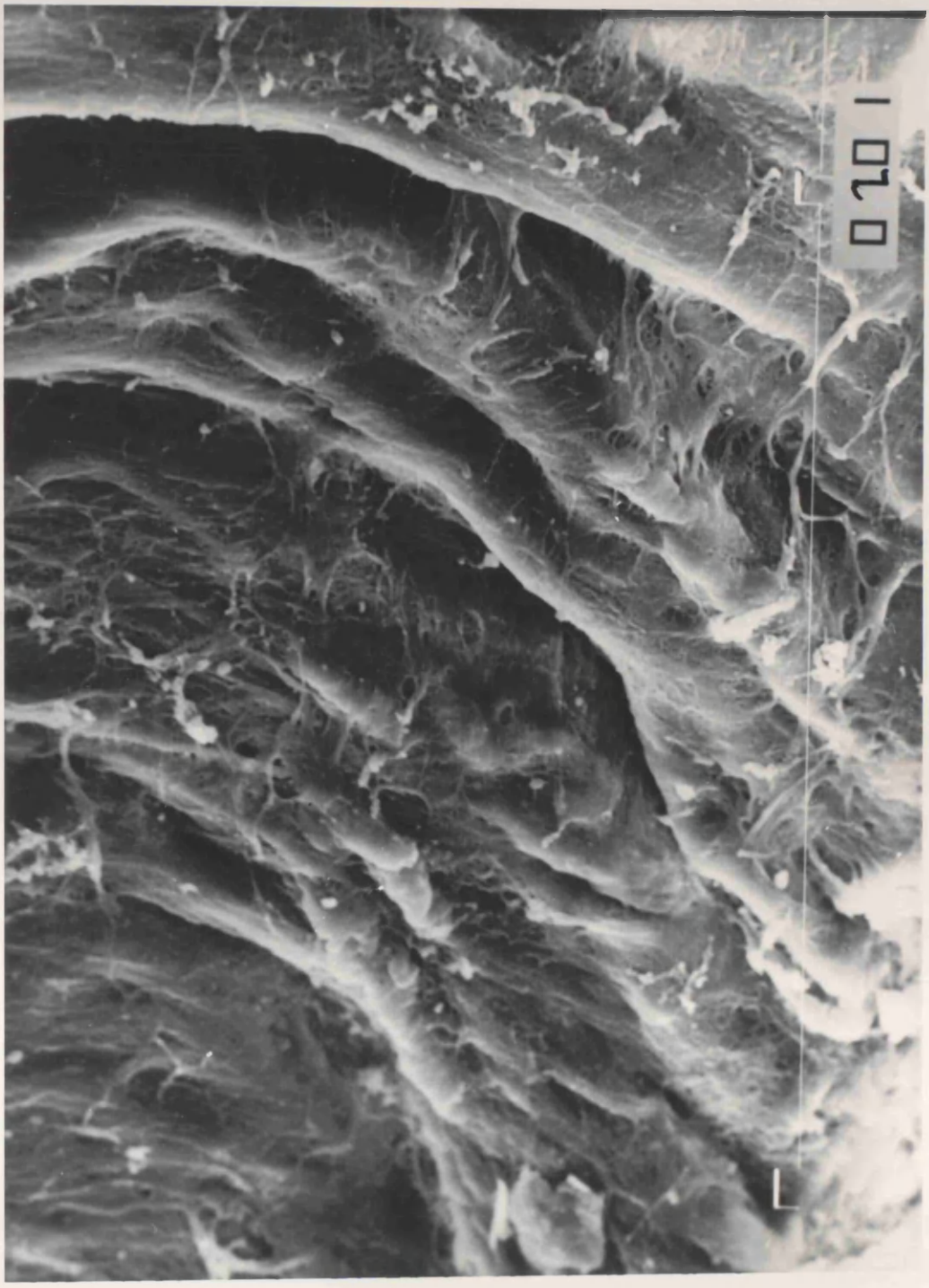
The samples were vortex-mixed and centrifuged (1000g; 5mins.; $4^{o}C$) to remove any non-acid-soluble tissue components. The cyclic GMP content of a fraction of the supernatant (400µl) was extracted by washing four times with three volumes of water-saturated ether. Any remaining ether was evaporated by placing the sample tubes in a water bath at $80^{o}C$ for 2 minutes. The samples (300µl) were neutralised with sodium acetate buffer (100µl; 200mM; pH6.2), vortex-mixed and stored at $4^{o}C$ until required.

(iii) Radioimmune assay

Tissue cyclic GMP levels were measured using a commercially available cyclic GMP radioimmune assay kit (Amersham International, UK). The assay was based on the competitive binding between unlabelled cyclic GMP and a fixed quantity of the tritium- ($[^{3}H]$)-labelled cyclic nucleotide to an antiserum which has high specificity and affinity for cyclic GMP.

Authentic cyclic GMP standard concentrations (0.06-8 pmoles/100µl) were prepared in sodium acetate buffer (50mM; pH5.0). Samples of both standard and unknown tissue concentrations of cyclic GMP (100µl) were acetylated by the addition of a freshly prepared mixture of acetic anhydride

Photograph 1.
A scanning electron micrograph of a segment of rat aorta, with intact endothelium, fixed in glutaraldehyde (1000 x magnification; luminal surface). Layers of smooth muscle cells are compressed, giving a folded appearance. This may be caused by shrinkage of the tissue in the fixant. Platelets are seen adhered to the endothelial surface.



Photograph 2.
A scanning electron micrograph of a segment of rat aorta, with damaged endothelium, fixed in glutaraldehyde (1000 x magnification; luminal surface). Again, the layers of smooth muscle cells are compressed, giving a folded appearance that may be caused by tissue shrinkage in the fixant. Remnants of the endothelium can be seen, although very few platelets remain adhered to the luminal surface (c.f. photograph 1).

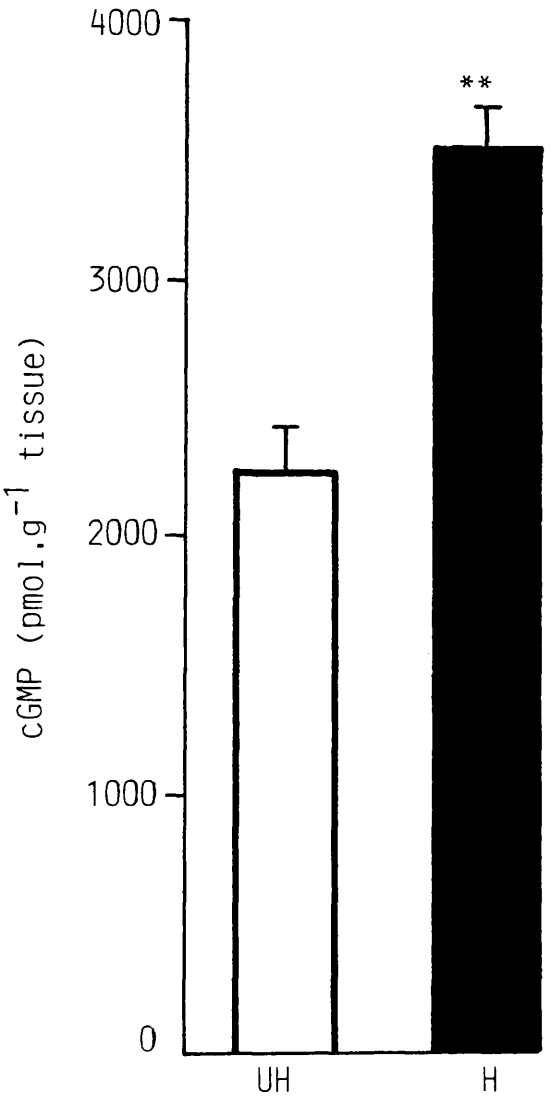


Figure 5.
Effect of sodium nitroprusside (10^{-6}M) on levels of cyclic GMP. Aortic rings were exposed to sodium nitroprusside alone, for 60s.. During acid extraction of cyclic GMP, tissues were either homogenised (shaded) or left unhomogenised (unshaded). The level of cyclic GMP induced by sodium nitroprusside was significantly higher in tissues that were homogenised than in tissues that were unhomogenised. (** $p<0.01$). ($n<6$).

and triethylamine, 1:2 by volume (5µl) (Frandsen and Krishna, 1976). The samples were immediately vortex-mixed. This acetylation process increased the sensitivity of the assay (see figure 7). [^{3}H]-labelled cyclic GMP (50µl) was then added to each sample, followed by anti-cyclic GMP antiserum (50µl). Samples were vortex-mixed and incubated for 16hrs. at 4^{o}C. Following this incubation period, ice-cold ammonium sulphate (1ml) was added to each sample and then mixed. The samples were allowed to stand for 5 minutes after the addition of ammonium sulphate to the last sample, before centrifuging (1000g; 5mins.; 4^{o}C). The supernatant was decanted and the assay tubes inverted on a tissue to drain. The necks of the assay tubes were carefully wiped to remove any remaining supernatant. Cold distilled water (1.1ml) was then added to each tube and vortex-mixed until the precipitate had dissolved. Samples (1ml) were transferred from the assay tubes to plastic counting vials containing a water-miscible scintillant (Ecoscint; 10ml), and antibody bound [^{3}H]-cyclic GMP was measured by counting for 5 minutes in a Packard Liquid Scintillation Counter. Standard calibration curves were determined in each experiment and plotted as radioactivity bound (c.p.m.) against concentration of cyclic GMP (figure 6) or as C_o/C_x against concentration of cyclic GMP where C_o and C_x represent the amount of [^{3}H]-cyclic GMP bound in the absence and presence of unlabelled cyclic GMP respectively (figure 7). The concentrations of cyclic GMP in unknown samples (100µl) were determined by reference to the calibration curve. The concentration of cyclic GMP in the original sample (1.6ml) was expressed in pmol.mg^{-1} of tissue, and

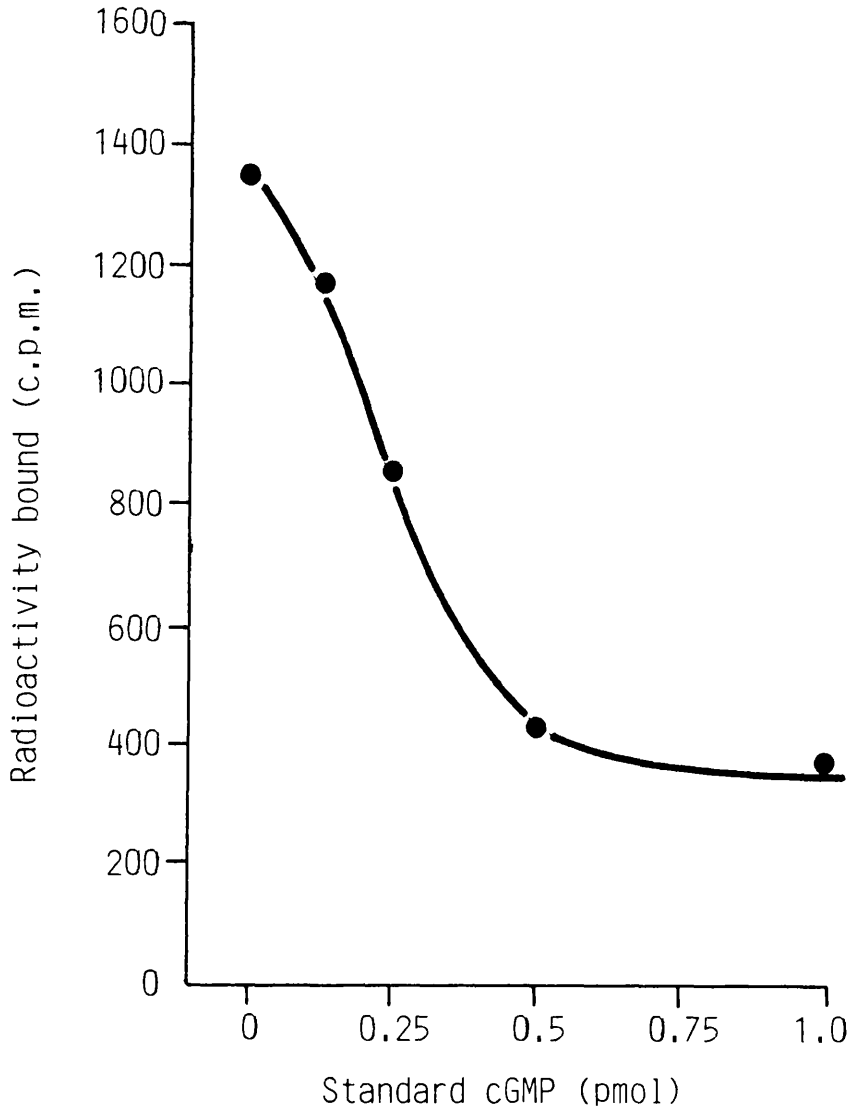


Figure 6.
Typical standard curve showing radioactivity bound (c.p.m.) for known concentrations of cyclic GMP during radioimmune assay (see text for explanation).

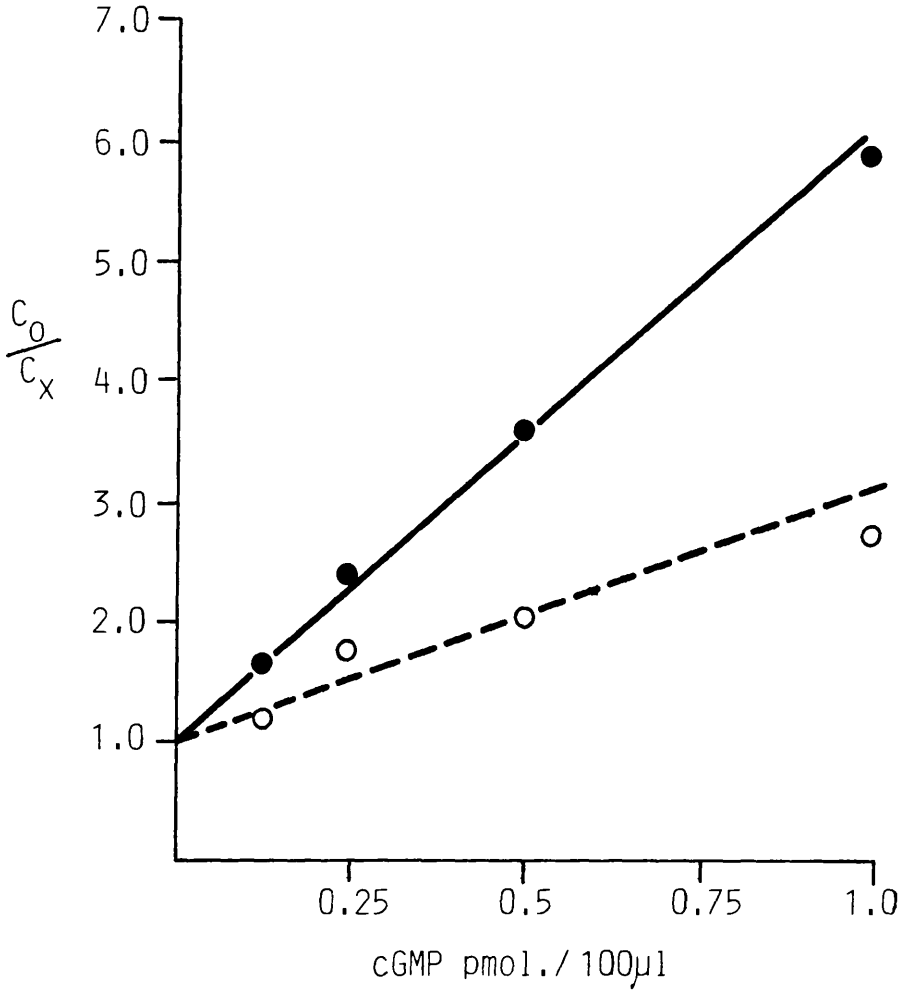


Figure 7.
Standard calibration graphs obtained for known concentrations of cyclic GMP during radioimmune assay. The abilities of acetylated cyclic GMP (●) and non-acetylated cyclic GMP (○) to compete with [^{3}H]-cyclic GMP for binding to specific anti-cyclic GMP antiserum were measured (see text for details). Acetylation of cyclic GMP increased the C_o/C_x ratio and the sensitivity of the assay.

calculated using the following method:

(1) concentration of cyclic GMP in 100µl multiplied by 16 gives concentration of cyclic GMP in sample tube ($pmol.tube^{-1}$).

(2) concentration of cyclic GMP in sample tube divided by weight of aortic ring (mg) gives concentration of cyclic GMP per weight of tissue ($pmol.mg^{-1}$).

Inositol Phospholipid studies

(i) Experimental

Agonist-induced hydrolysis of phospholipids was monitored by measuring the formation of [^{32}P]-Ptd OH in aortic rings prelabelled with [^{32}P]-orthophosphate (-Pi). Aortic rings were prepared as previously described and incubated (37^{o}C; 120mins.) in Ca^{2+}-free, phosphate-free Hepes' buffer containing carrier-free [^{32}P]-Pi ($2\mu Ci.ml^{-1}$). Hepes buffer was of the following composition; (mM): NaCl 111.4; KCl, 4.7; $MgCl_2$, 1.2; glucose 11.1; HEPES, 10.1; pH adjusted to 7.4 with NaOH (1N). Excess [^{32}P]-Pi was removed by washing each tissue once with Hepes' buffer (4^{o}C) and then a further three times with modified Krebs' buffer (4^{o}C). Preliminary experiments were conducted using Ca^{2+}-free Krebs' buffer. Reactions were initiated by the addition of agonist(s) or vehicle and terminated by rapidly freezing aortic rings in liquid nitrogen before transferring to glass tubes containing 4ml of chloroform/methanol/10M-HCl (25:50:4, by volume) at room temperature and vortex mixing (Lloyd et al., 1972). The aortic rings were then hand homogenised.

(ii) Extraction

Phospholipids were extracted using a modification of the method of Lloyd et al, (1972). Chloroform and distilled

water in equal volumes (1.25ml), were added to the homogenate and mixed thoroughly. The samples were centrifuged (1000g; 5mins.; 4^{o}C) which caused a partitioning of two distinct phases with a protein interface. The lower, organic phase was removed by Pasteur pipette to a drying bottle and dried under oxygen-free nitrogen at 45^{o}C. The drying bottle was then sealed and kept on ice for not more than 16 hrs..

(iii) Separation of lipids

The phospholipids were separated by two dimensional thin layer chromatography (t.l.c.) (Yavin and Zutra, 1977). Silica gel t.l.c. plates (10cm x 10cm) were marked 2cm x 2cm from the lower left edge and 5µg of carrier Ptd OH was spotted. The plates were run in the first dimension in a basic solvent system of chloroform/methanol/40% methylamine (130:60:15:, by volume). Acidic phospholipids such as Ptd Ins and Ptd OH were retarded by the basic nature of this solvent medium. The solvent front was allowed to run to a distance of 1-2cm from the top of the plate. The t.l.c. plates were removed from the solvent, dried under warm air, exposed to concentrated hydrochloric acid fumes to neutralise the methylamine and dried sequentially under warm and cool air. The plates were then washed in the second dimension in an acidic solvent comprising diethylether/ acetic acid (19:1, by volume) and dried under cool air. The lipids were separated in a solvent system of chloroform/acetone/methanol/acetic acid/distilled water (10:4:2:3:1, by volume) in the second dimension. The solvent front was allowed to run the same distance as the acidic wash.

(iv) Detection and measurement of phospholipids

The plates were dried under cool air for a period of not less than 30 minutes before placing in iodine vapour to visualise the phospholipids (see figure 8). The spots corresponding to Ptd OH were scraped into scintillation vials containing Ecoscint (5ml) and counted in a Packard Liquid Scintillation Counter. The levels of Ptd OH were expressed as c.p.m..mg^{-1} of tissue. Changes in [^{32}P]-Ptd OH reflect changes in Ptd OH concentration (Holmsen et al., 1984) and the DAG precursor of Ptd OH is probably derived from the hydrolysis of phosphoinositides (MacIntyre and Pollock, 1983).

Mesurement of rat blood pressure

Blood pressure measurements were conducted in the Blood Pressure Unit, Western Infirmary, Glasgow. Systolic blood pressure was measured in conscious animals by a tail-cuff method using a gartner cuff blood pressure recorder (model 800S, W and W Electronics, Basel, Switzerland).
Rats were familiarised with the recording equipment, and then housed in a constant temperature chamber (36^{o}C; 15mins.) prior to blood pressure measurement.

Materials

The following drugs and reagents were used in this study: acetylcholine chloride and carbamylcholine chloride (both Sigma); HEPES (Sigma); M&B 22948 (kindly supplied by Dr. W. Martin, Department of Pharmacology, The University, Glasgow); L-noradrenaline bitartrate (Koch-Light); 4α-phorbol 12,13-didecanoate (4α PDD), phorbol 12-myristate 13-acetate (PMA), and carrier phosphatidic acid (all Sigma); prazosin hydrochloride (Pfizer); and sodium nitroprusside

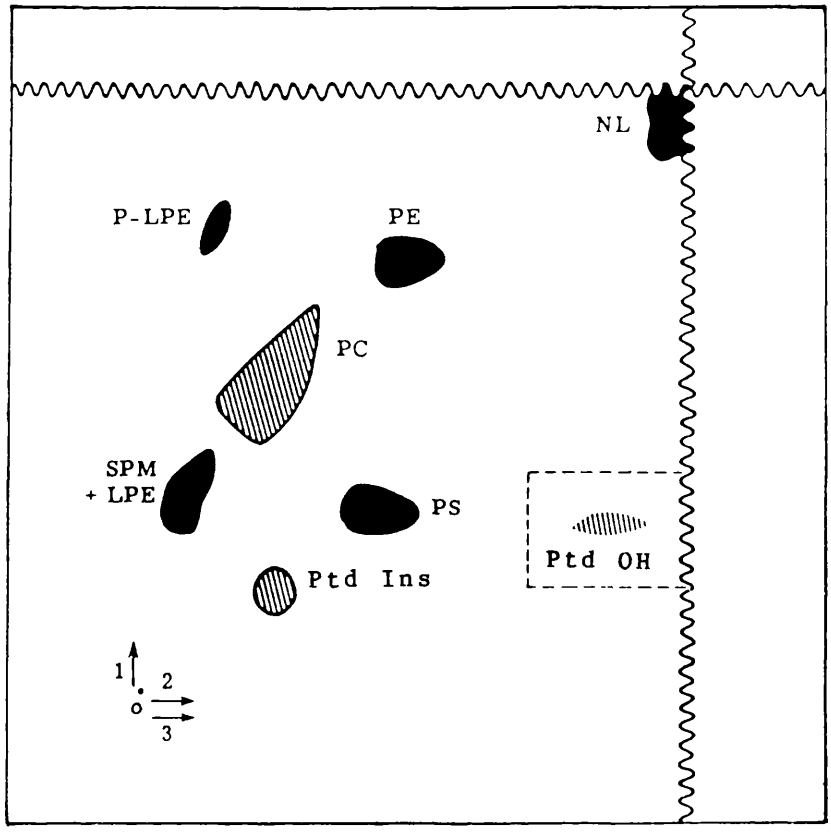


Figure 8.
Schematic representation of the major phospholipids separated by two-dimensional thin layer chromatography (see text for details).

Phospholipids visualised by iodine staining were: phosphatidic acid (Ptd OH); phosphatidylinositol (Ptd Ins); phosphatidylserine (PS); phosphatidylcholine (PC); phosphatidylethanolamine (PE); sphingomyelin (SPM); lyso-phosphatidylethanolamine (LPE); and neutral lipids (NL).

(Analar).

In general, drugs were dissolved in 0.9% saline and freshly made up as stock solutions of 10^{-2}M before each experiment. M&B 22948 has a low solubility in water (0.1%) and was dissolved (5%) in ethanol. PMA and 4αPDD were dissolved in dimethylsulphoxide (DMSO), giving stock solutions of 1.6 x 10^{-3}M, from which appropriate dilutions were made. The concentration of DMSO in the organ bath did not exceed 0.05%. Both PMA and 4αPDD were protected from light. Cyclic GMP radioimmune assay kits were supplied by Amersham International (Amersham U.K.), and carrier-free [^{32}P]-orthophosphate (P_i) was obtained from the Regional Isotope Dispensary, Western Infirmary, Glasgow, U.K.

Preparation of bovine inhibitory factor

The method used to prepare extracts of bovine inhibitory factor was essentially that described by Ambache *et al*, (1975) as modified by Gillespie and Martin (1980). Briefly, fresh bovine retractor penis muscles were minced, ground with glass beads in a chilled mortar and pestle and then stirred, while slowly adding methanol (5 volumes), in an M.S.E. blender. The extract was filtered (Whatman's no. 1 filter paper) under reduced pressure in a Buchner funnel, and the filtrate transferred to plastic centrifuge tubes, before chilling in liquid nitrogen to precipitate phospholipids. The precipitation of phospholipids was completed by centrifugation (4000g; 10mins.; 4^{o}C). The supernatant was decanted through a Sep. Pak., briefly degassed using a suction pump and then applied to a freshly packed Bio-Rad Anion exchange resin (200-400 mesh formate form). The exchange column was then washed with cold

distilled water. The extract was eluted from the column with sodium nitrate (150mM) and detected at 254nm by an absorbance detector, collected in weighed ampoules and freeze-dried overnight. At this stage the extract was a dry, white powder. The extract was then dissolved in cold distilled water to give a standard preparation of $20\mu g.ml^{-1}$. The bovine inhibitory factor requires acid activation for biological activity (Gillespie and Martin, 1980) and therefore the extract, which was an alkaline solution at this stage, was acidified to pH2.0 with HCl (1N) for 10 minutes, before neutralising with NaOH (1N). The extract was stored on ice and used within 4hrs..

Preparation of nitric oxide solution

Nitric oxide gas (0.2ml; British Gas) was removed by syringe from a silicone rubber tube connected to a cylinder at one end, and immersed in water at the other end. The gas was rapidly injected into a Clinbritic bottle, sealed with a silicone rubber septum and containing saline (59.6ml) which had been deoxygenated by bubbling with helium for 1 hr.. This gave a stock solution of 1.5×10^{-4}M that was stable for approximately 8 hrs..

Analysis of results

Results are expressed as mean $\pm$ standard error of the mean (S.E.M.). Groups of data were compared statistically using the student's t-test and probability (p) values of less than 0.05 were taken to be significant.

RESULTS

Section One: Vasorelaxaton induced by bovine inhibitory factor in rat aortic rings

The aim of this series of experiments was to characterise the nature of smooth muscle relaxation induced by the bovine inhibitory factor in rat isolated aortic rings. Vascular relaxation induced by the inhibitory factor, was compared with that induced by other vasorelaxants, in an attempt to determine the chemical identity of this inhibitory substance. The characteristics of vascular relaxation induced by the inhibitory factor were compared with those induced by the endothelium-dependent relaxant, acetylcholine, and the endothelium-independent relaxant, sodium nitroprusside. Two principal parameters were measured: (i) the degree of smooth muscle relaxation; and (ii) the tissue levels of cyclic GMP induced by the vasorelaxants.

Part One: Preliminary experiments on vascular smooth muscle contraction

Noradrenaline induced concentration-dependent contractions of aortic rings both in the presence and absence of endothelium (fig. 9). Noradrenaline induced greater contractions of aortic rings in the absence of endothelium. The EC_{50} concentration of noradrenaline for muscle contraction in the presence of endothelium was determined to be 1.6×10^{-8}M.

The time-course of muscle tone development induced by noradrenaline (1.6×10^{-8}M) in the presence of endothelium is shown in figure 10. Muscle tone stabilised after 5mins..

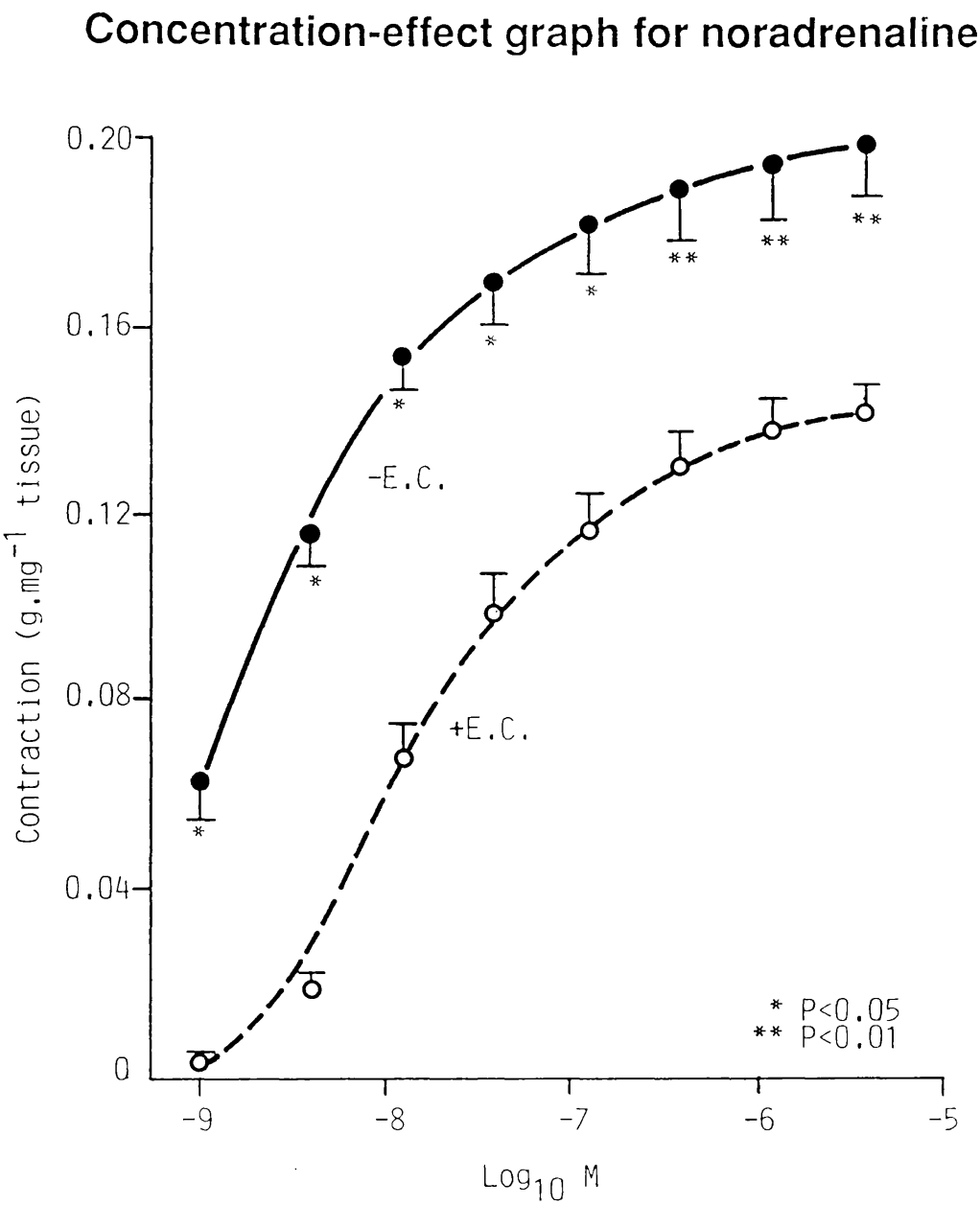


Figure 9.
Concentration-response graphs for noradrenaline in aortic rings in the presence (○), and absence (●) of endothelium. Muscle tension was recorded. Noradrenaline induced greater contractions of aortic rings in the absence of endothelium. (* $p<0.05$, ** $p<0.01$). (n=6).

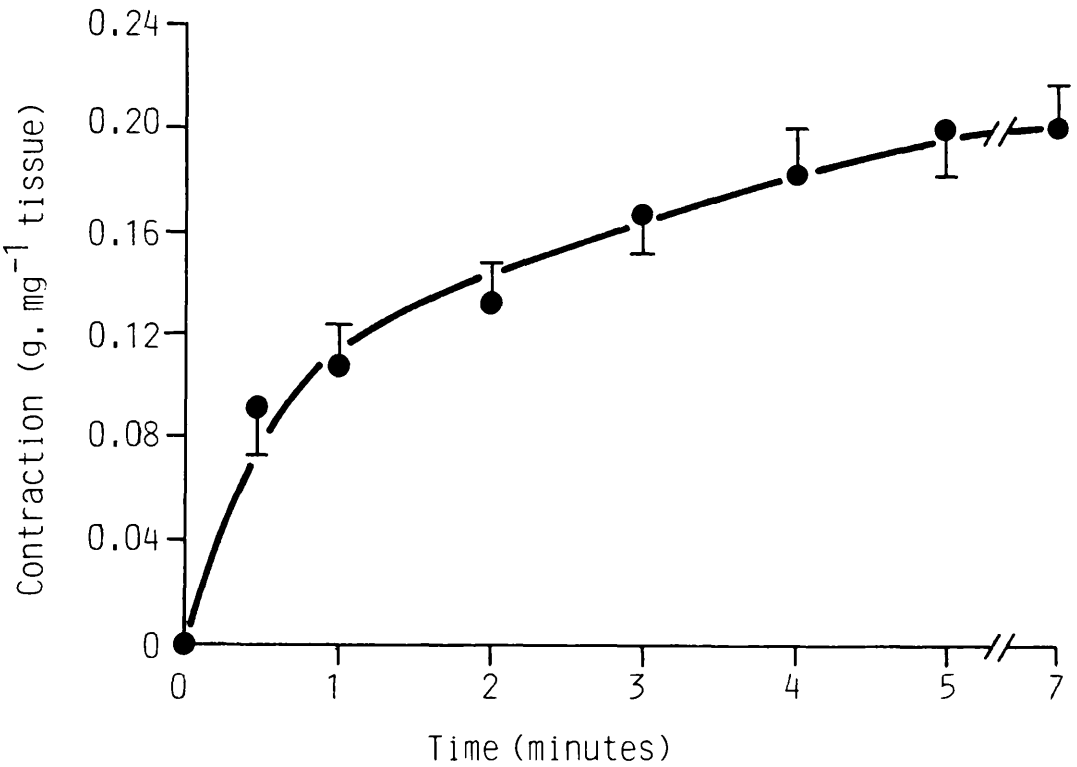


Figure 10.
Time-course of noradrenaline-induced contraction of aortic rings in the presence of endothelium. The EC_{50} concentration of noradrenaline was used (1.6×10^{-8}M). Muscle tone stabilised after 5 mins.. (n=6).

Part Two: Vascular relaxation induced by bovine inhibitory factor

Smooth muscle relaxations induced by acetylcholine, inhibitory factor and sodium nitroprusside were studied in aortic rings precontracted with noradrenaline (1.6×10^{-8}M) for 5mins., both in the presence and absence of endothelium (figure 11).

Acetylcholine-induced relaxations were confirmed to be endothelium-dependent, and inhibitory factor- and sodium nitroprusside-induced relaxations were confirmed to be endothelium-independent (figure 11).

Vascular relaxations induced by acetylcholine, inhibitory factor and sodium nitroprusside are shown quantitatively in concentration-response graphs in figures 12 and 13. Carbachol-induced relaxations are shown for comparison with those produced by acetylcholine. In the presence of endothelium (figure 12), acetylcholine was marginally more potent than carbachol at inducing muscle relaxation, although this effect was not statistically significant. Both drugs induced the same degree of maximum relaxation. Removal of the endothelium (figure 13), abolished relaxations induced by both acetylcholine and carbachol but did not affect relaxations induced by inhibitory factor and sodium nitroprusside.

The levels of cyclic GMP associated with drug-induced changes in muscle tone were measured in tissues in which the mechanical responses had been recorded. Noradrenaline (1.6×10^{-8}M; 5mins.) alone, did not significantly alter levels of cyclic GMP from those measured in control tissues (figure 14).

The effects of acetylcholine (10^{-5}M), sodium nitroprusside (10^{-6}M) and inhibitory factor (200µl) on levels of cyclic GMP, in relation to the degree of muscle relaxation, are shown in figures 15, 16, and 17 respectively.

Acetylcholine (10^{-5}M) (figure 15) induced almost complete inhibition of muscle tone induced by noradrenaline (1.6×10^{-8}M; 5mins.), and a 10-fold increase in the level of cyclic GMP. This maximum level of cyclic GMP was attained after 30s. exposure of aortic rings to acetylcholine (10^{-5}M). The development but not the maintenance of muscle relaxation was associated with increased levels of cyclic GMP.

Sodium nitroprusside (10^{-6}M) (figure 16) induced complete inhibition of muscle tone induced by noradrenaline (1.6×10^{-8}M; 5mins.), and a 100-fold increase in the level of cyclic GMP. This maximum level of cyclic GMP was attained after 60s. exposure of aortic rings to sodium nitroprusside (10^{-6}M). Both the development and maintenance of muscle relaxation were associated with increased levels of cyclic GMP.

Inhibitory factor (200µl) (figure 17) induced a 25% maximum relaxation of aortic rings precontracted with noradrenaline (1.6×10^{-8}M; 5mins.). This effect was not associated with significant changes in the levels of cyclic GMP.

Acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) induced approximately the same degree of vascular relaxation, although the level of cyclic GMP induced by sodium nitroprusside (10^{-6}M) was 10-fold higher than that induced by acetylcholine (10^{-5}M) (figure 18 and table 1).

Acetylcholine and sodium nitroprusside, at concentrations

that induced the same degree of relaxation as that induced by inhibitory factor (200µl), did not significantly increase levels of cyclic GMP (figures 19 and 20).

Sodium nitroprusside induced almost complete inhibition of muscle tone without significantly increasing levels of cyclic GMP (figure 20).

Levels of cyclic GMP induced by acetylcholine (10^{-5}M; 30s.) and sodium nitroprusside (10^{-6}M; 60s.) respectively, were not significantly different between tissues exposed to these relaxants alone, or precontracted with noradrenaline (1.6×10^{-8}M; 5mins.) (figure 21).

Noradrenaline induced concentration-dependent contractions of rat aortic strips (figure 22). The EC_{50} concentration of noradrenaline for muscle contraction was determined to be 1.2×10^{-8}M.

The degree of muscle relaxations induced by inhibitory factor were similar in both rat aortic rings and aortic strips, precontracted with noradrenaline (EC_{50} concentrations) (figure 23a).

Inhibitory factor (100µl) induced complete inhibition of muscle tone raised with noradrenaline (1.6×10^{-8}M) in rabbit aortic strips (figure 23b).

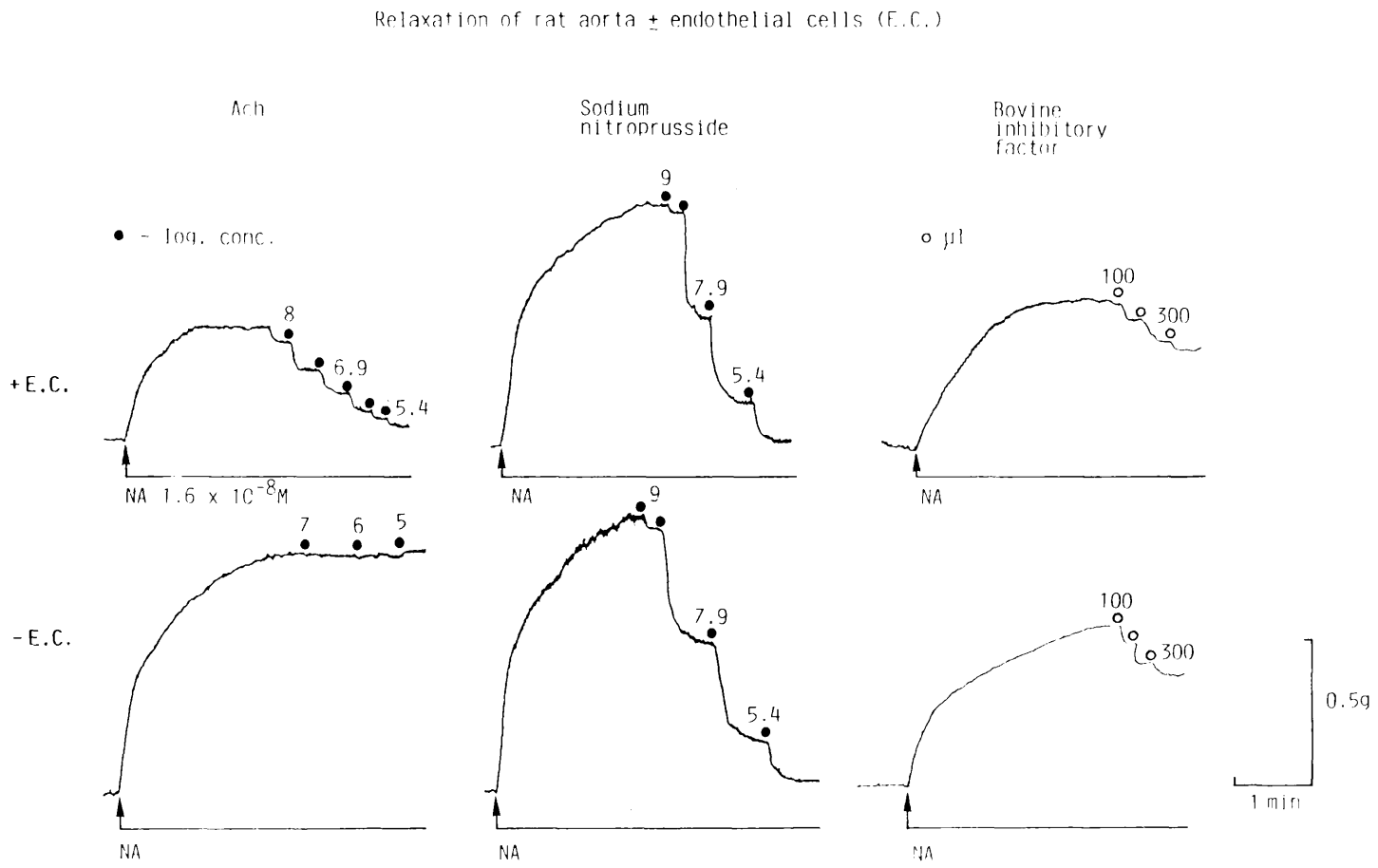


Figure 11.
Mechanical responses of aortic rings in the presence and absence of endothelium. Muscle tone was raised with noradrenaline (NA) (EC_{50}: 1.6 x 10^{-8}M). Acetylcholine (ACh), inhibitory factor and sodium nitroprusside were added successively to the organ baths, producing cumulative concentration-response graphs. Acetylcholine-induced relaxation was endothelium-dependent, whereas relaxations induced by inhibitory factor and sodium nitroprusside were endothelium-independent.

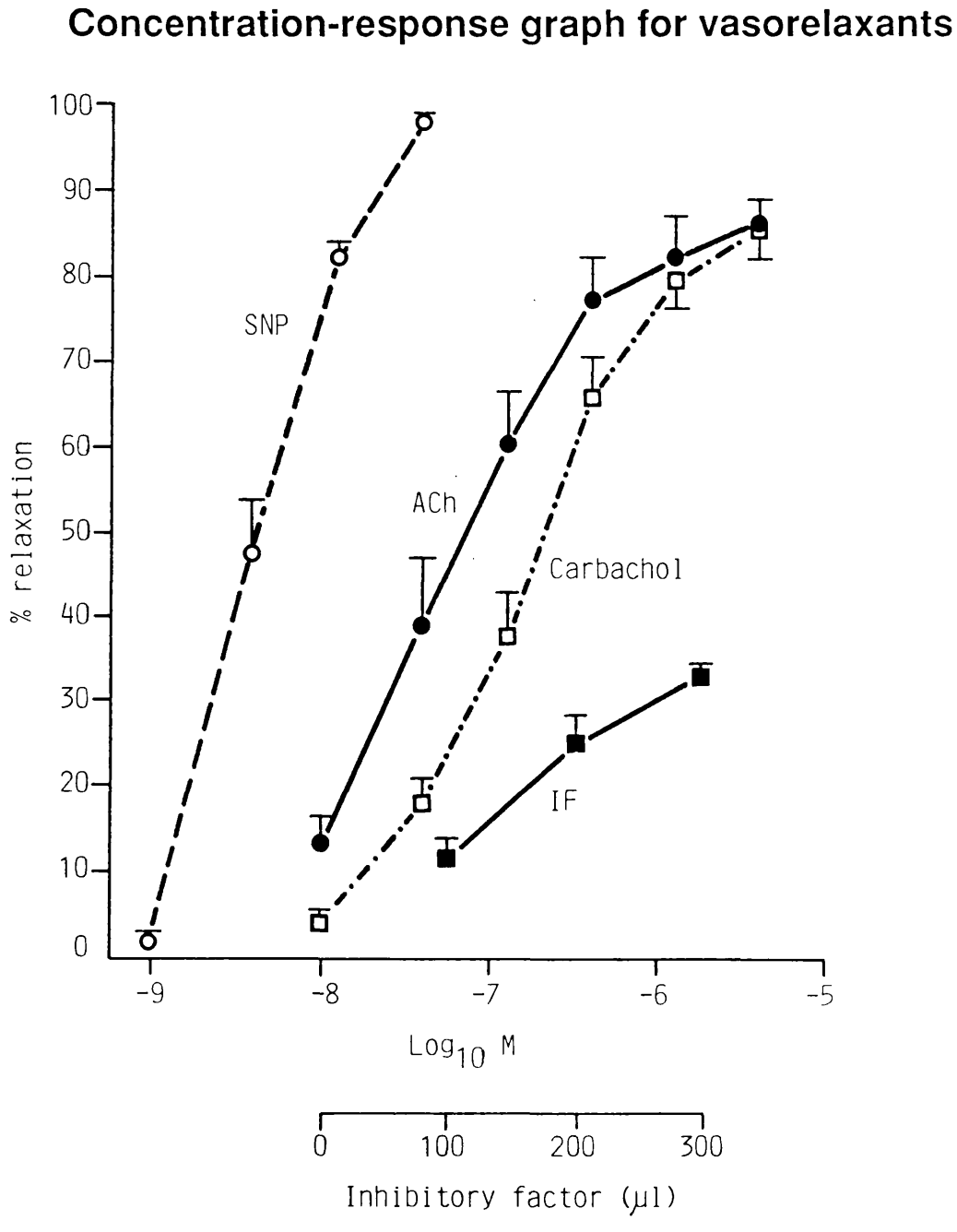


Figure 12.
Concentration-response graphs for vasorelaxants in the presence of endothelium. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M). The effects of acetylcholine (ACh) (●), carbachol (□), inhibitory factor (IF) (■), and sodium nitroprusside (SNP) (○) are shown. (n=6).

