INVESTIGATION OF

BLOOD VESSEL CONTRACTILITY & SECOND MESSENGER MECHANISMS IN HYPERTENSION

A thesis presented for the degree of Doctor of Philosophy

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by

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CHAPTER 3:

RESULTS

ABBREVIATIONS:

ADP	: adenosine 5' diphosphate
ATP	: adenosine 5' triphosphate
CDP-DG	: cytosine diphospho-diacylglycerol
cAMP	: cyclic adenosine 3'5'monophosphate
cGMP	: cyclic guanosine 3'5'monophosphate
$[Ca^{2+}]^i$	: cytosolic free calcium
CTL	: Control
DAG	: 1,2-diacylglycerol
DEAE	: diethylaminoethyl
EDTA	: ethylenediamine tetraacetic acid
G protein	: guanine nucleotide-binding regulatory protein
MLCK	: myosin light chain kinase
NAD^+	: nicotinamide adenine dinucleotide
P_i	: inorganic phosphate
GTP	: guanosine 5' triphosphate
IP_1	: inositol 1-phosphate
IP_2	: inositol 4,5-bisphosphate
IP_3	: inositol 1,4,5-trisphosphate
IP_4	: inositol 1,3,4,5-tetrakisphosphate
PA	: phosphatidic acid (Ptd OH)
PI	: phosphatidylinositol

PIP	: phosphatidylinositol 4-phosphate
PIP_2	: phosphatidylinositol 4,5-bisphosphate
PPI	: polyphosphoinositides
PKC	: protein kinase C
PLC	: phospholipase C
TCA	: trichloroacetic acid

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SUMMARY

This study examined the effects of drugs in vascular smooth muscle. Mechanical responses to vasoconstrictors and vasorelaxants were examined and correlated with the corresponding biochemical mechanisms that regulate vascular tone. Responses to drugs were examined in isolated segments of rat aorta and tail artery.

The main areas of research were:

1. A study of the mechanical responses to vasoconstrictors and vasorelaxants in isolated vasculature from Wistar rats and of how these responses are altered in vasculature from spontaneously hypertensive rats (SHR) and from the appropriate normotensive Wistar Kyoto (WKY) controls.

2. A study of the effect of vasoconstrictors and vasorelaxants on the second messengers (inositol phosphates, and cyclic nucleotides) involved in contraction and relaxation in vascular smooth muscle and of how these biochemical responses are altered in vasculature from SHR.

3. A study of the interactions between the second messengers involved in modulating tone in vasculature from Wistar rats and from SHR and WKY rats.

The principal methods used were:

1. Measurement of contractions and relaxations of isolated aortic rings and of pressor responses to vasoconstrictors in perfused isolated tail artery segments.

2. Radiochemical measurement of agonist-induced accumulation of (^{3}H)-inositol phosphates (IPs) in isolated aortic rings, pre-incubated in (^{3}H)-myo-inositol.

3. Radiochemical measurement by radio-immunoassay of drug-induced changes in the levels of cyclic adenosine monophospate (cAMP) and cyclic guanosine monophosphate (cGMP) in isolated arterial rings.

4. Measurement of blood pressure in the conscious rat by the tail cuff method.

The principal results obtained in this study are summarized below:

1. The vasoconstrictor, noradrenaline (NA, 10^{-10} -10^{-5}M) produced concentration-dependent contractions of rat isolated aortic rings. KCl (10^{-2} -10^{-1}M) also caused concentration- dependent contractions.

2. The endothelium-dependent vasorelaxnt, acetylcholine (ACh, $3x10^{-9}$ -$3x10^{-6}$M) and the endothelium-independent vasorelaxant, sodium nitroprusside (SNP, 10^{-10}

-$3x10^{-6}$M) relaxed isolated aortic rings that had been precontracted with NA (EC_{75}, $3x10^{-8}$M).

3. Isoprenaline (ISO, $3x10^{-9}$ -10^{-7}M) relaxed isolated aortic rings that had been precontracted with NA (EC_{75}, $3x10^{-8}$M).

4. NA (10^{-8} -10^{-3}M) increased the accumulation of (^{3}H)-inositol phosphates in aortic rings from Wistar rats in a concentration dependent manner.

5. The ability of NA (10^{-5}M) to increase the accumulation of (^{3}H)-inositol phosphates was inhibited by the $alpha_1$ -adrenoceptor antagonist, prazosin (PRAZ, 10^{-6}M). These results suggest that the ability of NA to increase the accumulation of inositol phosphates (IPs) in rat aortic rings is an $alpha_1$-adrenoceptor-mediated response.