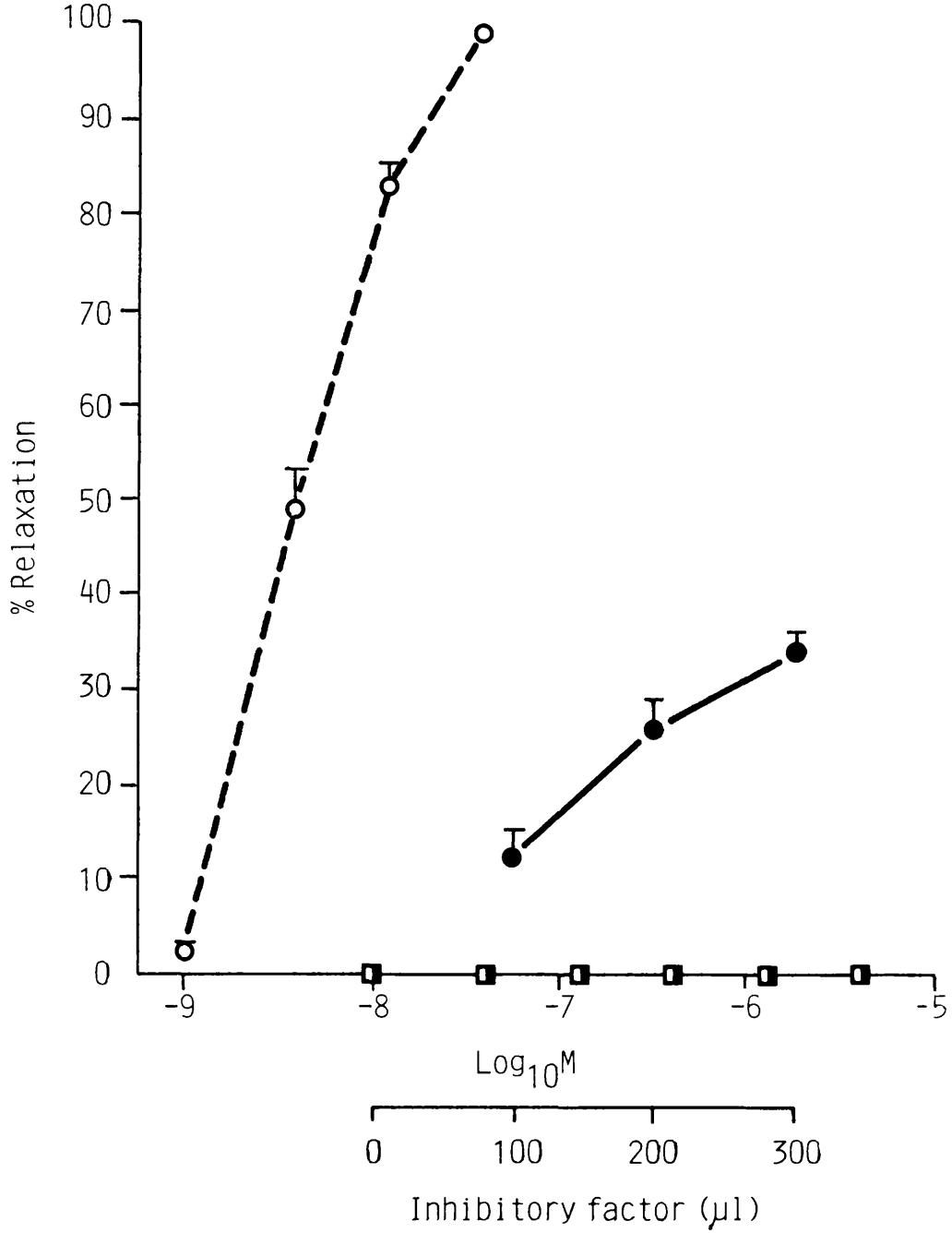


Figure 13.
Concentration-response graphs for vasorelaxants in the absence of endothelium. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M). The effects of acetylcholine (■), carbachol (□), inhibitory factor (●) and sodium nitroprusside (O) are shown. Removal of the endothelium abolished the relaxations induced by acetylcholine and carbachol. Relaxations induced by inhibitory factor and sodium nitroprusside were unaffected (c.f. figure 12). (n=6).

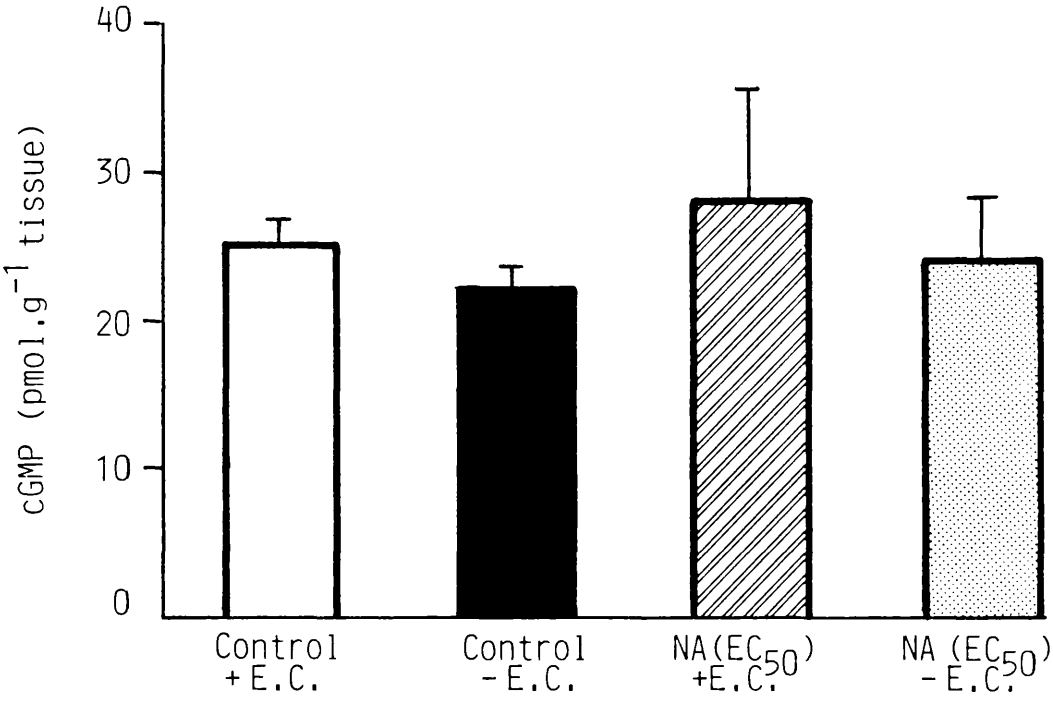


Figure 14.
Effect of noradrenaline (NA) (1.6×10^{-8}M) on levels of cyclic GMP in the presence (+EC) and absence (-EC) of endothelium. Control tissues were exposed to saline (5mins.) and test tissues to noradrenaline (5mins.). Noradrenaline did not significantly alter tissue levels of cyclic GMP. (n=6).

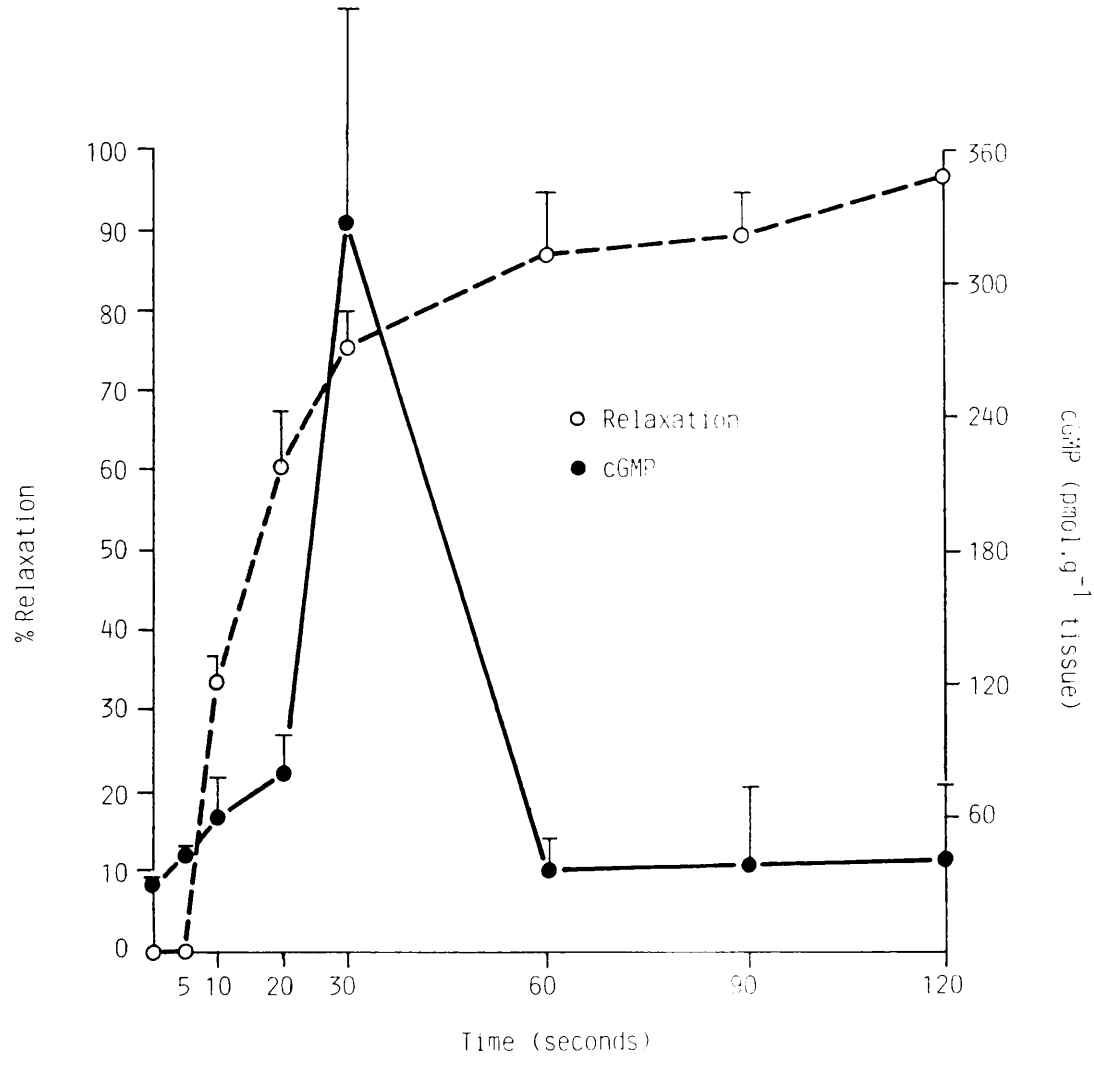


Figure 15.
Time-courses of vascular relaxation (○) and levels of cyclic GMP (●) induced by acetylcholine (10^{-5}M) in aortic rings in the presence of endothelium. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M). Vascular relaxation was preceded by a rise in the level of cyclic GMP. The development, but not the maintenance of relaxation was associated with increased levels of cyclic GMP. ($n \leq 6$).

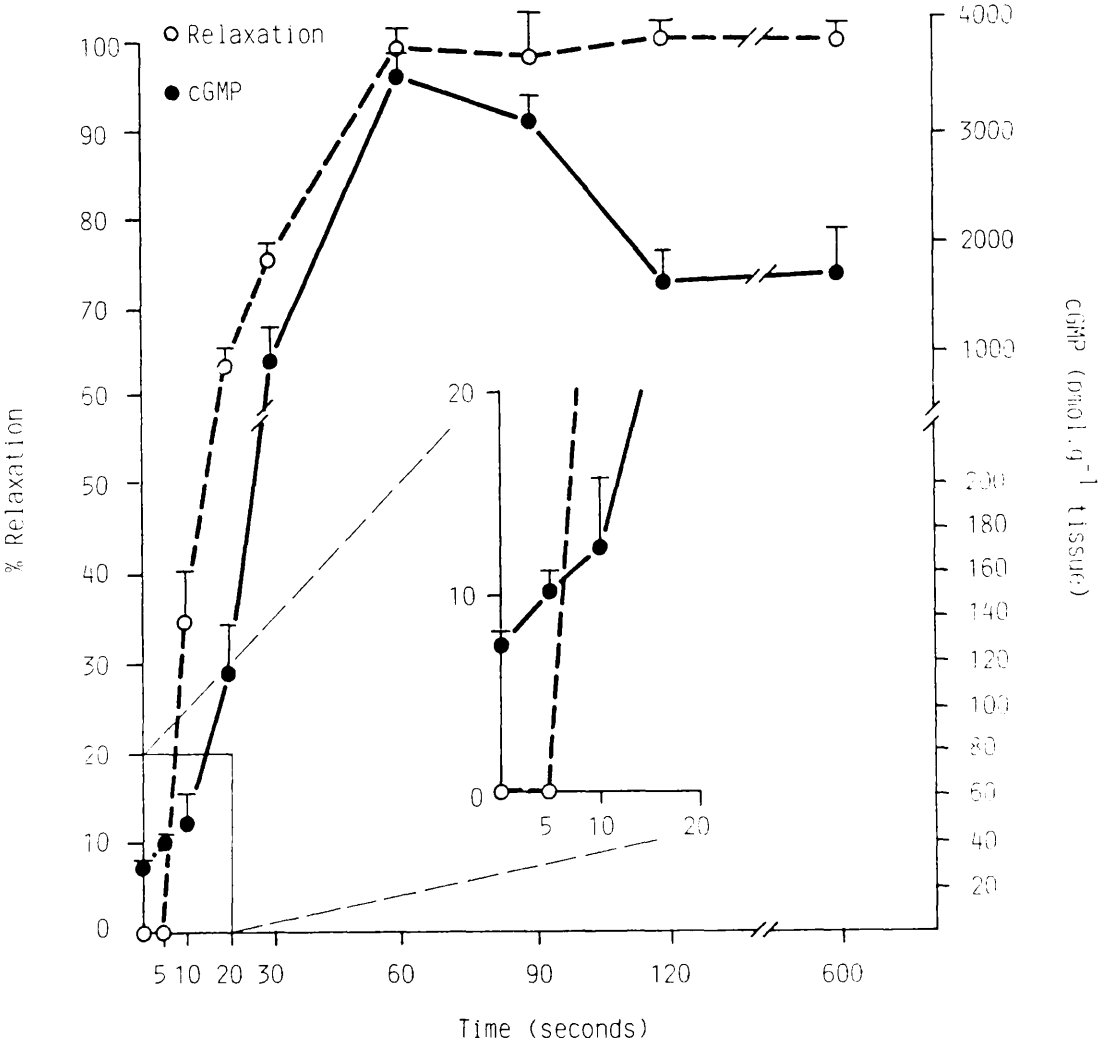


Figure 16.
Time-courses of vascular relaxation (○) and levels of cyclic GMP (●) induced by sodium nitroprusside (10^{-6}M) in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6 x 10^{-8}M). Vascular relaxation was preceded by a rise in the level of cyclic GMP (this effect is better seen in the inset which shows a magnification of vascular responses recorded in the first 20s. exposure of tissues to sodium nitroprusside). The development and maintenance of relaxation were associated with increased levels of cyclic GMP. (n≤6).

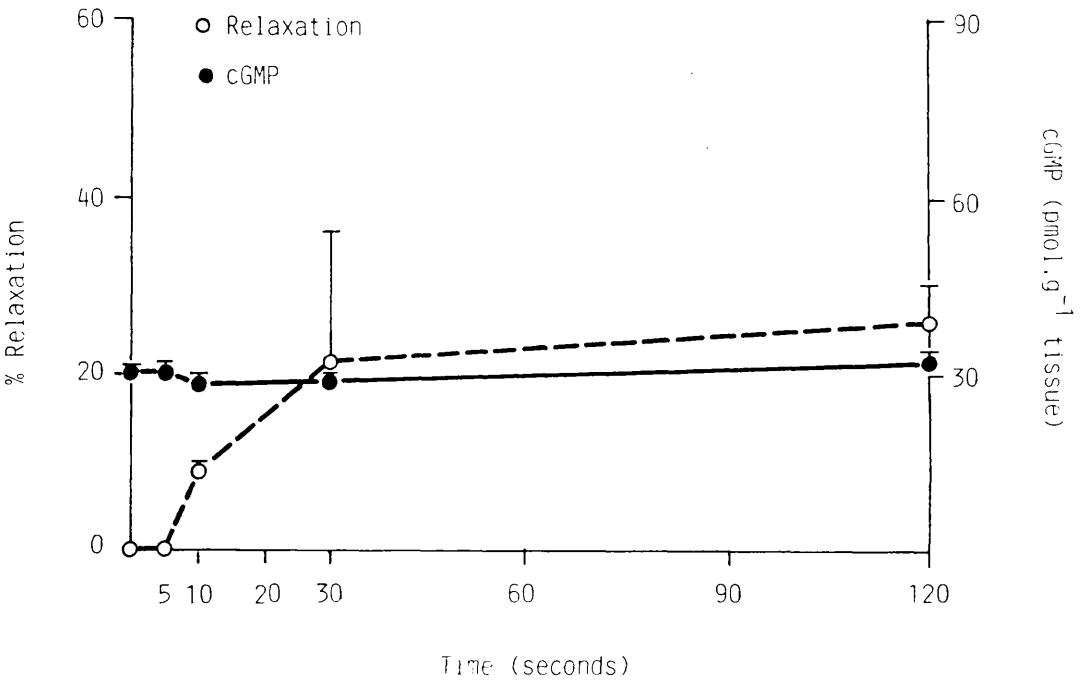


Figure 17.
Time-courses of vascular relaxation (○) and levels of cyclic GMP (●) induced by inhibitory factor (200µl of a standard preparation) in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M). The inhibitory factor induced a maximum relaxation of 25%. This effect was not associated with changes in the levels of cyclic GMP. ($n \leq 6$).

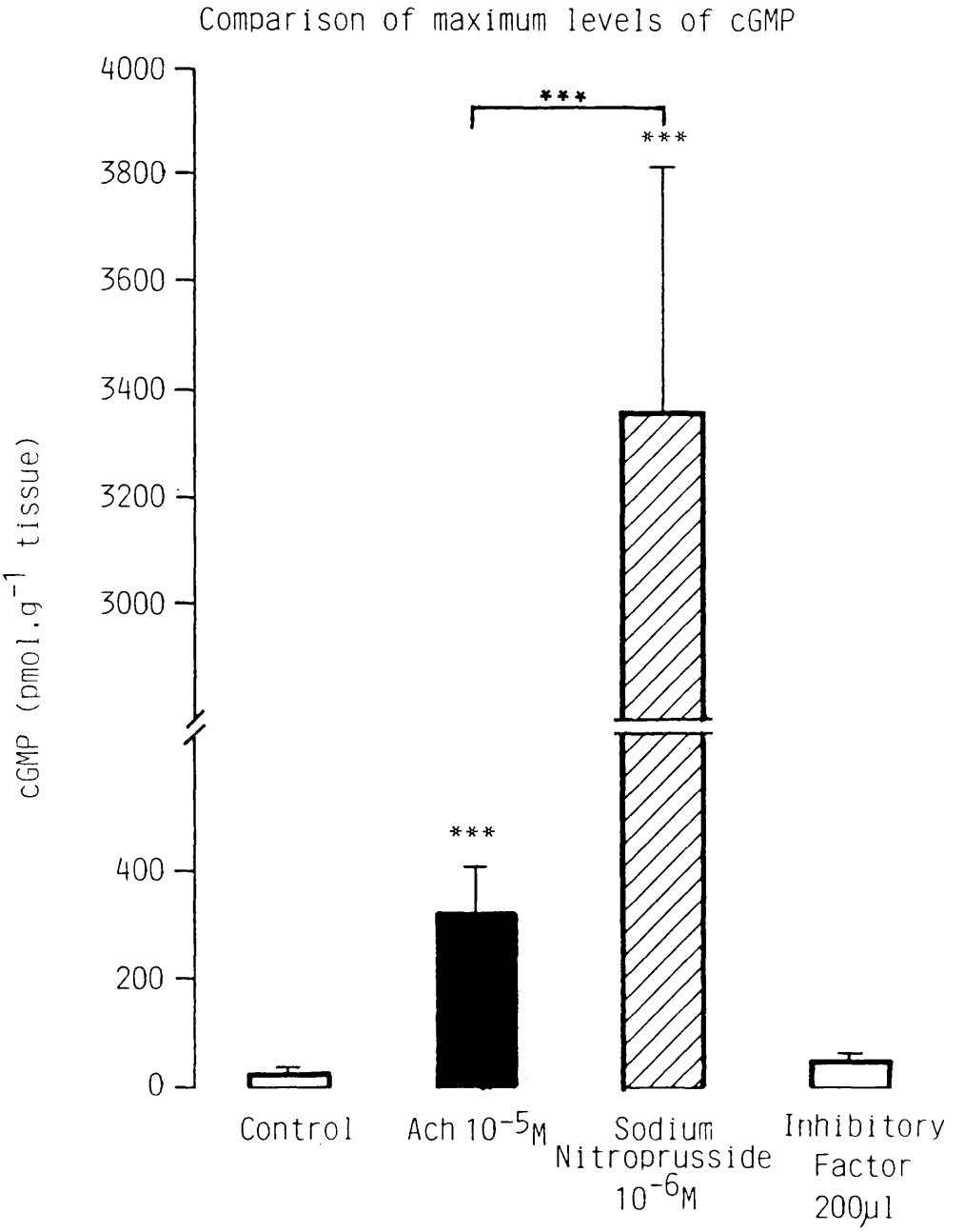


Figure 18.
Comparison of the maximum levels of cyclic GMP induced by acetylcholine (ACh) (10^{-5}M), inhibitory factor (200µl) and sodium nitroprusside (10^{-6}M). The levels of cyclic GMP induced by acetylcholine and sodium nitroprusside were significantly higher than those measured in control tissues (*** $p<0.001$). Sodium nitroprusside induced a significantly higher level of cyclic GMP than that induced by acetylcholine (*** $p<0.001$). ($n<6$).

Table 1.
Comparison of maximum degrees of relaxation and maximum levels of cyclic GMP induced by acetylcholine (ACh), sodium nitroprusside (SNP) and inhibitory factor (IF). Sodium nitroprusside (10^{-6}M) induced approximately the same degree of relaxation as that induced by acetylcholine (10^{-5}M). However, the levels of cyclic GMP induced by sodium nitroprusside were 10-fold higher than those induced by acetylcholine (*** $p<0.001$). ($n\leq 6$).

	MAXIMUM % RELAXATION	MAXIMUM CYCLIC GMP LEVELS
ACh(10^{-5}M)	96.8 ± 3.2	324.7 ± 435.3
SNP(10^{-6}M)	100.0 ± 0.9	3350.9 ± 437.5***
IF(200ul)	26.6 ± 3.8	32.6 ± 3.6

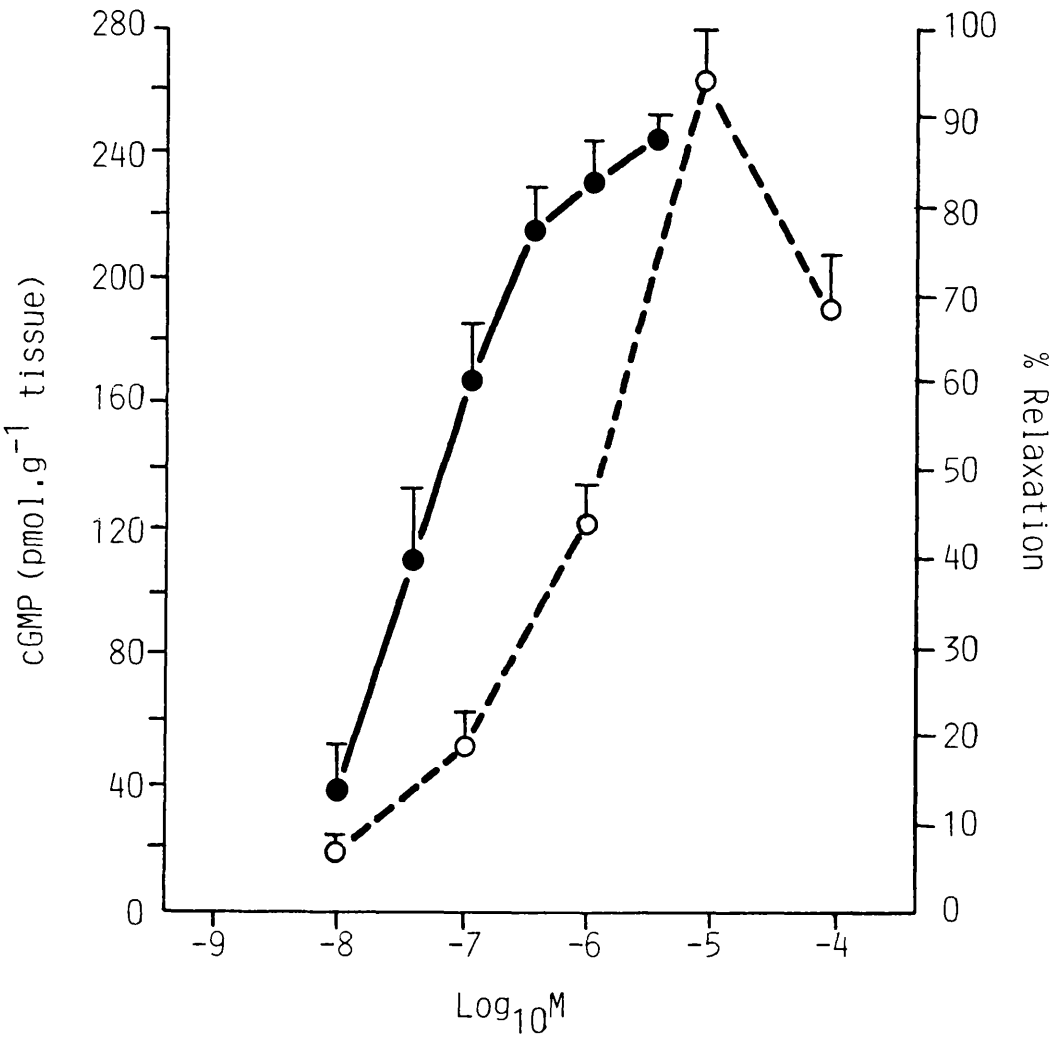


Figure 19.
Concentration-response graphs for acetylcholine in aortic rings in the presence of endothelium. The effects of acetylcholine on vascular relaxation (●) and levels of cyclic GMP (○) are shown. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6 x 10^{-8}M). (n≤6).

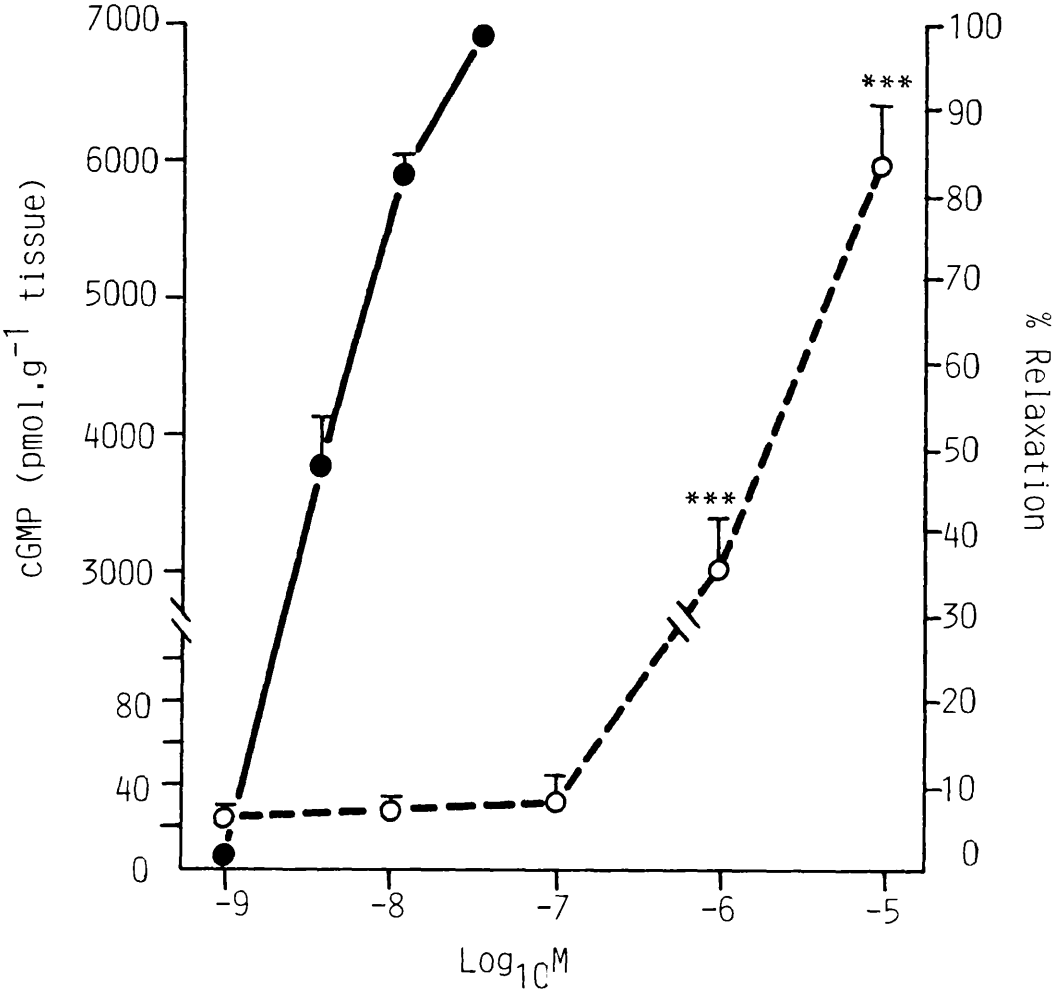


Figure 20.
Concentration-response graphs for sodium nitroprusside in aortic rings. The effects of sodium nitroprusside on vascular relaxation (●) and levels of cyclic GMP (○) are shown. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M). Sodium nitroprusside induced powerful relaxations at concentrations that did not significantly increase levels of cyclic GMP. ($n \leq 6$).

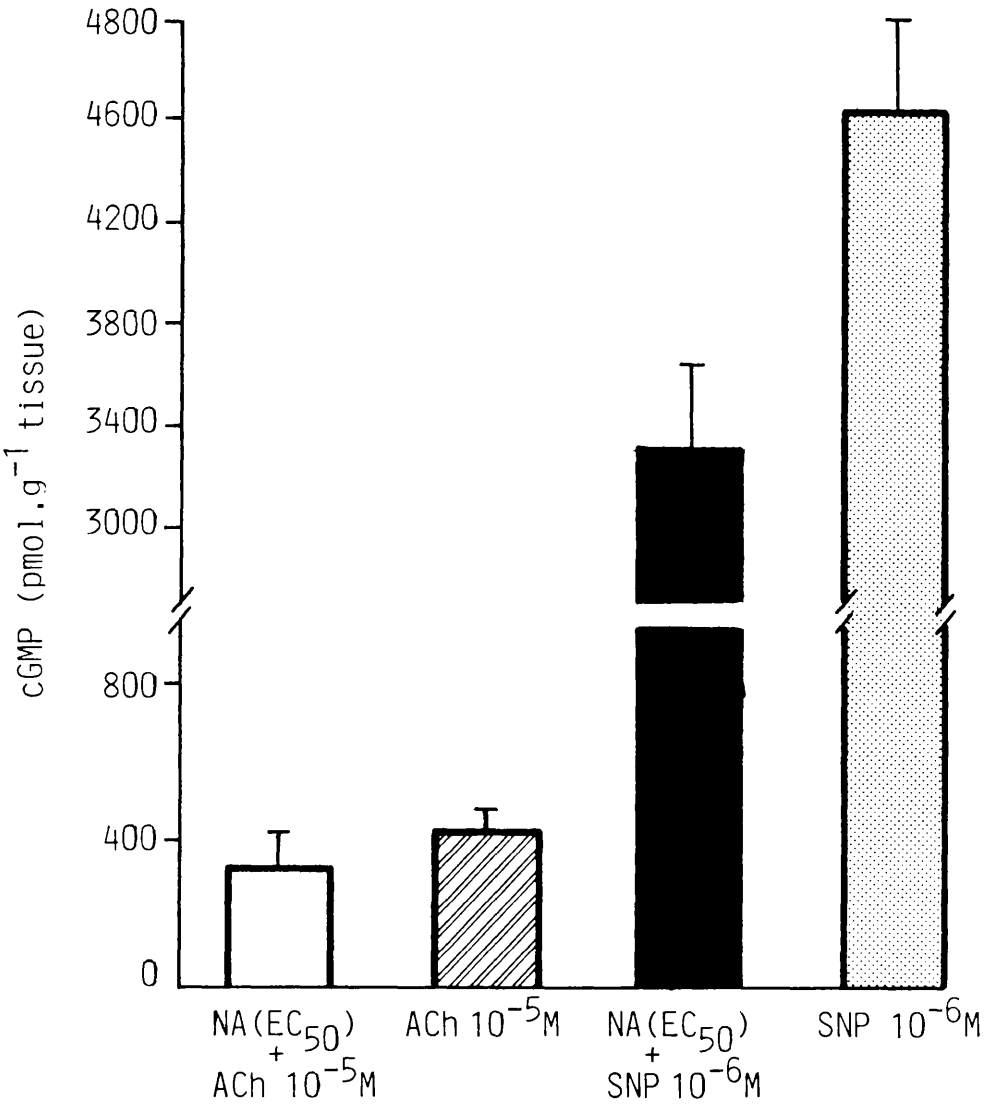


Figure 21.
Effects of acetylcholine (ACh) (10^{-5}M) and sodium nitroprusside (SNP) (10^{-6}M) on levels of cyclic GMP. Aortic rings were exposed to either acetylcholine or sodium nitroprusside alone, or after having been precontracted with noradrenaline (NA) (1.6×10^{-8}M). The abilities of acetylcholine and sodium nitroprusside to increase levels of cyclic GMP were not dependent on tissues being precontracted with noradrenaline. The endothelium was intact. (n=6).

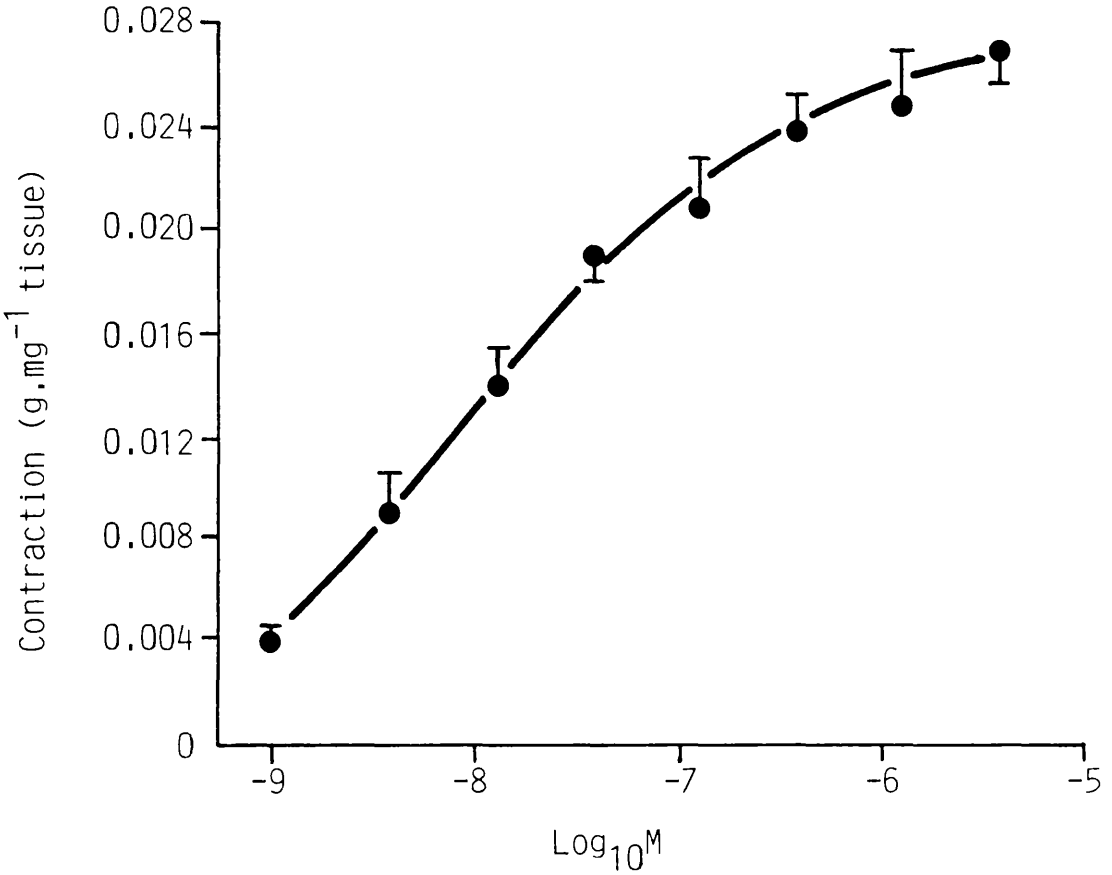


Figure 22.
Concentration-response graph for noradrenaline in aortic strips in the presence of endothelium. Muscle tension was recorded. (n=6).

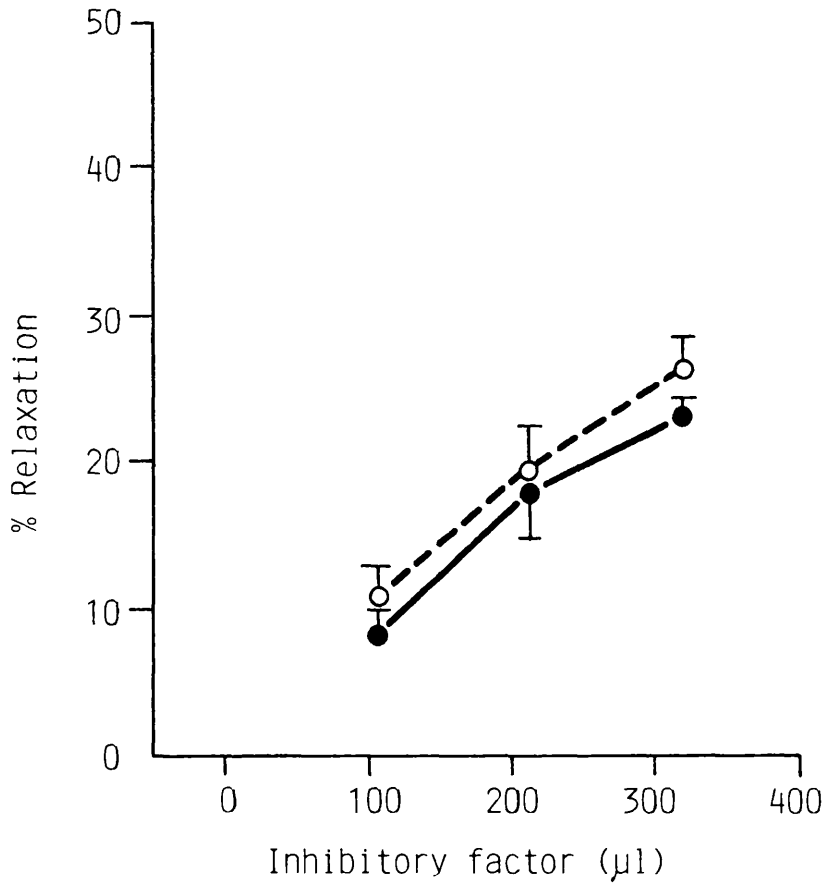


Figure 23a.
Concentration-response graphs for inhibitory factor in aortic rings (○) and aortic strips (●) in the presence of endothelium. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (EC_{50} concentrations). Inhibitory factor induced similar weak relaxations of both aortic rings and aortic strips. (n=6).

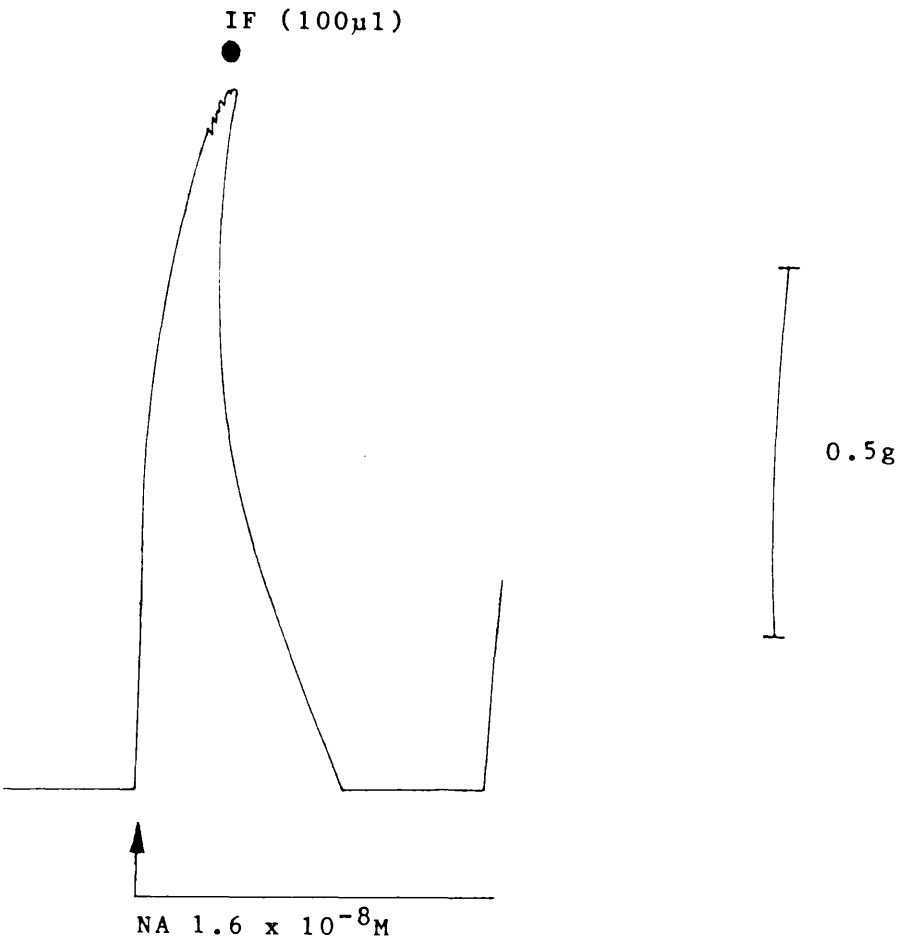


Figure 23b.
Typical mechanical response of rabbit aortic ring in the presence of endothelium. Muscle tone was raised with noradrenaline (1.6 x 10^{-8}M). Inhibitory factor (IF) (100µl) induced complete inhibition of muscle tone.