6. KCl ($3x10^{-2}$M) also increased the accumulation of IPs in isolated aortic rings from Wistar rats. This result suggests that the KCl-induced influx of Ca^{2+} may cause a PI-response, perhaps as a result of activation of phospholipase-C.

7. ACh (10^{-5}M) and SNP (10^{-6}M) caused concentration-dependent accumulation of cyclic guanosine monophosphate (cGMP) in aortic rings in the presence of NA (EC_{75}, $3x10^{-8}$M).

8. The beta-adrenoceptor agonist, ISO (10^{-8}M) and the directly-acting

relaxant, forskolin (FOR, 10^{-6}M) increased the accumulation of cAMP in isolated aortic rings from Wistar rats.

9. The cGMP-increasing drugs ACh (10^{-5}M) and SNP (10^{-6}M) and also the permeant cGMP analogue, 8-bromo-cGMP (0.3mM) inhibited the NA-induced PI-response in isolated aortic rings from Wistar rats.

10. ACh did not inhibit the PI-response in isolated aortic rings in which the endothelium had been removed mechanically by rubbing or chemically by Triton X-100 (0.1% V/V).

11. ISO (10^{-6}M), salbutamol (SAL, 10^{-6}M) and the permeant cAMP analogue, 8-Bromo-cAMP (0.3mM) also inhibited the NA-induced PI-response.

12. The combination of SNP (10^{-6}M), and FOR (10^{-6}M), additively inhibited the NA-induced PI-response in isolated aortic rings.

13. Isobutylmethylxanthine (IBMX, $1x10^{-6}$M) inhibited the NA-induced PI-response in rat isolated aortic rings.

14. Propranolol (PROP, $5x10^{-6}$M) antagonised the inhibitory effect of ISO (10^{-6}M) and SAL (10^{-6}M) on the NA-induced PI-response in rat isolated aortic rings.

15. The maximal inhibition of NA-induced tone produced by ACh ($1x10^{-6}$ M) was

80% and produced by SNP ($3x10^{-8}$M) was 100%.

16. ACh (10^{-6}M), SNP (10^{-6}M), ISO (10^{-8}M) and FOR (10^{-6}M) all inhibited the PI-response by less than 50% . These results indicate that at the concentrations in which these drugs can maximally inhibit the NA- induced tone, they can only partially inhibit NA-induced PI-response. These results suggest that cAMP and cGMP may have effects other than to inhibit the PI-response.

17. Nifedipine (NIF, 10^{-6}M) inhibited the PI-response in aortic rings from Wistar rats. This result suggests that extracellular Ca^{2+} may be required for the maintenance of the PI-response.

18. The average systolic blood pressure (190 $\pm$ 2 mm Hg) of SHR was significantly higher than that (109.5 $\pm$ 2.8 mm Hg) of WKY normotensive controls.

19. Contractile responses of aortic rings from SHR to NA and KCl were smaller than those obtained in rings from WKY.

20. Inhibitory responses of aortic rings from WKY and SHR to ACh were similar. In contrast, inhibitory responses to low concentrations of SNP ($3x10^{-10}$-10^{-9}M) were smaller in SHR than those obtained in WKY rings, but inhibitory responses to high concentrations of SNP ($3x10^{-8}$ -$3x10^{-7}$M) were larger in SHR

than in rings from WKY controls.

21. ISO ($3x10^{-10}$-10^{-7}M) caused concentration-dependent relaxation of NA-precontracted aortic rings from SHR and WKY rats. Responses to low concentrations of ISO ($3x10^{-10}$ -$1x10^{-8}$M) were smaller in rings from SHR than in rings from WKY controls, but responses to high concentrations of ISO ($3x10^{-8}$ -$1x10^{-7}$M) were not significantly different.

22. Low concentrations of NIF (10^{-9} -$3x10^{-8}$M) caused smaller inhibitions of NA-induced tone in aortic rings from SHR than in rings from WKY but higher concentrations (10^{-6} -$3x10^{-6}$ M) of NIF produced larger inhibitions in rings from SHR than in rings from WKY.

23. Low concentrations of NA (10^{-8} -10^{-6}M) produced a larger PI-response in isolated aortic rings from SHR than in rings from WKY, but with higher concentrations of NA (10^{-5} -10^{-3}M) there were no significant differences. These results conflict with the mechanical results.