Section Two: Effects of contractile agents on the degree of vascular smooth muscle relaxation

In this series of experiments, the effects of the contractile agents used to raise muscle tone on the degree of vascular relaxation were examined. Three parameters were measured: (i) the degree of smooth muscle relaxation; (ii) the tissue levels of cyclic GMP induced by the vasorelaxants; and (iii) the rates of phosphatidylinositol (Ptd Ins) hydrolysis associated with drug-induced muscle tone.

Part One: Preliminary experiments on vascular smooth muscle contractions induced by KCl

KCl induced concentration-dependent contractions of aortic rings in both the presence and absence of endothelium (figures 24 and 25). KCl induced muscle contractions over a narrower concentration range, and at higher concentrations, than noradrenaline (figure 24). In the presence of endothelium, the EC_{50} concentration of KCl for muscle contraction was determined to be 20mM (figure 24). Also, the EC_{70} concentration of KCl was determined to be 30mM. Removal of the endothelium did not significantly alter KCl-induced contractions of aortic rings (figure 25).

In general, prazosin (10^{-6}M) did not affect KCl-induced contractions of aortic rings (figure 26). However, prazosin did significantly reduce KCl-induced contractions at the highest concentration of KCl (90mM). Tissues were exposed to prazosin (10^{-6}M) for 10 minutes prior to the addition of KCl.

The time-course of muscle contraction induced by KCl (20mM) is shown in figure 27. Muscle tone stabilised after 5mins..

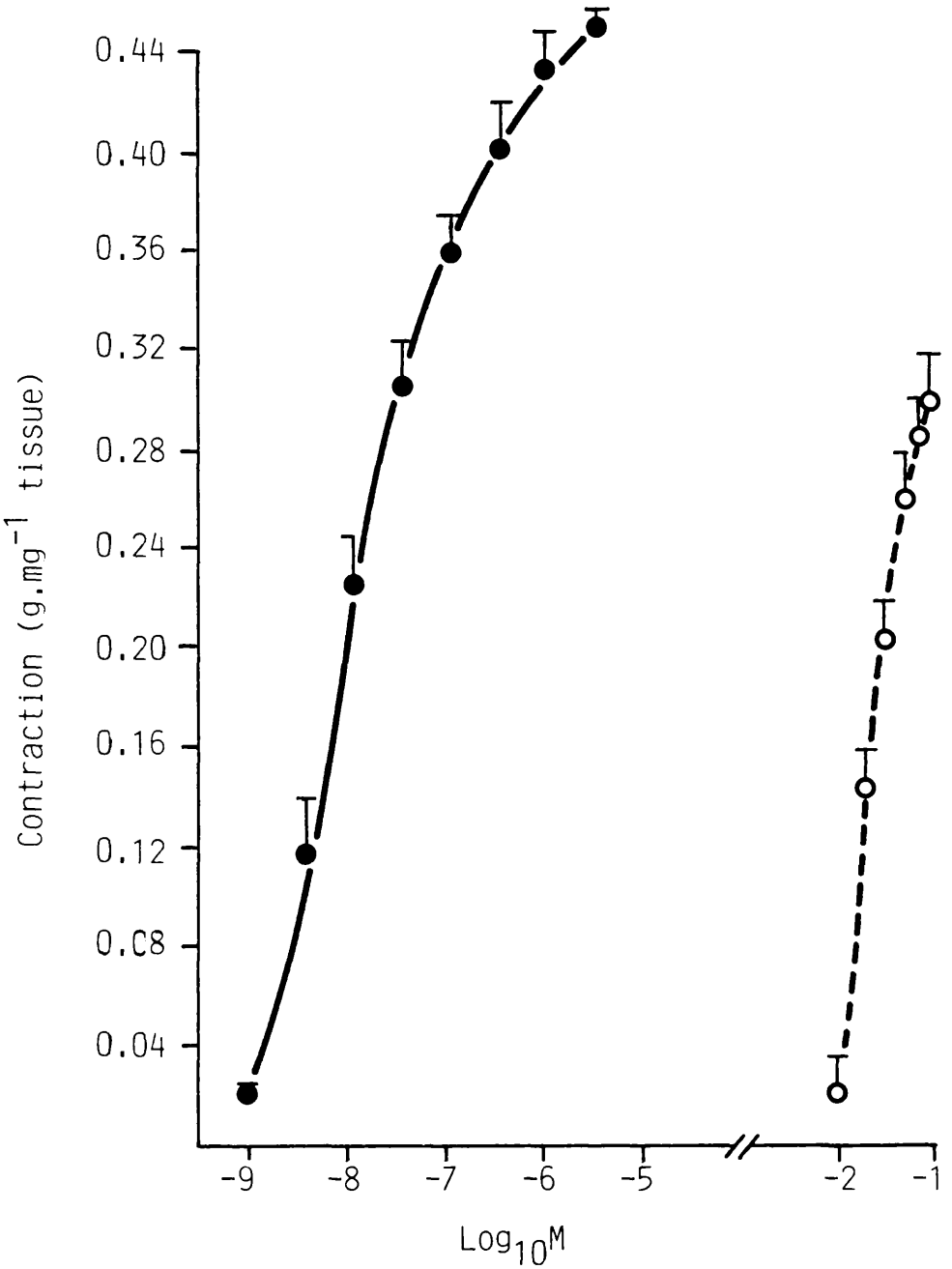


Figure 24.
Concentration-response graph for KCl (○) in aortic rings in the presence of endothelium. Muscle tension was recorded. The effect of noradrenaline (●) is shown for comparison. (n=6).

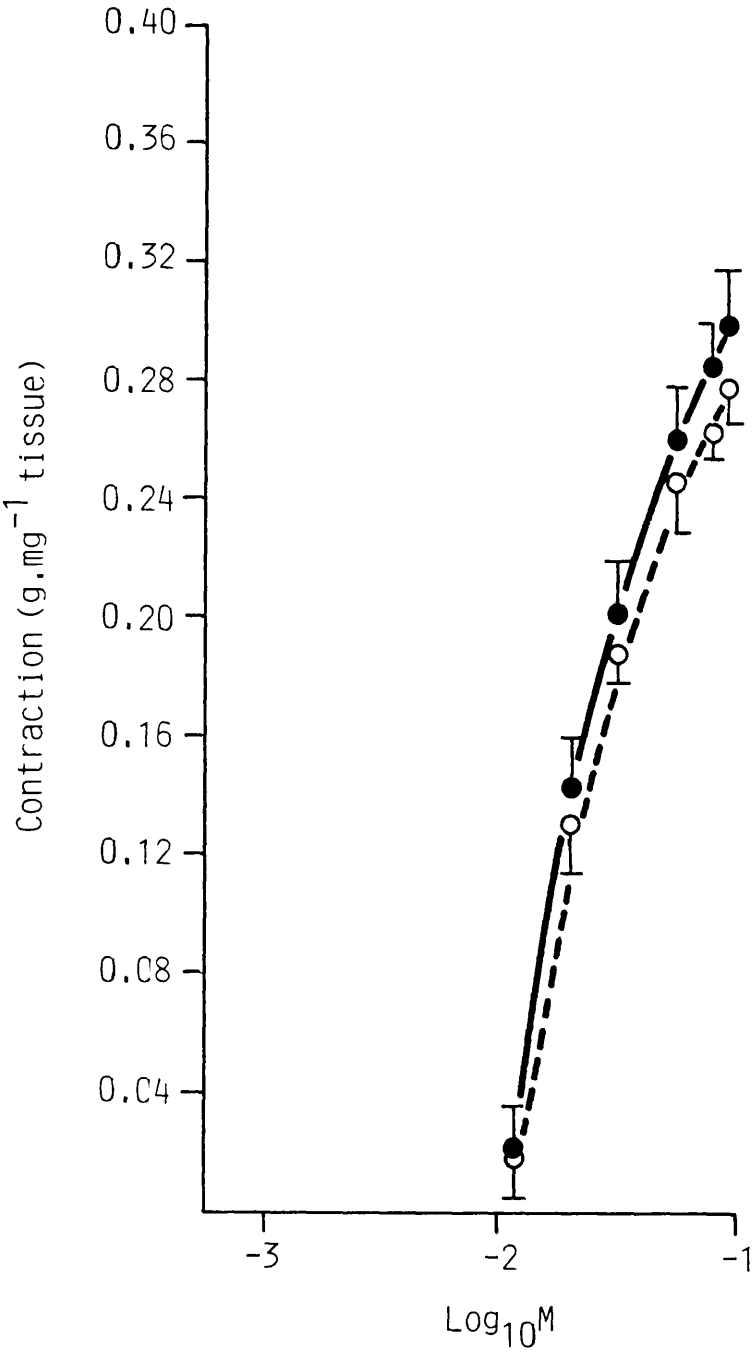


Figure 25.
Concentration-response graphs for KCl in aortic rings in the presence (●) and absence (○) of endothelium. Muscle tension was recorded. KCl-induced contractions of aortic rings were similar in the presence or absence of endothelium. (n=6).

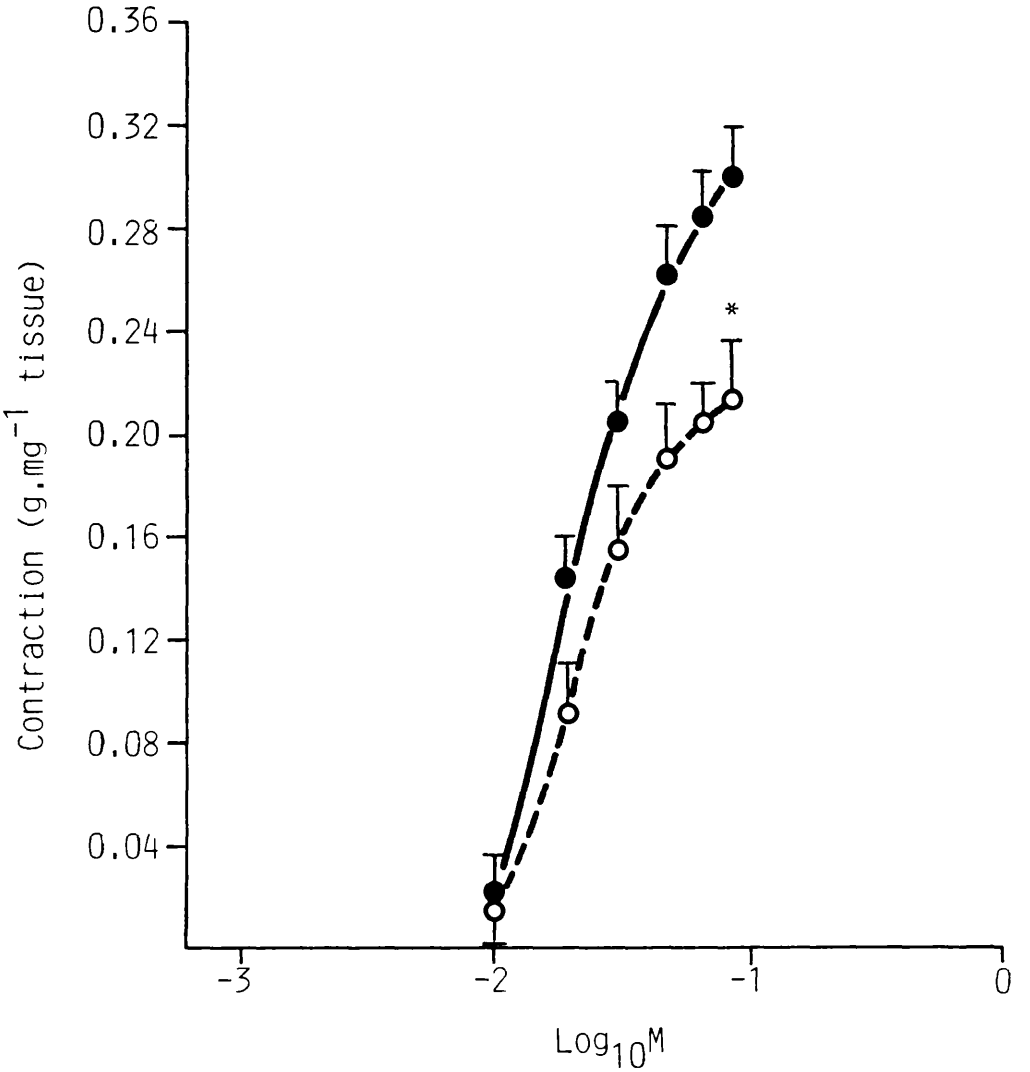


Figure 26.
Concentration-response graphs for KCl in aortic rings in the presence (○) and absence (●) of prazosin (10^{-6}M). Muscle tension was recorded. Prazosin only significantly reduced muscle contraction induced by the highest concentration of KCl. (* $p<0.05$). The endothelium was intact. (n=6).

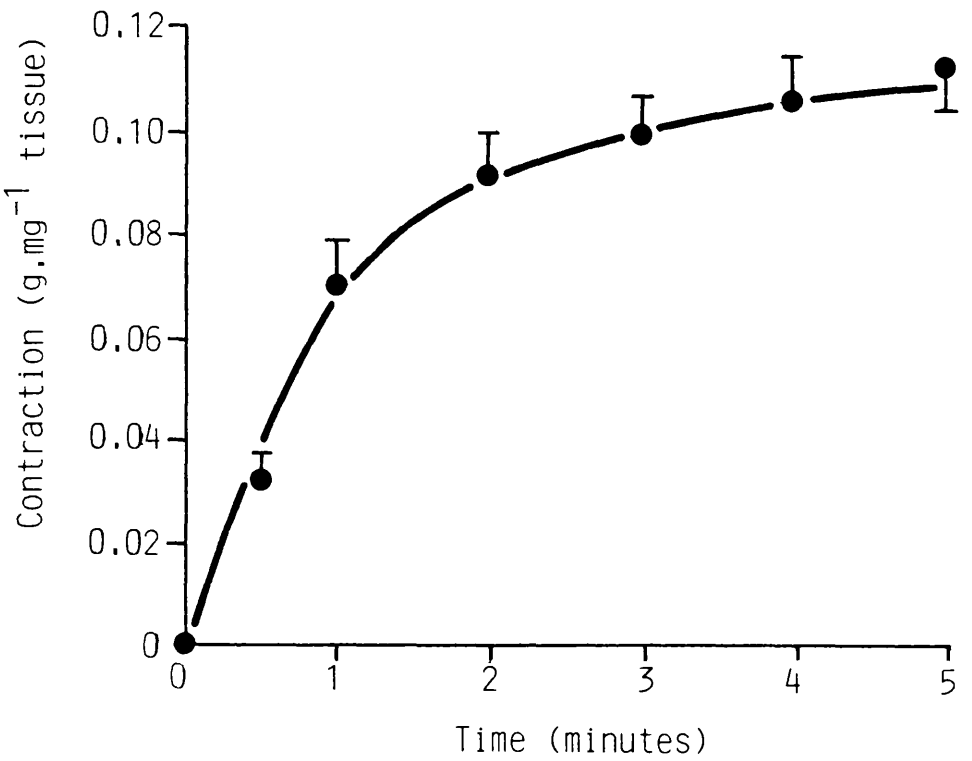


Figure 27.
Time-course of KCl-induced contraction of aortic rings in the presence of endothelium. The EC_{50} concentration of KCl was used (20mM). Muscle tone stabilised after 5mins.. (n=6).

Part Two: Vascular smooth muscle relaxations of aortic rings precontracted with KCl

Smooth muscle relaxations induced by acetylcholine and sodium nitroprusside were studied in aortic rings precontracted with KCl (20mM) for 5mins., in the presence of endothelium (figure 28).

Vascular relaxations induced by acetylcholine and sodium nitroprusside are shown quantitatively in concentration-response graphs (figures 29 and 30).

Acetylcholine and sodium nitroprusside induced significantly weaker relaxations of aortic rings precontracted with KCl (20mM) than of aortic rings precontracted with noradrenaline (1.6×10^{-8}M) in figures 29 and 30.

KCl (20mM; 5mins.) did not significantly alter levels of cyclic GMP from those measured in control tissues exposed to saline (5mins.), both in the presence and absence of endothelium (figure 31).

Acetylcholine (10^{-5}M; 30s.) significantly increased levels of cyclic GMP in aortic rings precontracted with noradrenaline (1.6×10^{-8}M; 5mins.), but not in tissues precontracted with KCl (20mM; 5mins.) (figure 32).

The level of cyclic GMP measured in tissues precontracted with KCl (20mM; 5mins.), then relaxed by acetylcholine (10^{-5}M; 30s.) was significantly smaller than in tissues precontracted with noradrenaline (1.6×10^{-8}M; 5mins.) alone (figure 32).

Sodium nitroprusside (10^{-6}M; 60s.) significantly increased levels of cyclic GMP both in aortic rings precontracted with noradrenaline (1.6×10^{-8}M; 5mins.) and aortic rings precontracted with KCl (20mM; 5mins) (figure 33).

The level of cyclic GMP induced by sodium nitroprusside (10^{-6}M; 60s.) was significantly smaller in tissues precontracted with KCl (20mM; 5mins.) than in tissues precontracted with noradrenaline (1.6 x 10^{-8}M; 5mins.) (figure 33).

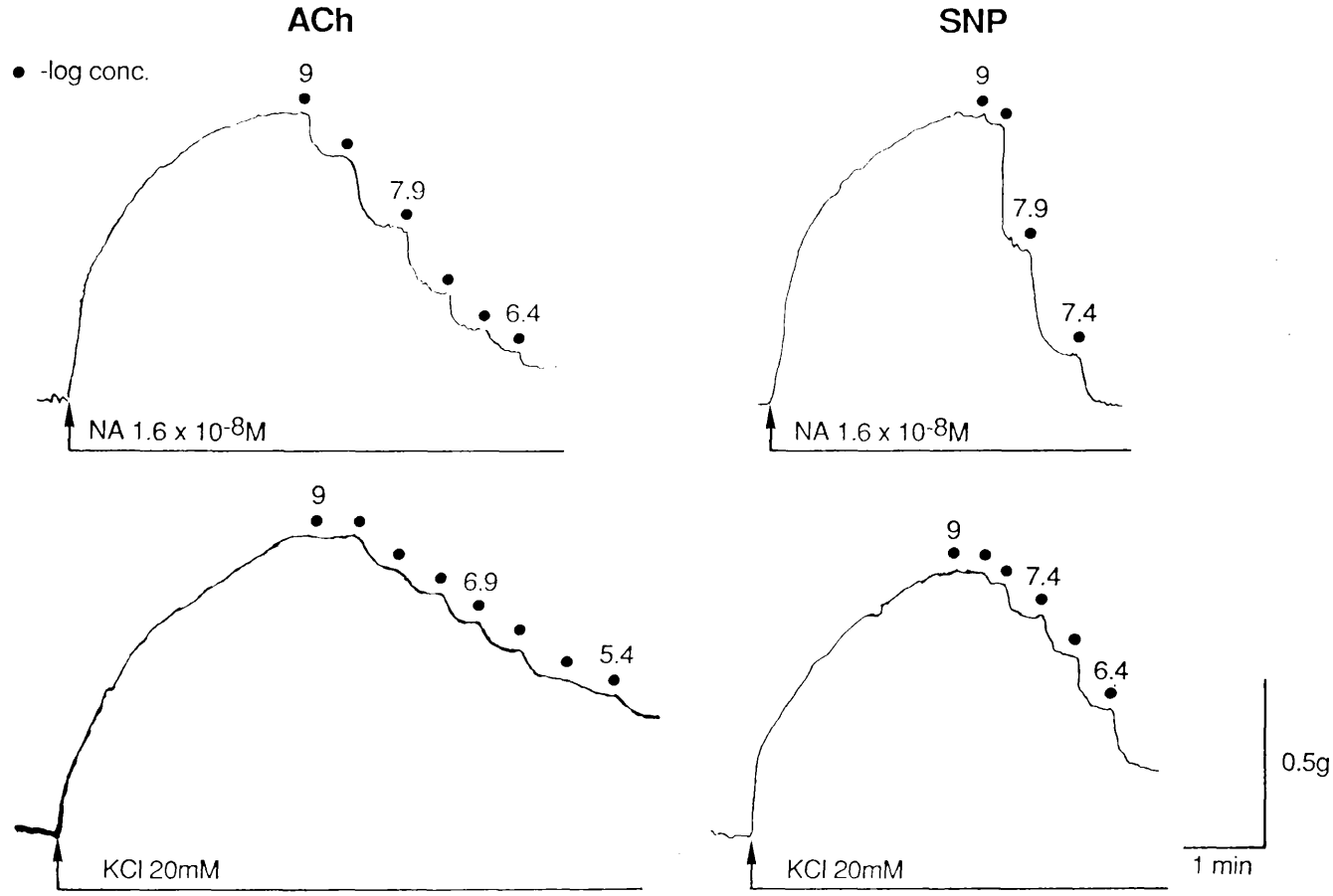


Figure 28.
Mechanical responses of aortic rings in the presence of endothelium. Muscle tone was raised with noradrenaline (NA) (EC_{50}: 1.6 x 10^{-8}M) (upper trace) or KCl (EC_{50}: 20mM) (lower trace). Acetylcholine (ACh) and sodium nitroprusside (SNP) were added successively to the organ baths, producing cumulative concentration-response graphs. Relaxations were greater in tissues precontracted with noradrenaline.

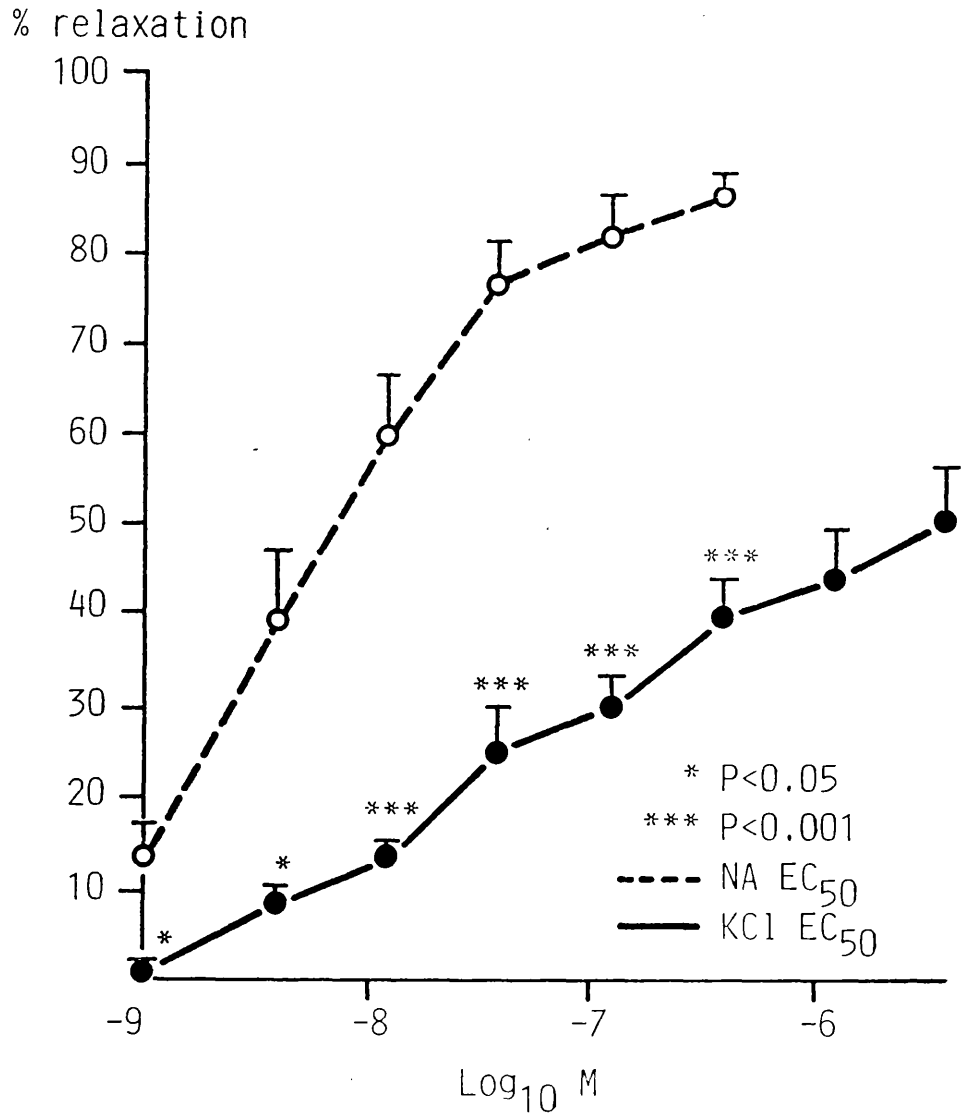


Figure 29.
Concentration-response graphs for acetylcholine in aortic rings in the presence of endothelium. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M) (○), or KCl (20mM) (●). Acetylcholine induced significantly weaker relaxations of aortic rings precontracted with KCl than of aortic rings precontracted with noradrenaline. (* $p<0.05$, *** $p<0.001$). (n=6).

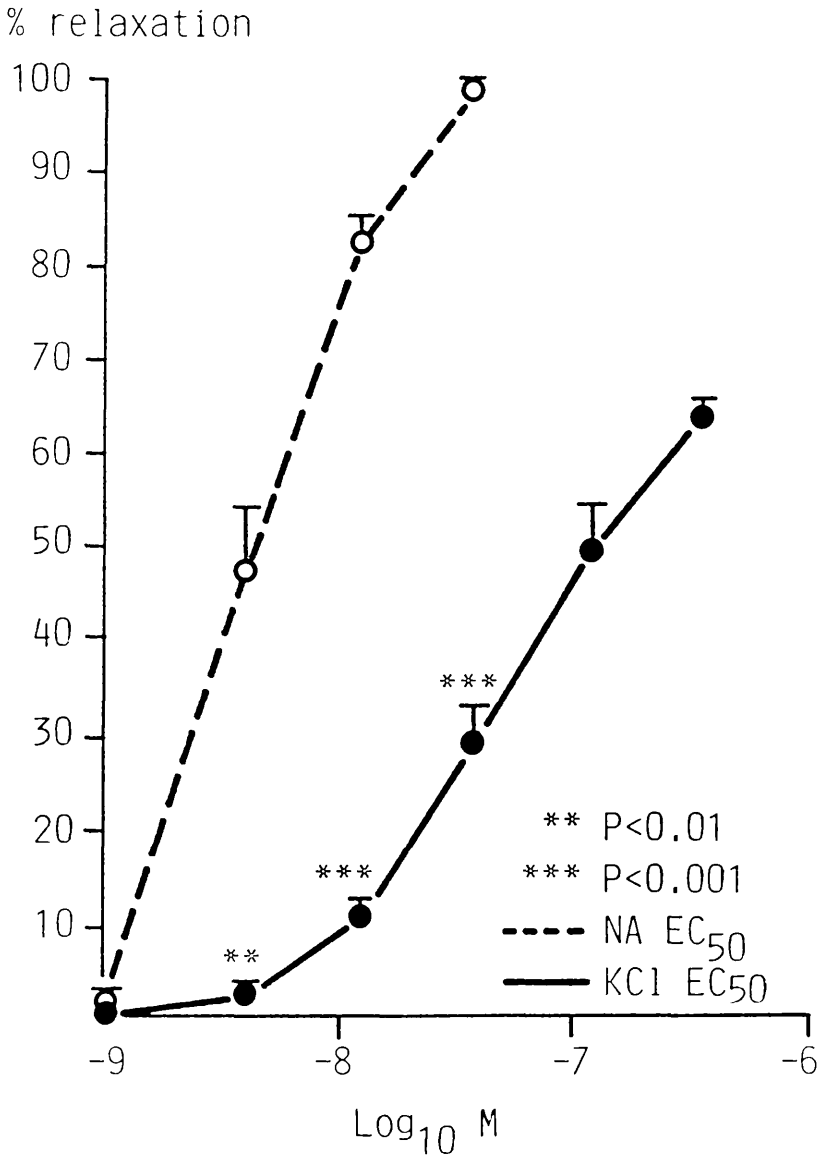


Figure 30.
Concentration-response graphs for sodium nitroprusside in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (1.6×10^{-8}M) (O) or KCl (20mM) (●). Sodium nitroprusside induced significantly weaker relaxations of aortic rings precontracted with KCl than of aortic rings precontracted with noradrenaline. (** $p<0.01$, *** $p<0.001$). (n=6).

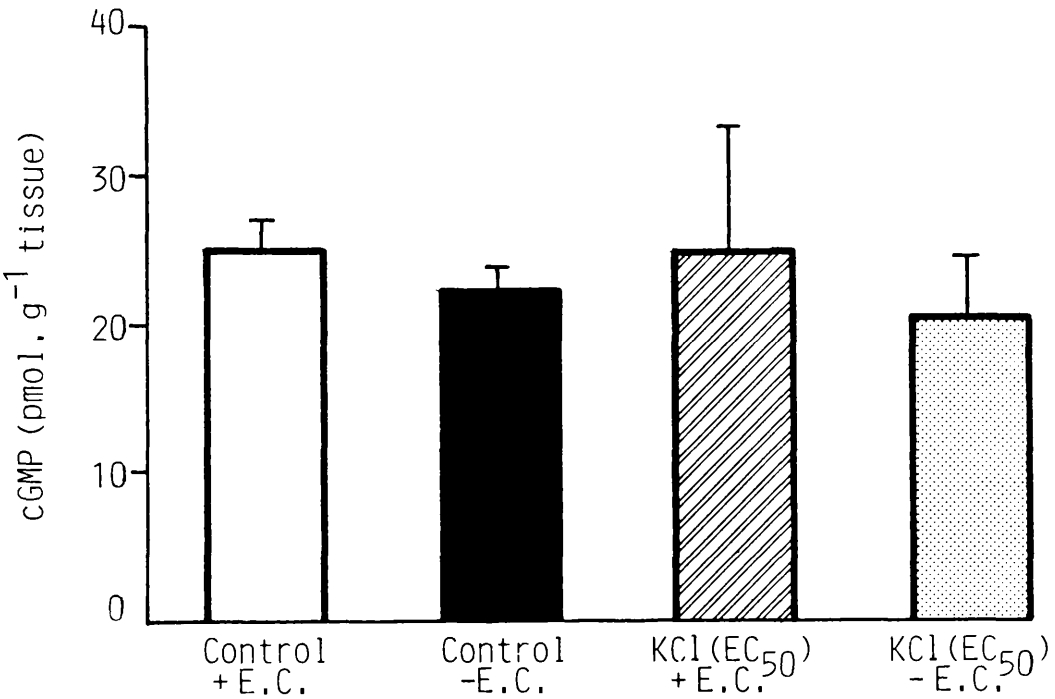


Figure 31.
Effect of KCl on levels of cyclic GMP in presence (+EC) and absence (-EC) of endothelium. Control tissues were exposed to saline (5mins.) and test tissues to KCl (5mins.). KCl did not significantly alter levels of cyclic GMP. (n=6).

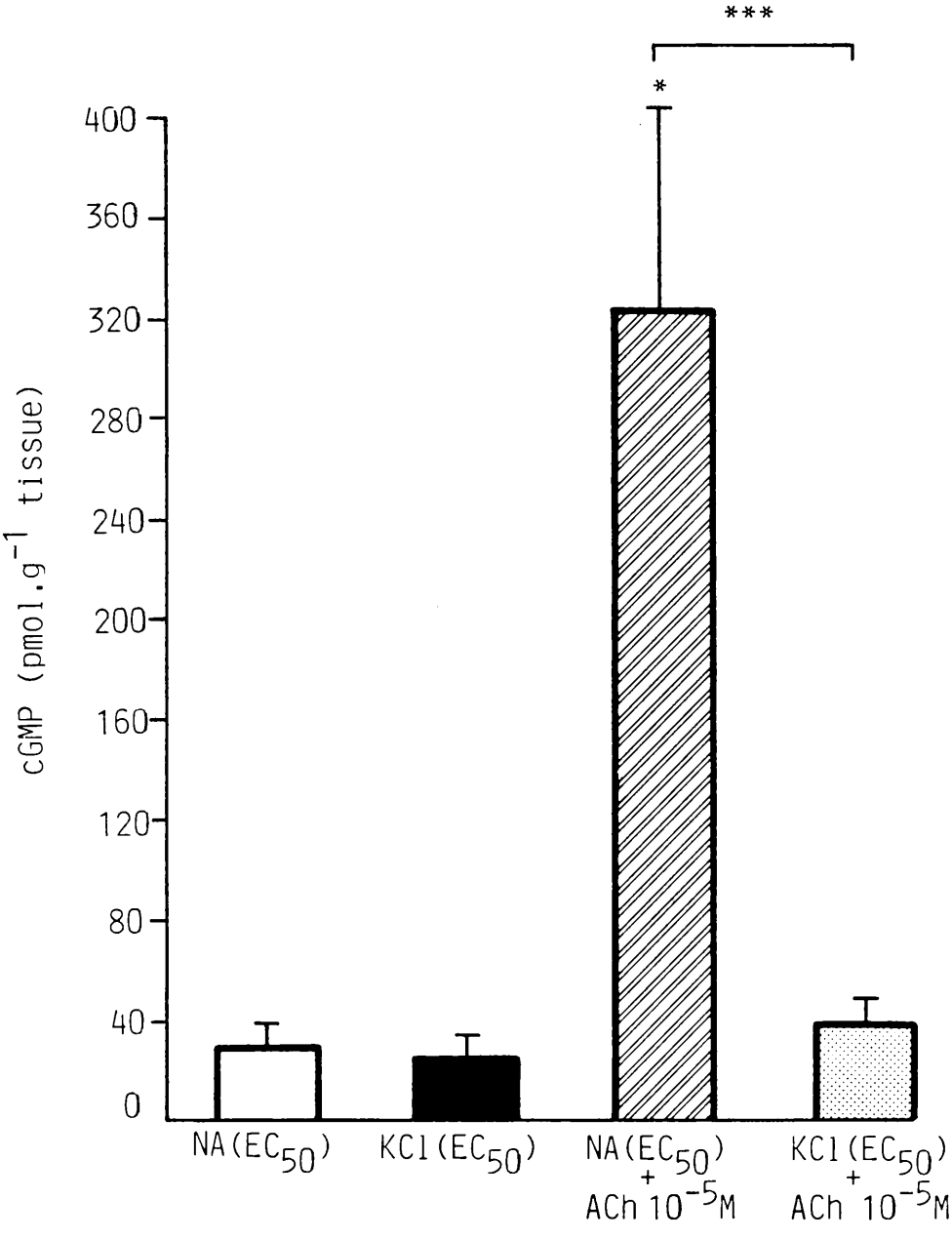


Figure 32.
Effects of acetylcholine (ACh) (10^{-5}M) on levels of cyclic GMP in aortic rings precontracted with noradrenaline (NA) (1.6×10^{-8}M) or KCl (20mM). Tissues were exposed to noradrenaline (5mins.), or KCl (5mins.), or noradrenaline (5mins.) followed by acetylcholine (30s.), or KCl (5mins.) followed by acetylcholine (30s.). Noradrenaline and KCl alone did not induce high levels of cyclic GMP. Acetylcholine significantly increased levels of cyclic GMP only in tissues precontracted with noradrenaline (* $p<0.05$). The level of cyclic GMP induced by acetylcholine was significantly smaller in tissues precontracted with KCl than in tissues precontracted with noradrenaline. (*** $p<0.001$). The endothelium was intact. (n=6).

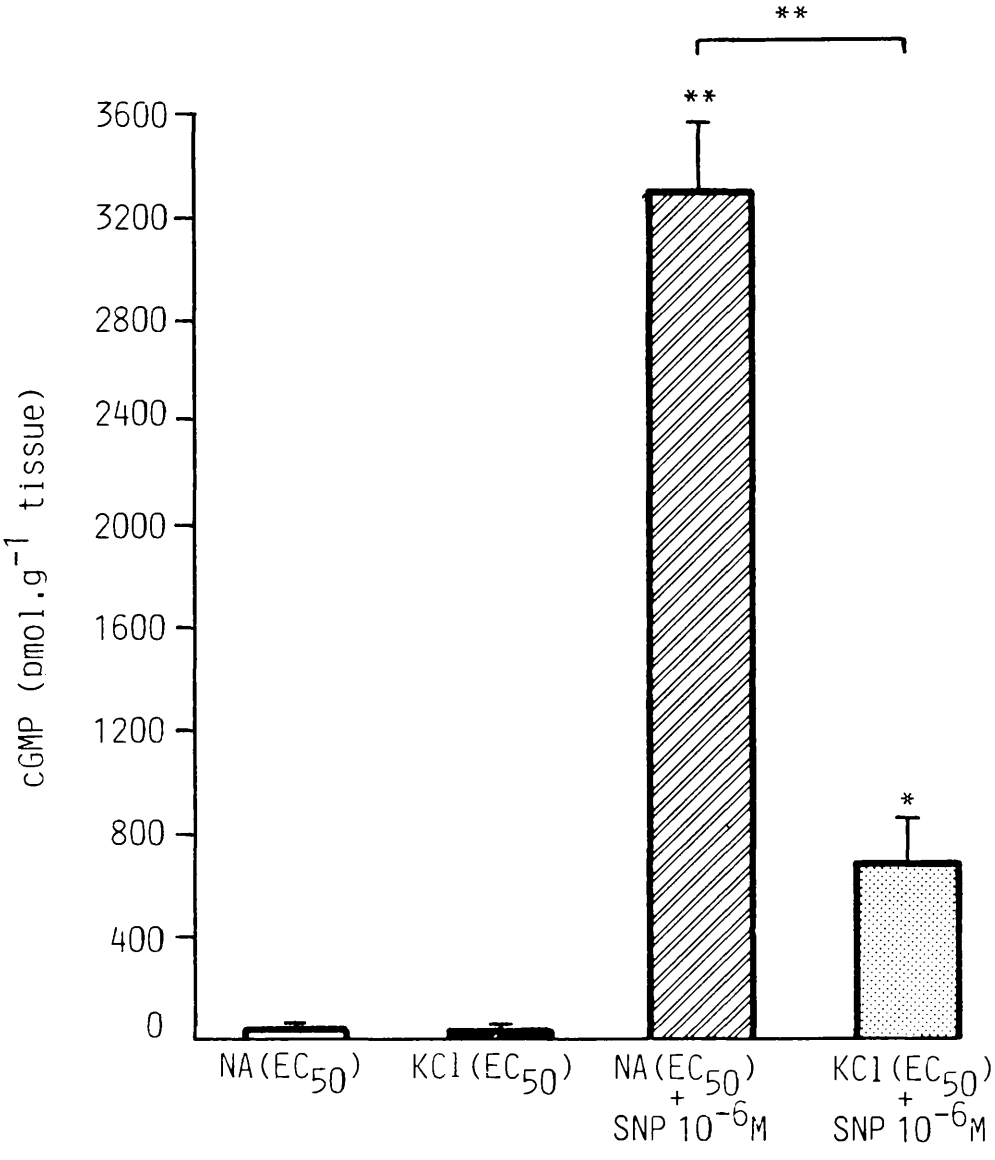


Figure 33.
Effects of sodium nitroprusside (SNP) (10^{-6}M) on levels of cyclic GMP in aortic rings precontracted with noradrenaline (NA) (1.6 x 10^{-8}M) or KCl (20mM). Tissues were exposed to noradrenaline (5mins.), or KCl (5mins.), or noradrenaline (5mins.) followed by sodium nitroprusside (60s.), or KCl (5mins.) followed by sodium nitroprusside (60s.). Noradrenaline and KCl alone did not induce high levels of cyclic GMP. Sodium nitroprusside significantly increased levels of cyclic GMP (* $p<0.05$, ** $p<0.01$). The increase in the level of cyclic GMP induced by sodium nitroprusside was significantly smaller in tissues precontracted with KCl than in tissues precontracted with noradrenaline (** $p<0.01$). (n=6).

Part Three: Effects of acetylcholine and sodium nitroprusside on the rates of Ptd Ins hydrolysis associated with smooth muscle contractions of aortic rings

The time-course of noradrenaline-induced formation of Ptd OH is shown in figure 34. Noradrenaline (10^{-4}M) induced an initial rapid formation of Ptd OH. The levels of Ptd OH stabilised after 5mins.

Noradrenaline induced a concentration-dependent increase in the levels of Ptd OH, both in the presence and absence of endothelium (figure 35). At any one concentration of noradrenaline, removal of the endothelium did not significantly increase the levels of Ptd OH (figure 35).

Removal of Ca^{2+} (2.5mM) from the bathing medium, Krebs bicarbonate buffer, did not significantly alter the levels of Ptd OH measured in control tissues exposed to saline (5mins.) or in test tissues exposed to noradrenaline (2×10^{-7}M; 5mins.) (figure 36). Noradrenaline (2×10^{-7}M; 5mins.) induced a significant increase in the levels of Ptd OH above the control values, both in the presence and absence of Ca^{2+} (2.5mM).

Acetylcholine (10^{-5}M; 30s.) significantly reduced the level of Ptd OH below that induced by noradrenaline (2×10^{-7}M; 5mins.) whether added after or before noradrenaline (figure 37).

Sodium nitroprusside (10^{-6}M; 60s.) significantly reduced the level of Ptd OH below that induced by noradrenaline (2×10^{-7}M; 5mins.) whether added after or before noradrenaline (figure 38).

KCl (30mM; 5mins.) did not significantly increase the level

of Ptd OH above that measured in control tissues exposed to saline (5mins.) (figure 39).

Acetylcholine (10^{-5}M; 30s.) and sodium nitroprusside (10^{-6}M; 60s.) respectively, did not significantly alter the levels of Ptd OH measured in aortic rings exposed to KCl (30mM; 5mins.) whether added after or before KCl (figure 39).

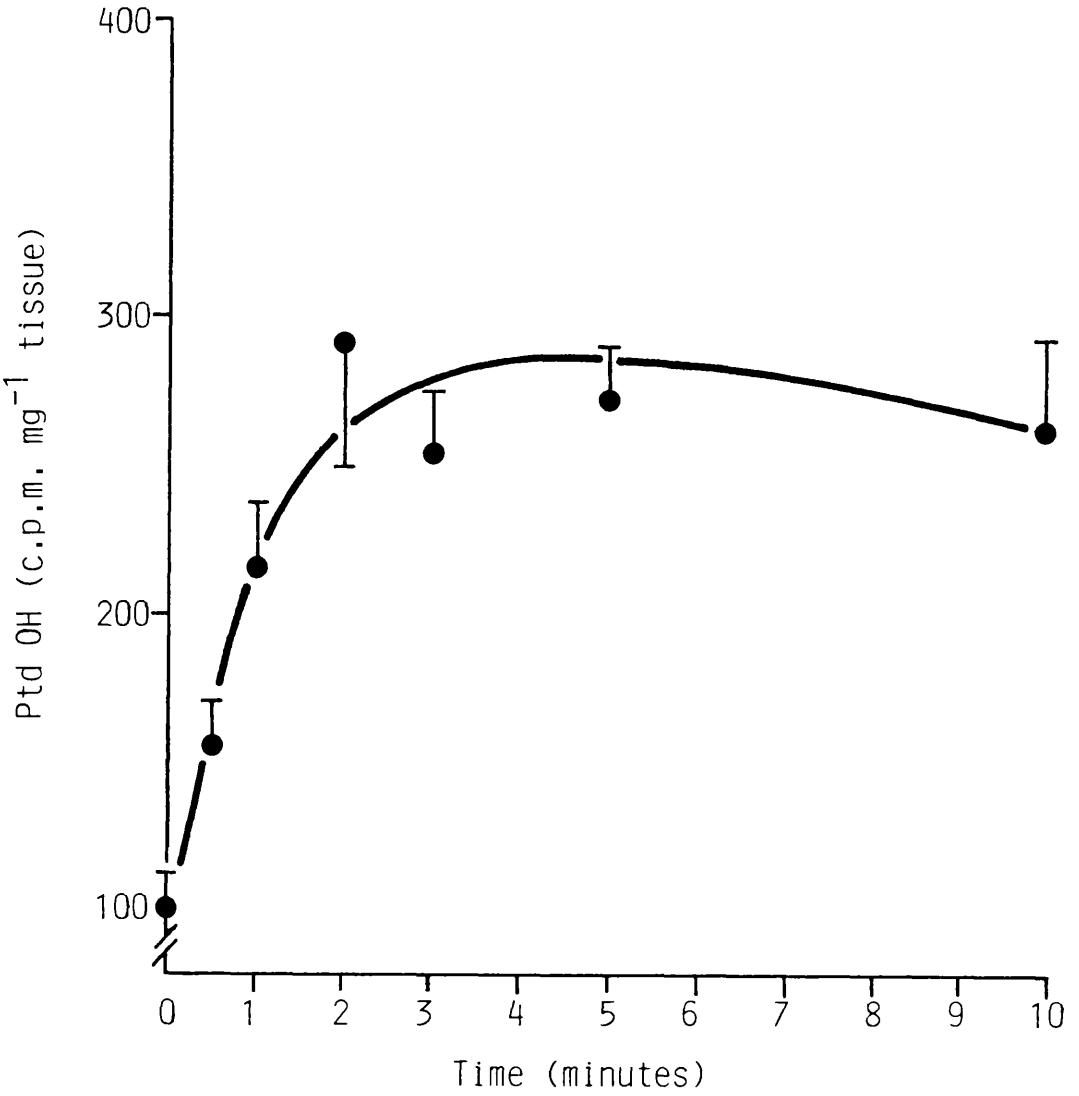


Figure 34.
Time-course of formation of Ptd OH induced by noradrenaline (10^{-4}M) in aortic rings in the presence of endothelium. Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. The level of Ptd OH stabilised after 5mins.. (n=4).