24. ACh (10^{-5}M) and SNP (10^{-6}M) increased the levels of cGMP in isolated aortic rings from SHR to a greater extent than in rings from WKY and also produced larger inhibitions of the NA-induced PI- response in rings from SHR than in rings from WKY rats.

25. ISO (10^{-8}M) produced a smaller inhibition of the PI-response in rings from SHR

than in those of WKY, and this was reflected in smaller ISO- induced increases in cAMP in rings from SHR.

26. FOR (10^{-6}M) also produced smaller increases in cAMP in isolated aortic rings from SHR than in rings from WKY.

27. NA ($3x10^{-9}$ -10^{-5}M) produced concentration-dependent contractions in isolated tail artery rings from WKY and SHR rats. Lower concentrations of NA ($3x10^{-9}$ -10^{-6}M) produced responses that were not significantly different in rings from SHR and WKY, but at higher concentrations ($3x10^{-6}$-10^{-5}M) tail artery rings from SHR were significantly more responsive to NA than those from WKY control rats. When these results were expressed as percentages of the maximum response, there were no significant differences in the responses of rings from SHR and WKY rats.

28. KCl (10^{-2} -10^{-1}M) produced concentration-dependent contractions in isolated tail artery rings from WKY and SHR rats. Responses to KCl were not significantly different in rings from SHR and WKY controls. When the results were expressed as percentages of the maximum response, there were no significant differences in the responses of rings from SHR and WKY rats.

29. NA ($3x10^{-9}$ -10^{-5}M) produced concentration-dependent contractions in isolated perfused tail artery segments from SHR and WKY rats. Lower concentrations of NA ($3x10^{-9}$ -$3x10^{-6}$M) produced responses that were not significantly different in

segments from SHR and WKY rats, but at the highest concentration examined (10^{-5}M) tail artery segments from SHR were significantly more responsive to NA than those from WKY control rats. When the results were expressed as percentages of the maximum response, most of the responses obtained, except those produced by the highest and lowest concentrations, were significantly smaller in segments from SHR than in those from WKY rats.

30. KCl (10^{-2} -$5x10^{-1}$ M) produced concentration-dependent contractions in isolated perfused tail artery segments from WKY and SHR rats. There were no significant differences between the responses to KCl in rings from SHR and WKY control rats. When the results were expressed as percentages of the maximum response, there was no significant difference between the responses obtained in rings from SHR and WKY rats.

31. NA (10^{-6} M, 10^{-5}M) produced concentration-dependent PI-hydrolysis in tail artery segments from SHR and WKY control rats. The basal (control) level of PI-hydrolysis in segments from the SHR was significantly higher than in segments from the WKY rats.The PI response to NA (10^{-6}M) was not significantly different in segments from SHR and WKY, but a higher concentration of NA (10^{-5}M) induced significantly greater PI-hydrolysis in segments from SHR than in those from WKY controls.

32. The results obtained in this study indicate that the changes that occur in SHR are complex and cannot be attributed to a single alteration in a particular biochemical

coupling mechanism. Instead, it seems likely that complex, sequential changes occur in SHR and our ability to identify these depends upon several factors, which include the age of the animal examined, the biochemical parameter determined, the way mechanical responses of the isolated segments of artery are measured and even the way the results are calculated.

CHAPTER ONE

INTRODUCTION

SECTION 1:HYPERTENSION

I. EPIDEMIOLOGY OF HYPERTENSION:

Hypertension is a worldwide health problem. It is a major determinant of coronary heart disease, which in industrialized countries is the commonest cause of death. It is also the chief cause of cerebrovascular disease. It has become the commonest reason for initiating lifetime medication, with a correspondingly large consumption of medical resources (Benowitz & Bourne, 1989; Ganten & Mulrow, 1990).