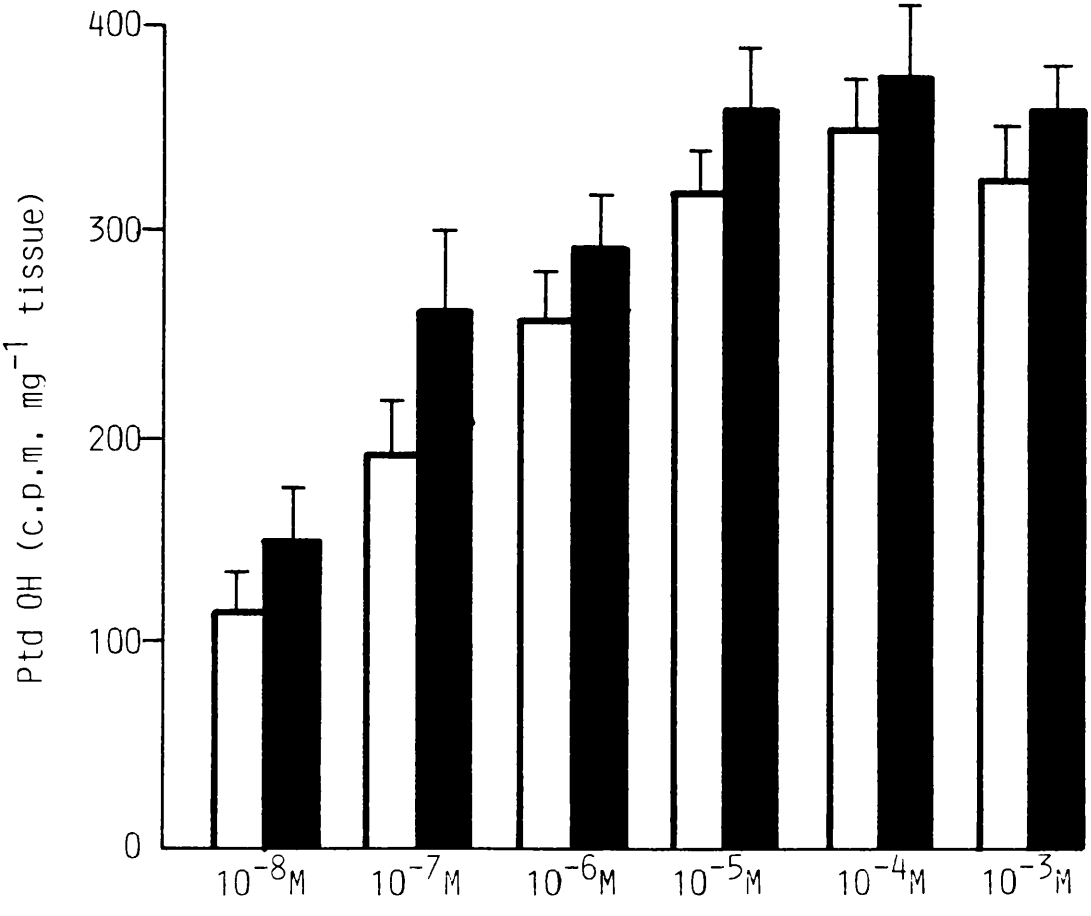


Figure 35.
Concentration-response histogram for noradrenaline in the presence (unshaded) and absence (shaded) of endothelium. Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Tissues were exposed to noradrenaline for 5mins.. Noradrenaline induced a concentration-dependent increase in the levels of Ptd OH. At any one concentration of noradrenaline, removal of endothelium did not significantly increase the level of Ptd OH. (n=4).

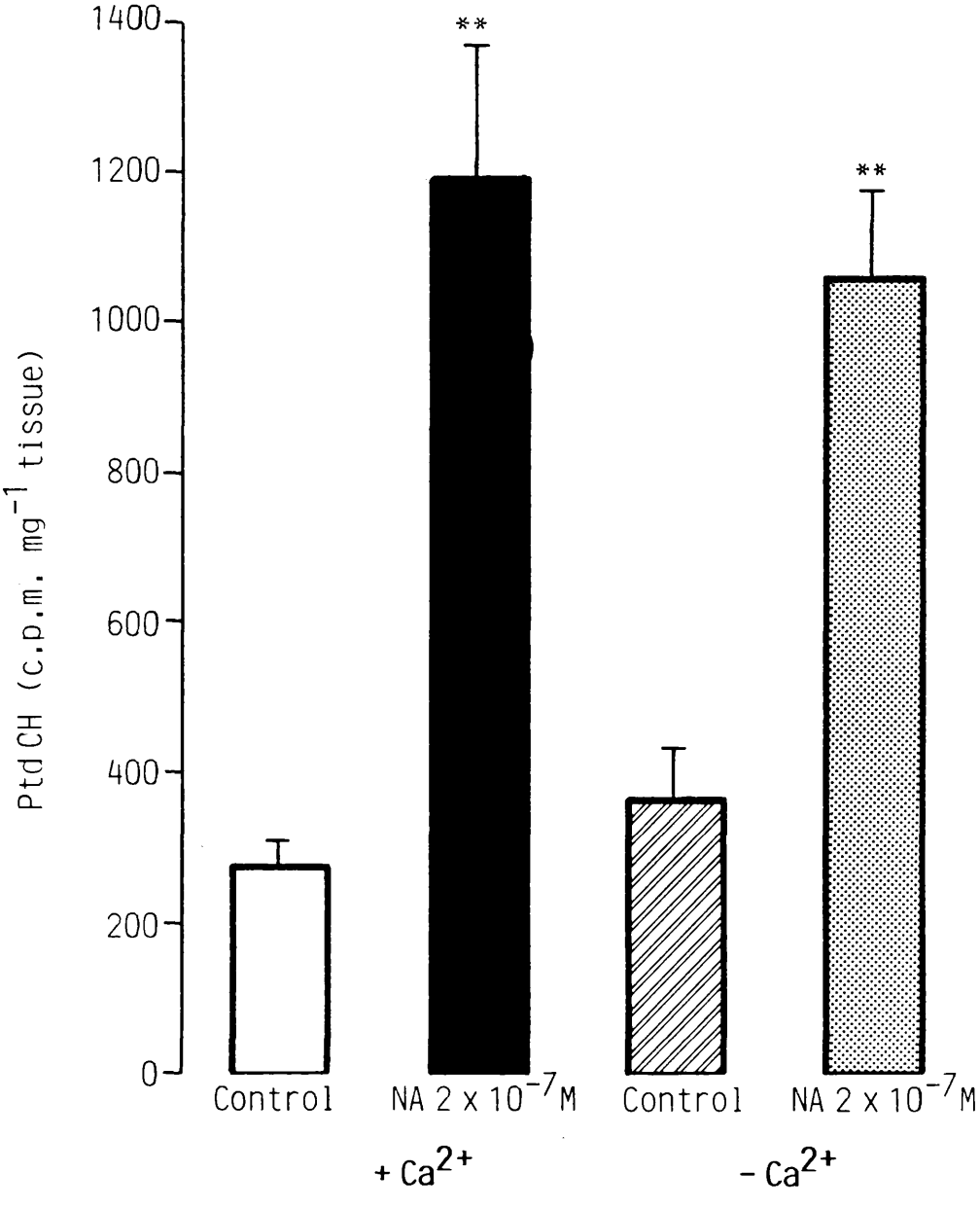


Figure 36.
Effects of noradrenaline (EC_{70}: 2 x 10^{-7}M) on the rate of Ptd Ins hydrolysis in the presence and absence of Ca^{2+} (2.5mM). Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Tissues were exposed to noradrenaline for 5mins.. Both in the presence and absence of Ca^{2+}, noradrenaline induced significant increases in the levels of Ptd OH above control values. Removal of Ca^{2+} from the bathing medium did not significantly alter the control, or noradrenaline-induced Ptd OH levels. (** $p<0.01$). (n=4).

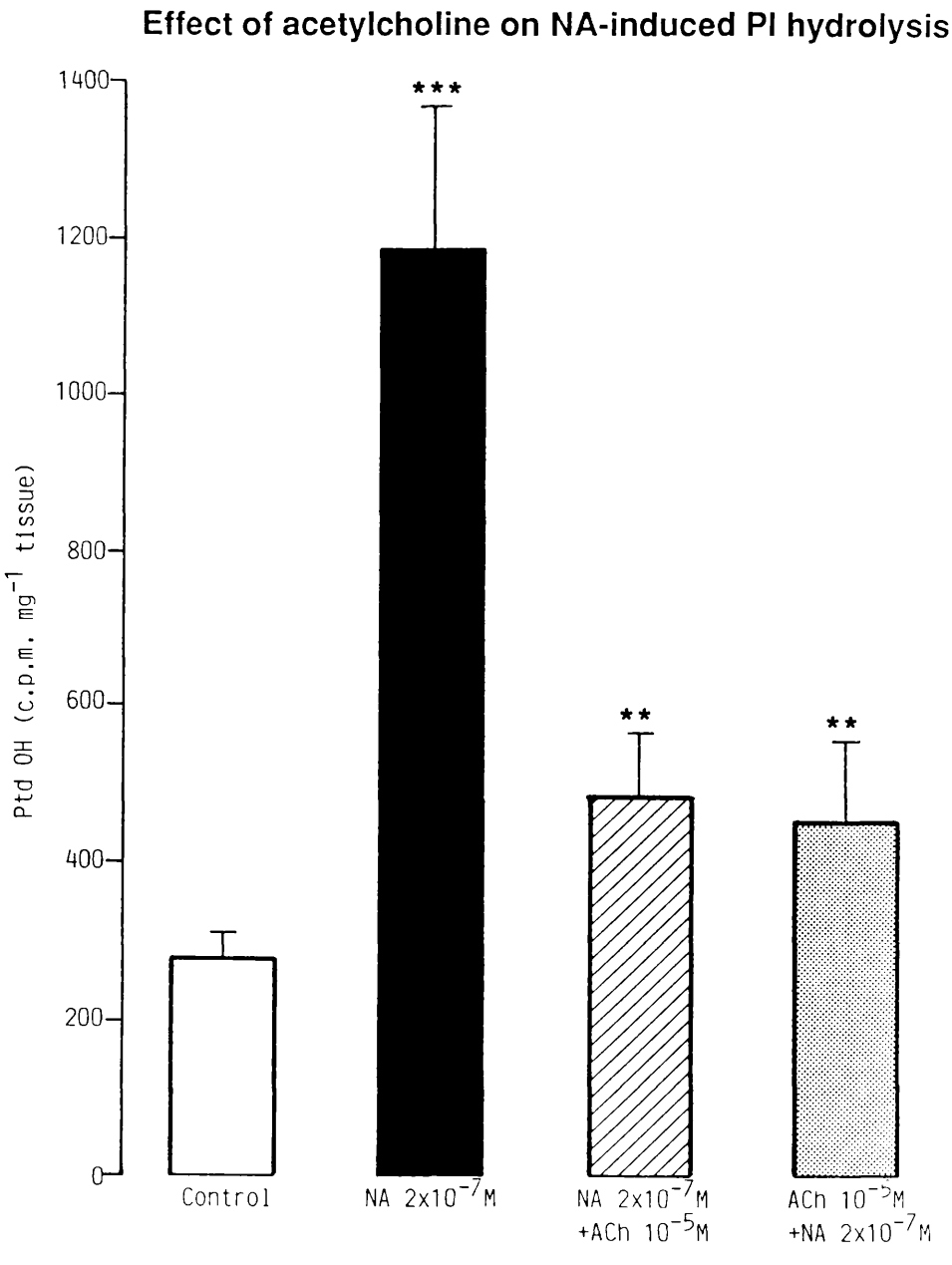


Figure 37.
Effect of acetylcholine (ACh) (10^{-5}M) on the rate of Ptd Ins hydrolysis induced by noradrenaline (NA) (2 x 10^{-7}M). Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Control tissues were exposed to saline (5mins.), and test tissues to noradrenaline (5mins.) or noradrenaline (5mins.) and acetylcholine (30sec.). Noradrenaline alone significantly increased the level of Ptd OH above the control value (*** $p<0.001$). Acetylcholine, whether added after or before noradrenaline, significantly reduced the level of Ptd OH below that induced by noradrenaline alone. (** $p<0.01$). The endothelium was intact. (n=4).

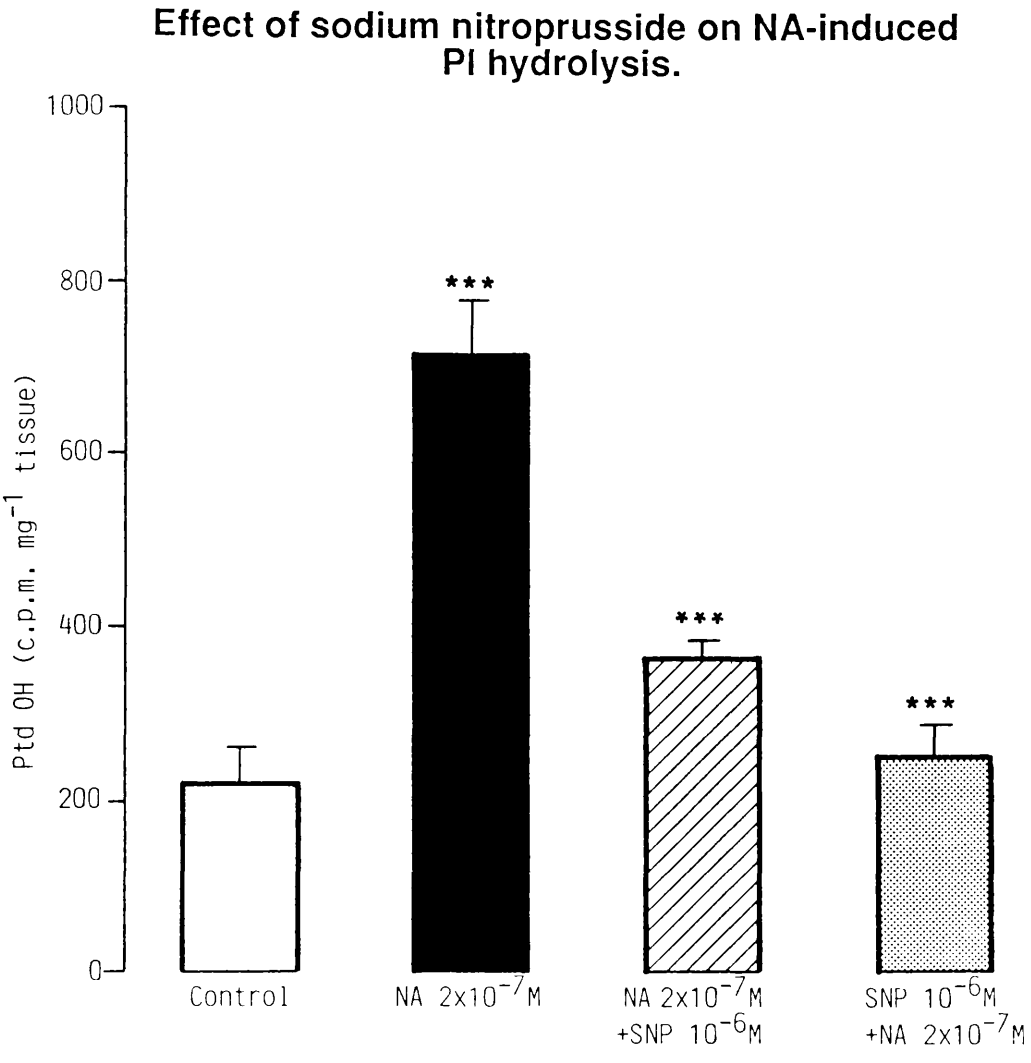


Figure 38.
Effect of sodium nitroprusside (SNP) (10^{-6}M) on the rate of Ptd Ins hydrolysis induced by noradrenaline (NA) (2 x 10^{-7}M). Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Control tissues were exposed to saline (5mins.), and test tissues to noradrenaline (5mins.) or noradrenaline (5mins.) and sodium nitroprusside (60sec.). Noradrenaline alone significantly increased the level of Ptd OH above the control value (*** $p<0.001$). Sodium nitrprusside, whether added after or before noradrenaline, significantly reduced the level of Ptd OH below that induced by noradrenaline alone (*** $p<0.001$). (n=4).

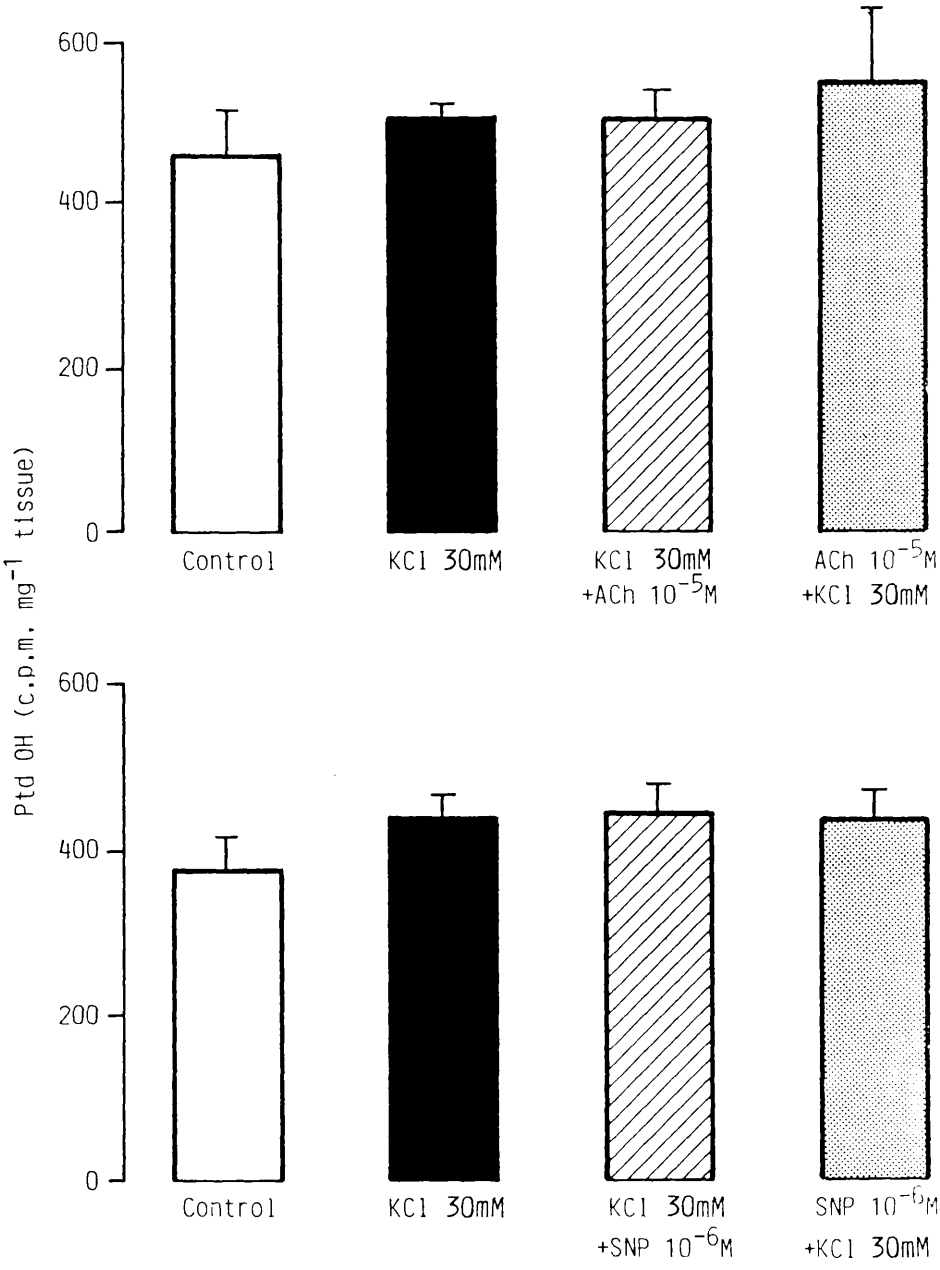


Figure 39.
Effects of acetylcholine (ACh) (10^{-5}M) and sodium nitroprusside (SNP) (10^{-6}M) on the rate of Ptd Ins hydrolysis induced by KCl (EC_{70}: 30mM). Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Control tissues were exposed to saline (5mins.), and test tissues to KCl (5mins.) or KCl (5mins.) and either acetylcholine (30sec.) or sodium nitroprusside (60sec.). KCl alone did not significantly increase the level of Ptd OH. Acetylcholine and sodium nitroprusside, whether added after or before KCl, did not significantly reduce the level of Ptd OH below that induced by KCl alone. The endothelium was intact. (n=4).

Part Four: Preliminary experiments on the effects of phorbol esters on vascular smooth muscle tone

Noradrenaline induced concentration-dependent contractions of aortic rings both in the absence and presence of DMSO, 0.05% (figure 40). DMSO did not significantly alter contractions of aortic rings induced by noradrenaline. The EC_{70} concentration of noradrenaline for muscle contraction in the presence of DMSO, 0.05% was determined to be 2×10^{-7}M. DMSO is the vehicle for the phorbol esters PMA and 4αPDD.

Both noradrenaline, in the presence of DMSO 0.05%, and PMA induced concentration-dependent contractions of aortic rings in the presence and absence of endothelium (figures 41 and 42). The EC_{80} concentration of PMA for muscle contraction in the presence of endothelium was determined to be 5×10^{-7}M. In the presence of endothelium, the EC_{70} concentration of noradrenaline in the presence of DMSO, 0.05%, and the EC_{80} concentration of PMA were equipotent (figure 41).

Removal of the endothelium did not significantly alter muscle contractions induced by PMA (figure 42).

PMA (5×10^{-7}M) induced a slowly-developing contraction of aortic rings in the presence of endothelium (figure 43). Muscle tone stabilised after approximately 45mins. (figures 43 and 44).

Noradrenaline induced concentration-dependent contractions of aortic rings in the presence of DMSO, 0.05%, and in the presence of 4αPDD (10^{-6}M) (figure 45). 4αPDD did not significantly alter noradrenaline-induced muscle contractions of aortic rings.

PMA significantly reduced noradrenaline-induced contractions

of aortic rings in a concentration-dependent manner (figure 46). PMA, at higher concentrations, increased muscle tone. Noradrenaline-induced contractions were measured from the level of tone induced by PMA alone.

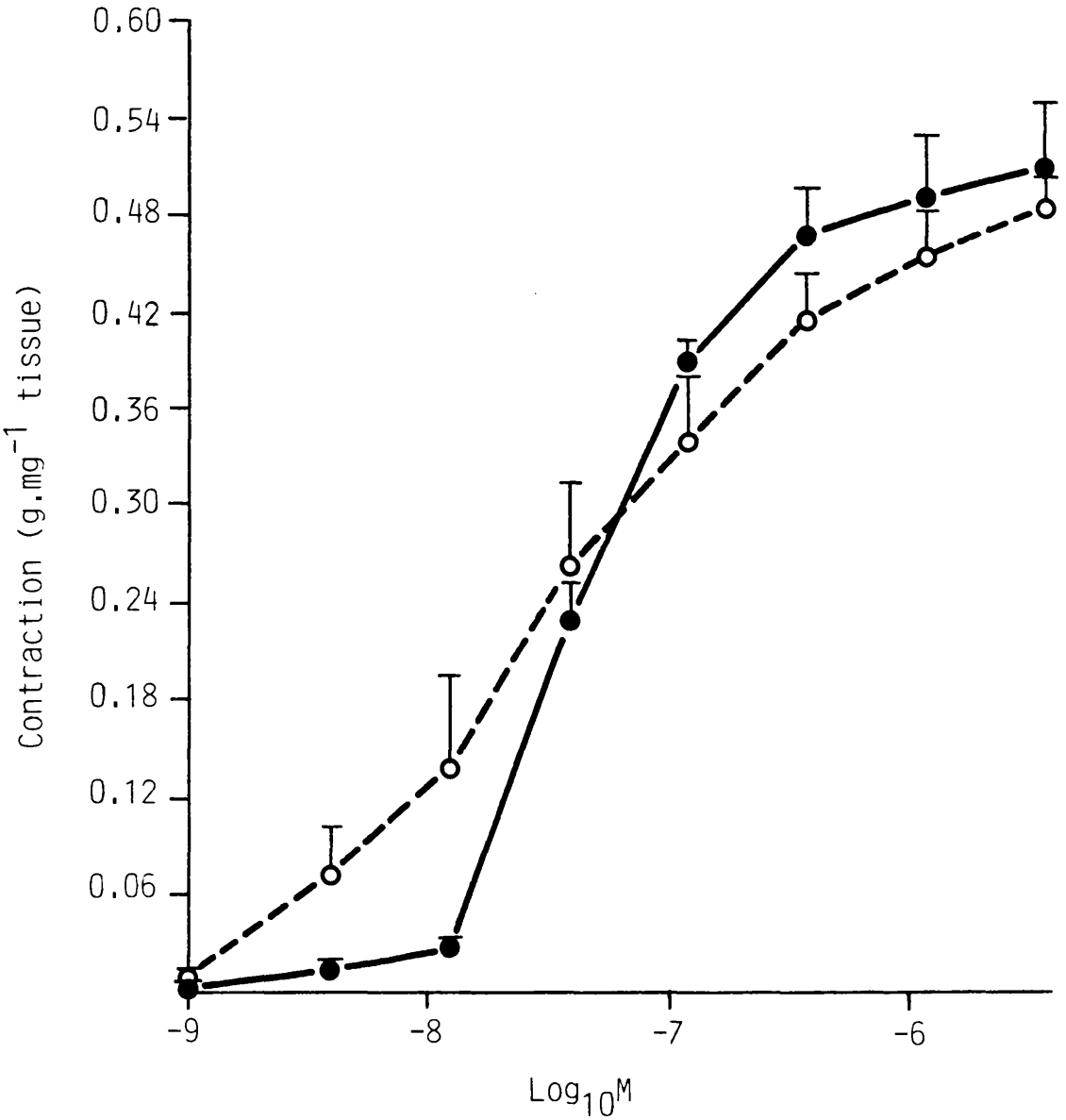


Figure 40.
Concentration-response graphs for noradrenaline in aortic rings in the presence (○) and absence (●) of DMSO, 0.05%. Muscle tension was recorded. DMSO did not affect noradrenaline-induced contractions of aortic rings. The endothelium was intact. (n=4).

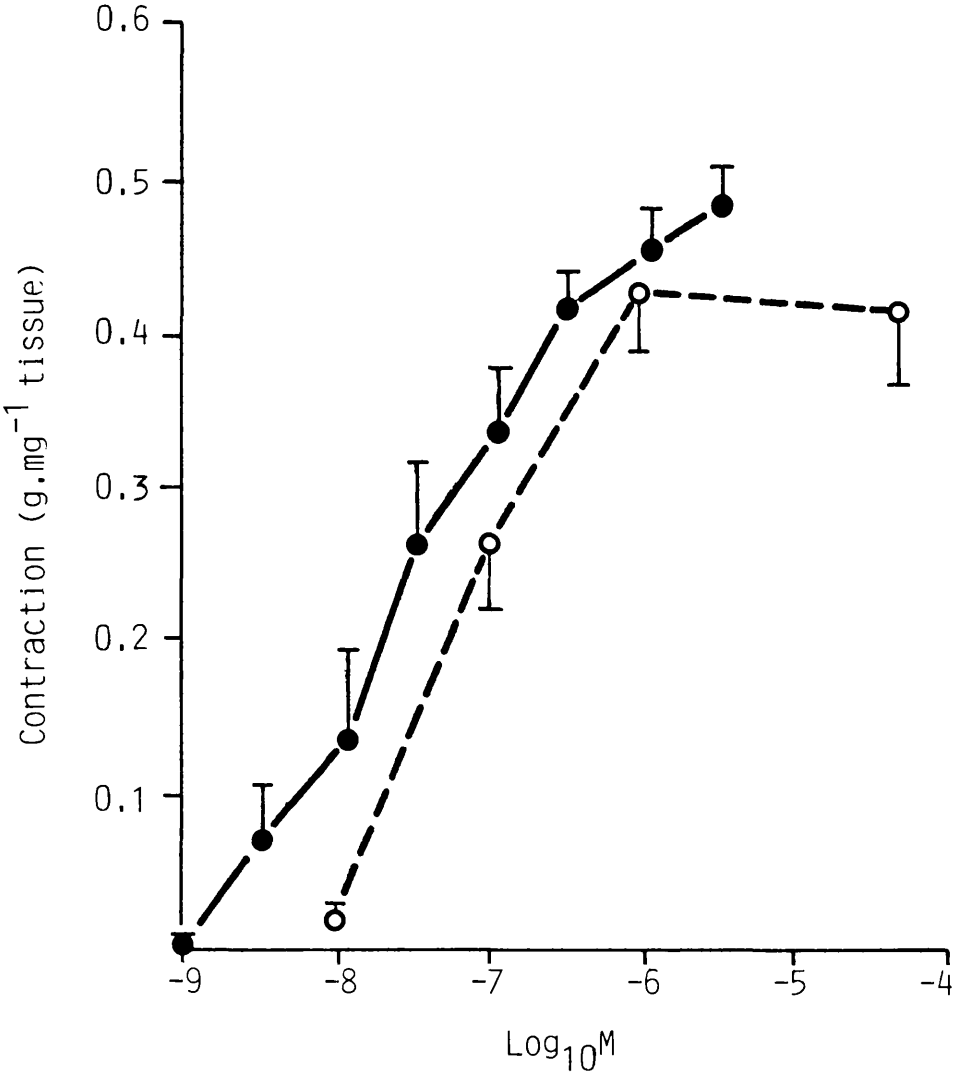


Figure 41.
Concentration-response graphs for noradrenaline in aortic rings in the presence of DMSO, 0.05%(●) and for PMA (○). DMSO is the vehicle for PMA. Muscle tension was recorded. The endothelium was intact. (n=4).

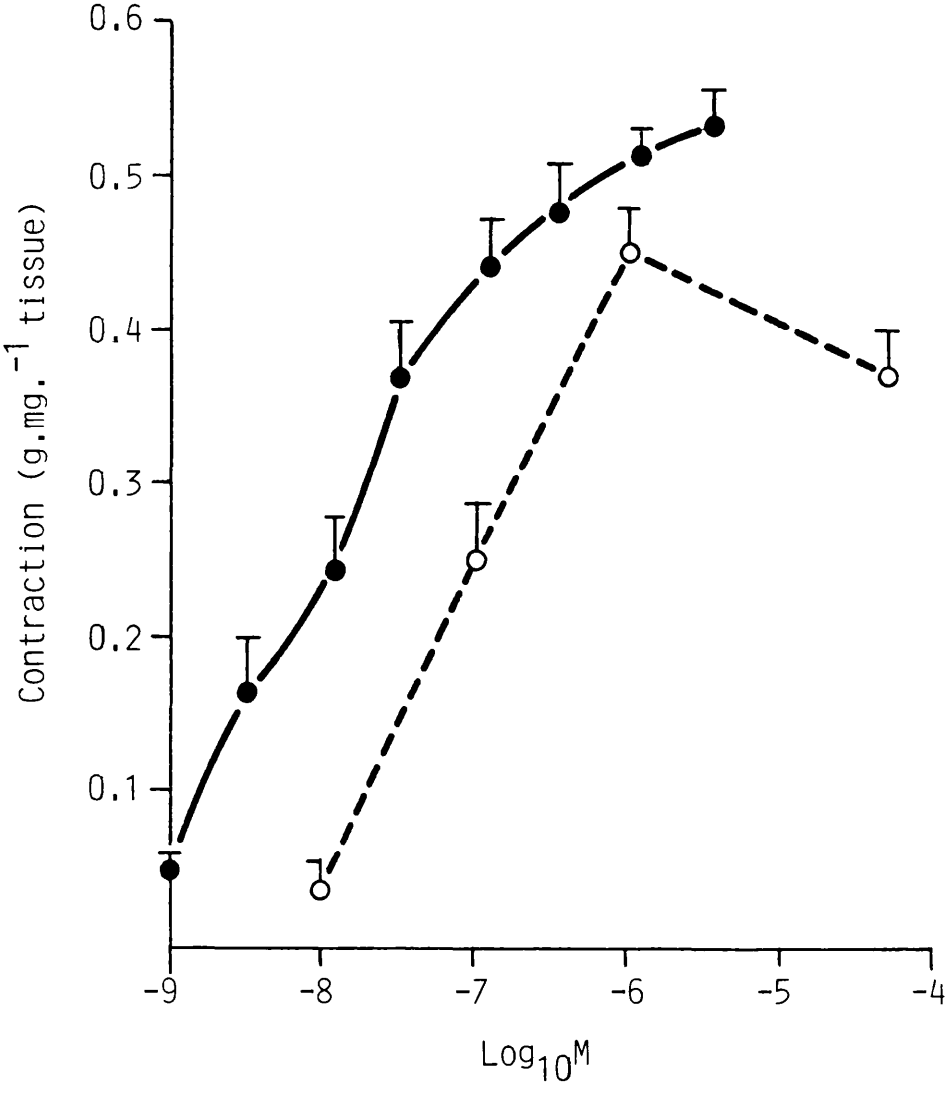


Figure 42.
Concentration-response graphs for noradrenaline in aortic rings in the presence of DMSO, 0.05% (●) and for PMA (○). DMSO is the vehicle for PMA. Muscle tension was recorded. The endothelium was removed. Removal of the endothelium did not significantly alter contractions induced by noradrenaline or PMA (c.f. figure 41). (n=4).

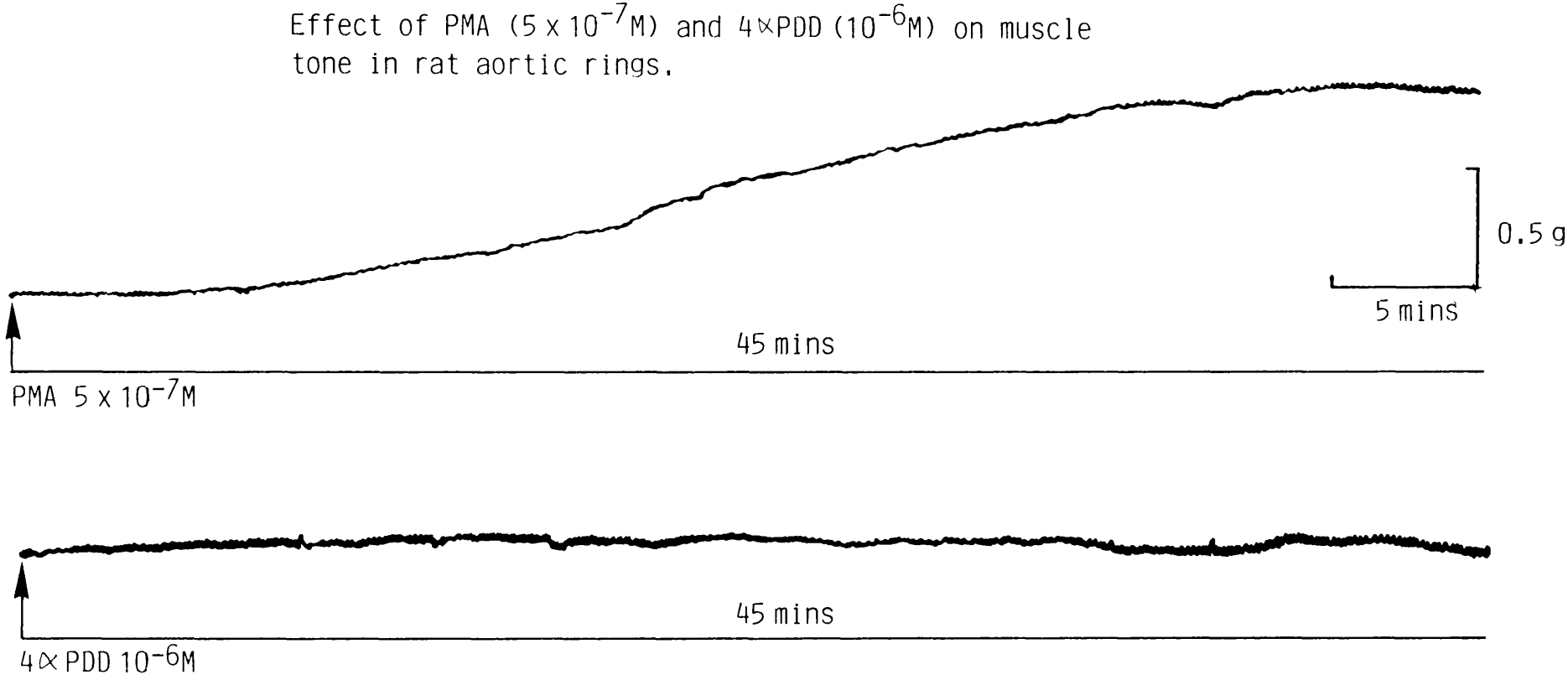


Figure 43.
Mechanical responses of aortic rings precontracted with PMA (EC_{80}: 5×10^{-7}M) (upper trace), and 4∝PDD (10^{-6}M) (lower trace). PMA induced a slowly-developing contraction, but 4∝PDD did not induce tone above the basal level. The endothelium was intact.

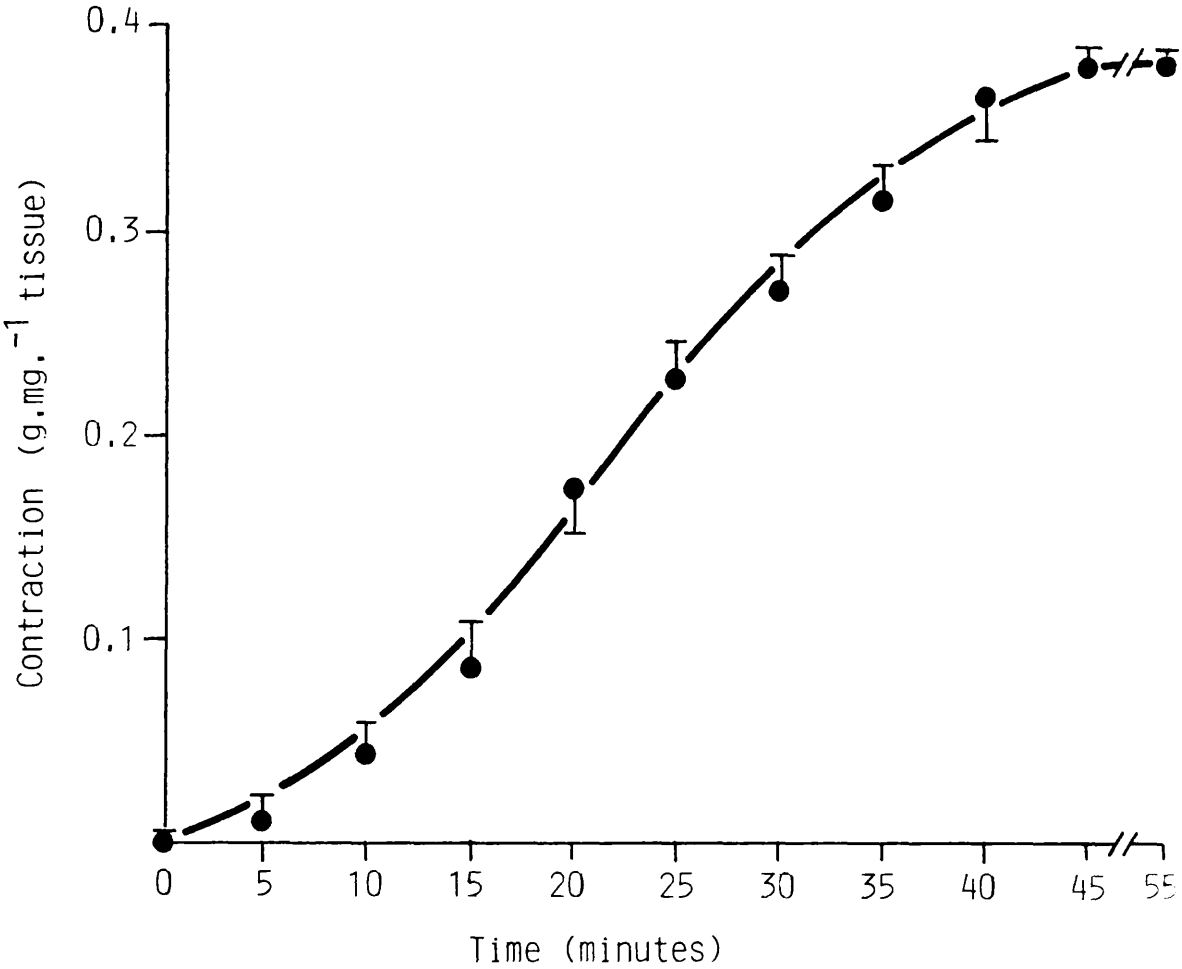


Figure 44.
Time-course of PMA-induced contraction of aortic rings. The EC_{80} concentration of PMA was used (5×10^{-7}M). Muscle tone stabilised after 45mins.. The endothelium was intact. (n=6).

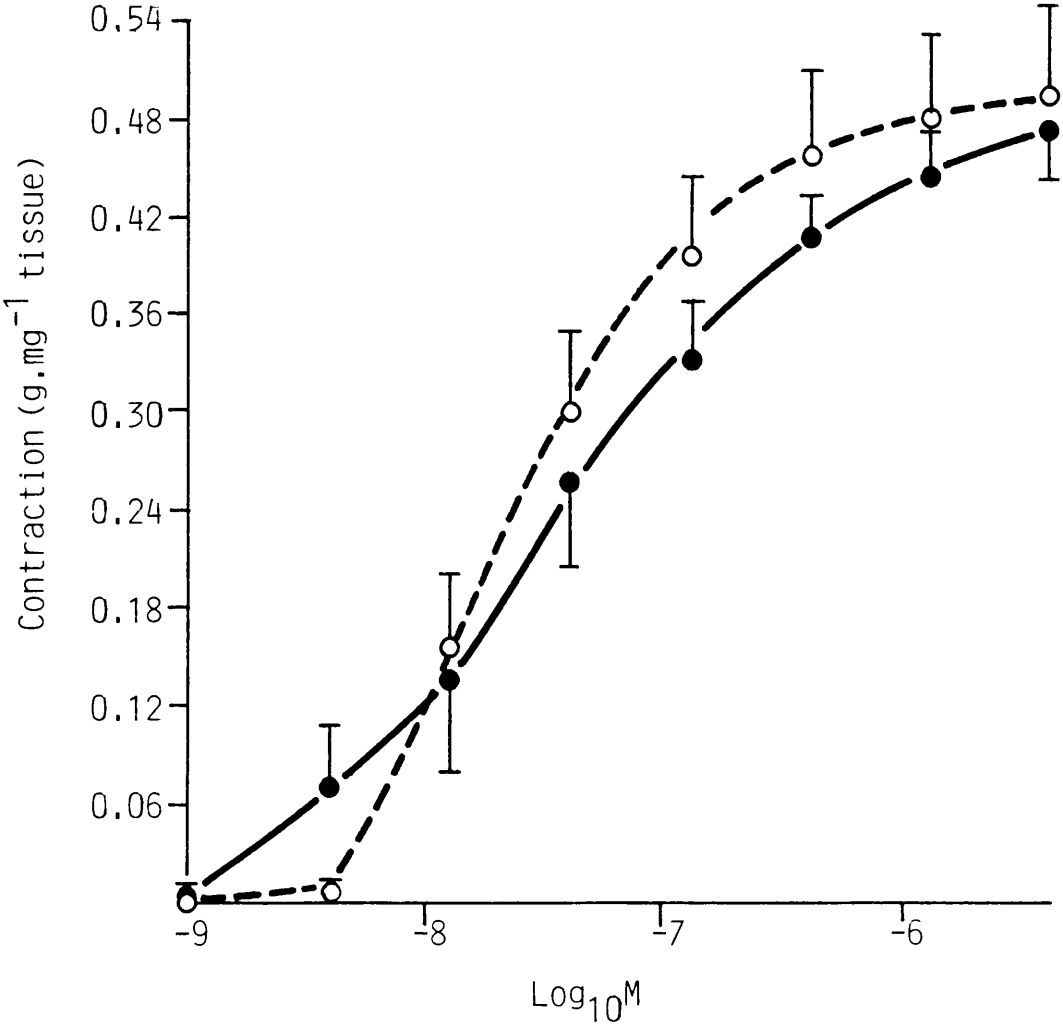


Figure 45.
Concentration-response graphs for noradrenaline in the presence of DMSO, 0.05% (●) or 4∝PDD (10^{-6}M) (○). Muscle tension was recorded. 4∝PDD did not affect noradrenaline-induced contractions of aortic rings. The endothelium was intact. (n=6).