Hypertension occurs in 10% to 20% of the population. This figure varies depending on the level of blood pressure at which a person is classified as hypertensive and on the technical procedures of blood pressure measurement. Hypertension occurs more frequently in European countries, Japan and North America than in less industrialized countries and rural societies. By some estimates, the arterial blood pressure of 15% of the American population is elevated to a degree that requires medical treatment (Benowitz & Bourne, 1989; Ganten & Murlow, 1990; Geber & Nies, 1990). Untreated, malignant hypertension causes the death of 90% of patients within a year (Ganten & Murlow, 1990; Geber & Nies, 1990). The problem may not be revealed until end organ damage is severe and perhaps irreversible, usually after the occurrence of a serious complication. Sustained arterial hypertension damages blood vessels in the kidney, heart, and brain and leads to an increased incidence of renal failure, coronary

disease and stroke. Epidemiological studies indicate that the risk of damage to kidney, heart and brain are directly related to the extent of blood pressure elevation. Even mild hypertension in young or middle-age adults increases the risk of eventual end organ damage at any level of blood pressure or age. The risk is greater in black people and relatively less in premenopausal women than in men. Other positive risk factors include a family history of cardiovascular disease, smoking, hyperlipidemia, diabetes, psychological stress, environmental and dietary factors (increased salt and perhaps decreased calcium intake) may contribute to the development of hypertension (Benowitz & Bourne, 1989; Ganten & Mulrow, 1990).

II. DEFINITION & CLASSIFICATION OF HYPERTENSION:

Hypertension can be classified in different ways, such as by etiology (primary or essential and secondary) or according to severity such as mild, moderate, labile, stable or malignant hypertension.

The World Health Organization has defined blood pressure categories:

A: Normotension: systolic less than 140 mm Hg and diastolic less than 90 mm Hg.

B: Borderline hypertension: systolic from 140 to 160 mm Hg and diastolic between 90 and 95 mm Hg.

C: Hypertension: systolic greater than 160 mm Hg and/or diastolic greater than 95 mm Hg.

These are consensus numbers and do not take into account all the other factors

which influence morbidity due to an elevated blood pressure (Rose, 1986; Ganten & Mulrow, 1990).

III. ETIOLOGY OF HYPERTENSION:

A specific cause of hypertension can be established in perhaps no more than 10% of patients. It is important to consider specific causes, however, because some of them such as renal artery constriction, coarctation of aorta and phaeochromocytoma, are amenable to surgical treatment. Patients in whom no specific cause of hypertension can be found are said to have essential hypertension. In most cases, elevated blood pressure is associated with an overall increase in resistance to flow of blood through arterioles, while cardiac output is usually normal. Elevated blood pressure is usually caused by a combination of several abnormalities (i.e.multifactorial) (Rose, 1986; Benowitz & Bourne, 1989).

SECTION 2: REGULATION OF BLOOD PRESSURE:

Arterial blood pressure (BP) is directly proportional to the product of the blood flow (cardiac output, CO) and the resistance to the passage of blood through precapillary arterioles (peripheral vascular resistance, PVR):

$$BP = CO \times PVR$$

In both normal and hypertensive individuals, blood pressure is maintained by moment-to-moment regulation of CO and PVR, controlled at three sites; arterioles,

postcapillary venules (capacitance vessels) and the heart. A fourth site, the kidney, contributes to the maintenance of blood pressure by regulating the volume of the intravascular fluid. The baroreflex, mediated by sympathetic and parasympthetic nerves, acts in combination with humoral mechanisms, including the renin-angiotensin- aldosterone system, to co-ordinate these four control sites and to maintain normal blood pressure.

Blood pressure in a hypertensive patient is controlled by the same mechanisms that operate in normotensive subjects, but the baroreceptors and the renal blood volume pressure control system appear to be set at a higher level of blood pressure. (Benowitz & Bourne, 1989).

Regulation of vascular resistance to the flow of blood serves two quite different purposes. On the one hand, this resistance must be regulated to establish an arterial pressure that is sufficient to deliver an adequate blood flow to all the tissues of the body, although it must not so high that it damages the arteries. This function, is centrally regulated. The second purpose of the regulation of vascular resistance is that of adjusting blood flow to individual organs to meet the demand of their varying metabolic requirements.

The resistance to blood flow and hence the energy or pressure required to force blood through the vessels is dependent on the blood viscosity and on the nature of the vessels through which it is forced. The vascular characteristics that oppose the flow of blood are called "vascular hindrance" (Bohr & Webb, 1986).

I. VASCULAR HINDRANCE:

The physiological regulation of resistance is affected by changes in the physical properties of the vessel through which it flows. There are several important physiological parameters which can alter vascular resistance, yet for practical purposes this regulation is mainly carried out by changes in the cross-sectional area of the vascular lumen. These changes are caused either by structural or functional alterations in the vessel wall. Functional changes are those caused by contraction or relaxation of the vascular smooth muscle.

A: LUMEN RADIUS:

The lumen, as it reflects the cross-sectional area of the vessel through which blood flows, is the major determinant of resistance to flow. This parameter is adjusted in the physiological regulation of resistance and is also altered in pathological changes in vascular resistance. The quantitative relationship between the radius of the lumen of the vessel and the resistance to flow was defined by Hagen in 1839 and is referred to as the "Hagen-poiseuille law". It is expressed as:

$$R = \frac{8L\eta}{\pi r^4}$$

Where R=resistance, L=length, η=blood viscosity, r=radius, with this dependence of resistance on the fourth power of the radius, a 10% decrease in the radius causes approximately a 50% increase in resistance to flow (Bowman &

Rand, 1980; Guyton, 1986; Bohr & Webb, 1986).

B: OTHER STRUCTURAL DETERMINANTS:

As defined by the Hagen-Poiseuille Law, the resistance to flow also increases as a direct function of the length of vessel. However, there is no physiological process that regulates vascular resistance by changing vessel length (Bohr & Webb, 1986).

Wall thickness also plays an important role in determining the magnitude of the change in resistance produced by vascular smooth muscle contraction or relaxation. When vascular smooth muscle contracts, the circumference of the vessel is reduced. If the vessel wall is thick, the tissue of the wall inside the contracting muscle will be forced into the lumen, reducing its cross-sectional area and thereby amplifying the effect of the smooth muscle contraction. Folkow (1978) has defined the quantitative significance of this determinant of vascular resistance.

The other key site for regulation of PVR and in turn blood pressure is the vascular smooth muscle (VSM).

II. VASCULAR SMOOTH MUSCLE:

The basic structure of an artery is shown in (Fig. 1.1). All blood vessels have this fundamental arrangement, a particular blood vessel being characterised by the proportion of each constituent. Vascular smooth muscle has been