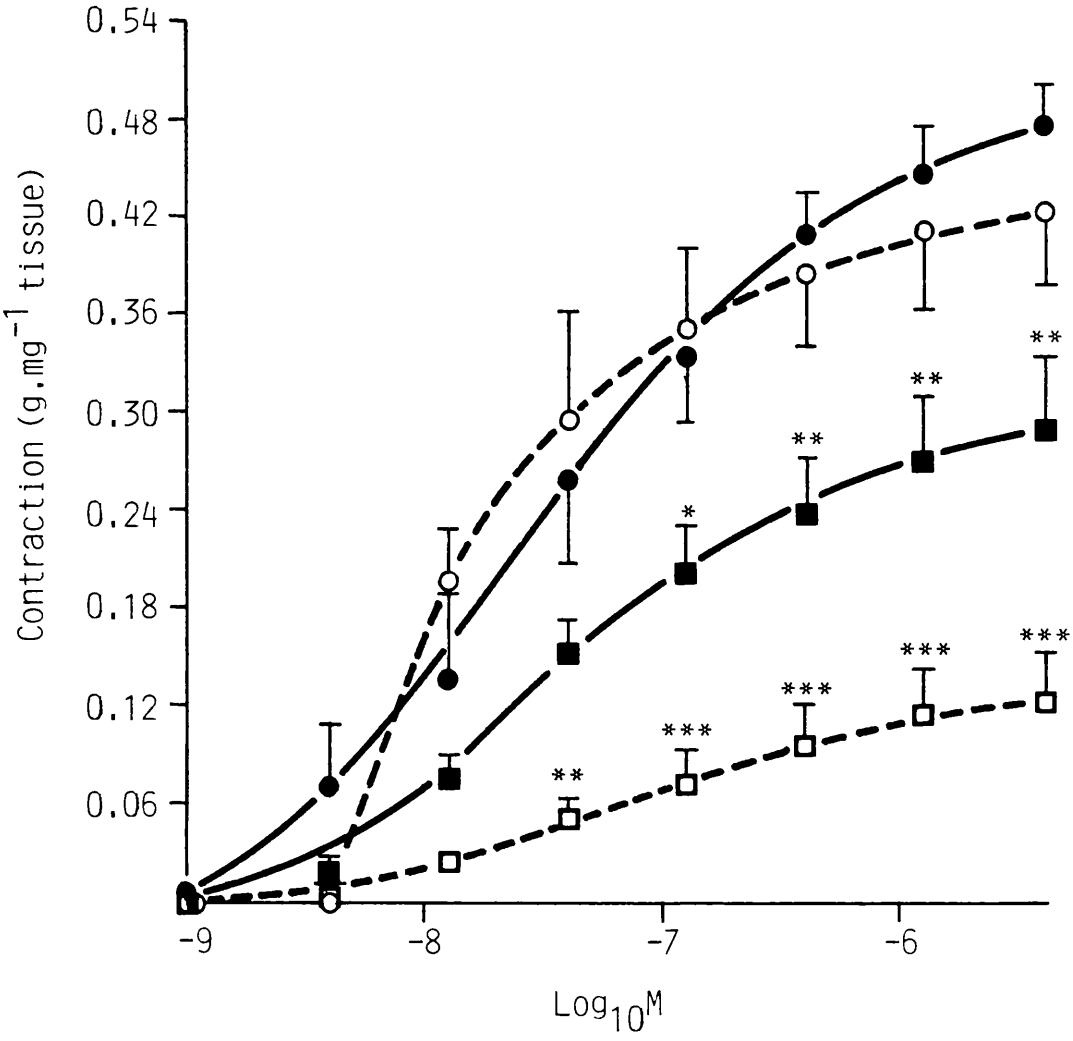


Figure 46.
Concentration-response graphs for noradrenaline in the presence of DMSO, 0.05% (●) or PMA (10^{-8}M)(○), or PMA (10^{-7}M) (■), or PMA (10^{-6}M) (□). Muscle tension was recorded. Noradrenaline induced smaller contractions of aortic rings with increasing concentration of PMA. The endothelium was intact. (n=6; * $p<0.05$, ** $p<0.01$, *** $p<0.001$; p values indicate statistical differences between noradrenaline-induced contractions in the presence of PMA and noradrenaline-induced contractions in the presence of DMSO, 0.05%).

Part Five: Vascular smooth muscle relaxations in aortic rings precontracted with PMA

Acetylcholine induced concentration-dependent relaxations of aortic rings precontracted with noradrenaline (2×10^{-7}M) both in the absence and presence of DMSO, 0.05% (figure 47). DMSO did not significantly alter muscle relaxations induced by acetylcholine.

Similarly sodium nitroprusside induced concentration-dependent relaxations of aortic rings precontracted with noradrenaline (2×10^{-7}M) both in the absence and presence of DMSO, 0.05% (figure 48). DMSO did not significantly alter muscle relaxations induced by sodium nitroprusside.

Vascular smooth muscle relaxations induced by acetylcholine and sodium nitroprusside were studied in aortic rings precontracted with PMA (5×10^{-7}M) in the presence of endothelium (figure 49).

Vascular relaxations induced by acetylcholine and sodium nitroprusside are shown quantitatively in concentration-response graphs in figures 50 and 51.

Acetylcholine induced significantly weaker relaxations of aortic rings precontracted with PMA (5×10^{-7}M) than of aortic rings precontracted with noradrenaline (2×10^{-7}M) (figure 50). Acetylcholine induced 25% maximum relaxation of aortic rings.

Sodium nitroprusside induced significantly weaker relaxations of aortic rings precontracted with PMA (5×10^{-7}M) than of aortic rings precontracted with noradrenaline (2×10^{-7}M) (figure 51). Sodium nitroprusside induced 60% relaxation of aortic rings.

Acetylcholine (10^{-5}M; 30s.) significantly increased levels

of cyclic GMP in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.) (figure 52), but not in tissues precontracted with PMA (5×10^{-7}M; 45mins.). The level of cyclic GMP measured in tissues precontracted with PMA (5×10^{-7}M; 45mins.) followed by exposure to acetylcholine (10^{-5}M; 30s.) was significantly smaller than that induced by acetylcholine (10^{-5}M; 30s.) in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.).

Sodium nitroprusside (10^{-6}M; 60s.) significantly increased levels of cyclic GMP both in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.) and in tissues precontracted with PMA (5×10^{-7}M; 45mins.) (figure 52). The level of cyclic GMP induced by sodium nitroprusside (10^{-6}M; 60s.) was significantly smaller in tissues precontracted with PMA (5×10^{-7}M; 45mins.) than in tissues precontracted with noradrenaline (2×10^{-7}M) (figure 52).

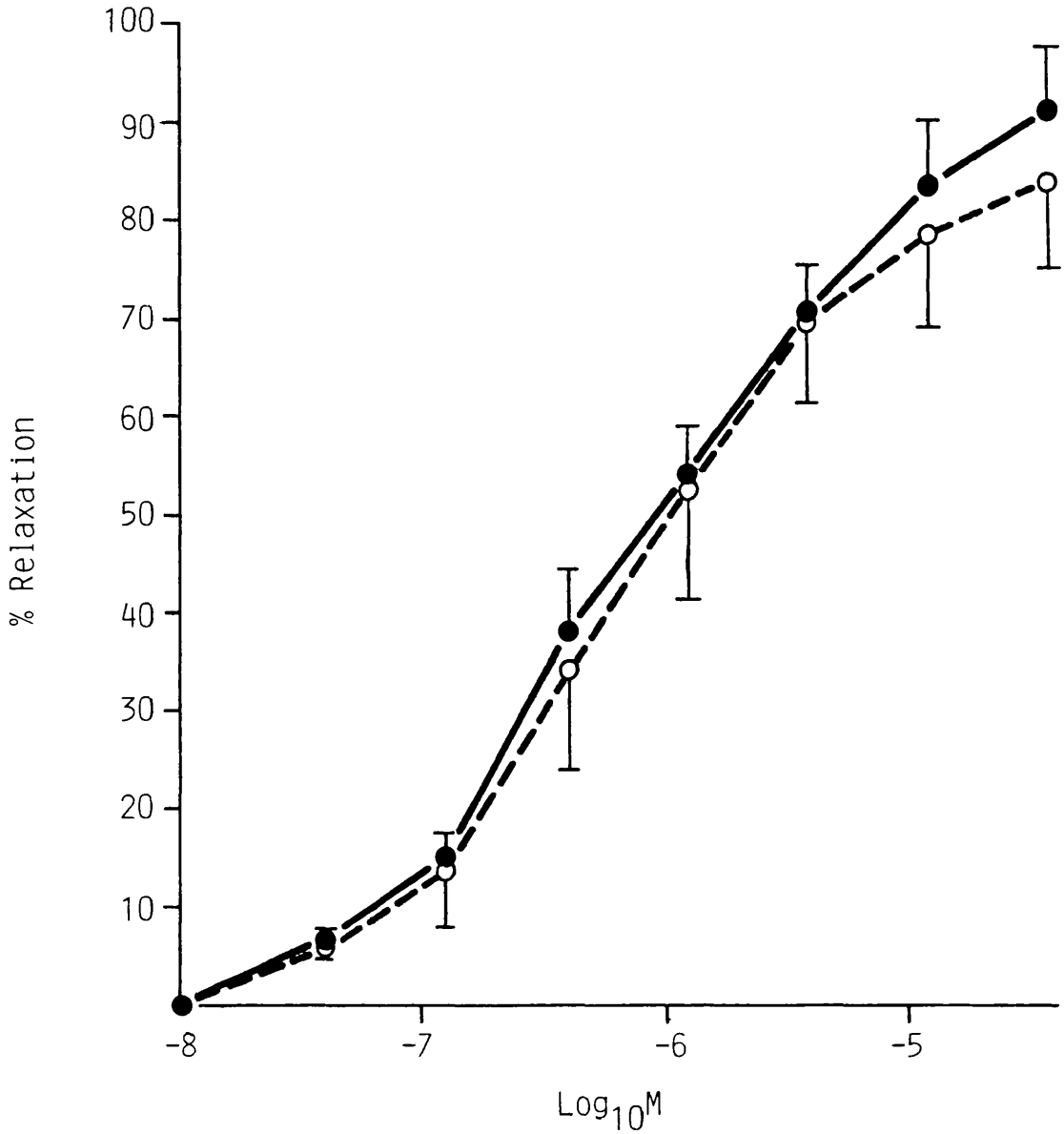


Figure 47.
Concentration-response graphs for acetylcholine in the presence (○) and absence (●) of DMSO, 0.05%. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M). DMSO did not affect acetylcholine-induced relaxation. The endothelium was intact. (n=6).

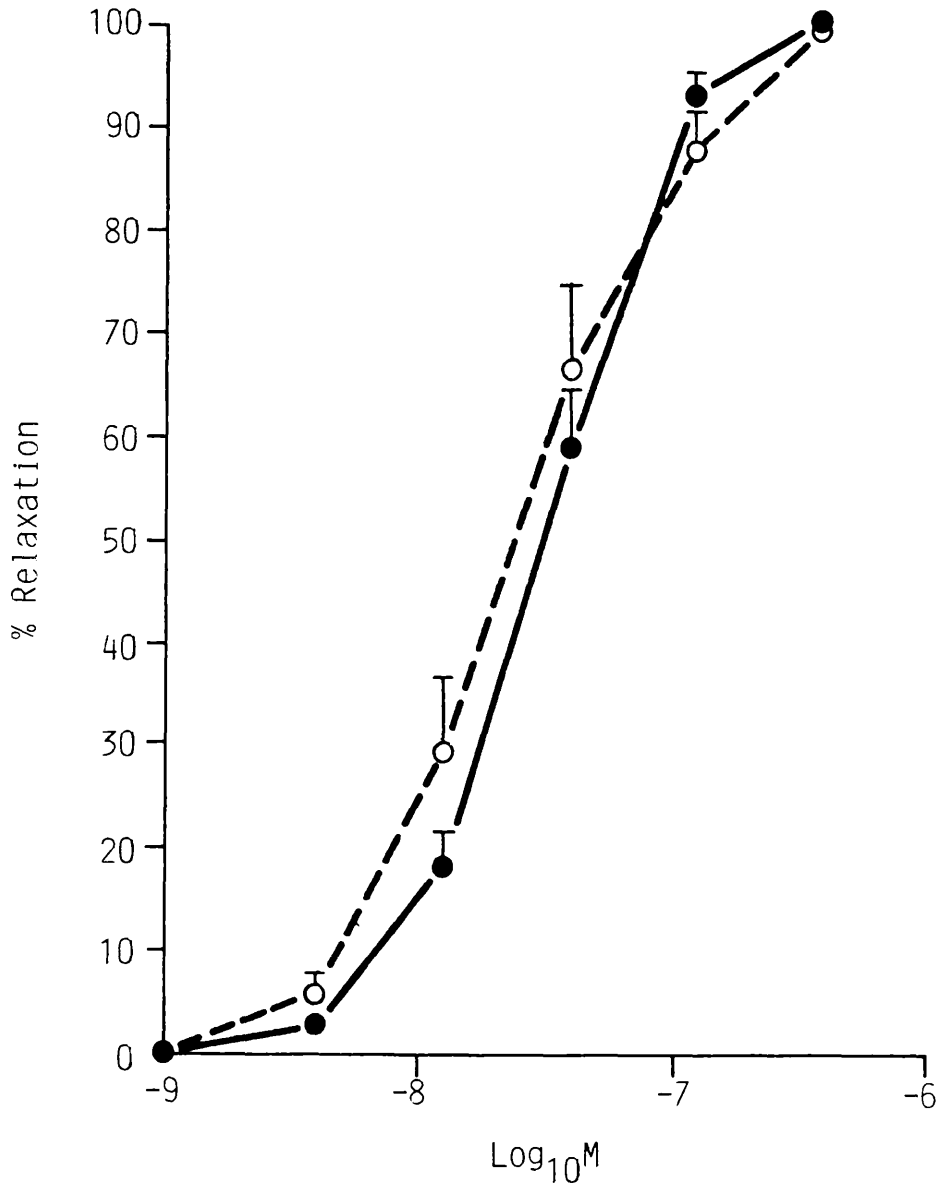


Figure 48.
Concentration-response graphs for sodium nitroprusside in presence (○) and absence (●) of DMSO, 0.05%. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M). DMSO did not affect sodium nitroprusside-induced relaxation. (n=6).

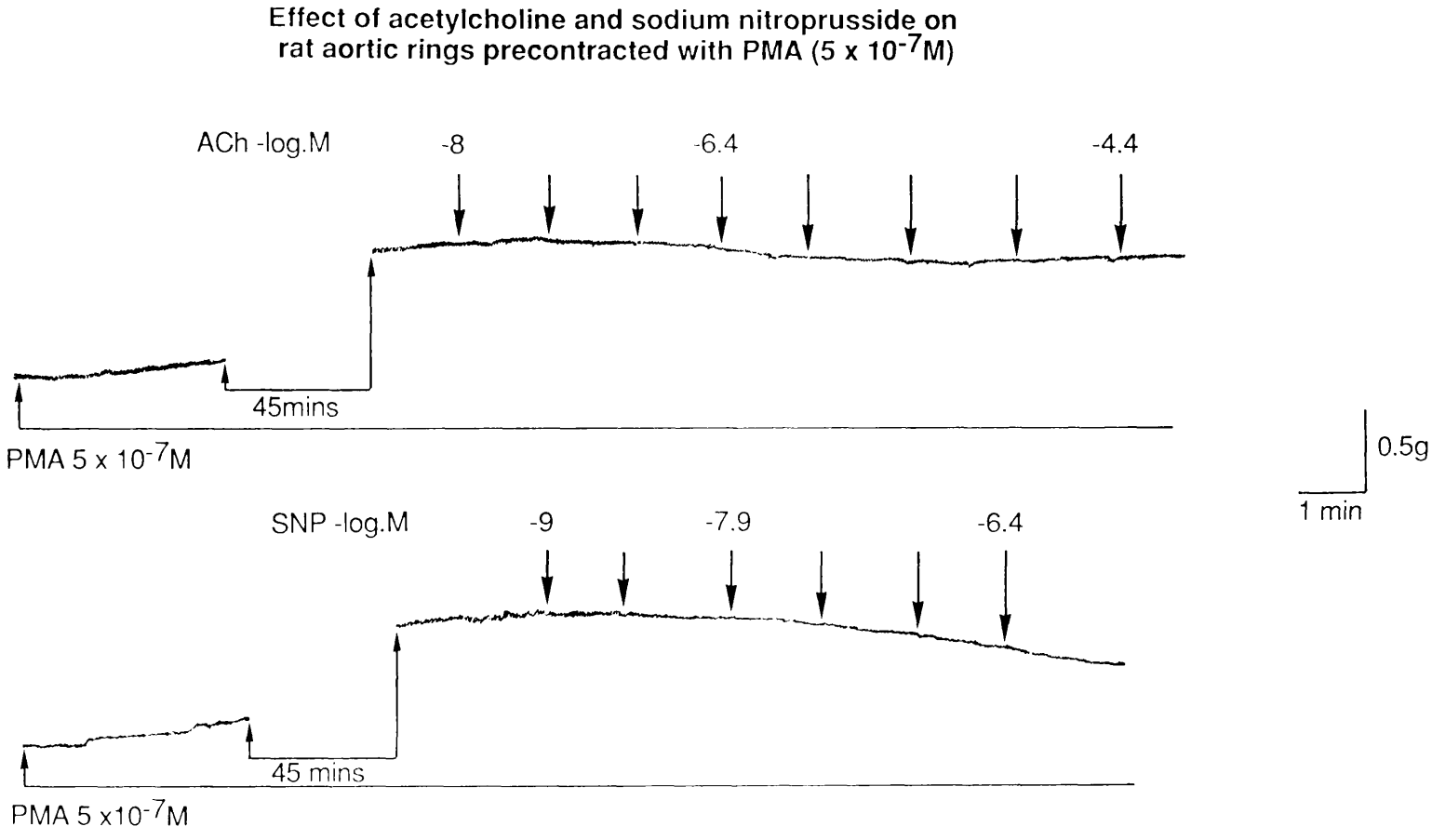


Figure 49.
Mechanical responses of aortic rings in the presence of endothelium. Muscle tone was raised with PMA (5 x 10^{-7}M). PMA induced slowly-developing contractions of aortic rings, and muscle tone stabilised after 45mins.. The traces have been shortened to fit the page. Acetylcholine (ACh) (upper trace) and sodium nitroprusside (SNP) (lower trace) were added successively to the organ baths, producing cumulative concentration-response graphs.

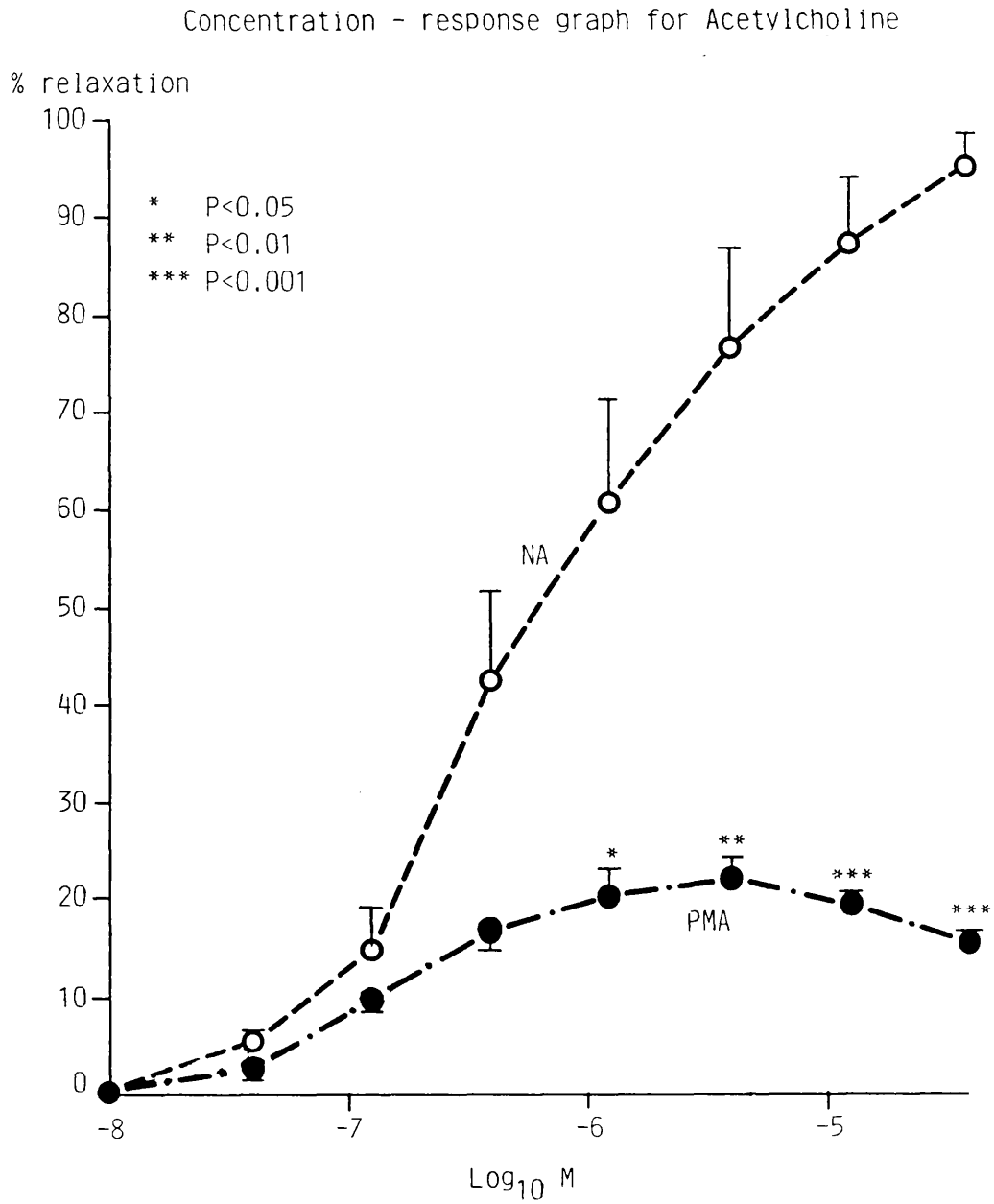


Figure 50.
Concentration-response graphs for acetylcholine in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M) (○), or PMA (5×10^{-7}M) (●). Acetylcholine induced significantly weaker relaxations of aortic rings precontracted with PMA than of aortic rings precontracted with noradrenaline. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). The endothelium was intact. (n=6).

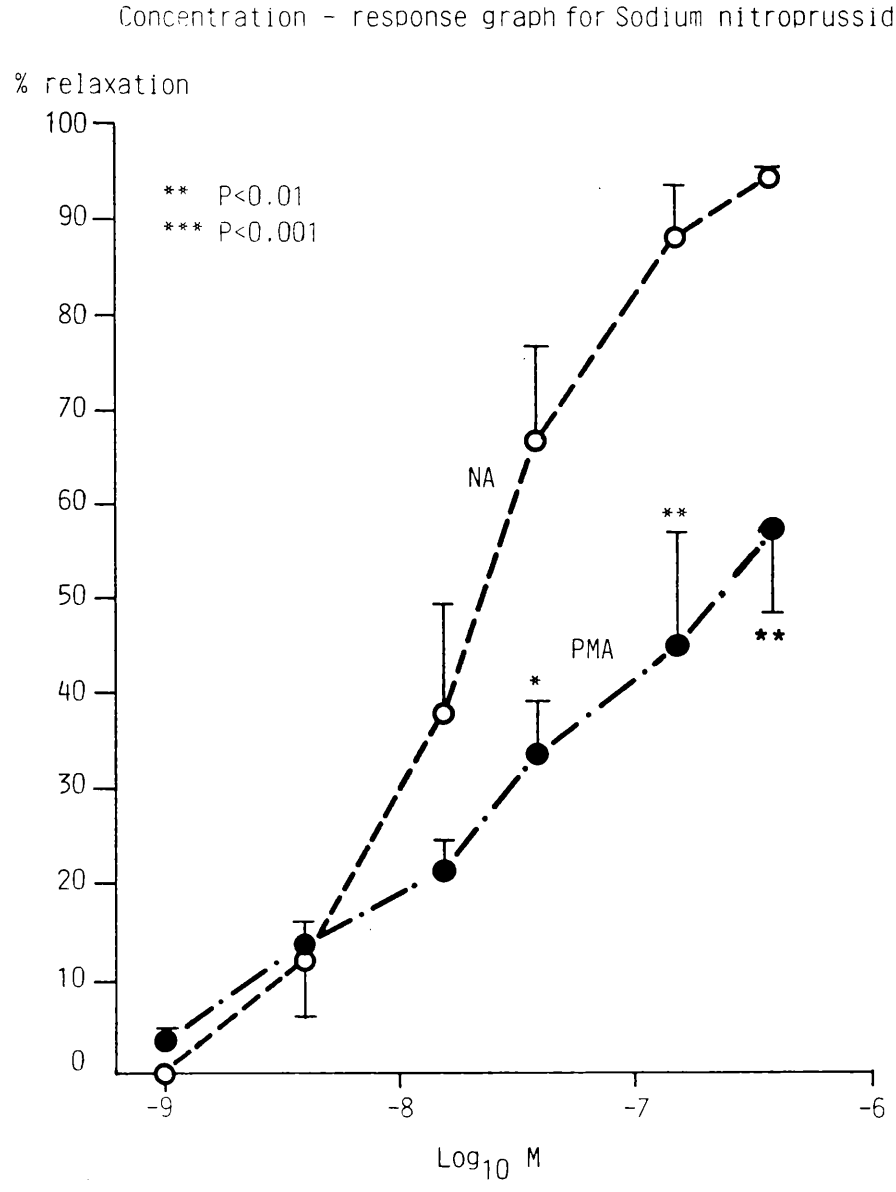


Figure 51.
Concentration-response graphs for sodium nitroprusside in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M) (○), or PMA (5×10^{-7}M) (●). Sodium nitroprusside induced significantly weaker relaxations of aortic rings precontracted with PMA than of aortic rings precontracted with noradrenaline. (* $p<0.05$, ** $p< 0.01$). (n=6).

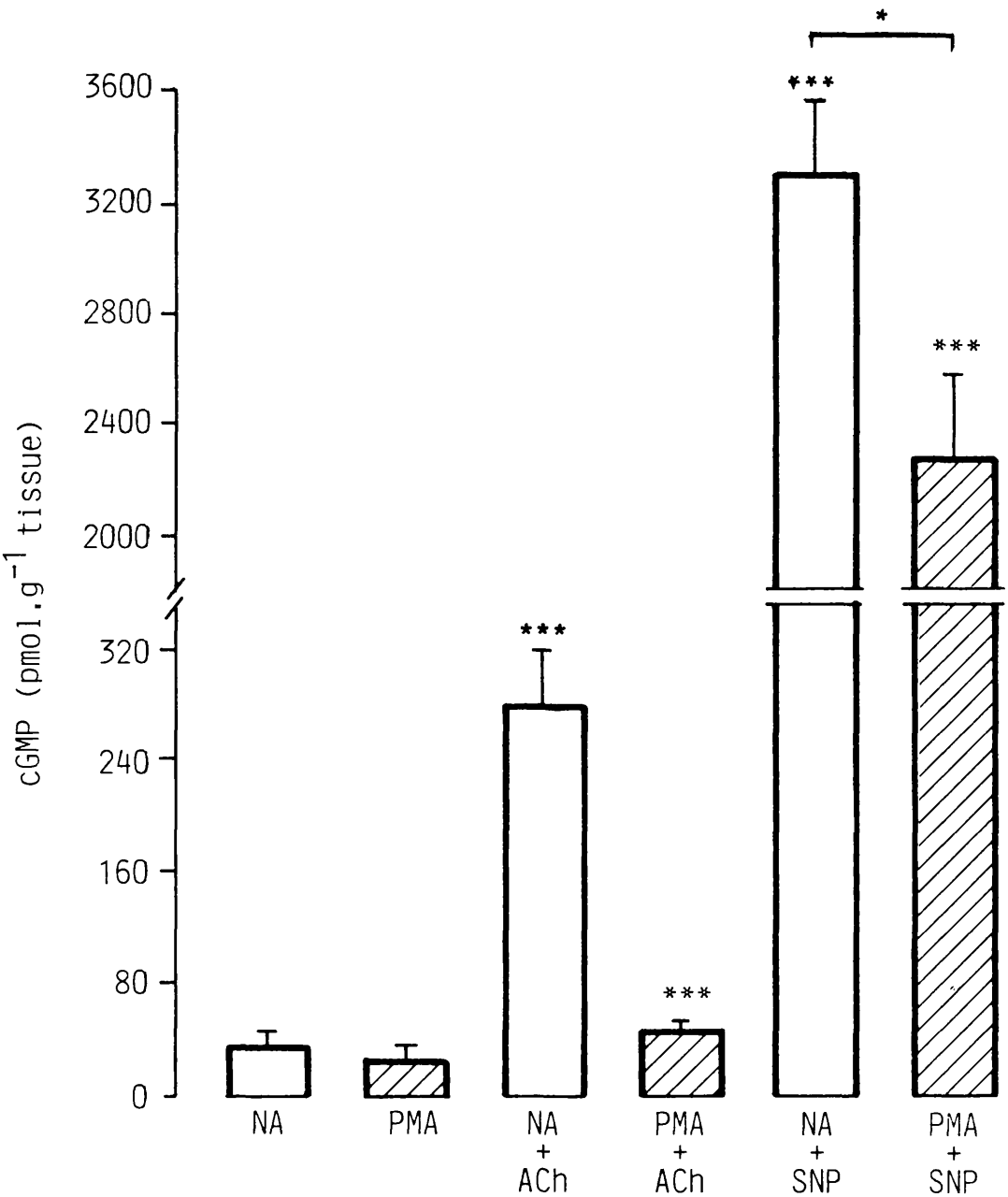


Figure 52.
Effects of acetylcholine (ACh) (10^{-5}M) and sodium nitroprusside (SNP) (10^{-6}M) on levels of cyclic GMP in aortic rings precontracted with noradrenaline (NA) (2×10^{-7}M) or PMA (5×10^{-7}M). Tissues were exposed to noradrenaline (5mins.), or PMA (45mins.), or noradrenaline (5mins.) followed by either acetylcholine (30s.) or sodium nitroprusside (60s.), or PMA (45mins.) followed by either acetylcholine (30s.) or sodium nitroprusside (60s.). Noradrenaline and PMA did not induce high levels of cyclic GMP. Acetylcholine significantly increased levels of cyclic GMP only in tissues precontracted with noradrenaline (*** $p<0.001$). The level of cyclic GMP induced by acetylcholine was significantly smaller in tissues precontracted with PMA than in tissues precontracted with noradrenaline (*** $p<0.001$). Sodium nitroprusside significantly increased levels of cyclic GMP (*** $p<0.001$). The level of cyclic GMP induced by sodium nitroprusside was significantly smaller in tissues precontracted with PMA than in tissues precontracted with noradrenaline (* $p<0.05$).(n=6).

Part Six: Effects of acetylcholine and sodium nitroprusside on the rates of Ptd Ins hydrolysis associated with PMA-induced contraction of aortic rings

PMA (5×10^{-7}M; 45mins.) did not significantly increase the level of Ptd OH above that measured in control tissues exposed to DMSO (0.05%; 45mins.) (figure 53). Acetylcholine (10^{-5}M; 30s.) and sodium nitroprusside (10^{-6}M; 60s.) respectively, did not significantly reduce the level of Ptd OH below that measured in tissues exposed to PMA (5×10^{-7}M; 45mins.) alone (figure 53).

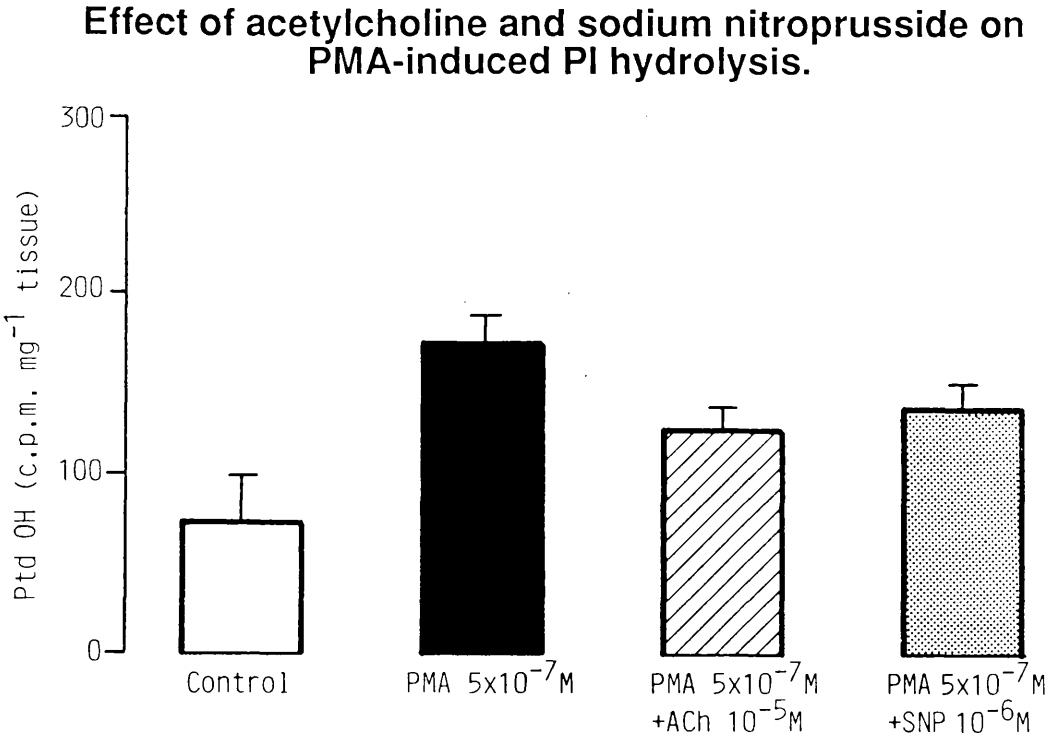


Figure 53.
Effects of acetylcholine (ACh) (10^{-5}M) and sodium nitroprusside (SNP) (10^{-6}M) on the rate of Ptd Ins hydrolysis induced by PMA (5 x 10^{-7}M). Hydrolysis of Ptd Ins was monitored by measuring levels of Ptd OH. Control tissues were exposed to DMSO, 0.05% (45mins.) and test tissues to PMA (45mins.), or PMA (45mins.) and either acetylcholine (30sec.) or sodium nitroprusside (60sec.). PMA alone did not significantly increase the level of Ptd OH above the control value. Acetylcholine and sodium nitroprusside did not significantly alter the level of Ptd OH induced by PMA. The endothelium was intact. (n=4).

Part Seven: Vascular relaxations induced by acetylcholine and nitric oxide in aortic rings precontracted with noradrenaline or PMA

In aortic rings precontracted with PMA (5×10^{-7}M), acetylcholine induced significantly weaker relaxations than those induced by sodium nitroprusside (figure 54).

Vascular relaxations induced by acetylcholine and nitric oxide were studied in aortic rings precontracted with noradrenaline (2×10^{-7}M) or PMA (5×10^{-7}M) in the presence of endothelium (figures 55 and 56).

Nitric oxide induced rapidly-developing but transient relaxations of aortic rings precontracted with noradrenaline (2×10^{-7}M) (figure 55).

Nitric oxide induced slowly-developing and transient relaxations of aortic rings precontracted with PMA (5×10^{-7}M) (figure 56).

Vascular relaxations induced by acetylcholine and nitric oxide are shown quantitatively in concentration-response graphs (figure 57).

Both acetylcholine and nitric oxide induced powerful concentration-dependent relaxations of aortic rings precontracted with noradrenaline (2×10^{-7}M) (figure 57). Over the concentration range studied, nitric oxide-induced relaxations were not significantly different from those induced by acetylcholine. Both acetylcholine and nitric oxide induced significantly weaker relaxations of aortic rings precontracted with PMA (5×10^{-7}M). Again, over the concentration range studied, nitric oxide-induced relaxations were not significantly different from those induced by acetylcholine.

Nitric oxide-induced relaxations of aortic rings precontracted with either noradrenaline (2×10^{-7}M) or PMA (5×10^{-7}M), were not significantly altered by removal of the endothelium (figure 58).

Acetylcholine (10^{-5}M; 30s.) significantly increased levels of cyclic GMP in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.) (figure 59). However, acetylcholine (10^{-5}M) did not significantly increase levels of cyclic GMP in tissues precontracted with PMA (5×10^{-7}M; 45mins.). The level of cyclic GMP measured in tissues precontracted with PMA (5×10^{-7}M; 45mins.) was significantly smaller than in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.). In comparison, nitric oxide (3×10^{-6}M; 20s.) significantly increased levels of cyclic GMP both in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.) or PMA (5×10^{-7}M; 45mins.). However, the level of cyclic GMP induced by nitric oxide (3×10^{-6}M; 20s.) was significantly smaller in tissues precontracted with PMA (5×10^{-7}M; 45mins.) than in tissues precontracted with noradrenaline (2×10^{-7}M; 5mins.).

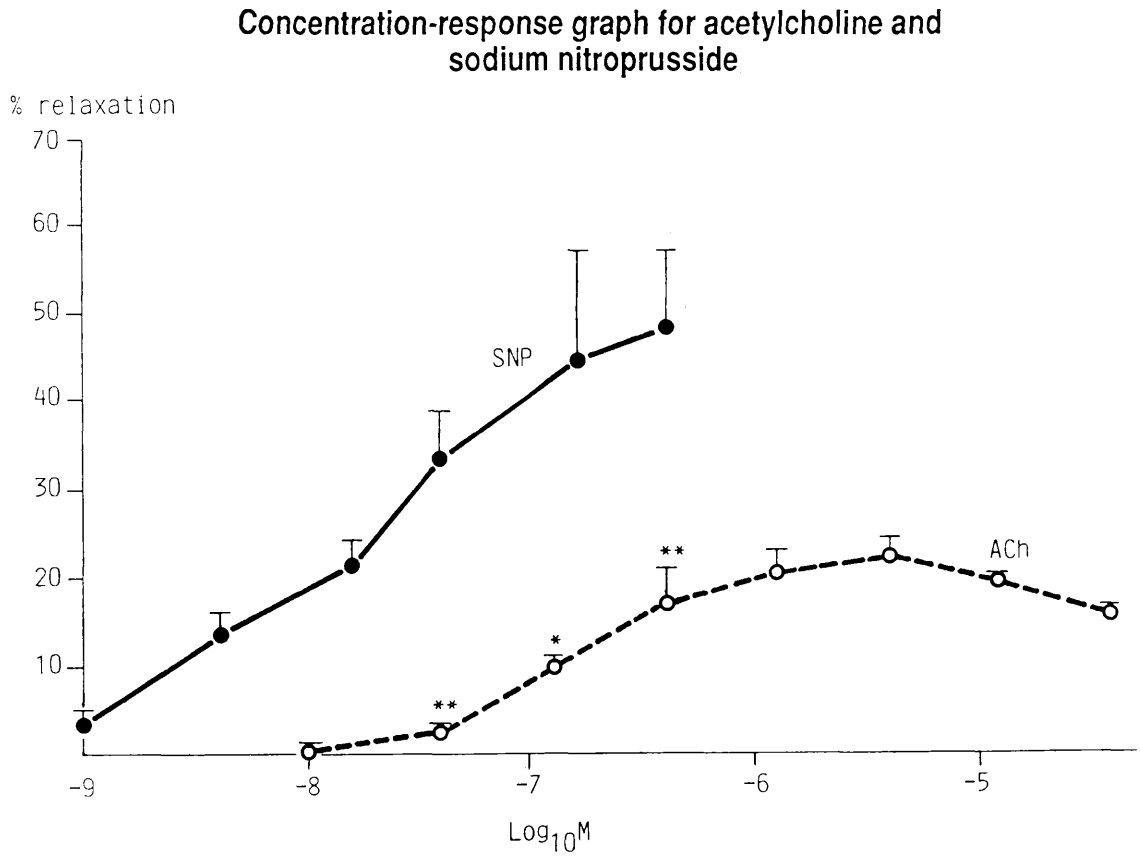


Figure 54.
Concentration-response graphs for acetylcholine (ACh) (○) and sodium nitroprusside (SNP) (●) in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by PMA (5×10^{-7}M). Acetylcholine induced significantly weaker relaxations than sodium nitroprusside. (* $p<0.05$, ** $p<0.01$). The endothelium was intact. (n=6).

Effect of acetylcholine and nitric oxide on rat aortic rings precontracted with NA (2 x 10^{-7}M)

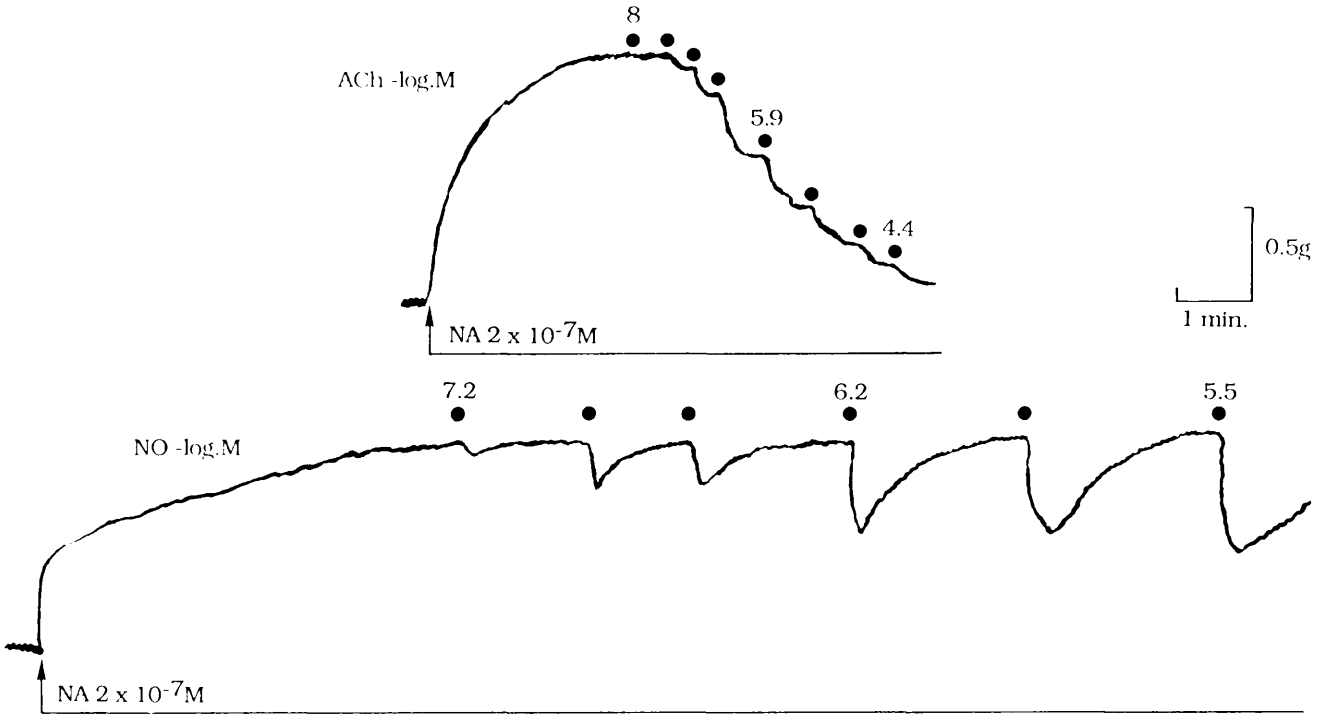


Figure 55.
Mechanical responses of aortic rings in the presence of endothelium. Muscle tone was raised with noradrenaline (NA) (2 x 10^{-7}M). Acetylcholine (ACh) (upper trace) and nitric oxide (NO) (lower trace) were added successively to the organ baths, producing cumulative concentration-response graphs.