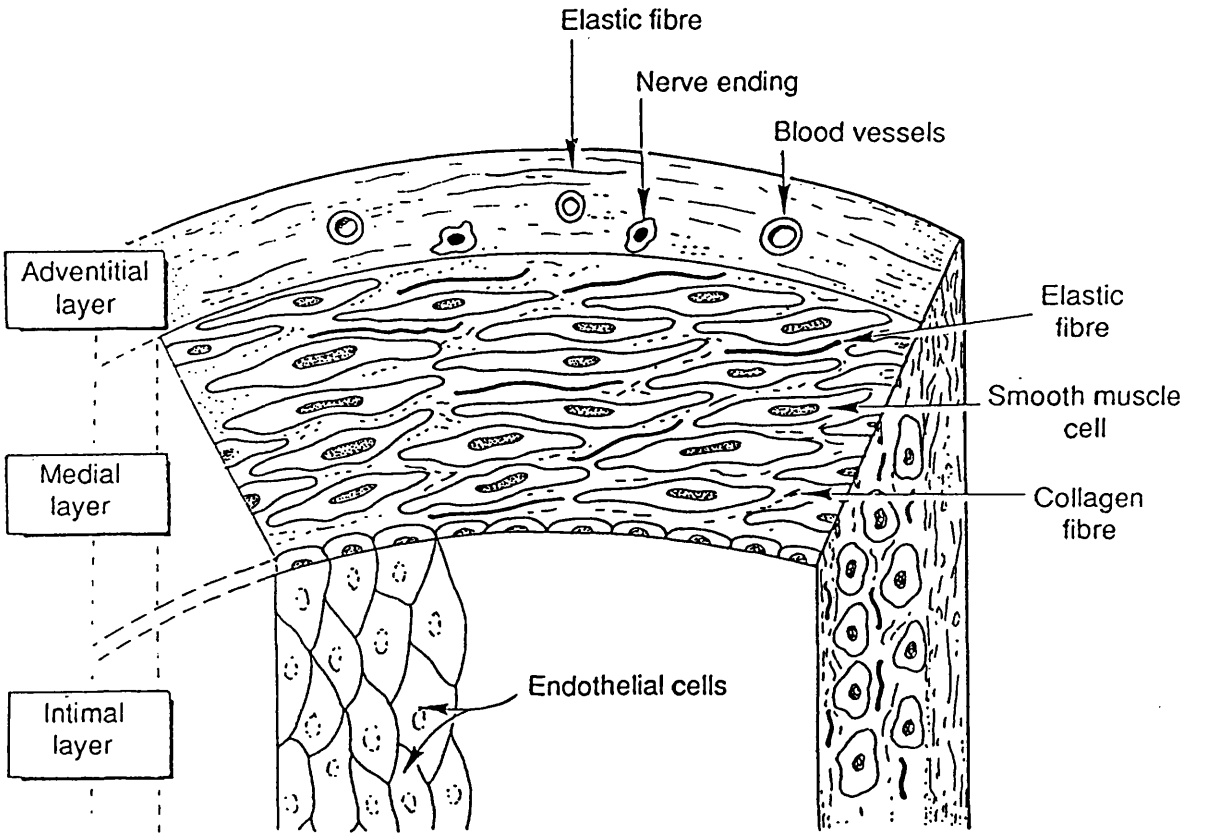

Fig. 1.1.
General structure diagram of a typical blood vessel wall. This diagram represents the layers of a blood vessel wall including intima, media and adventitia

comprehensively reviewed; Furchgott, 1955; Bohr, 1964; Somlyo & Somlyo, 1968; and Gabella, 1981. 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 tone, and in contrast with skeletal muscle, it is well suited for maintaining tension for long periods of time with only minimum energy expenditure (Johansson, 1986; Bohr & Webb, 1986). Vascular tone may be defined as the degree of muscle contraction maintained by the blood vessel (Morgan, 1987) and is influenced by many factors (Vanhoutte, 1978a). 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 neurotransmitter, vasoactive substances and drugs. Intrinsic tone however, is produced by factors such as the structure of the vessel wall. (Morgan, 1987).

The ultimate determinant of vascular smooth muscle tone is the rate or magnitude of calcium ion (Ca^{2+}) transport across the membrane (Bohr, 1963), 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; Karaki, 1989). The effects of all vasoconstrictor and vasodilator stimuli may ultimately be mediated through alterations in the $[Ca^{2+}]_i$ available to the contractile proteins (Bohr, 1963; Karaki, 1989).

Indeed, in cardiovascular disorders such as hypertension and myocardial ischaemia, increased vascular tone is associated with an elevated $[Ca^{2+}]_i$ (Cohen, 1983). The factors that affect tone may either be physical or chemical in nature. The physical factors include the structure and arrangement of the blood vessel wall,

the length-tension relationship of smooth muscle, the temperature and osmolarity of the bathing medium. Chemical factors are more numerous and can be neuronal or humoral in origin. The vascular endothelium also plays an important role in the modulation of vascular smooth muscle reactivity (Furchgott, 1984).

A: PHYSICAL FACTORS:

The principal physical influences on vascular tone are the structural 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 elastic elements which allow 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 also influences the access of neurotransmitters and vasoactive agents to receptor sites and to the mechanisms of deactivation (Vanhoutte, 1978).

B: CHEMICAL FACTORS:

1: INNERVATION OF BLOOD VESSELS:

a: SYMPATHETIC NERVES:

The major regulator of vascular resistance is the autonomic nervous system. It has been shown that in the conscious rat, sympathetic blockade resulted in a 33 mmHg fall in arterial pressure (Hiwatari et al., 1985). Blood vessels are innervated predominantly by sympathetic nerves. Exceptionally, some blood vessels such as the precapillary arterioles in skeletal muscle in many species, including man, receive a cholinergic sympathetic innervation (Rand et al., 1976; Guyton, 1986); these fibres release acetylcholine, therefore sympathetic nerves carry both vasoconstrictor and vasodilator fibres, but the most important of these are sympathetic vasoconstrictor fibres, which are distributed to all areas of the circulation. This distribution is however, greater in some tissues than in others. It is less important in skeletal and cardiac muscle and in the brain, whereas it has a powerful influence in the kidneys, gut, spleen and the skin (Guyton, 1986).

There are variations in the density of nerve distribution and in the degree of penetration of nerve endings into the smooth muscle layers. In most arteries the nerves are restricted to the adventitial- medial border, in veins they usually penetrate into the media (Vanhoutte & Luscher, 1986). Both in animals and man, the density of adrenergic innervation of blood vessels decreases progressively with age (Frewin et al., 1971; Waterson et al., 1974; Gerke et al., 1975). However, in some diseases, vascular tone is mainly regulated by sympathetic nerves (Bevan

& Su, 1973).