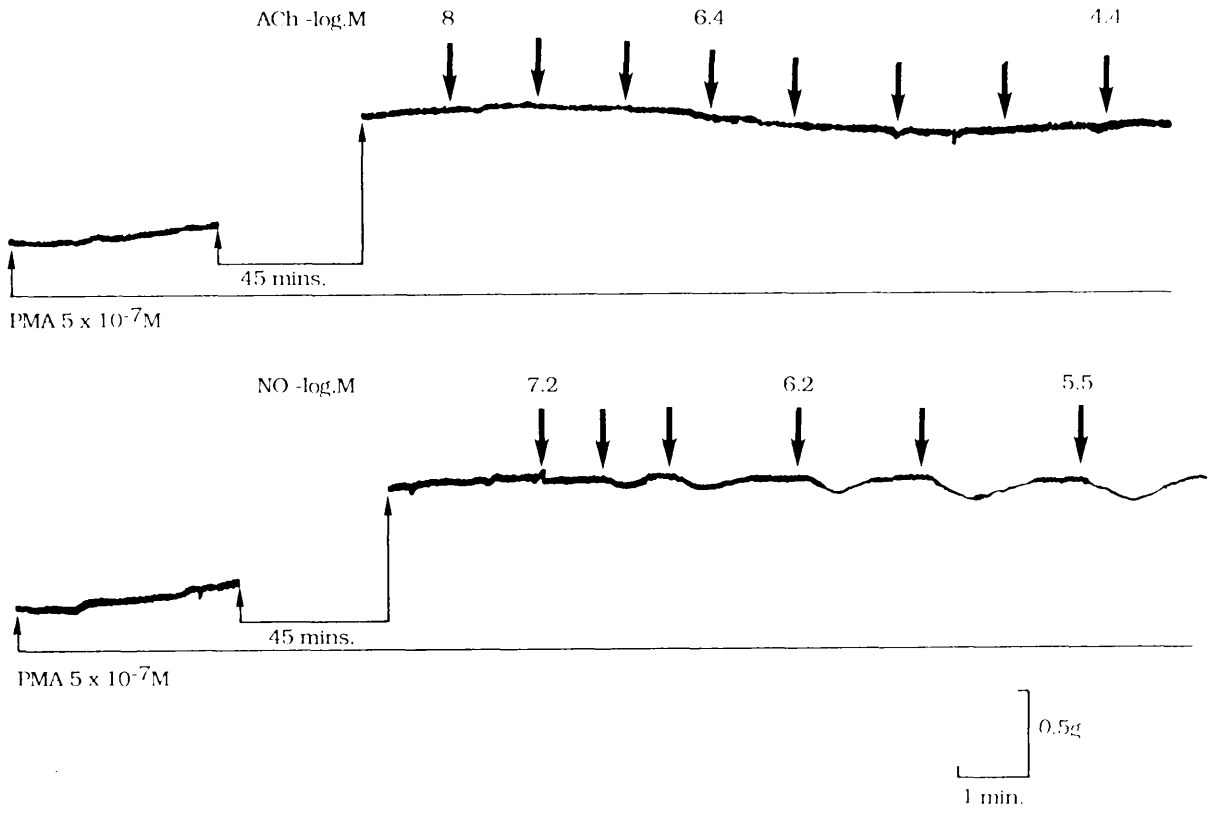


Figure 56.
Mechanical responses of aortic rings in the presence of endothelium. Muscle tone was raised with PMA (5 x 10^{-7}M). Acetylcholine (ACh) (upper trace) and nitric oxide (NO) (lower trace) were added successively to the organ baths, producing cumulative concentration-response graphs.

Concentration-response graph for acetylcholine and nitric oxide.

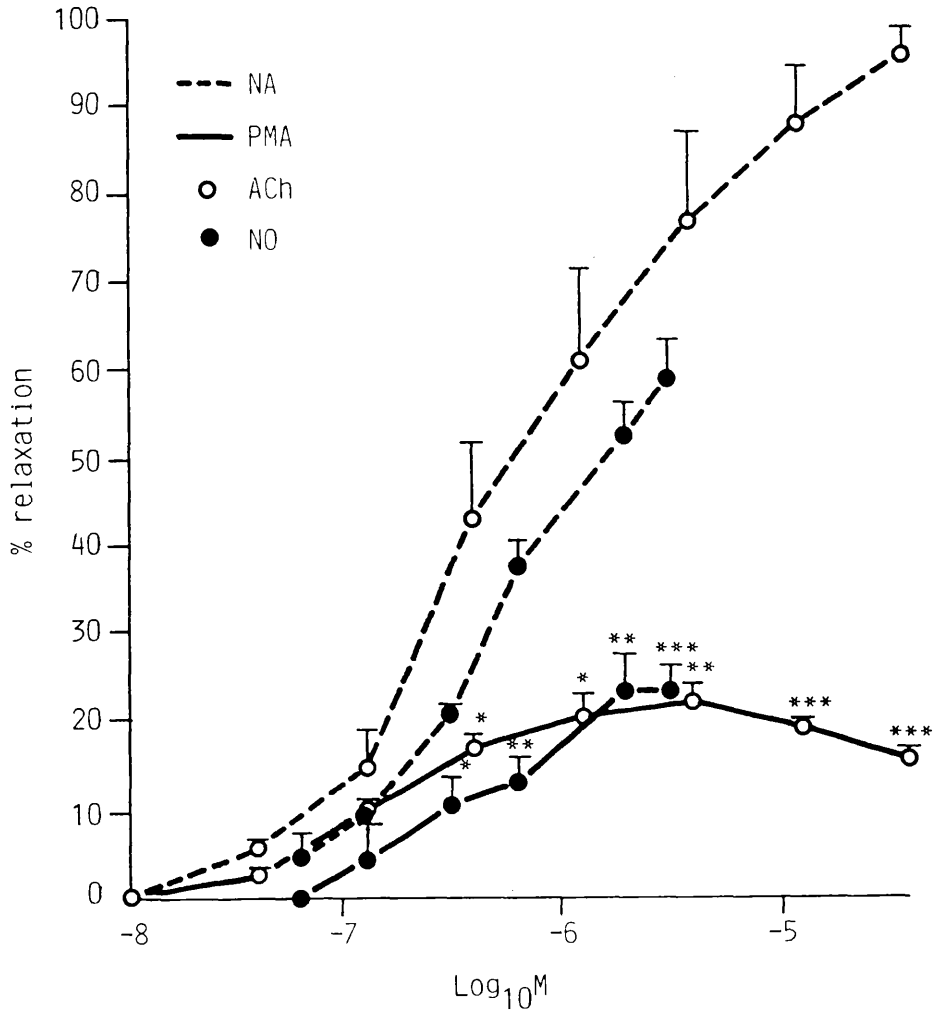


Figure 57.
Concentration-response graphs for acetylcholine (ACh) (○) and nitric oxide (NO) (●) in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M) (- - -), or PMA (5×10^{-7}M) (——). The effect of nitric oxide was similar to that of acetylcholine. Acetylcholine and nitric oxide induced significantly weaker relaxations of tissues precontracted with PMA than of tissues precontracted with noradrenaline. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). The endothelium was intact. (n=6).

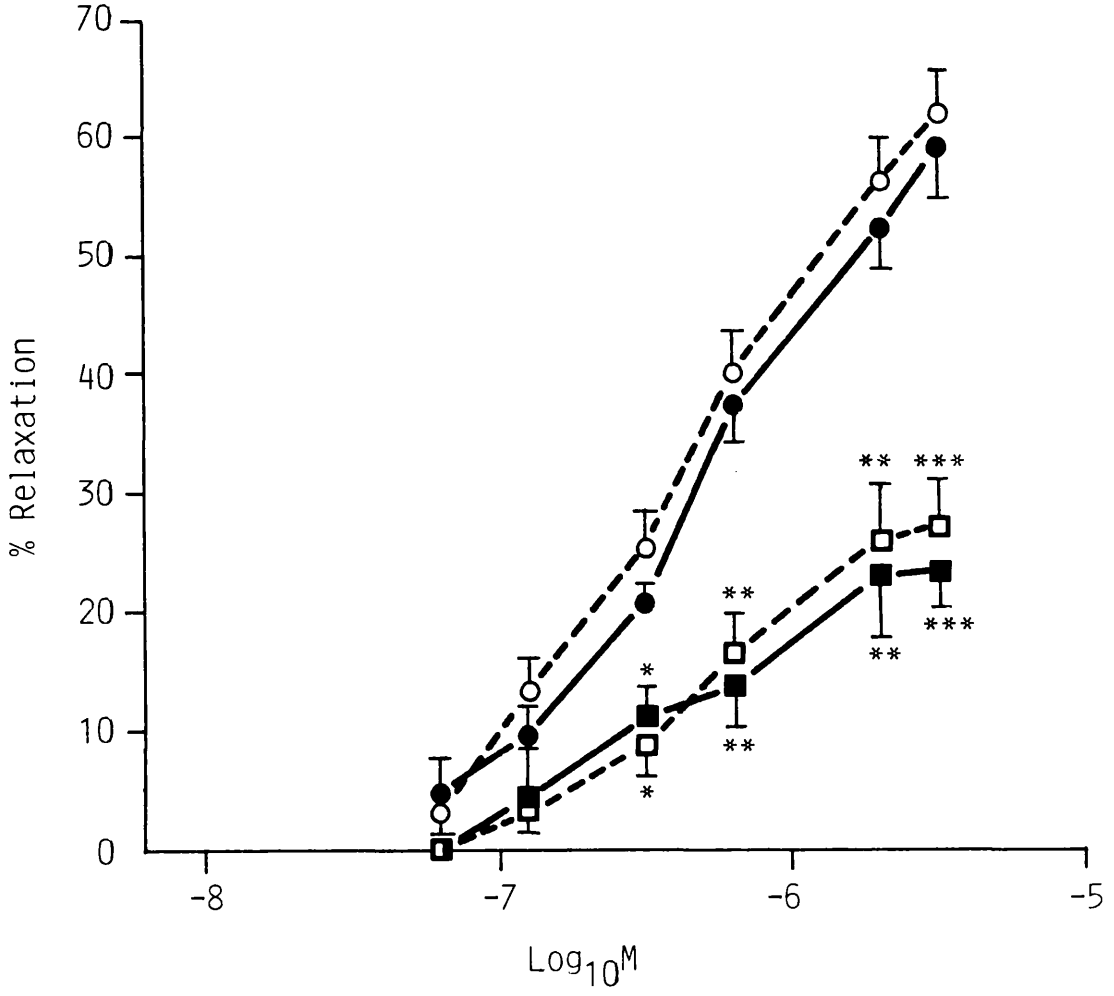


Figure 58.
Concentration-response graphs for nitric oxide in aortic rings. The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (2×10^{-7}M) or PMA (5×10^{-7}M). Muscle tone was raised with either noradrenaline, in the presence (●) or absence (○) of endothelium, or PMA, in the presence (■) or absence (□) of endothelium. Nitric oxide-induced relaxation was endothelium-independent. Nitric oxide was significantly less able to relax aortic rings precontracted with PMA than aortic rings precontracted with noradrenaline. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). (n=6).

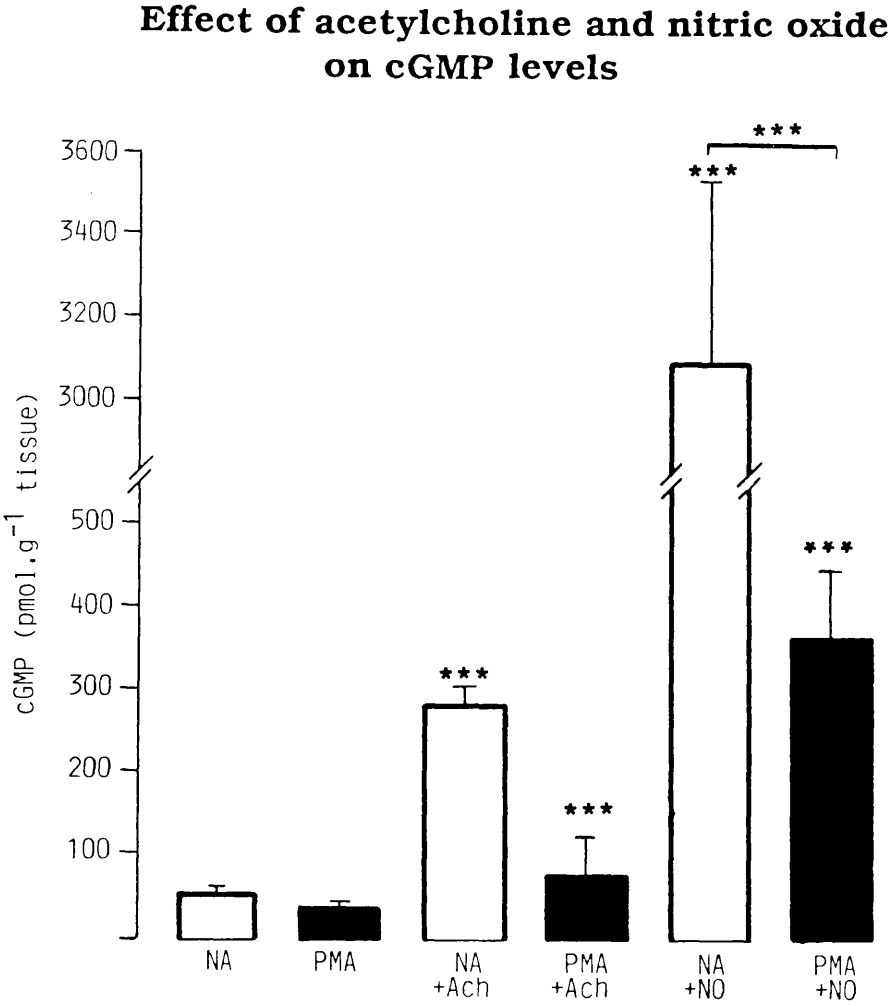


Figure 59.
Effects of acetylcholine (ACh) (10^{-5}M) and nitric oxide (NO) (3×10^{-6}M) on levels of cyclic GMP in aortic rings precontracted with noradrenaline (NA) (2×10^{-7}M) or PMA (5×10^{-7}M). Noradrenaline and PMA alone, did not induce high levels of cyclic GMP. Acetylcholine significantly increased levels of cyclic GMP only in tissues precontracted with noradrenaline (*** $p<0.001$). Nitric oxide significantly increased levels of cyclic GMP (*** $p<0.001$). The levels of cyclic GMP induced by acetylcholine and nitric oxide were significantly smaller in tissues precontracted with PMA than in tissues precontracted with noradrenaline. (*** $p<0.001$). The endothelium was intact. (n=6).

Part Eight: Effects of nitric oxide on the rate of Ptd Ins hydrolysis associated with vascular smooth muscle contraction induced by noradrenaline or PMA

Nitric oxide (3×10^{-6}M; 20s.) significantly reduced the level of Ptd OH below that measured in the presence of noradrenaline (2×10^{-7}M; 5mins.), whether added after or before noradrenaline (figure 60).

Nitric oxide (3×10^{-6}M; 20s.) did not significantly reduce the level of Ptd OH below that measured in the presence of PMA (5×10^{-7}M; 45mins.), whether added after or before PMA (figure 61).

Effect of nitric oxide on NA-induced PI hydrolysis.

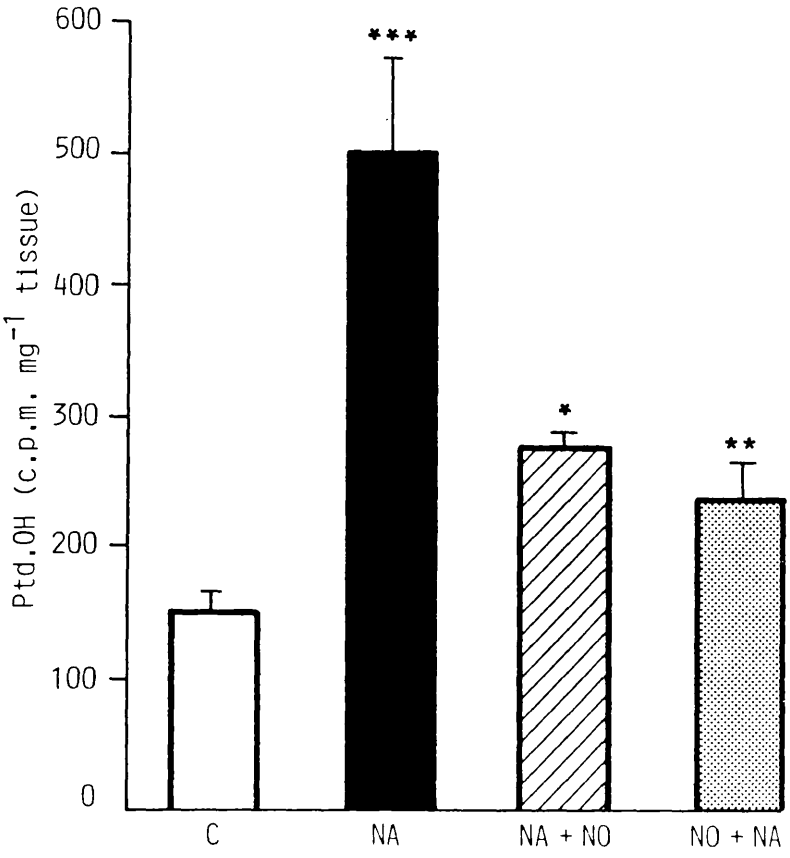


Figure 60.
Effect of nitric oxide (NO) (3 x 10^{-6}M) on the rate of Ptd Ins hydrolysis induced by noradrenaline (NA) (2 x 10^{-7}M). Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Control tissues were exposed to saline (5mins.), and test tissues to noradrenaline (5mins.) or noradrenaline (5mins.) and nitric oxide (20sec.). Noradrenaline alone, significantly increased the level of Ptd OH above the control value (*** $p<0.001$). Nitric oxide, whether added after or before noradrenaline, significantly reduced the level of Ptd OH below that induced by noradrenaline alone (* $p<0.05$, ** $p<0.01$). (n=4).

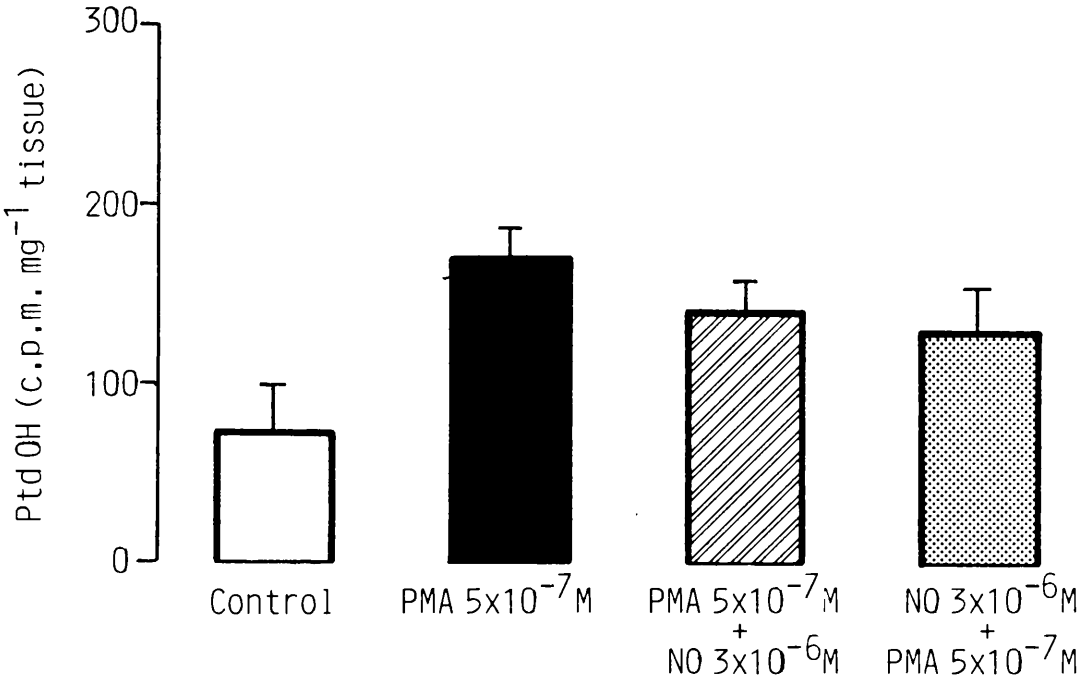


Figure 61.
Effect of nitric oxide (NO) (3 x 10^{-6}M) on the rate of Ptd Ins hydrolysis in the presence of PMA (5 x 10^{-7}M). Hydrolysis of Ptd Ins was monitored by measuring the level of Ptd OH. Control tissues were exposed to DMSO, 0.05% (45 mins.). Test tissues were exposed to PMA (45mins.) and nitric oxide (20s.). PMA alone did not significantly increase the level of Ptd OH above the control value. Nitric oxide, whether added after or before PMA, did not significantly reduce the level of Ptd OH below that measured in the presence of PMA alone. (n=4).

Section Three: Vascular relaxation of aortic rings from spontaneously hypertensive rats (SHR)

In this series of experiments, vascular relaxation was studied in aortic rings from spontaneously hypertensive rats (SHR) and normotensive wistar kyoto (WKY) rats. Three parameters were measured: (i) the degree of smooth muscle relaxation; (ii) the levels of cyclic GMP induced by the vasorelaxants; and (iii) the rates of Ptd Ins hydrolysis induced by noradrenaline.

These experiments were carried out with the assistance of Miss Fiona M^{c}Kenzie.

Part One: Measurement of blood pressures of SHR and WKY rats

The average systolic blood pressure of SHR was significantly higher than that of WKY rats (table 2).

Table 2.
Systolic blood pressure measured in conscious rats using the tail-cuff method.

RAT	BLOOD PRESSURE (mmHg) (± S.E.M.)
SHR	210.0 ± 6.5***
WKY	146.6 ± 7.2

The blood pressure measured in SHR was significantly higher than that measured in WKY normotensive rats (*** $p<0.001$). ($n\leq 4$).

Part Two: Preliminary experiments on vascular smooth muscle contractions induced by noradrenaline and KCl in aortic rings from SHR and WKY rats

The basal tone developed by aortic rings from SHR was higher than that developed by aortic rings from WKY rats.

Noradrenaline induced concentration-dependent contractions of aortic rings from both SHR and WKY rats (figure 62). The EC_{50} concentration of noradrenaline for muscle contraction was determined to be 10^{-7}M. Noradrenaline induced significantly smaller contractions of aortic rings from SHR, than from WKY rats (figure 62).

KCl induced concentration-dependent contractions of aortic rings from both SHR and WKY rats (figure 63). KCl induced significantly smaller contractions of aortic rings from SHR, than from WKY rats (figure 63).

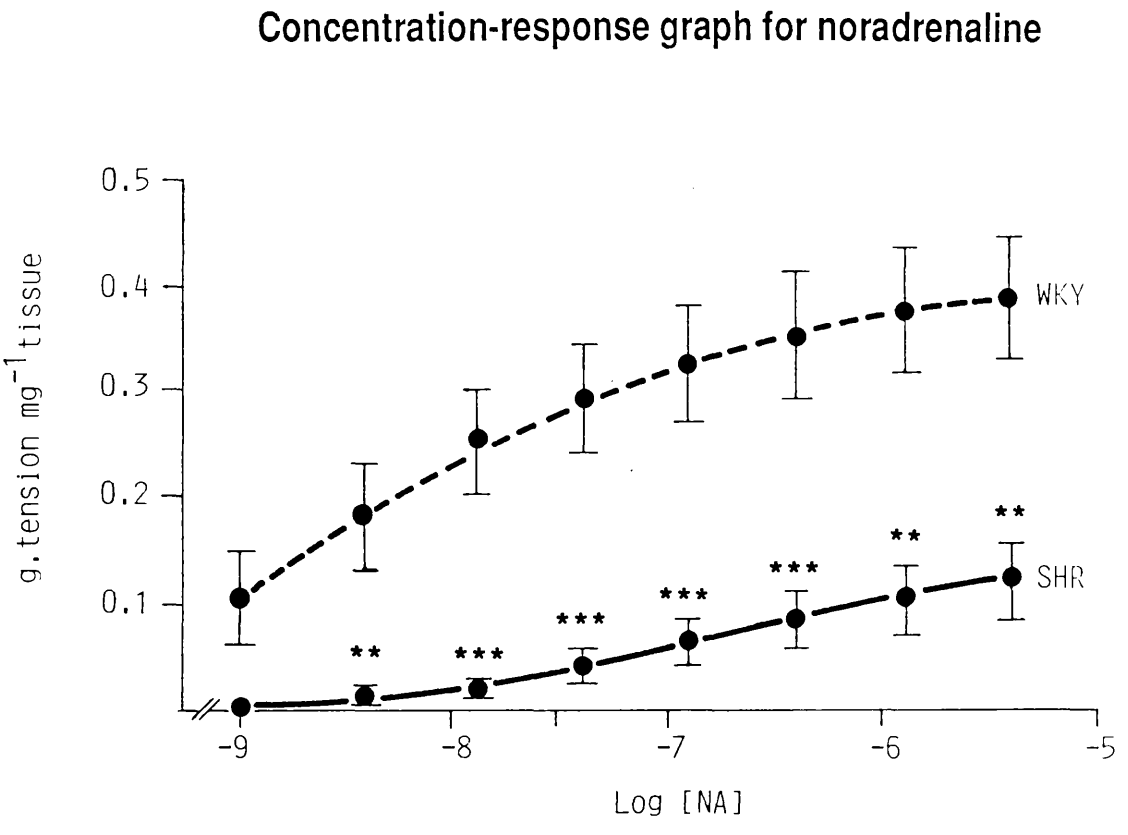


Figure 62.
Concentration-response graphs for noradrenaline in aortic rings from SHR (—) and WKY (--) rats. Muscle tension was recorded. Noradrenaline induced significantly smaller contractions in tissues from SHR than in tissues from WKY rats. (** $p<0.01$, *** $p<0.001$). The endothelium was intact. (n=6).

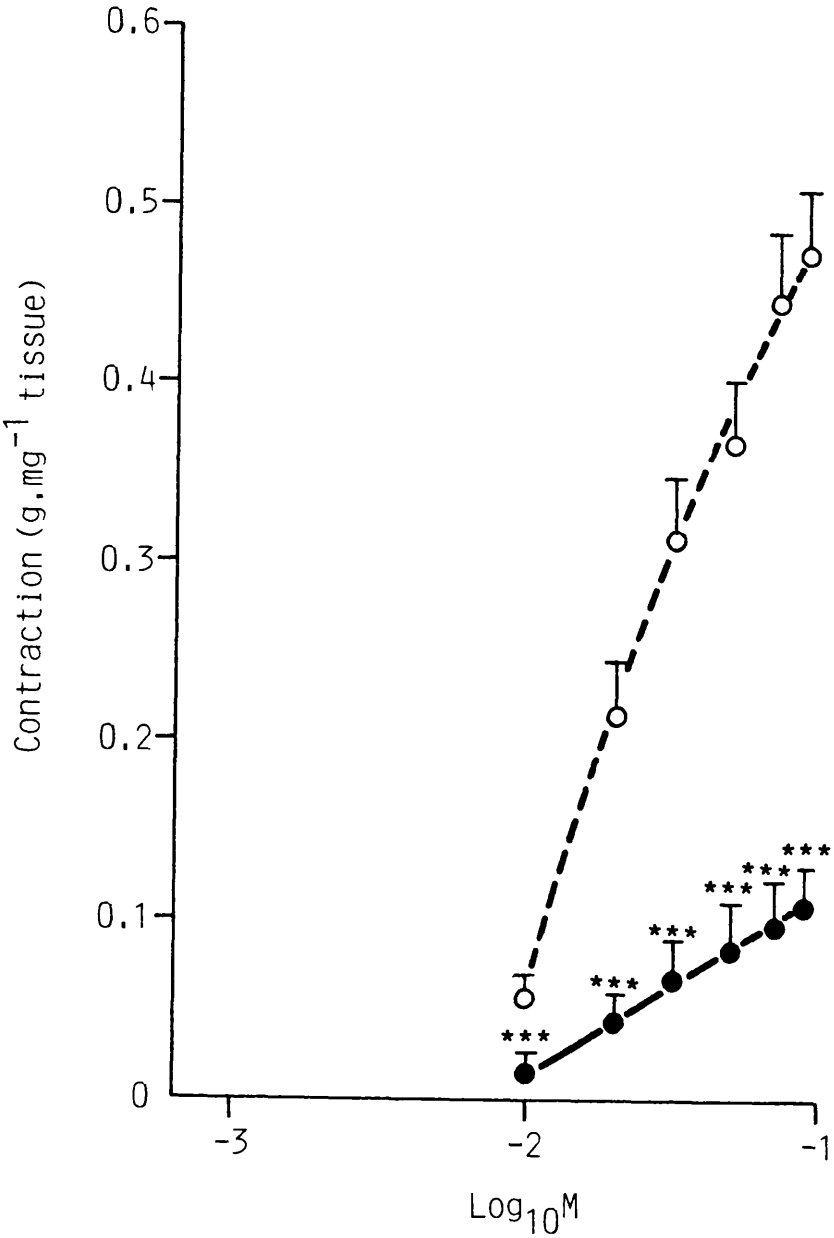


Figure 63.
Concentration-response graphs for KCl in aortic rings from SHR (●) and WKY (O) rats. Muscle tension was recorded. KCl induced significantly smaller contractions in tissues from SHR than in tissues from WKY rats. (*** $p<0.001$). The endothelium was intact. (n=6).

Part Three: Vascular relaxations of aortic rings from SHR and WKY rats

Acetylcholine induced concentration-dependent relaxations of aortic rings precontracted with noradrenaline (10^{-7}M) from both SHR and WKY rats (figure 64). Acetylcholine-induced relaxations of aortic rings were not significantly different between tissues from SHR and WKY rats.

Sodium nitroprusside induced concentration-dependent relaxations of aortic rings, precontracted with noradrenaline (10^{-7}M), from both SHR and WKY rats (figure 65). At low concentrations (10^{-8}M), sodium nitroprusside induced significantly weaker relaxations of aortic rings from SHR, than of aortic rings from WKY rats. However, at higher concentrations (10^{-6}M), sodium nitroprusside induced significantly greater relaxations of aortic rings from SHR than of aortic rings from WKY rats.

Sodium nitroprusside induced concentration-dependent relaxations of aortic rings from SHR both in the presence and absence of M&B 22948 (10^{-5}M) (figure 66). M&B 22948 did not significantly alter relaxations induced by sodium nitroprusside. Ethanol, the vehicle for M&B 22948, did not affect relaxations induced by sodium nitroprusside (not shown). In aortic rings, precontracted with noradrenaline, from both SHR and WKY rats, sodium nitroprusside (10^{-6}M; 60s.) significantly increased levels of cyclic GMP above those measured in control tissues exposed to noradrenaline alone (figure 67). However, the level of cyclic GMP induced by sodium nitroprusside (10^{-6}M) in aortic rings from SHR was significantly greater than that induced in aortic rings from WKY rats.

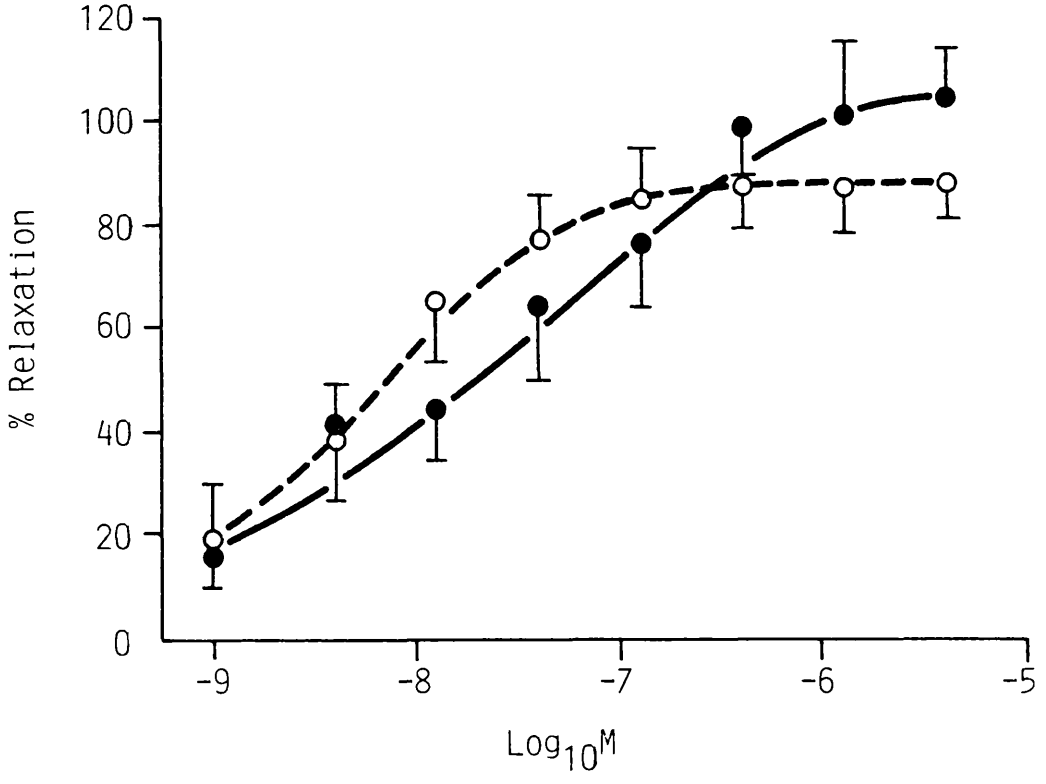


Figure 64.
Concentration-response graphs for acetylcholine in aortic rings from SHR (●) and WKY rats (○). The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (EC_{50}: 10^{-7}M). Acetylcholine-induced relaxations were not significantly different between aortic rings from SHR and WKY rats. (n=6)

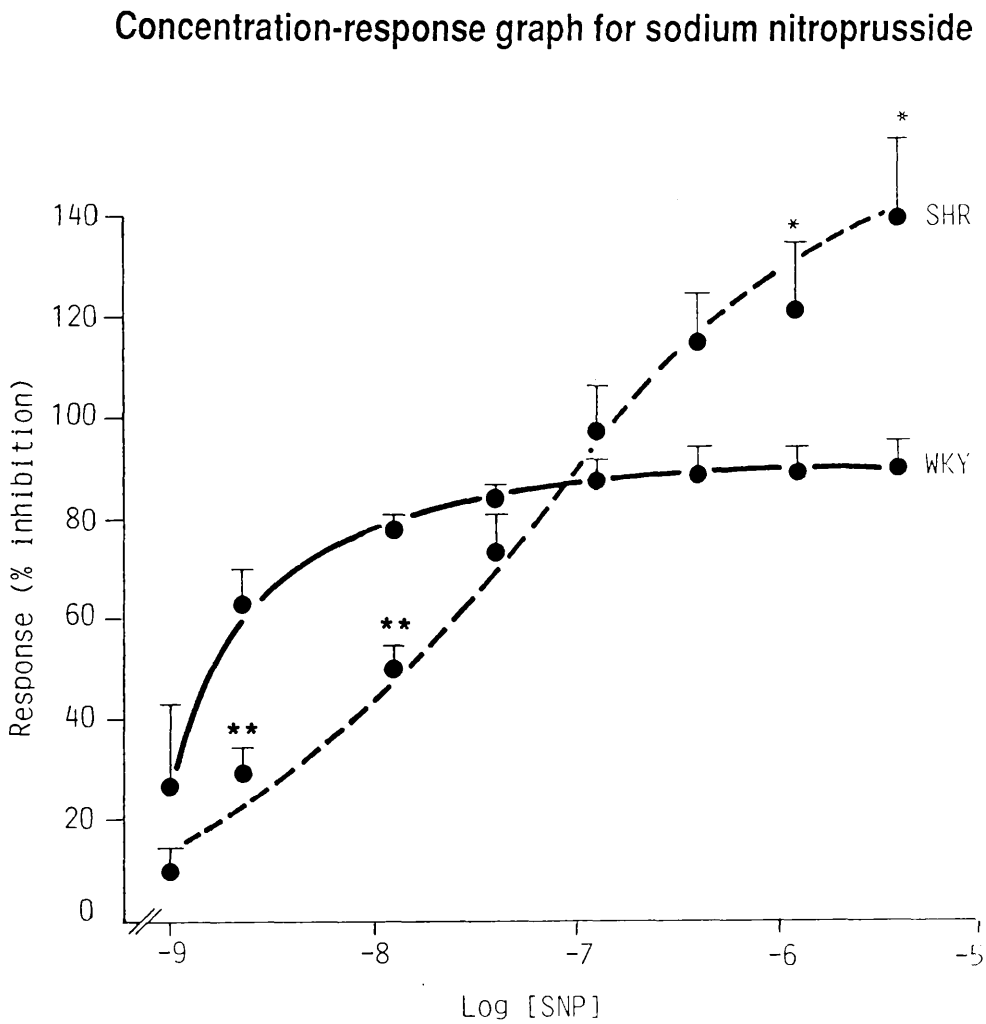


Figure 65.
Concentration-response graphs for sodium nitroprusside in aortic rings from SHR (---) and WKY rats (—). The degree of muscle relaxation was recorded and expressed as a % of the contraction elicited by noradrenaline (EC_{50}: 10^{-7}M). At low concentrations, sodium nitroprusside induced significantly weaker relaxations in SHR than in WKY tissues (** $p<0.01$). However, at higher concentrations, sodium nitroprusside induced significantly greater relaxations in SHR than in WKY tissues. (* $p<0.05$). (n=6).

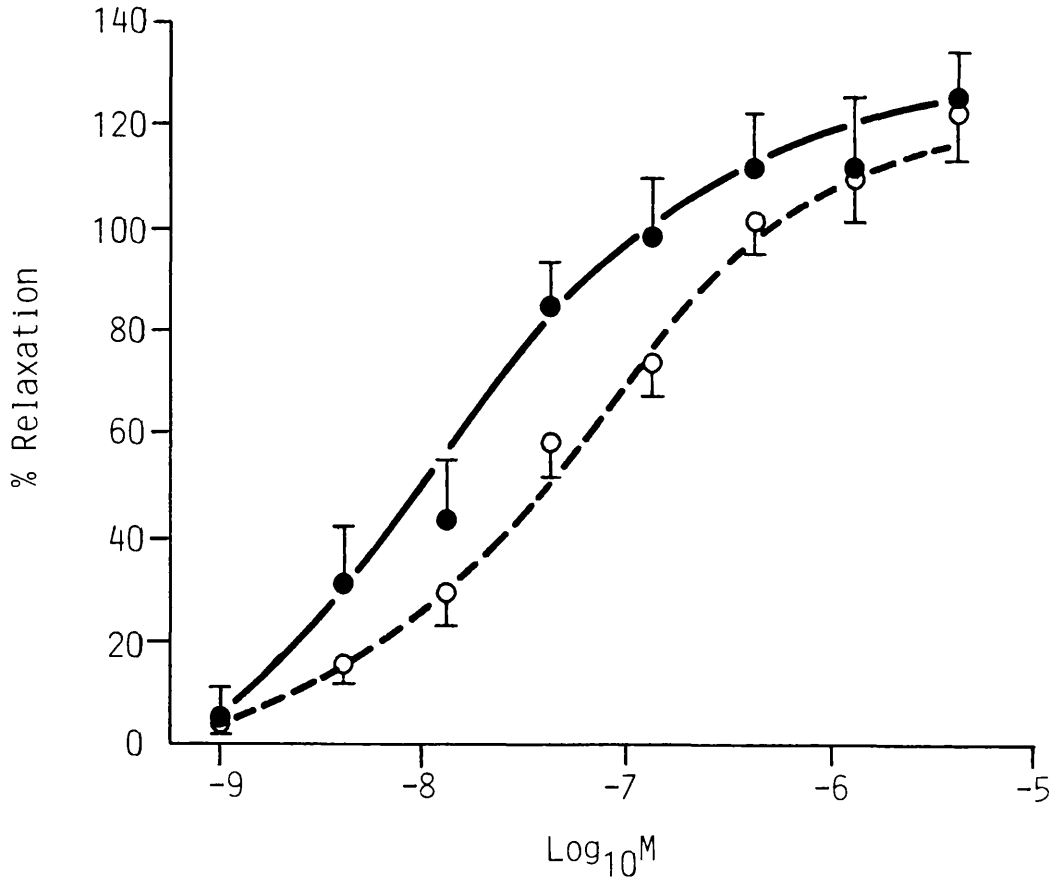


Figure 66.
Concentration-response graphs for sodium nitroprusside in aortic rings from SHR, in the absence (●) and presence (○) of M&B 22948 (10^{-5}M). Sodium nitroprusside-induced relaxations were unaffected by M&B 22948. (n=6).

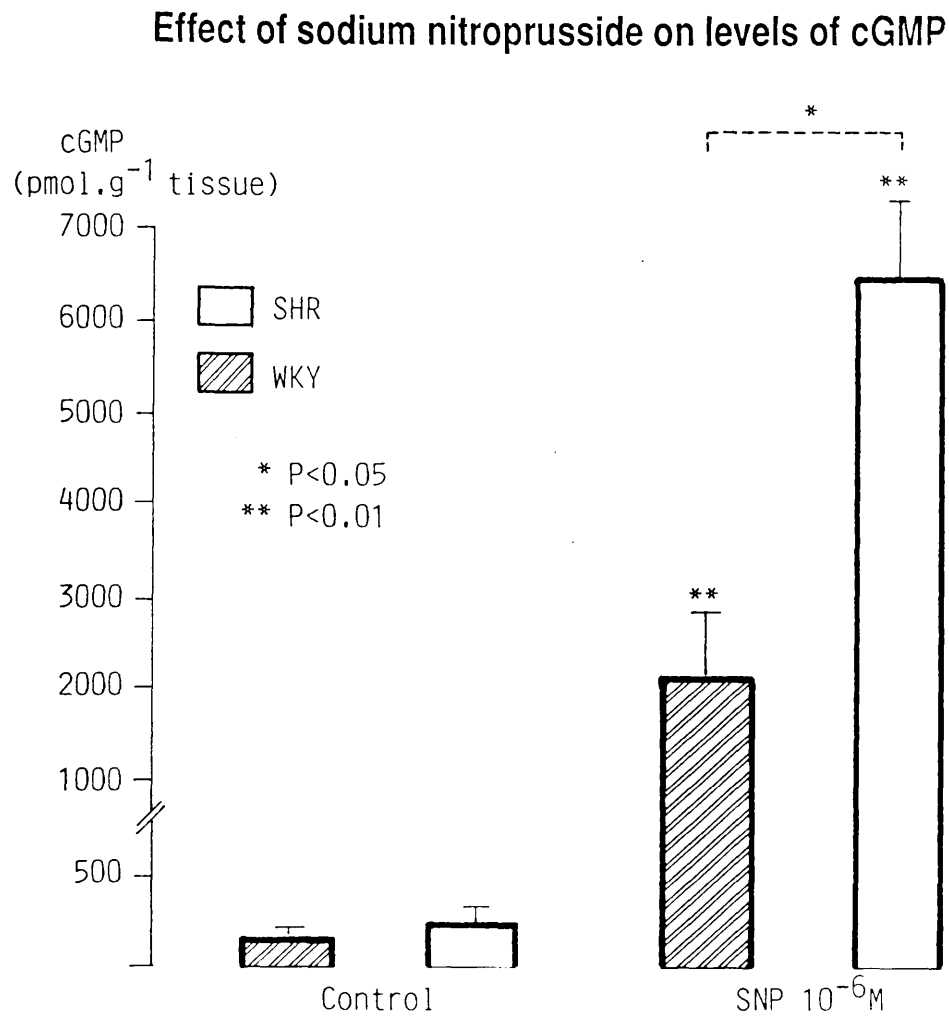


Figure 67.
Effect of sodium nitroprusside on levels of cyclic GMP in aortic rings, from SHR and WKY rats, precontracted with noradrenaline (10^{-7}M). Sodium nitroprusside significantly increased levels of cyclic GMP above control values in tissues from both SHR and WKY rats (** $p<0.01$). However, sodium nitroprusside induced a significantly higher level of cyclic GMP in tissues from SHR than in tissues from WKY rats (* $p<0.05$). (n=6).