Functional changes in vascular smooth muscle associated with hypertension, may be initiated by changes that occur in this neurogenic control of vascular reactivity (Vanhoutte et al., 1981; Winquist et al., 1982). Noradrenaline (NA) is the principal neurotransmitter by which the effects of the sympathetic nervous system are mediated to alter vascular smooth muscle tone. Vascular smooth muscle has various adrenoceptor subtypes on which NA will act. The distribution of these receptors varies greatly. For example, small coronary arteries have more beta (ß) than alpha (α)-adrenoceptors, hence these small vessels respond to NA administration or sympathetic nerve stimulation with a dilation. In contrast, the smooth muscle of large coronary arteries has more α than ß-adrenoceptors, hence they are constricted by NA (Mulvany & Halpern, 1976). On the basis of the activities of specific agonists and antagonists, α-adrenoceptors have been classified into α_1 and α_2 subtypes. Pre-junctional α-adrenoceptors regulate transmitter release from adrenergic nerve endings, whereas post-junctional α- adrenoceptors mediate contraction of vascular smooth muscle (Somlyo & Somlyo, 1970; Stjarne & Gripe, 1973; Starke et al., 1975; McGrath et al., 1989). Pre-junctional α-adrenoceptors were originally classified as α_2-adrenoceptors and the post-junctional α-adrenoceptors may be either α_1- or α_2-adrenoceptors or both (Langer, 1974; Starke et al., 1975; Wikberg, 1979; Fitzgerald, 1988).

b: NON-ADRENERGIC NON-CHOLINERGIC (NANC) NERVES:

At the end of the 1960s the existence of NANC nerves was recognised by Burnstock and his colleagues (Burnstock, 1969). They demonstrated the existence of NANC nerves in the gastro-intestinal tract of all vertebrates including man, and in the urogenital, respiratory and cardiovascular systems. Unlike, the adrenergic and cholinergic divisions of the autonomic nervous system, which were named according to the neurotransmitters released from the nerves, the NANC nerves could not be named in this way because of the multiplicity of possible transmitters that mediate the responses of NANC nerves, and therefore these nerves were named non-adrenergic non-cholinergic.

In 1970, Burnstock and his co-workers concluded that among many substances, ATP is the substance which is most likely to be the NANC transmitter in the intestine and bladder, and thus the purinergic nerve hypothesis was formulated (Burnstock, 1972). Since then, considerable evidence has accumulated in support of this hypothesis, although there have also been some reports that oppose it (Burnstock, 1981).

It was generally believed that each type of autonomic nerve releases only one transmitter. In 1976, Burnstock also suggested that some nerve cells store and release more than one neurotransmitter. The co-transmission hypothesis is now widely accepted and it is recognised that many nerves contain more than one transmitter (Burnstock, 1986). There is evidence for the co-release of ATP and NA from some sympathetic nerves (Burnstock, 1983; Sneddon & Burnstock, 1984).

2. ENDOTHELIUM AND MODULATION OF TONE:

The vascular endothelium is the layer of squamous epithelial cells that is in direct contact with blood and endothelial cells line the entire circulatory system. In addition to its important functions, which include, a: Capillary transport and exchange between blood and tissue, b: Regulation of plasma lipids, c: Participation in the control of haemostasis, the endothelium modulates the reactivity of vascular smooth muscle (Robertson & Rosen, 1978; Vanhoutte et al., 1986). This regulatory role involves several mechanisms which include a: The endothelium acts as a physical barrier that prevents hormones and other vasoactive agents that circulate in blood acting on vascular smooth muscle, b: The endothelium extracts and metabolizes or inactivates vasoactive substances such as NA, serotonin and kinins and thereby prevents or diminishes their activity in vascular smooth muscle; c: The endothelium activates enzymes of plasma constituents such as angiotensin-II by converting enzyme; d: The endothelium secretes vasoactive agents such as prostacyclin and other inhibitory and excitatory mediators in response to vasoactive stimuli (Vanhoutte et al., 1986; Vanhoutte & Lüscher, 1986; Greenberg & Diecke 1988).

a: ENDOTHELIUM DERIVED RELAXING FACTOR (EDRF):