Part Four: Effects of noradrenaline on the rates of Ptd Ins hydrolysis in aortic rings from SHR and WKY rats

Noradrenaline (2×10^{-7}M) induced a significant increase in the level of Ptd OH above the control value in aortic rings from WKY rats (figure 68). The level of Ptd OH was significantly higher in control tissues exposed to saline, from SHR, than the level of Ptd OH measured in control tissues from WKY rats. Noradrenaline did not significantly increase the level of Ptd OH above the control value in SHR.

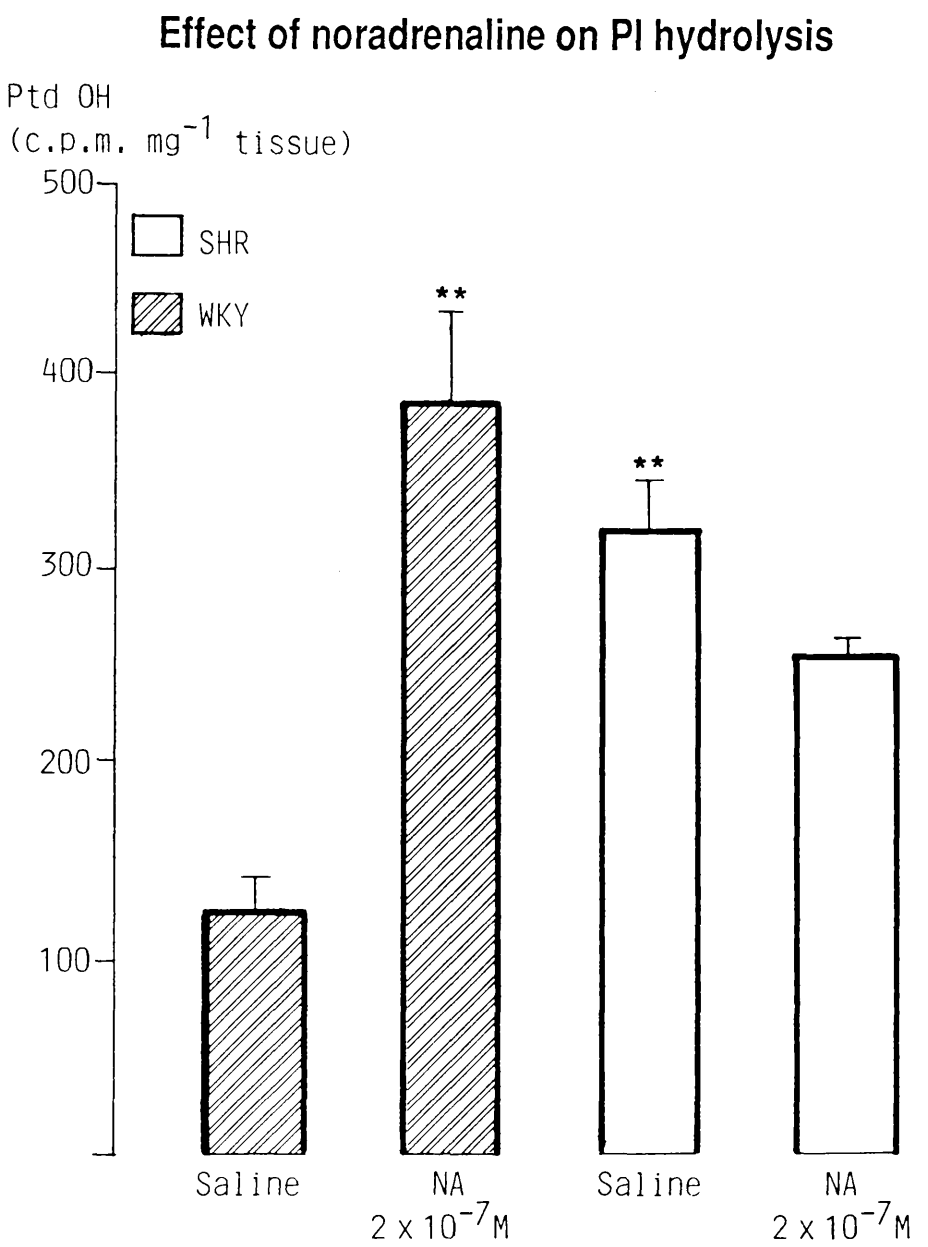


Figure 68.
Effect of noradrenaline (NA) (2 x 10^{-7}M) on the rate of Ptd Ins hydrolysis in aortic rings from SHR and WKY rats. Hydrolysis of Ptd Ins was monitored by measuring the levels of Ptd OH. Control tissues were exposed to saline (5mins.), and test tissues to noradrenaline (5mins.). Noradrenaline induced a significant increase in the level of Ptd OH above the control value in tissues from WKY rats (** $p<0.01$). The level of Ptd OH in SHR control tissues was significantly higher than that in WKY control tissues (** $p<0.01$). Noradrenaline did not increase the level of Ptd OH above the control value in SHR tissues. (n=4).

DISCUSSION

The degree of vascular smooth muscle tone exerted by a blood vessel, is the resultant of both smooth muscle contraction and relaxation. The detailed mechanisms of vascular relaxation however, are poorly understood, and present opportunities for further investigation.

In this study, rat isolated aortic rings were used to examine smooth muscle relaxation, since this tissue provided a convenient system for both mechanical recording and biochemical measurements. Throughout this study, both smooth muscle contraction and relaxation were recorded, and related to the intracellular biochemical changes that accompanied the development or inhibition of muscle tone. In most experiments, the biochemical correlates of muscle tension were measured in tissues in which the mechanical responses had been recorded. This approach allowed a closer analysis of the relationship between muscle tension and the causative intracellular events.

This study was divided into three general sections, although interpretation of data and subsequent development of ideas were not confined to any one particular series of experiments. The areas of research were:

(i) vascular relaxation induced by the bovine inhibitory factor;

(ii) the effects of the contractile agents used to raise muscle tone on the degree and nature of vascular relaxation; and

(iii) vascular relaxation of isolated aortic rings from spontaneously hypertensive rats.

Section One

It is now well established that vascular smooth muscle relaxation induced by nitrovasodilators and endothelium-dependent relaxants, is mediated by cyclic GMP (reviewed by Rapoport and Murad, 1983b). Furthermore, an inhibitory factor extracted from the bovine retractor penis muscle has been shown to relax various arterial smooth muscle preparations in numerous species, including the rat (Bowman et al., 1981). This relaxant effect may also be mediated by cyclic GMP (Bowman and Drummond, 1984). The results presented in this current study, to a large extent confirm these previous findings, although important and fundamental differences are reported.

Acetylcholine and sodium nitroprusside were confirmed to induce endothelium-dependent and endothelium-independent relaxations respectively, of rat isolated aortic rings precontracted with noradrenaline (figure 11). These relaxant effects were associated with increased levels of cyclic GMP in a concentration- and time-dependent manner, and are in agreement with the common hypothesis for vascular smooth muscle relaxation (figures 12, 13, 15 and 16). However, the bovine inhibitory factor, a smooth muscle relaxant, induced much weaker relaxations of both rat aortic rings and strips precontracted with noradrenaline, but induced complete inhibition of muscle tone raised with noradrenaline in rabbit aortic strips (figures 12, 13, 23a and 23b). The weak relaxant effects of the inhibitory factor in rat aorta contrast with other studies in which the inhibitory factor induced potent, concentration-dependent relaxations of rat aortic strips (Bowman et al., 1981).

Furthermore, relaxations of rat aortic rings induced by the inhibitory factor were not associated with increased levels of cyclic GMP (figure 17). This result was unexpected since inhibitory factor-induced relaxations of the bovine retractor penis muscle were associated with increased levels of cyclic GMP (Bowman and Drummond, 1984).

Several explanations may account for the weak relaxations of rat aortic rings induced by the inhibitory factor in this study, and the apparent inability to increase levels of cyclic GMP associated with smooth muscle relaxation. Firstly, the efficacy of the inhibitory factor may be species dependent. In this study, identical samples from an aliquot of the same extract produced weak relaxations of rat aortic rings and strips, but induced complete inhibition of muscle tone raised with noradrenaline in rabbit aortic strips (figures 23a and 23b). Since the conditions in each experiment were apparently identical, this heterogeneity of vascular responsiveness to the inhibitory factor may be species-related. The differential reactivity to the inhibitory factor in the rat and the rabbit may be explained by differences in the activities of the enzymes guanylate cyclase and cyclic GMP phosphodiesterases in the two species. There is some evidence to suggest that the activities of these enzymes differ in the rat and the rabbit: in rat aorta, acetylcholine induced levels of cyclic GMP that were not maintained with prolonged relaxation (figure 15; Rapoport et al., 1983), whereas in rabbit aorta, acetylcholine increased cyclic GMP levels that remained elevated with continued relaxation (Furchgott et al., 1984). This suggests that possibly the activities of cyclic GMP

phosphodiesterases are higher in rat than in rabbit aorta, and this may prevent increased levels of cyclic GMP and muscle relaxation induced by the inhibitory factor. However, the relaxant effects of acetylcholine and sodium nitroprusside would also be influenced by the actions of cyclic GMP phosphodiesterases. It would therefore be interesting to repeat the present series of experiments using rabbit aortic rings to determine if the potent relaxations induced by the inhibitory factor are associated with increased levels of cyclic GMP and exhibit a temporal relationship.

Although inhibitory factor-induced relaxation of bovine retractor penis muscle was associated with increased levels of cyclic GMP (Bowman and Drummond, 1984), it may be unreasonable to expect that the inhibitory factor would also increase levels of cyclic GMP associated with relaxation of arterial smooth muscle preparations. The inhibitory factor may be the endogenous inhibitory transmitter of the NANC innervation of the bovine retractor penis muscle (Ambache et al., 1975) and therefore may be expected to be more stable in this tissue than in rat aorta, since the function of a neurotransmitter is dependent on at least transient stability. Furthermore, there is strong evidence, from other work, to suggest that the inhibitory factor is nitrite from which nitric oxide is released on acid activation (Martin et al., 1988) and the low efficacy of the inhibitory factor in rat aorta may be due to the ability of this tissue to generate free radicals such as superoxide (O_2^-) that can oxidise and inactivate the inhibitory factor. It would therefore be interesting to compare the relaxant effects of

the inhibitory factor in rat and rabbit aortic rings both in the absence and presence of superoxide dismutase, a scavenger of superoxide that catalyses the rapid dismutation of superoxide to oxygen and hydrogen peroxide.

The technique used to measure tissue levels of cyclic GMP may have determined the results obtained. In this study, the levels of cyclic GMP in aortic rings were measured by radioimmune assay using a commercially available kit. In most experiments, cyclic GMP levels were measured in tissues in which the mechanical responses had been recorded, and although this technique may permit clearer interpretations of the relationship between muscle tension and levels of cyclic GMP, since both parameters were measured in the same tissues, practical considerations may limit the extent to which the analysis can be developed. For example, prior to the process of cyclic GMP extraction, aortic rings in which muscle tension had been recorded, were rapidly detached from force transducers and removed from organ baths before freezing in liquid nitrogen. The transfer of tissues was carried out in less than 2s., but this time period may have been sufficient for cyclic GMP phosphodiesterases to hydrolyse cyclic GMP to 5'-GMP, before being inactivated by freezing. However, this was a standard procedure used throughout the study and applied to other vasorelaxants that induced detectably high levels of cyclic GMP. A comparison between different relaxants therefore, is valid. The information derived from these experiments however, would have been more representative of the actual changes in tissue cyclic GMP levels if instantaneous freezing of aortic rings at the desired time, had occurred. Perhaps the

adoption of "drop-down" organ baths that allow rapid freezing of aortic rings while they are still attached to force transducers and exerting a particular degree of muscle tension (eg. Ignarro et al., 1984), would have been an improvement on the technique used.

In this study, extraction of cyclic GMP was demonstrated to be more efficient following tissue homogenisation (figure 5). Homogenisation of aortic rings was thought to increase the surface area across which the acid extractant could permeate and thus increase the yield of detectable cyclic GMP. However, this result contrasts with the findings of Bowman and Drummond, 1984, who reported that tissue homogenisation during acid extraction did not significantly increase the yield of cyclic GMP. Therefore, extraction of cyclic GMP, in this present study, under apparently optimal conditions, did not result in increased levels of cyclic GMP in response to the inhibitory factor during vascular relaxation.

It must be appreciated that the total tissue cyclic GMP content exists in distinct intracellular compartments (Waldman and Murad, 1987). Furthermore, tissue homogenisation and acid extraction may yield concentrations of cyclic GMP that originate from different pools. Therefore, although the extraction method used may maximise the yield of cyclic GMP obtained, it does not distinguish between different pools of cyclic GMP and this may obscure the actual changes in cyclic GMP formation if the changes occur in only one of the compartments.

Throughout this study, tissue levels of cyclic GMP were measured by a radioimmune assay based on the competitive

binding between unlabelled cyclic GMP and [^{3}H]-labelled cyclic GMP to an antiserum with high affinity and specificity for cyclic GMP. The levels of cyclic GMP measured in all experiments were within the detection limits specified for the assay kit. Furthermore, the standard radioimmune assay procedure was modified in that acetylation of cold cyclic GMP increased the sensitivity of the assay (figure 7). By using [^{3}H], a β emitter, as the isotope label, the radioimmune assay provided a convenient, highly reproducible and safe means of measuring cyclic GMP. [^{125}I]-labelled cyclic GMP is commonly used in the measurement of cyclic GMP levels, but although [^{125}I] is a more energetic emitter of radioactivity, giving rise to a more sensitive assay, the absolute resolution of tissue levels of cyclic GMP would not be enhanced. Furthermore, the use of [^{125}I]-labelled cyclic GMP in the measurement of tissue levels of cyclic GMP has been reported to give variable reproducibility (Shaw, personal communication).

Despite the inability of the inhibitory factor to increase levels of cyclic GMP associated with vascular relaxation of rat aortic rings, a role for cyclic GMP cannot be completely excluded from the interpretation of the results. This study presents results that suggest that the absolute levels of cyclic GMP measured during vascular relaxation of rat aortic rings may be of less importance than some other mechanistic indicator such as the rate of turnover of cyclic GMP. Also, other work has shown that there may be a close link between membrane hyperpolarisation and the formation of cyclic GMP during vascular relaxation (Rapoport *et al.*, 1985), and this also requires consideration.

16). However, only the initial development, but not maintenance of smooth muscle relaxation induced by acetylcholine, closely paralleled increased levels of cyclic GMP (figure 15). This confirms other reports (Rapoport et al., 1983), and suggests that increased levels of cyclic GMP may mediate the early development of muscle relaxation. However, the rapid decrease in cyclic GMP levels while acetylcholine-induced relaxation was well maintained (figure 15), suggests that intracellular mechanisms, following the formation of cyclic GMP are responsible for maintaining relaxation. Other possible explanations for the dramatic decrease in cyclic GMP levels after 30s. exposure to acetylcholine are: (i) cyclic GMP was being extruded from the smooth muscle cells; and (ii) cyclic GMP was hydrolysed by highly active phosphodiesterases. The first of these two explanations seems unlikely since cyclic GMP would probably take several minutes to traverse the plasma membrane (eg. Rashatwar et al., 1987) and falls in the levels of cyclic GMP are measured after only 30s. (figure 15). The second possibility is a more feasible explanation of the results and requires further investigation. It is tempting to speculate on the effect of cyclic GMP phosphodiesterase inhibition on acetylcholine-induced relaxations and cyclic GMP levels in rat aortic rings: inhibition of cyclic GMP phosphodiesterases may reduce the EC_{50} concentration of acetylcholine for muscle relaxation and increase the levels of cyclic GMP, which may remain elevated for a longer period. The work of Souness et al., (1987) appears to support this hypothesis.

In contrast with the effects of acetylcholine, the levels of

cyclic GMP induced by sodium nitroprusside (10^{-6}M), closely paralleled both the development, and for some time, the maintenance of vascular relaxation (figure 16). Sodium nitroprusside is a potent stimulator of guanylate cyclase activity and the large increases in levels of cyclic GMP and their prolonged elevation may be accounted for by saturation of cyclic GMP phosphodiesterases which were unable to hydrolyse the high concentration of cyclic GMP.
The difference in the levels of cyclic GMP induced by sodium nitroprusside and acetylcholine may also be accounted for by differences in the mechanisms leading to stimulation of guanylate cyclase activity. The relaxant effects of acetylcholine are believed to be receptor-mediated (Furchgott and Zawadzki, 1980a) and involve the synthesis and/or release of the EDRF. It may be proposed that this is an ordered process that is regulated at various sites by other intracellular mechanisms. Sodium nitroprusside-induced relaxations however, involve the diffusion of the relaxant across the plasma membrane, after which nitric oxide is thought to be spontaneously generated via an S-nitrosothiol intermediate (Ignarro and Kadowitz, 1985). This process would appear to be a more rapid but crude means of stimulating guanylate cyclase activity and may partially explain the different potencies of acetylcholine and sodium nitroprusside in increasing levels of cyclic GMP.

Section Two

Relaxation of isolated arterial smooth muscle preparations is usually studied in tissues which have been precontracted with some contractile agent or procedure. Furthermore,

vascular tone is a resultant of the two opposing mechanical processes of muscle contraction and relaxation. Therefore, the effects of the contractile agents used to raise muscle tone on the degree and nature of vascular relaxation of rat aortic rings were studied. Using this approach, it was hoped to further elucidate the mechanisms of smooth muscle relaxation.

In this study, three contractile agents were compared: noradrenaline, KCl and the tumour-promoting phorbol ester PMA.

Noradrenaline-induced contractions of rat aortic rings were associated with enhanced hydroylsis of Ptd Ins (figures 34-38). This effect was consistent with the literature and is believed to be mediated by $alpha_1$-adrenoceptors (Villalobos-Molina et al., 1982). However, the actions of noradrenaline on post-junctional $alpha_2$-adrenoceptors and beta-adrenoceptors must also be considered. Stimulation of $alpha_2$-adrenoceptors, located on vascular smooth muscle, by noradrenaline, elicits a Ca^{2+} influx through receptor-operated channels without any change in the membrane potential (Hondeghem and Katzung, 1986), but is not associated with accelerated Ptd Ins hydrolysis (Rüegg, 1988). In rat aorta, it has been proposed that the post-junctional alpha-adrenoceptors mediating vasoconstriction may possess characteristics of both $alpha_1$- and $alpha_2$-adrenoceptors (Ruffolo et al., 1981; 1982). However, more recent work in this field suggests that post-junctional alpha-adrenoceptors in rat aorta should be classified as a homogeneous population of $alpha_1$-adrenoceptors (Beckeringh et al., 1984; Chiu et al., 1986). Noradrenaline-induced

contractions of rat aortic rings may therefore be regarded to be mediated by $alpha_1$-adrenoceptors. $Alpha_1$-adrenoceptor-mediated contractions will, however, be counteracted to some degree by smooth muscle relaxation mediated by $beta_2$-adrenoceptor activation by noradrenaline. This effect is mediated by cyclic AMP (Hardman, 1981). This study did not measure tissue levels of cyclic AMP since levels of this cyclic nucleotide did not change during vascular relaxation induced by nitrovasodilators and endothelium-dependent relaxants (Rapoport et al., 1983). However, it would be interesting to measure the levels of cyclic AMP associated with noradrenaline-induced contractions of aortic rings in the presence and absence of a beta-adrenoceptor antagonist to determine the role of beta-adrenoceptors in contractions of aortic rings induced by noradrenaline. In order to minimise any non-$alpha_1$-adrenoceptor-mediated effects on the tension developed by rat aortic rings, it would have been more prudent to use a selective $alpha_1$-adrenoceptor agonist eg. phenylephrine instead of noradrenaline.

In this study, the muscle tension developed by rat aortic rings was recorded isometrically using force displacement transducers (figure 4) and standard methods commonly cited in the literature, were employed in the preparation of aortic rings or strips for mechanical recording (eg. Martin et al., 1986). However, certain of these experimental procedures may have influenced the results obtained, and therefore require further discussion.

True isometric tension is recorded in tissues that contract without shortening of the muscle fibres. This is attained when the opposing force, the applied tension, exceeds that

tension developed by the tissue, without over-stretching the muscle fibres. In this study, resting tensions of 2g and 1g were applied to aortic rings and strips respectively, and these exceeded the tension developed by the tissues (figures 9, 22, 25, 41 and 42).

Another factor which may have influenced the experimental results was the contractile viability of the tissue when suspended between two wire hooks in an organ bath for mechanical recording. It was important during the preparation of tissues for both mechanical and biochemical measurements, to maintain a functional endothelium, unless otherwise desired. It is to be expected that no matter how carefully the tissues were prepared, some damage to the vascular endothelium would occur when the tissues were suspended between wire hooks in the organ baths. However, this was a standard procedure used throughout the study and applied to all experiments, and therefore a valid comparison between experiments was possible. Furthermore it has been reported that only a fraction of the endothelial layer need be intact for complete relaxation of aortic rings induced by endothelium-dependent relaxants (Peach et al., 1985). It may also be assumed therefore, that a similar fraction of endothelial cells would influence noradrenaline-induced contractions. Therefore, minor damage to the vascular endothelium would not significantly affect the results obtained.

Removal of the endothelium, by rubbing with a wooden stick, was found to be a satisfactory method as confirmed by electron microscopy (photographs 1 and 2) and the abolishment of acetylcholine-induced relaxations (figures 11

and 13). However, during rubbing of aortic rings, care was taken to avoid damage to the smooth muscle layers, which may have resulted in diminished contractions in response to noradrenaline or other contractile agents.

Equilibration of tissues for 60mins. prior to the start of experiments was necessary to allow the re-establishment of ion concentration gradients and metabolic processes, following dissection of the tissues from the animal. Noradrenaline induced variable contractions when tissues were not allowed to equilibrate (not shown).

In this study, contractions of aortic rings were also induced by KCl and PMA. These contractions were not receptor-mediated (Jones, 1981; Chatterjee and Tejada, 1986) and were not associated with enhanced hydrolysis of Ptd Ins (figures 39 and 53). KCl-induced contractions were, for the most part, unaffected by the $alpha_1$-adrenoceptor antagonist, prazosin (10^{-6}M) (figure 26). However, prazosin did significantly reduce contractions elicited by the highest concentration of KCl (90mM). This contrasts with findings that contractions of aortic rings induced by KCl at low concentrations (8-12mM) were diminished by the $alpha_1$-adrenoceptor antagonist, phentolamine, but were unaffected at higher concentrations of KCl (>16mM) (Soltis and Katovich, 1985). This effect may be attributed to non-specific actions of phentolamine. From the present study therefore, it can be concluded that KCl, at physiological concentrations, did not release noradrenaline from adrenergic nerve terminals by depolarising the nerve membrane. Consistent with this view is the low rate of Ptd Ins hydrolysis measured in response to KCl (figure 39) since

noradrenaline alone, accelerated Ptd Ins hydrolysis (eg. figure 35). Furthermore, the rat thoracic aorta has a sparse adrenergic innervation (Kuchii et al., 1973), and therefore any KCl-induced release of noradrenaline would produce only a minor contribution to the contractions recorded. Therefore, subsequent experiments involving KCl-induced contractions of aortic rings were not conducted in the presence of prazosin.

The method used in this study to monitor agonist-induced hydrolysis of phosphoinositides, was the measurement of [^{32}P]-orthophosphate incorporation into Ptd OH. This technique gave an indirect measure of decreases in the levels of the parent inositol phospholipid, Ptd Ins, PIP, or PIP_2. However, the limitations of this technique must be recognised:

(i) the increase in levels of [^{32}P]-Ptd OH is a secondary event following the initial hydrolysis of inositol phospholipids. Furthermore, Ptd OH is an intermediate metabolite, being formed from DAG, and in turn serving as a precursor for the formation of Ptd Ins. The levels of Ptd OH may therefore fluctuate.

(ii) Ptd OH is mainly formed by the action of phospholipase C on the phosphoinositides. However, two minor mechanisms, independent of the phosphoinositide cycle, may affect the measured levels of [^{32}P]-Ptd OH: a) an enzyme, triglyceride lipase, can also generate DAG, from which Ptd OH is formed; and b) de novo synthesis of Ptd OH may occur, although this is believed to be a relatively slow process (Neufeld and Majerus, 1983) and may not interfere with the levels of [^{32}P]-Ptd OH measured in this study.

It may therefore be assumed that in this study increased levels of [^{32}P]-Ptd OH reflected increased mass of Ptd OH, and that accumulation of [^{32}P]-Ptd OH represented agonist-stimulated hydrolysis of phosphoinositides (Holmsen et al., 1984).

An increasingly popular technique for monitoring the hydrolysis of phosphoinositides, is the measurement of inositol phosphates (Berridge et al., 1983). This is a simpler technique, and when used in the presence of lithium to inhibit the enzyme inositol phosphate phosphatase, gives a direct measure of the hydrolytic products of the phosphoinositides. It would be worthwhile to repeat certain experiments carried out in this study using this alternative technique for the measurement of phosphoinositide hydrolysis.

The results presented in this study, clearly show that acetylcholine and sodium nitroprusside induced weaker relaxations of aortic rings precontracted with KCl, than of aortic rings precontracted with noradrenaline (figures 28-30). This finding is consistent with the work of Godfraind, 1986, who showed that an analogue of cyclic GMP, 8-bromo cyclic GMP, did not inhibit the influx of Ca^{2+} through potential-operated channels activated by KCl during muscle contraction. Also consistent with this view are the observations in this study that KCl-induced contractions of aortic rings and associated levels of cyclic GMP were unaffected by the removal of the endothelium (figures 25 and 31). This suggests that basal release of the EDRF (Martin et al., 1986), acting via cyclic GMP, does not affect the intracellular mechanisms mediating KCl-induced contractions.

The weak relaxations induced by acetylcholine and sodium nitroprusside were reflected in small rises in cyclic GMP and this finding is in accordance with the view that it is the absolute level of cyclic GMP that determines the degree of vascular relaxation. This contradicts previous results in this study (figures 17, 19 and 20), that suggested that other intracellular mechanisms may mediate smooth muscle relaxation.

Similarly, acetylcholine and sodium nitroprusside induced weaker relaxations of aortic rings precontracted with PMA, than of aortic rings precontracted with noradrenaline (figures 49-51). Again these weaker relaxations were associated with smaller increases in the levels of cyclic GMP (figure 52). Furthermore, the low rate of Ptd Ins hydrolysis measured in response to KCl or PMA was not altered by either acetylcholine (10^{-5}M) or sodium nitroprusside (10^{-6}M) (figures 39 and 53). However, accelerated Ptd Ins hydrolysis induced by noradrenaline was inhibited by both acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) (figures 37 and 38), suggesting that vascular relaxation may be caused by inhibition of the Ptd Ins response by cyclic GMP and is in agreement with the work of Rapoport (1986). Furthermore, in the absence of endothelium, noradrenaline induced greater contractions of aortic rings (figure 9) but this effect was not associated with decreased levels of cyclic GMP (figure 14). Indeed, removal of the vascular endothelium, the source of the EDRF, did not, significantly enhance the rate of Ptd Ins hydrolysis (figure 35).

There is evidence in the literature to suggest that the

basal release of the EDRF, acting via cyclic GMP, exerts an inhibitory effect on vascular tone (Martin et al., 1986). Removal of the endothelium removes the source of the EDRF and therefore abolishes the inhibitory effect on noradrenaline-induced contractions. Mechanical results obtained in this study (figure 9) partially corroborate this explanation. However, since noradrenaline-induced contractions of aortic rings, both in the presence and absence of endothelium, were not associated with changes in the levels of cyclic GMP, the possibility exists that basal release of the EDRF does not exert an inhibitory effect on smooth muscle tone by increasing cyclic GMP levels. Consistent with this view was the measurement of similar levels of cyclic GMP in control tissues exposed to saline in the presence and absence of endothelium (figure 14).
It may be proposed from these results that for potent vascular relaxation, associated with substantial increases in the levels of cyclic GMP, to be demonstrated in rat aortic rings, enhanced hydrolysis of Ptd Ins is first necessary. Consistent with this hypothesis is the observation in pig aortic smooth muscle that phosphoinositides were required for maximal stimulation of the plasmalemmal Ca^{2+}-Mg^{2+}-ATPase by protein kinase G, activated by cyclic GMP, in reducing $[Ca^{2+}]_i$ during muscle relaxation (Vrolix et al., 1988).
Inhibition of noradrenaline-induced muscle tone by cyclic GMP, may occur via multiple pathways. It has been proposed that cyclic GMP may inhibit $alpha_1$-adrenoceptor mediated vascular contraction by: (i) phosphorylating and inhibiting myosin light chain kinase (MLCK) via cyclic GMP-dependent

protein kinase (Nishikawa et al., 1984); and (ii) reducing $[Ca^{2+}]_i$ by acceleration of Ca^{2+} extrusion through the sarcolemma (Popescu et al., 1985), or inhibition of Ca^{2+} release from intracellular Ca^{2+} stores (Rapoport, 1986), or inhibition of Ca^{2+} influx into the cells (Taylor and Meisheri, 1986). In tissues precontracted with either KCl or PMA, the weaker relaxations recorded may be accounted for by a limited number of sites of inhibition for cyclic GMP. For example, in tissues precontracted with KCl, increased muscle tone is predominantly caused by an influx of Ca^{2+} through potential-operated channels. These channels are insensitive to cyclic GMP (Godfraind, 1986), and therefore the weaker relaxations recorded may be caused only by cyclic GMP-stimulated extrusion of Ca^{2+} through the sarcolemma and inhibition of MLCK resulting in dephosphorylation of myosin light chains. Similarly, in tissues precontracted with PMA, muscle relaxation may be due to inhibition of MLCK by cyclic GMP-dependent protein kinase since contraction is mediated by phosphorylation of the light chains by protein kinase C (Aksoy et al., 1983). It is uncertain whether cyclic GMP would affect Ca^{2+} metabolism in tissues precontracted with PMA, since there is some doubt as to increased $[Ca^{2+}]_i$ associated with PMA-induced contraction (Sybertz et al., 1986; Khalil and van Breemen, 1988).

The weak relaxations induced by acetylcholine and sodium nitroprusside of aortic rings precontracted with KCl or PMA are consistent with the current understanding of smooth muscle contraction and relaxation. However, the reasons for the associated small rises in cyclic GMP levels remain obscure. It may be speculated that KCl, or PMA, during

muscle contraction, gave rise to conditions within the cells that altered the activities of the enzymes guanylate cyclase and cyclic GMP phosphodiesterases, and thus prevented increases in cyclic GMP. In this respect, elevation of $[Ca^{2+}]_i$ (in the μM range) has been shown to directly inhibit guanylate cyclase (Greutter et al., 1980). This explanation however, requires further investigation. Therefore, lower levels of cyclic GMP together with a limited number of sites of inhibitory action for cyclic GMP, due to the nature of the mechanisms of contraction, may explain the weak relaxations observed in tissues precontracted with KCl or PMA.

However, in contrast with this explanation are the observations that acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) increased levels of cyclic GMP to the same extent in tissues not precontracted with noradrenaline as in tissues precontracted with noradrenaline (figure 21). Furthermore, acetylcholine (10^{-5}M) and sodium nitroprusside (10^{-6}M) inhibited noradrenaline-induced Ptd Ins hydrolysis in aortic rings when added to the assay tubes prior to noradrenaline (figures 37 and 38).

Throughout this study, it was noted that sodium nitroprusside induced more powerful relaxations of aortic rings than those induced by acetylcholine (figures 11, 12, 28-30, and 54). This effect was particularly interesting in tissues precontracted with PMA (figure 54) and may be accounted for by the following explanations (i) sodium nitroprusside exhibits dual vasorelaxant actions; in addition to being a potent stimulator of guanylate cyclase, sodium nitroprusside has been shown to hyperpolarise

vascular smooth muscle cell membranes and/or activate the Na^+-K^+-ATPase (Rapoport et al., 1985); and/or (ii) synthesis and/or release of the EDRF may be inhibited by tumour-promoting phorbol esters by a mechanism that involves activation of endothelial protein kinase C and phosphorylation of the GTP binding protein, G_i (Weinheimer et al., 1986).

This latter possibility was investigated in the current study. The results obtained suggest that the synthesis and/or release of the EDRF induced by acetylcholine may have been inhibited by PMA, but this effect did not involve stimulation of endothelial protein kinase C. This can be concluded from experiments comparing the abilities of acetylcholine and nitric oxide to relax aortic rings precontracted with either noradrenaline or PMA. Nitric oxide, identified as one of the EDRF's (Palmer et al., 1987), was confirmed to induce endothelium-independent relaxations (figure 58). Both acetylcholine and nitric oxide induced powerful relaxations of aortic rings precontracted with noradrenaline (figures 55 and 57). These relaxations were associated with increased levels of cyclic GMP (figure 59). Nitric oxide induced transient relaxations that were rapid in onset (figure 55). This may be explained by the presence of free radicals (eg. superoxide) present in the tissues and organ baths that inactivated nitric oxide. However, in tissues precontracted with PMA, acetylcholine and nitric oxide induced weaker relaxations (figures 56 and 57) that were associated with significantly smaller increases in the levels of cyclic GMP (figure 59). This is consistent with other results in this study. Again nitric

oxide induced transient relaxations that were slower in onset (figure 56). If PMA were inhibiting the synthesis and/or release of the EDRF stimulated by acetylcholine, via activation of endothelial protein kinase C, it might be expected that acetylcholine-induced relaxations would be diminished, but relaxations induced by nitric oxide would be unaffected by PMA. However, both acetylcholine- and nitric oxide-induced relaxations were inhibited to approximately the same extent (figure 57), and so the effects of PMA appear to be exerted in vascular smooth muscle cells rather than in the endothelium. It cannot be completely excluded from these results however, that PMA was stimulating an endothelial protein kinase C, since this effect may have been masked by the more predominant actions of PMA in vascular smooth muscle cells. The possibility that PMA was inhibiting the actions of nitric oxide in the endothelium can be excluded however, since similar results were obtained in both the presence and absence of endothelium (figure 58). Since the relaxant effects of sodium nitroprusside are mediated by nitric oxide (Ignarro and Kadowitz, 1985), and nitric oxide-induced relaxations were diminished to approximately the same extent as those relaxations induced by acetylcholine, in aortic rings precontracted with PMA (figures 56 and 57), it may be proposed that part of the sodium nitroprusside-induced relaxations of aortic rings precontracted with PMA was mediated by an alternative mechanism, such as membrane hyperpolarisation (Rapoport et al., 1985). However, for a more comprehensive analysis of this phenomenon, a detailed comparison between relaxations induced by sodium nitroprusside and nitric oxide is

necessary. In this respect, it is interesting to speculate whether the bovine inhibitory factor, which did not increase levels of cyclic GMP in rat aorta, may induce vascular relaxation via membrane hyperpolarisation.

Section Three

Essential hypertension is associated with elevated systemic arterial blood pressure and is caused by an increased peripheral resistance. The mechanisms mediating this increase in peripheral resistance, however, are poorly understood and have given rise to conflicting reports on the mechanisms of hypertension. For example, supersensitivity and subsensitivity have both been recorded in response to vasoconstrictor and vasodilator agents in hypertensive animals (reviewed by Winquist et al., 1982). In most of these studies only mechanical responses were recorded and the causative intracellular events were not measured. It is clear from these studies that the variability of results obtained may be due to the model and time-course of hypertension chosen, as well as the tissue and technique used for recording. The mechanisms of action of the agonists investigated may also have affected the results obtained. The current series of experiments therefore, examined the mechanisms of hypertension in only one model of hypertension, and related the mechanical responses of tissues to the underlying biochemical changes. Using this approach, it was hoped to gain a better understanding of vascular responsiveness in hypertension. The model of hypertension chosen for study was that observed in spontaneously hypertensive rats (SHR), which were

genetically predisposed to this condition and were indeed confirmed to be hypertensive in relation to normotensive wistar kyoto (WKY) control rats (table 2).

Noradrenaline induced much weaker contractions of aortic rings from SHR than of aortic rings from normotensive WKY control rats (figure 62). This finding is consistent with the work of Templeton et al, (1987). However, other results have shown a supersensitivity to noradrenaline (Kubo, 1979). The weak contractions elicited by noradrenaline in aortic rings from SHR, may be related to the higher resting tension developed by these tissues compared with that recorded in aortic rings from WKY control rats. It may be proposed therefore, that SHR aortic rings were incapable of developing further substantial tension in response to noradrenaline since the tissues were already partially contracted. This view is consistent with the high basal rate of Ptd Ins hydrolysis measured in SHR aortic rings, and the inability of noradrenaline to further enhance this rate of hydrolysis (figure 68).

Similarly, KCl induced much weaker contractions of SHR aortic rings than of WKY aortic rings. Although contractions of aortic rings elicited by KCl, as observed in tissues from male wistar rats, were not associated with an enhanced rate of Ptd Ins hydrolysis (figure 39), the weak contractions of SHR aortic rings induced by KCl, may also be explained by the high resting tension and elevated rate of Ptd Ins hydrolysis. Therefore, in the "resting" condition of SHR aortic rings, the contractile proteins may have been partially phosphorylated and KCl, acting via potential-operated Ca^{2+} channels, could not further activate the

contractile apparatus. However, it would have been interesting to measure the effect of KCl on the rate of Ptd Ins hydrolysis in both SHR and WKY aortic rings.

It is not known if the enhanced rate of Ptd Ins hydrolysis in SHR aortic rings in the "resting" state is a cause or an effect of hypertension. It is possible that increased $[Ca^{2+}]_i$ associated with hypertension would stimulate Ptd Ins hydrolysis, since the activity of phospholipase C is Ca^{2+}-sensitive (Abdel-Latif, 1986). Furthermore, it is interesting to speculate as to the existence of an endogenous ligand that, under the conditions of hypertension, stimulates this accelerated hydrolysis of Ptd Ins. A possible candidate is the platelet-derived growth factor (PDGF) which has been shown to stimulate Ptd Ins hydrolysis in Swiss 3T3 cells (Berridge *et al.*, 1984) and induce vascular smooth muscle cell proliferation *in vitro* (eg. Ross *et al.*, 1974).

Increased peripheral resistance observed in hypertension is believed to be partially caused by increased blood vessel wall thickness associated with smooth muscle hypertrophy (reviewed by Folkow, 1978). This characteristic of hypertension may be of importance in the method used to record mechanical responses of vascular tissues from hypertensive animals. Indeed many conflicting reports on vascular reactivity in hypertensive animals may be explained by the nature of the mechanical measurements recorded and not the pathophysiological and biochemical changes in the tissues. For example, supersensitivity to contractile agents was reported in many studies that recorded perfusion pressure in whole animal, or isolated vascular preparations

(eg. Collis and Vanhoutte, 1977). The perfusion pressure recorded in a blood vessel is determined by the law of Laplace, which states:

$$P=T/r,$$

where P is the perfusion pressure; T is the wall tension; and r is the radius of the lumen. In hypertension there is an increase in the wall thickness:lumen ratio, due mainly to smooth muscle hypertrophy. The diameter of the blood vessel is therefore decreased and there is a greater change in resistance produced by a given degree of muscle contraction in the vessel wall. However, when muscle tension is recorded directly in isolated vascular preparations, the contraction process of hypertrophic tissues from hypertensive animals may be less efficient, resulting in smaller increases in tension. It would therefore be interesting to compare vascular responsiveness in both perfused blood vessels and isolated tissue preparations from the same animal. Sodium nitroprusside induced concentration-dependent relaxations of both SHR and WKY aortic rings precontracted with noradrenaline (figure 65). At low concentrations, (10^{-9}M) sodium nitroprusside induced weaker relaxations of SHR aortic rings than of WKY tissues. It may be suggested that the relaxant effect of sodium nitroprusside at low concentrations was attenuated by highly active cyclic GMP phosphodiesterases in SHR aortic rings that rapidly hydrolysed cyclic GMP. However, the effect of phosphodiesterase inhibition on the levels of cyclic GMP induced by sodium nitroprusside at low concentrations in SHR aortic rings remains to be determined. However, in this study the cyclic GMP phosphodiesterase inhibitor M&B 22948

(10^{-5}M) did not affect sodium nitroprusside-induced relaxations of SHR aortic rings precontracted with noradrenaline (figure 66).

At higher concentrations, (10^{-6}M), sodium nitroprusside induced more powerful relaxations of SHR aortic rings than of WKY aortic rings (figure 65). This effect was associated with a significantly greater increase in the levels of cyclic GMP in SHR aortic rings than in WKY tissues (figure 67). It is possible that this greater increase in cyclic GMP levels alone, accounted for the more powerful relaxations induced by sodium nitroprusside in SHR aortic rings. However, a hyperpolarising effect of sodium nitroprusside on the cell membrane must also be considered since Rapoport et al, (1985) showed that inhibition of NA^+-K^+ATPase decreased the rise in cyclic GMP levels induced by sodium nitroprusside (10^{-6}M), but not the levels of cyclic GMP induced by sodium nitroprusside (10^{-7}M). Furthermore, this effect was dependent on tissue precontraction with noradrenaline. These findings suggest that activation of Na^+-K^+ATPase and/or membrane hyperpolarisation occurred at high concentrations of sodium nitroprusside, 10^{-6}M, and was in some way linked with the formation of cyclic GMP. Nitrovasodilators however, have been shown not to hyperpolarise vascular smooth muscle (Kuriyama et al., 1982).

Throughout this study, mechanical responses of rat aortic rings were related to underlying biochemical changes. This approach allowed a wider interpretation of the results obtained and raised many more questions on the mechanisms

involved in the regulation of vascular tone. The importance of membrane permeability in the regulation of cyclic GMP synthesis during vascular relaxation has now been fully recognised (Rapoport et al., 1985), and only when mechanical, biochemical and electro-pharmacological measurements are related to each other, will a more complete understanding of vasular smooth muscle reactivity be attained.

BIBLIOGRAPHY

Abdel-Latif A.A. (1986).
Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers.
Pharmacol. Rev. 38, 227-272.

Adelstein R.S. and Klee C.B. (1981).
Purification and characterization of smooth muscle myosin light chain kinase.
J. Biol. Chem. 256, 7501-7509.

Aksoy M.O., Mras S., Kamm K.E. and Murphy R.A. (1983).
Ca^{2+}, cAMP, and changes in myosin phosphorylation during contraction of smooth muscle.
Am. J. Physiol. 245, C255-C270.

Allan G., Brook C.D., Cambridge D. and Hladkiwskyj J. (1983).
Enhanced responsiveness of vascular smooth muscle to vasoconstrictor agents after removal of endothelial cells.
Br. J. Pharmacol. 79, 334P.

Altura B.T. and Altura B.M. (1978).
Factors affecting vascular responsiveness. In:
Microcirculation, vol II, eds. Altura B.M. and Kaley G.
pp 547-615. Baltimore; University Park.

Altura B.M. and Chand N. (1981).
Bradykinin-induced relaxation of renal and pulmonary arteries is dependent upon intact endothelial cells.
Br. J. Pharmacol. 74, 10-11.

Ambache N., Killick S.W. and Aboo Zar M. (1975).
Extraction from ox retractor penis of an inhibitory substance which mimics its atropine-resistant neurogenic relaxation.
Br. J. Pharmacol. 54, 409-410.

Angus J.A., Campbell G.R., Cocks T.M. and Manderson J.A. (1983).
Vasodilatation by acetylcholine is endothelium-dependent: a study by sonomicrometry in canine femoral artery in vivo.
J. Physiol. 344, 209-222.

Baez S. (1969).
Simultaneous measurements of radii and wall thickness of microvessels in the anesthetized rat.
Circ. Res. 25, 315-329.

Batty I.R., Nahorski S.R. and Irvine R.F. (1985).
Rapid formation of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortical slices.
Biochem. J. 232, 211-215.

Bayliss W.M. (1902).
On the local reactions of the arterial wall to changes of internal pressure.
J. Physiol. 28, 220-231.

Beckeringh J.J., Thoolen M.J.M.C., de Jonge A., Wilffert B., Timmermans P.B.M.W.M. and van Zwieten P.A. (1984).
The contractions induced in rat and guinea-pig aortic strips by the α_2-adrenoceptor selective agonists B-HT 920 and UK 14,304 are mediated by α_1-adrenoceptors.
Eur. J. Pharmacol. 104, 197-203.

Benton A.M., Gerrard J.M., Michiel T. and Kindom S.E. (1982).
Are lysophosphatidic acids or phosphatidic acids involved in stimulus activation coupling in platelets ?
Blood 60, 642-649.

Bernard C. (1852).
Sur les effects de la section de la portion céphalique de grand sympathique.
Compte Rendu des Scéances de la Société de Biologie. Novembre, 168-170. Cited by Detweiler D.K. (1978). In: Physiological basis of medical practice, ed. Brobeck J.R. pp 3.1-3.262. Baltimore London; Williams and Wilkins.

Berridge M.J. (1981).
Phosphatidylinositol hydrolysis: a multifunctional transducing mechanism.
Mol. Cell. Endo. 24, 115-140.

Berridge M.J. (1984).
Inositol trisphosphate and diacylglycerol as second messengers.
Biochem. J. 220, 345-360.

Berridge M.J. (1985).
The molecular basis of communication within the cell.
Scientific American 253, 96-106.

Berridge M.J., Dawson R.M.C., Downes C.P., Heslop J.P. and Irvine R.F. (1983).
Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides.
Biochem. J. 212, 473-482.

Berridge M.J., Heslop J.P., Irvine R.F. and Brown K.D. (1984).
Inositol trisphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor.
Biochem. J. 222, 195-201.

Berridge M.J. and Irvine R. F. (1984).
Inositol trisphosphate, a novel second messenger in cellular signal transduction.
Nature 312, 315-321.

Bevan J.A. and Su C. (1973).
Sympathetic mechanisms in blood vessels: nerve and muscle relationships.
Ann. Rev. Pharmacol. 13, 269-285.

Bingham Smith J., Smith L. and Higgins B.L. (1985).
Temperature and nucleotide dependence of calcium release by myo-inositol 1,4,5-trisphosphate in cultured vascular smooth muscle cells.
J. Biol. Chem. 260, 14413-14416.

Birnbaumer L. (1987).
Which G protein subunits are the active mediators in signal transduction ?
Trends Pharmacol. Sci. 8, 209-211.

Blackmore P.F., Bocckino S.B., Waynick L.E. and Exton J.H. (1985).
Role of a guanine nucleotide-binding regulatory protein in the hydrolysis of hepatocyte phosphatidylinositol 4,5-bisphosphate by calcium-mobilizing hormones and the control of cell calcium. Studies utilizing aluminum fluoride.
J. Biol. Chem. 260, 14477-14483.

Blaustein M.P. (1974).
The interrelationship between sodium and calcium fluxes across cell membranes.
Rev. Physiol. Biochem. Pharmacol. 70, 33-82.

Bohr D.F. (1963).
Vascular smooth muscle: dual effect of calcium.
Science 139, 597-599.

Bohr D.F. (1964).
Electrolytes and smooth muscle contraction.
Pharmacol. Rev. 16, 85-111.

Bolton T.B. (1979).
Mechanisms of action of neurotransmitters and other substances on smooth muscle.
Physiol. Rev. 59, 606-718.

Borle A.B. (1981).
Control, modulation, and regulation of cell calcium.
Rev. Physiol. Biochem. Pharmacol. 90, 13-153.

Bowman A. and Drummond A.H. (1984).
Cyclic GMP mediates neurogenic relaxation in the bovine retractor penis muscle.
Br. J. Pharmacol. 81, 665-674.

Bowman A., Gillespie J.S. and Martin W. (1981).
Actions on the cardiovascular system of an inhibitory material extracted from the bovine retractor penis.
Br. J. Pharmacol. 72, 365-372.

Bowman W.C. and Rand M.J. (1980).
Textbook of pharmacology, 2nd ed. Chapter 23. Oxford London Edinburgh Boston Melbourne; Blackwell Scientific Publications.

Brain S.D., Williams T.J., Tippins J.R., Morris H.R. and MacIntyre I. (1985).
Calcitonin gene-related peptide is a potent vasodilator.
Nature 313, 54-56.

Braughler M., Mittal C.K. and Murad F. (1979).
Purification of soluble guanylate cyclase from rat liver.
Proc. Natl. Acad. Sci. USA 76, 219-222.

Buckley J.T. and Hawthorne J.N. (1972).
Erythrocyte membrane polyphosphoinositide metabolism and the regulation of calcium binding.
J. Biol. Chem. 247, 7218-7223.

Bülbring E. and den Hertog A. (1980).
The action of isoprenaline on the smooth muscle of the guinea-pig taenia coli.
J. Physiol. 304, 277-296.

Butcher R.W. and Sutherland E.W. (1962).
Adenosine 3',5'-phosphate in biological materials. I.
Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine.
J. Biol. Chem. 237, 1244-1250.

Capponi A.M., Lew P.D. and Vallotton M.B. (1985).
Cytosolic free calcium levels in monolayers of cultured rat aortic smooth muscle cells. Effects of angiotensin II and vasopressin.
J. Biol. Chem. 260, 7836-7842.

Carrier G.O. and White R.E. (1985).
Enhancement of alpha-1 and alpha-2 adrenergic agonist-induced vasoconstriction by removal of endothelium in rat aorta.
J. Pharmacol. Exp. Ther. 232, 682-687.

Castagna M., Takai Y., Kaibuchi K., Sano K., Kikkawa U. and Nishizuka Y. (1982).
Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters.
J. Biol. Chem. 257, 7847-7851.

Cauvin C., Loutzenhiser R., Hwang O. and van Breemen C. (1982).
α_1-adrenoceptors induce Ca influx and intracellular Ca release in isolated rabbit aorta.
Eur. J. Pharmacol. 84, 233-235.

Chand N. and Altura B.M. (1981).
Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: role in lung vascular diseases.
Science 213, 1376-1379.

Chatterjee M. and Tejada M. (1986).
Phorbol ester-induced contraction in chemically skinned vascular smooth muscle.
Am. J. Physiol. 251, C356-C361.

Cherry P.D., Furchgott R.F., Zawadzki J.V. and Jothianandan D. (1982).
Role of endothelial cells in relaxation of isolated arteries by bradykinin.
Proc. Natl. Acad. Sci. USA 79, 2106-2110.

Chiu A.T., McCall D.E., Thoolen M.J.M.C. and Timmermans P.B.M.W.M. (1986).
Ca^{++} utilization in the constriction of rat aorta to full and partial alpha-1 adrenoceptor agonists.
J. Pharmacol. Exp. Ther. 238, 224-231.

Cockcroft S. (1987).
Polyphosphoinositide phosphodiesterase: regulation by a novel guanine nucleotide binding protein, G_p.
Trends Biochem. Sci. 12, 75-78.

Cocks T.M. and Angus J.A. (1983).
Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin.
Nature 305, 627-630.

Cohen R.A., Shepherd J.T. and Vanhoutte P.M. (1983a).
Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets.
Science 221, 273-274.

Cohen R.A., Shepherd J.T. and Vanhoutte P.M. (1983b).
5-hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries.
Am. J. Physiol. 245, H1077-1080.

Cohn J.N. (1983).
Calcium, vascular smooth muscle, and calcium entry blockers in hypertension.
Annal. Inter. Med. 98, 806-809.

Collis M.G. and Vanhoutte P.M. (1977).
Vascular reactivity of isolated perfused kidneys from male and female spontaneously hypertensive rats.
Circ. Res. 41, 759-767.

Davies J.M. and Williams K.I. (1984).
Endothelial-dependent relaxant effects of vaso-active intestinal polypeptide and arachidonic acid in rat aortic strips.
Prostaglandins 27, 195-202.

Dawson A.P., Comerford J.G. and Fulton D.V. (1986).
The effect of GTP on inositol 1,4,5-trisphosphate-stimulated Ca^{2+} efflux from a rat liver microsomal fraction. Is a GTP-dependent protein phosphorylation involved ?
Biochem. J. 234, 311-315.

DeFeo T.T. and Morgan K.G. (1985).
Calcium-force relationships as detected with aequorin in two different vascular smooth muscles of the ferret.
J. Physiol. 369, 269-282.

DeForrest J.M. and Hollis T.M. (1978).
Shear stress and aortic histamine synthesis.
Am. J. Physiol. 234, H701-H705.

de Lanerolle P., Nishikawa M., Yost D.A. and Adelstein R.S. (1984).
Increased phosphorylation of myosin light chain kinase after an increase in cyclic AMP in intact smooth muscle.
Science 223, 1415-1417.

De Mey J.G., Claeys M. and Vanhoutte P.M. (1982).
Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery.
J. Pharmacol. Exp. Ther. 222, 166-173.

De Mey J.G. and Vanhoutte P.M. (1982).
Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium.
Circ. Res. 51, 439-447.

Deth R. and van Breemen C. (1977).
Agonist induced release of intracellular Ca^{2+} in the rabbit aorta.
J. Memb. Biol. 30, 363-380.

Eglen R.M. and Whiting R.L. (1985).
Determination of the muscarinic receptor subtype mediating vasodilatation.
Br. J. Pharmacol. 84, 003-005.

Endo T., Naka M. and Hidaka H. (1982).
Ca^{2+}-phospholipid dependent phosphorylation of smooth muscle myosin.
Biochem. Biophys. Res. Commun. 105, 942-948.

Fain J.N. and Berridge M.J. (1979).
Relationship between hormonal activation of phosphatidylinositol hydrolysis, fluid secretion and calcium flux in the blowfly salivary gland.
Biochem. J. 178, 45-58.

Fiscus R.R. and Dyer D.C. (1981).
Cyclic nucleotides and contractility in human and sheep umbilical arteries.
Eur. J. Pharmacol. 73, 283-291.

Folkow B. (1978).
Cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension.
Clin. Sci. Mol. Med. 55, 3s-22s.

Fortes Z.B., Garcia Leme J. and Scivoletto R. (1983).
Vascular reactivity in diabetes mellitus: role of the endothelial cell.
Br. J. Pharmacol. 79, 771-781.

Frandsen E.K. and Krishna G. (1976).
A simple ultrasensitive method for the assay of cyclic AMP and cyclic GMP in tissues.
Life Sci. 18, 529-542.

Frangos J.A., Eskin S.G., McIntire L.V. and Ives C.L. (1985).
Flow effects on prostacyclin production by cultured human endothelial cells.
Science 227, 1477-1479.

Furchgott R.F. (1955).
The pharmacology of vascular smooth muscle.
Pharmacol. Rev. 7, 183-265.

Furchgott R.F. (1983).
Role of endothelium in responses of vascular smooth muscle.
Circ. Res. 53, 557-573.

Furchgott R.F. (1987).
Studies on the relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid-activatable inhibitory factor from bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide. In: Mechanisms of vasodilatation ed. Vanhoutte P.M. Chapter 1. New York; Raven Press.

Furchgott R.F., Cherry P.D., Zawadzki J.V. and Jothianandan D. (1984).
Endothelial cells as mediators of vasodilation of arteries.
J. Cardio. Pharmacol. 6, S336-S343.

Furchgott R.F. and Zawadzki J.V. (1980a)
The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.
Nature 288, 373-376.

Furchgott R.F. and Zawadski J. (1980b).
Acetylcholine relaxes arterial smooth muscle by releasing a relaxing substance from endothelial cells.
Fed. Proc. 39, 581.

Gabella G. (1981).
Structure of smooth muscles. In: Smooth muscle: an assessment of current knowledge, eds. Bülbring E., Brading A., Jones A.W. and Tomita T. pp 1-46. London; Edward Arnold.

Garbers D.L. (1979).
Purification of soluble guanylate cyclase from rat lung.
J. Biol. Chem. 254, 240-243.

Gillespie J.S. (1966).
Tissue binding of noradrenaline.
Proc. R. Soc. B 166, 1-10.

Gillespie J.S. (1987).
Searching for the non-adrenergic non-cholinergic autonomic transmitter. In: Pharmacology, Proc. 10th IUPHAR, Excerpta Medica International congress series 750, eds. Rand M.J. and Raper C. pp 161-170. Amsterdam New York Oxford; Elsevier Science Publishers B.V.

Gillespie J.S. and Martin W. (1980).
A smooth muscle inhibitory material from the bovine retractor penis and rat anococcygeus muscles.
J. Physiol. 309, 55-64.

Gilman A.G. (1984).
G proteins and dual control of adenylate cyclase.
Cell 36, 577-579.

Gimble J.M., Waisman D.M., Gustin M., Goodman D.B.P. and Rasmussen H. (1982).
Studies of the Ca^{2+} transport mechanism of human erythrocyte inside-out membrane vesicles.
J. Biol. Chem. 257, 10781-10788.

Godfraind T. (1986).
EDRF and cyclic GMP control gating of receptor-operated calcium channels in vascular smooth muscle.
Eur. J. Pharmacol. 126, 341-343.

Godfraind T., Egleme C. and Al Osachie I. (1985).
Role of endothelium in the contractile response of rat aorta to α-adrenoceptor agonists.
Clin. Sci. 68 (suppl. 10), 65s-71s.

Goldstein A. (1949).
The interactions of drugs and plasma proteins.
Pharmacol. Rev. 1, 102-165.

Gordon J.L. and Martin W. (1983a).
Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of ^{86}Rb efflux from isolated endothelial cells.
Br. J. Pharmacol. 79, 531-541.

Gordon J.L. and Martin W. (1983b).
Stimulation of endothelial prostacyclin production plays no role in endothelium-dependent relaxation of the pig aorta.
Br. J. Pharmacol. 80, 179-186.

Gore R.W. (1972).
Wall stress: a determinant of regional differences in response of frog microvessels to norepinephrine.
Am. J. Physiol. 222, 82-91.

Griffith T.M., Edwards D.H., Lewis M.J., Newby A.C. and Henderson A.H. (1984).
The nature of endothelium-derived vascular relaxant factor.
Nature 308, 645-647.

Gruetter D.Y., Gruetter C.A., Barry B.K., Baricos W.H., Hyman A.L., Kadowitz P.J. and Ignarro L.J. (1980).
Activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside, and nitrosoguanidine- inhibition by calcium, lanthanum, and other cations, enhancement by thiols.
Biochem. Pharmacol. 29, 2943-2950.

Guharay F. and Sachs F. (1984).
Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle.
J. Physiol. 352, 685-701.

Guimarães S., Azevedo I., Cardoso W. and Oliveira M.C. (1975).
Relation between the amount of smooth muscle of venous tissue and the degree of supersensitivity to isoprenaline caused by inhibition of catechol-O-methyl transferase.
Naunyn-Schmiedeberg's Arch. Pharmacol. 286, 401-412.

Hardman J.G. (1981).
Cyclic nucleotides and smooth muscle contraction: some conceptual and experimental considerations. In: Smooth muscle: an assessment of current knowledge, eds. Bülbring E., Brading A., Jones A.W. and Tomita T. pp 249-262. London; Edward Arnold.

Hardman J.G. (1984).
Cyclic nucleotides and regulation of vascular smooth muscle.
J. Cardio. Pharmacol. 6 (suppl. 4), S639-S645.

Harvey W. (1628).
An anatomical discourse on the motion of the heart and blood in animals. Cited by Bowman W.C. and Rand M.J. (1980). In: Textbook of pharmacology, 2nd ed. p 23.1. Oxford London Edinburgh Boston Melbourne; Blackwell Scientific Publications.

Haslam R.J. and Davidson M.M. (1984).
Receptor-induced diacylglycerol formation in permeabilized platelets; possible role for a GTP-binding protein.
J. Recept. Res. 4, 605-629.

Helmreich E.J.M. and Pfeuffer T. (1985).
Regulation of signal transduction by β-adrenergic hormone receptors.
Trends Pharmacol. Sci. 6, 438-443.

Herlihy J.T. and Murphy R.A. (1973).
Length-tension relationship of smooth muscle of the hog carotid artery.
Circ. Res. 33, 275-283.

Hickey K.A., Rubanyi G., Paul R.J. and Highsmith R.F. (1985).
Characterization of a coronary vasoconstrictor produced by cultured endothelial cells.
Am. J. Physiol. 248, C550-C556.

Hidaka H., Yamaki T., Ochiai Y., Asano T. and Yamabe H. (1977).
Cyclic 3':5'-nucleotide phosphodiesterase determined in various human tissues by DEAE-cellulose chromatography.
Biochim. Biophys. Acta. 484, 398-407.

Hildebrandt J.D., Codina J. and Birnbaumer L. (1984).
Interaction of the stimulatory and inhibitory regulatory proteins of the adenylyl cyclase system with the catalytic component of cyc⁻ S49 cell membranes.
J. Biol. Chem. 259, 13178-13185.

Hokin L.E. (1985).
Receptors and phosphoinositide-generated second messengers.
Ann. Rev. Biochem. 54, 205-235.

Holmsen H., Dangelmaier C.A. and Rongved S. (1984).
Tight coupling of thrombin-induced acid hydrolase secretion and phosphatidate synthesis to receptor occupancy in human platelets.
Biochem. J. 222, 157-167.

Hondeghem L.M. and Katzung B.G. (1986).
Control of vascular smooth muscle contractility and the action of calcium channel blockers. In: Regulation of heart function. Basic concepts and clinical applications, ed. Rupp H. pp 38-52. Stuttgart New York; Thieme.

Houston D.S., Shepherd J.T. and Vanhoutte P.M. (1985).
Adenine nucleotides, serotonin, and endothelium-dependent relaxations to platelets.
Am. J. Physiol. 248, H389-H395.

Houston D.S. and Vanhoutte P.M. (1985).
Direct contraction and endothelium-mediated relaxation of isolated canine coronary arteries by aggregating human platelets.
Fed. Proc. 44, 1562.

Ignarro L.J., Burke T.M., Wood K.S., Wolin M.S. and Kadowitz P.J. (1984).
Association between cyclic GMP accumulation and acetylcholine-elicited relaxation of bovine intrapulmonary artery.
J. Pharmacol. Exp. Ther. 228, 682-690.

Ignarro L.J. and Kadowitz P.J. (1985).
The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation.
Ann. Rev. Pharmacol. Toxicol. 25, 171-191.

Irvine R.F. (1986).
Calcium transients: mobilization of intracellular Ca^{2+}.
Br. Med. Bull. 42, 369-374.

Irvine R.F., Letcher A.J. and Dawson R.M.C. (1984).
Phosphatidylinositol-4,5-bisphosphate phosphodiesterase and phosphomonoesterase activities of rat brain. Some properties and possible control mechanisms.
Biochem. J. 218, 177-185.

Irvine R.F. and Moor R.M. (1986).
Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}.
Biochem. J. 240, 917-920.

Jones A.W. (1981).
Vascular smooth muscle and alterations during hypertension.
In: Smooth muscle: an assessment of current knowledge, eds. Bülbring E., Brading A., Jones A.W. and Tomita T. pp 397-429. London; Edward Arnold.

Jones L.M. and Michell R.H. (1975).
The relationship of calcium to receptor-controlled stimulation of phosphatidylinositol turnover. Effects of acetylcholine, adrenaline, calcium ions, cinchocaine and a bivalent cation ionophore on rat parotid-gland fragments.
Biochem. J. 148, 479-485.

Kamitani T., Katamoto M., Tatsumi M., Katsuta K., Ono T., Kikuchi H. and Kumada S. (1984).
Mechanism(s) of the hpotensive effect of synthetic 1-O-octadecyl-2-O-acetyl-glycero-3-phosphorylcholine.
Eur. J. Pharmacol. 98, 357-366.

Katada T., Bokoch G.M., Smigel M.D., Ui M. and Gilman A.G. (1984).
The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Subunit dissociation and the inhibition of adenylate cyclase in S49 lymphoma cyc^- and wild type membranes.
J. Biol. Chem. 259, 3586-3595.

Katsuki S., Arnold W., Mittal C. and Murad F. (1977).
Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine.
J. Cyclic Nucleotide Res. 3, 23-35.

Katusic Z.S., Shepherd J.T. and Vanhoutte P.M. (1984a).
Vasopressin causes endothelium-dependent relaxations of the canine basilar artery.
Circ. Res. 55, 575-579.

Katusic Z., Shepherd J.T. and Vanhoutte P.M. (1984b).
Oxytocin induces endothelium-dependent relaxation of canine basilar arteries.
Physiologist 27, 282.

Keatinge W.R. and Torrie C. (1976).
Action of sympathetic nerves on inner and outer muscle of sheep carotid artery, and effect of pressure on nerve distribution.
J. Physiol. 257, 699-712.

Khalil R.A. and van Breemen C. (1988).
Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation ?
J. Pharmacol. Exp. Ther. 244, 537-542.

Kikkawa U., Takai Y., Tanaka Y., Miyake R. and Nishizuka Y. (1983).
Protein kinase C as a possible receptor protein of tumor-promoting phorbol esters.
J. Biol Chem. 258, 11442-11445.

Kimura H., Mittal C.K. and Murad F. (1975).
Activation of guanylate cyclase from rat liver and other tissues by sodium azide.
J. Biol. Chem. 250, 8016-8022.

Kimura H. and Murad F. (1975).
Subcellular localization of guanylate cyclase.
Life Sci. 17, 837-843.

Kowarski D., Shuman H., Somlyo A.P. and Somlyo A.V. (1985).
Calcium release by noradrenaline from central sarcoplasmic reticulum in rabbit main pulmonary artery smooth muscle.
J. Physiol. 366, 153-175.

Kramer G.L. and Hardman J.G. (1980).
Cyclic nucleotides and blood vessel contraction. In: Handbook of physiology: the cardiovascular system II, vascular smooth muscle, eds. Bohr D.F., Somlyo A.P. and Sparks H.V. pp 179-199. Bethesda; The American Physiology Society.

Krebs H.A. and Henseleit K. (1932).
Unter suchungen uber die harnstoffbildung im tierkorper.
Hoppe-Seylers Z. Physiol. Chem. 210, 33-66.

Ku D.D. (1982).
Coronary vascular reactivity after acute myocardial ischemia.
Science 218, 576-578.

Kubo T. (1979).
Increased pressor responses to pressor agents in spontaneously hypertensive rats.
Can. J. Physiol. Pharmacol. 57, 59-64.

Kuchii M., Shibata S. and Mori J. (1973).
Pharmacological nature of adrenergic innervation in rat aorta.
Comp. Gen. Pharmacol. 4, 131-138.

Kukovetz W.R., Holzmann S. and Pöch G. (1982).
Function of cyclic GMP in acetylcholine-induced contraction of coronary smooth muscle.
Naunyn-Schmiedeberg's Arch. Pharmacol. 319, 29-33.

Kuno M. and Gardner P. (1987).
Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes.
Nature 326, 301-304.

Kuo J.F., Andersson R.G.G., Wise B.C., Mackerlova L., Salomonsson I., Brackett N.L., Katoh N., Shoji M. and Wrenn R.W. (1980).
Calcium-dependent protein kinase: widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipid, calmodulin, and trifluoperazine.
Proc. Natl. Acad. Sci. USA 77, 7039-7043.

Kuriyama H., Ito Y., Suzuki H., Kitamura K. and Itoh T. (1982).
Factors modifying contraction-relaxation cycle in vascular smooth muscles.
Am. J. Physiol. 243, H641-H662.

Langer S.Z. (1974).
Presynaptic regulation of catecholamine release.
Biochem. Pharmacol. 23, 1793-1800.

Lansman J.B. (1988).
Going with the flow.
Nature 331, 481-482.

Lansman J.B., Hallam T.J. and Rink T.J. (1987).
Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers ?
Nature 325, 811-813.

Lee T.J. (1982).
Cholinergic mechanism in the large cat cerebral artery.
Circ. Res. 50, 870-879.

Lewicki J.A., Brandwein H.J., Waldman S.A. and Murad F. (1980).
Purified guanylate cyclase: characterization, iodination and preparation of monoclonal antibodies.
J. Cyclic Nucleotide Res. 6, 283-296.

Lincoln T.M. and Fisher-Simpson V. (1983).
A comparison of the effects of forskolin and nitroprusside on cyclic nucleotides and relaxation in the rat aorta.
Fed. Proc. 42, 898.

Litosch I. and Fain J.F. (1986).
Regulation of phosphoinositide breakdown by guanine nucleotides.
Life Sci. 39, 187-194.

Ljung B., Bevan J.A., Pegram B.L., Purdy R.E. and Su M. (1975).
Vasomotor nerve control of isolated arteries and veins.
Acta. physiol. scand. 94, 506-516.

Lloyd J.V., Nishizawa E.E., Haldar J. and Mustard J.F. (1972).
Changes in ^{32}P-labelling of platelet phospholipids in response to ADP.
Br. J. Haematol. 23, 571-585.

MacIntyre D.E. and Pollock W.K. (1983).
Platelet-activating factor stimulates phosphatidylinositol turnover in human platelets.
Biochem. J. 212, 433-437.

Marcus A.J., Ullman H.L. and Safier L.B. (1969).
Lipid composition of subcellular particles of human blood platelets.
J. Lipid Res. 10, 108-114.

Martin W., Furchgott R.F., Villani G.M. and Jothianandan D. (1986).
Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor.
J. Pharmacol. Exp. Ther. 237, 529-538.

Martin W., Smith J.A., Lewis M.J. and Henderson A.H. (1988).
Evidence that inhibitory factor extracted from bovine retractor penis is nitrite, whose acid-activated derivative is stabilized nitric oxide.
Br. J. Pharmacol. 93, 579-586.

Meisheri K.D. and van Breemen C. (1982).
Effects of β-adrenergic stimulation on calcium movements in rabbit aortic smooth muscle: relationship with cyclic AMP.
J. Physiol. 331, 429-441.

Mekata F. and Keatinge W.R. (1975).
Electrical behaviour of inner and outer smooth muscle of sheep carotid artery.
Nature 258, 534-535.

Michell B. (1986).
Inositol phosphates. Profusion and confusion.
Nature 319, 176-177.

Michell R.H., Kirk C.J., Jones L.M., Downes C.P. and Creba J.A. (1981).
The stimulation of inositol lipid metabolism that accompanies calcium mobilization in stimulated cells: defined characteristics and unanswered questions.
Phil. Trans. R. Soc. Lond. B 296, 123-138.

Miller V.M. and Vanhoutte P.M. (1984).
Muscular and endothelial responsiveness to alpha$_2$-adrenergic activation in canine blood vessels.
Physiologist 27, 282.

Mittal C.K. and Murad F. (1977a).
Properties and oxidative regulation of guanylate cyclase.
J. Cyclic Nucleotide Res. 3, 381-391.

Mittal C.K. and Murad F. (1977b).
Formation of adenosine 3':5'-monophosphate by preparations of guanylate cyclase from rat liver and other tissues.
J. Biol. Chem. 252, 3136-3140.

Morgan K.G. (1987).
Calcium and vascular smooth muscle tone.
Am. J. Med. 82 (suppl 3B), 9-15.

Morgan J.P. and Morgn K.G. (1984).
Alteration of cytoplasmic ionized calcium levels in smooth muscle by vasodilators in the ferret.
J. Physiol. 357, 539-551.

Muallem S., Schoeffield M., Pandol S. and Sachs G. (1985).
Inositol trisphosphate modification of ion transport in rough endoplasmic reticulum.
Proc. Natl. Acad. Sci. USA 82, 4433-4437.

Mueller E. and van Breemen C. (1979).
Role of intracelluar Ca^{2+} sequestration in β-adrenergic relaxation of a smooth muscle.
Nature 281, 682-683.

Murad F., Mittal C.K., Arnold W.P., Katsuki S. and Kimura H. (1978).
Guanylate cyclase: activation by azide, nitro compounds, nitric oxide, and hydroxyl radical and inhibition by hemoglobin and myoglobin.
Adv. Cyclic Nucleotide Res. 9, 145-158.

Murray J.L., Fridovich I., Makhoul R.G. and Hagen P.O. (1986).
Stabilization and partial characterization of endothelium-derived relaxing factor from cultured bovine aortic endothelial cells.
Biochem. Biophys. Res. Commun. 141, 689-696.

Nabika T., Velletri P.A., Lovenberg W. and Beaven M.A. (1985).
Increase in cytosolic calcium and phosphoinositide metabolism induced by angiotensin II and [Arg]vasopressin in vascular smooth muscle cells.
J. Biol. Chem. 260, 4661-4670.

Naka M., Nishikawa M., Adelstein R.S. and Hidaka H. (1983).
Phorbol ester-induced activation of human platelets is associated with protein kinase C phosphorylation of myosin light chains.
Nature 306, 490-492.

Neufeld E.J. and Majerus P.W. (1983).
Arachidonate release and phosphatidic acid turnover in stimulated human platelets.
J. Biol. Chem. 258, 2461-2467.

Niggli V., Sigel E. and Carafoli E. (1982).
The purified Ca^{2+} pump of human erythrocyte membranes catalyzes an electroneutral Ca^{2+}-H^{+} exchange in reconstituted liposomal systems.
J. Biol. Chem. 257, 2350-2356.

Nishikawa M., de Lanerolle P., Lincoln T.M. and Adelstein R.S. (1984).
Phosphorylation of mammalian myosin light chain kinases by the catalytic subunit of cyclic AMP-dependent protein kinase and by cyclic GMP-dependent protein kinase.
J. Biol. Chem. 259, 8429-8436.

Nishizuka Y. (1983).
Phospholipid degradation and signal translation for protein phosphorylation.
Trends Biochem. Sci. 8, 13-16.

Olesen S.P., Clapham D.E. and Davies P.F. (1988).
Haemodynamic shear stress activates a K^{+} current in vascular endothelial cells.
Nature 331, 168-170.

Owen M.P. and Bevan J.A. (1985).
Acetylcholine induced endothelial-dependent vasodilation increases as artery diameter decreases in the rabbit ear.
Experientia 41, 1057-1058.

Palmer R.M.J., Ferrige A.G. and Moncada S. (1987)
Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor.
Nature 327, 524-526.

Park S. and Rasmussen H. (1985).
Activation of tracheal smooth muscle contraction: synergism between Ca^{2+} and activators of protein kinase C.
Proc. Natl. Acad. Sci. USA 82, 8835-8840.

Peach M.J., Singer H.A. and Loeb A.L. (1985).
Mechanisms of endothelium-dependent vascular smooth muscle relaxation.
Biochem. Pharmacol. 34, 1867-1874.

Peiper U., Schmidt E., Laven R. and Griebel L. (1973).
Length-tension relationships in resting and activated vascular smooth muscle fibres.
Pflügers Arch. 340, 113-122.

Pfeuffer E., Dreher R.M., Metzger H. and Pfeuffer T. (1985).
Catalytic unit of adenlyate cyclase: purification and identification by affinity crosslinking.
Proc. Natl. Acad. Sci. USA 82, 3086-3090.

Popescu L.M., Panoiu C., Hinescu M. and Nutu O. (1985).
The mechanism of cGMP-induced relaxation in vascular smooth muscle.
Eur. J. Pharmacol. 107, 393-394.

Powis G. (1973).
Binding of catecholamines to connective tissue and the effect upon the reponses of blood vessels to noradrenaline and to nerve stimulation.
J. Physiol. 234, 145-162.

Prentki M., Janjic D., Biden T.J., Blondel B. and Wollheim C.B. (1984).
Regulation of Ca^{2+} transport by isolated organelles of a rat insulinoma. Studies with endoplasmic reticulum and secretory granules.
J. Biol. Chem. 259, 10118-10123.

Putney Jr. J.W. (1986).
A model for receptor-regulated calcium entry.
Cell Calcium 7, 1-12.

Rand M.J., McCulloch M.W. and Story D.F. (1976).
Pre-junctional modulation of noradrenergic transmission by noradrenaline, dopamine and acetylcholine. In: Central action of drugs in blood pressure regulation, eds. Davies D.S. and Reid J.L. pp 94-132. Baltimore; University Park.

Rapoport R.M. (1986).
Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidylinositol hydrolysis in rat aorta.
Circ. Res. 58, 407-410.

Rapoport R.M., Draznin M.B. and Murad F. (1982).
Sodium nitroprusside-induced protein phosphorylation in intact rat aorta is mimicked by 8-bromo cyclic GMP.
Proc. Natl. Acad. Sci. USA 79, 6470-6474.

Rapoport R.M., Draznin M.B. and Murad F. (1983).
Endothelium-dependent vasodilator- and nitrovasodilator-induced relaxation may be mediated through cyclic GMP formation and cyclic GMP-dependent protein phosphorylation.
Trans. Assoc. Amer. Physicians 96, 19-30.

Rapoport R.M., Draznin M.B. and Murad F. (1984).
Mechanisms of adenosine triphosphate-, thrombin-, and trypsin-induced relaxation of rat thoracic aorta.
Circ. Res. 55, 468-479.

Rapoport R.M. and Murad F. (1983a).
Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP.
Circ. Res. 52, 352-357.

Rapoport R.M. and Murad F. (1983b).
Endothelium-dependent and nitrovasodilator-induced relaxation of vascular smooth muscle: role of cyclic GMP.
J. Cyclic Nucleotide Protein Phosphorylation Res. 9, 281-296.

Rapoport R.M., Schwartz K. and Murad F. (1985).
Effect of sodium-potassium pump inhibitors and membrane-depolarizing agents on sodium nitroprusside-induced relaxation and cyclic guanosine monophosphate accumulation in rat aorta.
Circ. Res. 57, 164-170.

Rashatwar S.S., Cornwell T.L. and Lincoln T.M. (1987).
Effects of 8-bromo-cGMP on Ca^{2+} levels in vascular smooth muscle cells: possible regulation of Ca^{2+}-ATPase by cGMP-dependent protein kinase.
Proc. Natl. Acad. Sci. USA 84, 5685-5689.

Rasmussen H. (1983).
Cellular calcium metabolism.
Annal. Int. Med. 98, 809-816.

Rasmussen H. and Barrett P.Q. (1984).
Calcium messenger system: an integrated view.
Physiol. Rev. 64, 938-984.

Regoli D., Mizrahi J., D'orléans-Juste P. and Caranikas S. (1982).
Effects of kinins on isolated blood vessels. Role of endothelium.
Can. J. Physiol. Pharmacol. 60, 1580-1583.

Reuter H. (1983).
Calcium channel modulation by neurotransmitters, enzymes and drugs.
Nature 301, 569-574.

Ringer S. (1883).
A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart.
J. Physiol. 4, 29-42.

Robertson Jr. A.L. and Rosen L.A. (1978).
The arterial endothelium. In: Microcirculation, vol II, eds. Altura B.M. and Kaley G. pp 145-309. Baltimore; University Park.

Ross R., Glomset J., Kariya B. and Harker L. (1974).
A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro.
Proc. Natl. Acad. Sci. USA 71, 1207-1210.

Rubanyi G.M. and Vanhoutte P.M. (1985a).
Muscarinic receptor subtypes mediating the release of endothelium-derived relaxing factor(s) in canine femoral arteries.
Physiologist 28, 325.

Rubanyi G. and Vanhoutte P.M. (1985b).
Endothelium-removal decreases relaxations of canine coronary arteries caused by β-adrenergic agonists and adenosine.
J. Cardio. Pharmacol. 7, 139-144.

Rubanyi G.M. and Vanhoutte P.M. (1987).
Nature of endothelium-derived relaxing factor: are there two relaxing mediators ?
Circ. Res. 61 (suppl II), II61-II67.

Rüegg J.C. (1988).
Vertebrate smooth muscle. In: Calcium in muscle activation, pp 201-238. Berlin Heidelberg New York London Paris Tokyo; Springer-Verlag.

Ruffolo Jr. R.R., Waddell J.E. and Yaden E.L. (1981).
Postsynaptic alpha adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of alpha-2 adrenergic receptors in rat aorta.
J. Pharmacol. Exp. Ther. 217, 235-240.

Ruffolo Jr. R.R., Waddell J.E. and Yaden E.L. (1982).
Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas.
J. Pharmacol. Exp. Ther. 221, 309-314.

Satoh H. and Inui J. (1984).
Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery.
Eur. J. Pharmacol. 97, 321-324.

Scheid C.R., Honeyman T.W. and Fay F.S. (1979).
Mechanism of β-adrenergic relaxation of smooth muscle.
Nature 277, 32-36.

Schultz J.E. and Klumpp S. (1980).
Guanylate cyclase in the excitable ciliary membrane of paramecium.
FEBS Lett. 122, 64-66.

Senter P.D., Eckstein F., Mülsch A. and Böhme E. (1983).
The stereochemical course of the reaction catalyzed by soluble bovine lung guanylate cyclase.
J. Biol. Chem. 258, 6741-6745.

Shaw A.M. (1988)
Department of Child Health, University of Dundee
Personal communication.

Singer H.A. and Peach M.J. (1983).
Endothelium-dependent relaxation of rabbit aorta. I. Relaxation stimulated by arachidonic acid.
J. Pharmacol. Exp. Ther. 226, 790-795.

Smallwood J.I., Waisman D.M., Lafreniere D. and Rasmussen H. (1983).
Evidence that the erythrocyte calcium pump catalyzes a Ca^{2+}:nH^{+} exchange.
J. Biol. Chem. 258, 11092-11097.

Soltis E.E. and Katovich M.J. (1985).
Phentolamine and rat aortic smooth muscle responsiveness to potassium chloride, isoproterenol and norepinephrine.
Pharmacology 31, 67-71.

Somlyo A.V., Bond M., Somlyo A.P. and Scarpa A. (1985).
Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle.
Proc. Natl. Acad. Sci. USA 82, 5231-5235.

Somlyo A.P. and Somlyo A.V. (1968).
Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics.
Pharmacol. Rev. 20, 197-272.

Somlyo A.P. and Somlyo A.V. (1970).
Vascular smooth muscle. II. Pharmacology of normal and hypertensive vessels.
Pharmacol. Rev. 22, 249-353.

Souness J.E., Brazdil R.R. and Diocee B.K. (1987).
Smooth muscle relaxant actions of cGMP phosphodiesterase inhibitors.
Proc. 10th IUPHAR., P985. (Abstract).

Speden R.N. (1960).
The effect of initial strip length on the noradrenaline-induced isometric contraction of arterial strips.
J. Physiol. 154, 15-25.

Starke K., Endo T. and Taube H.D. (1975).
Relative pre- and postsynaptic potencies of α-adrenoceptor agonists in the rabbit pulmonary artery.
Naunyn-Schmiedeberg's Arch. Pharmacol. 291, 55-78.

Stephenson J.A. and Summers R.J. (1987).
Autoradiographic analysis of receptors on vascular endothelium.
Eur. J. Pharmacol. 134, 35-43.

Stjärne L. and Gripe K. (1973).
Prostaglandin-dependent and -independent feedback control of noradrenaline secretion in vasoconstrictor nerves of normotensive human subjects. A preliminary report.
Naunyn-Schmiedeberg's Arch. Pharmacol. 280, 441-446.

Strada S.J. and Thompson W.J. (1978).
Multiple forms of cyclic nucleotide phosphodiesterases: anomalies or biologic regulators ?
Adv. Cyclic Nucleotide Res. 9, 265-283.

Streb H., Irvine R.F., Berridge M.J. and Schulz I. (1983).
Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate.
Nature 306, 67-69.

Sybertz E.J., Desiderio D.M., Tetzloff G. and Chiu P.J.S. (1986).
Phorbol dibutyrate contractions in rabbit aorta: calcium dependence and sensitivity to nitrovasodilators and 8-BR-cyclic GMP.
J. Pharmacol. Exp. Ther. 239, 78-83.

Takai Y., Kishimoto A., Kikkawa U., Mori T. and Nishizuka Y. (1979).
Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system.
Biochem. Biophys. Res. Commun. 91, 1218-1224.

Taylor C.J. and Meisheri K.D. (1986).
Inhibitory effects of a synthetic atrial peptide on contractions and ^{45}Ca fluxes in vascular smooth muscle.
J. Pharmacol. Exp. Ther. 237, 803-808.

Taylor C.W. and Merritt J.E. (1986).
Receptor coupling to polyphosphoinositide turnover: a parallel with the adenylate cyclase system.
Trends Pharmacol. Sci. 7, 238-242.

Templeton A., Drew G.M. and M^{c}Grath J.C. (1987).
Endothelium-dependent vasodilator responses to acetylcholine in hypertension and diabetes.
Br. J. Pharmacol. 92, 766P

Toda N. (1984).
Endothelium-dependent relaxation induced by angiotensin II and histamine in isolated arteries of dog.
Br. J. Pharmacol. 81, 301-307.

Tyson C.A., Vande Zande H. and Green D.E. (1976).
Phospholipids as ionophores.
J. Biol. Chem. 251, 1326-1332.

Uchida E., Bohr D.F. and Hoobler S.W. (1967).
A method for studying isolated resistance vessels from rabbit mesentery and brain and their responses to drugs.
Circ. Res. 21, 525-536.

van de Voorde J. and Leusen I. (1983).
Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine.
Eur. J. Pharmacol. 87, 113-120.

Vanhoutte P.M. (1977).
Cholinergic inhibition of adrenergic transmission.
Fed. Proc. 36, 2444-2449.

Vanhoutte P.M. (1978).
Heterogeneity in vascular smooth muscle. In:
Microcirculation, vol II, eds. Altura B.M. and Kaley G. pp 181-309. Baltimore; University Park.

Vanhoutte P.M. (1980).
Physical factors of regulation. In: Handbook of physiology: cardiovascular system II, vascular smooth muscle, eds. Bohr D.F., Somlyo A. and Sparks Jr. H.V. pp 443-513. Bethesda; The American Physiological Society.

Vanhoutte P.M. (1987).
The end of the quest ?
Nature 327, 459-460.

Vanhoutte P.M. and Houston D.S. (1985).
Platelets, endothelium, and vasospasm.
Circulation 72, 728-734.

Vanhoutte P.M., Rubanyi G.M., Miller V.M. and Houston D.S. (1986).
Modulation of vascular smooth muscle contraction by the endothelium.
Ann. Rev. Physiol. 48, 307-320.

Villalobos-Molina R., Uc M., Hong E. and García-Sáinz J.A. (1982).
Correlation between phosphatidylinositol labeling and contraction in rabbit aorta: effect of alpha-1 adrenergic activation.
J. Pharmacol. Exp. Ther. 222, 258-261.

Vrolix M., Raeymaekers L., Wuytack F., Eggermont J., Hofmann F. and Casteels R. (1988).
Stimulation of the (Ca^{2+}+Mg^{2+})-ATPase by cGMP dependent protein kinase: an effect possibly involving phosphoinositides.
Eur. Symposium on Smooth Muscle, A25. (Abstract).

Waldman S.A., Lewicki J.A., Chang L.Y. and Murad F. (1983).
Highly purified particulate guanylate cyclase from rat lung: characterization and comparison with soluble guanylate cyclase.
Mol. Cell. Biochem. 57, 155-166.

Waldman S.A. and Murad F. (1987).
Cyclic GMP synthesis and function.
Pharmacol. Rev. 39, 163-196.

Waldman S.A., Rapoport R.M. and Murad F. (1984).
Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues.
J. Biol. Chem. 259, 14332-14334.

Walker J.W., Somlyo A.V., Goldman Y.E., Somlyo A.P. and Trentham D.R. (1987).
Kinetics of smooth and skeletal muscle activation by laser pulse photolysis of caged inositol 1,4,5-trisphosphate.
Nature 327, 249-252.

Weinheimer G., Wagner B. and Osswald H. (1986).
Interference of phorbolesters with endothelium-dependent vascular smooth muscle relaxation.
Eur. J. Pharmacol. 130, 319-322.

Wikberg J. (1979).
The pharmacological classification of adrenergic α_1 and α_2 receptors and their mechanisms of action.
Acta. physiol. scand. (suppl) 468, 1-89.

Wilson D.B., Bross T.E., Sherman W.R., Berger R.A. and Majerus P.W. (1985a).
Inositol cyclic phosphates are produced by cleavage of phosphatidylphosphoinositols (polyphosphoinositides) with purified sheep seminal vesicle phospholipase C enzymes.
Proc. Natl. Acad. Sci. USA 82, 4013-4017.

Wilson D.B., Connolly T.M., Bross T.E., Majerus P.W., Sherman W.R., Tyler A.N., Rubin L.J. and Brown J.E. (1985b).
Isolation and characterization of the inositol cyclic phosphate products of polyphosphoinositide cleavage by phospholipase C. Physiological effects in permeabilized platelets and limulus photoreceptor cells.
J. Biol. Chem. 260, 13496-13501.

Winquist R.J. (1985).
The relaxant effects of atrial natriuretic factor on vascular smooth muscle.
Life Sci. 37, 1081-1087.

Winquist R.J., Faison E.P., Waldman S.A., Schwartz K., Murad F. and Rapoport R.M. (1984).
Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle.
Proc. Natl. Acad. Sci. USA 81, 7661-7664.

Winquist R.J., Webb R.C. and Bohr D.F. (1982).
Vascular smooth muscle in hypertension.
Fed. Proc. 41, 2387-2393.

Yanagisawa M., Kurihara H., Kimura S., Tomobe Y., Kobayashi M., Mitsui Y., Yazaki Y., Goto K. and Masaki T. (1988).
A novel potent vasoconstrictor peptide produced by vascular endothelial cells.
Nature 332, 411-415.

Yavin E. and Zutra A. (1977).
Separation and analysis of ^{32}P-labeled phospholipids by a simple and rapid thin-layer chromatographic procedure and its application to cultured neuroblastoma cells.
Anal. Biochem. 80, 430-437.

Zwiller J., Basset P. and Mandel P. (1981).
Rat brain guanylate cyclase. Purification, amphiphilic properties and immunological characterization.
Biochim. Biophys. Acta. 658, 64-75.