Furchgott studied the paradoxical finding that acetylcholine (ACh) caused vasodilation in vivo but, not in vitro. Furchgott and Zawadzki (1980) discovered

that ACh-induced relaxation of isolated arteries depends on the presence of endothelial cells. Although initially described in the rabbit aorta, it was soon found that this is the case in all the isolated arteries from all mammalian species tested so far, including man (Vanhoutte & Lüscher 1986). The ACh-induced vasorelaxation was mediated by muscarinic receptors on the endothelial cells. When the endothelium is exposed to ACh it releases a diffusable substance with inhibitory effects on vascular smooth muscle. This substance was termed Endothelium-Derived Relaxing Factor (EDRF), to differentiate it from known vasoactive substances such as prostacyclin and Endothelium Derived Contracting Factor (EDCF). In addition to ACh, various substances release EDRF. These include adenosine triphosphate (ATP) and adenosine diphosphate (ADP), arachidonic acid, the calcium ionophore A-23187, substance P, histamine, platelet activating factor, serotonin, thrombin, bradykinin and vasopressin (Furchgott & Zawadzki, 1980; Griffith et al., 1984; Vanhoutte et al., 1986; Greenberg & Diecke 1988; Newby & Henderson, 1990). Some of these substances exhibit endothelium-dependent relaxation only or predominantly in some vascular beds and not in others. For example, vasopressin causes endothelium-dependent relaxation of basilar arteries, but causes contraction in systemic blood vessels (Katusic et al,. 1984). Other studies have indicated that substances that release EDRF also produce endothelium-dependent hyperpolarisation (Bolton & Clapp, 1986). This transient hyperpolarisation appears to be independent of the stimulation of guanylate cyclase by EDRF and may be due to activation of smooth muscle Na^+/K^+ -ATPase or K^+ -channels (Feletou & Vanhoutte, 1988) by a substance other than EDRF (Vanhoutte, 1987). One substance which has been shown to activate K^+

-channels has been termed Endothelium-Dependent Hyperpolarizing Factor (EDHF) (Komori & Suzuki, 1987; Komori et al., 1988).

The chemical structure of EDRF has been investigated, despite the problems arising from its short half-life (6 sec). Many attempts have been made to implicate a metabolite of arachidonic acid (AA), arising from cyclo-oxygenation, lipo-oxygenation, mono-oxygenation or free radical formation, but these have largely been unsuccessful (Furchgott, 1983; Gryglewski et al., 1988). Furchgott (1986) first proposed that EDRF is nitric oxide (NO) (cited by Furchgott, 1988), and this view has been supported by others (Palmer et al., 1987; Ignarro et al., 1987; Ignarro, 1990). It has also been reported that the source of NO is L-arginine (Ignarro et al., 1987; Collier & Vallance, 1989).

L-arginine is the natural substrate for NO synthase and arginine analogues can act as either inhibitors or alternative substrates for this enzyme (Palmer et al., 1988), One such analogue, N^G-monomethyl-L- arginine (L-NMMA), which was initially shown to be an inhibitor of the formation of NO^{2-} and NO^{3-} from L-arginine in the macrophage (Hibbs et al., 1987), has been widely used as a tool to study this pathway in a variety of biological systems. Blockade of NO synthesis by L-NMMA is stereospecific (Stuehr et al., 1989) and is competitively reversed by L-arginine but not by D-arginine in all systems so far studied (Collier & Vallance, 1989).

Nitrovasodilators and endogenous NO act by stimulating soluble guanylate cyclase (Murad et al., 1978). The effect of NO depends on its site of release. If the generator cell is the vascular endothelium, NO (EDRF) released by ACh or bradykinin, activates guanylate cyclase in adjacent smooth muscle cells which then relax. In addition, NO inhibits adhesion and aggregation of platelets (Collier &

Vallance, 1989).

b: ENDOTHELIUM DERIVED CONTRACTING FACTOR (EDCF):

In addition to producing EDRF and prostacyclin, the endothelium also releases EDCF. It has been shown that removal of the endothelium, depressed the contractile responses of canine mesentric veins to ACh (Greenberg & Tanaka, 1982), and of canine pulmonary and splenic veins to NA (De Mey & Vanhoutte 1981,1982 a,b.). Moreover, the contractile responses to substances such as histamine were enhanced. Thus, it was suggested that some vasoconstrictor agents (NA and ACh) stimulate the release of a vasoconstrictor substance from the endothelium of some canine arteries and veins (De Mey & Vanhoutte 1981,1982; Greenberg & Tanaka, 1982; Lüscher & Vanhoutte, 1986). EDCF(s) are also released from the endothelium in response to stretch (Katusic et al., 1986), distension and anoxia (De Mey & Vanhoutte, 1983; Rubanyi & Vanhoutte, 1985) and appear to play a major role in the facilitation of vascular constrictor responses to other substances rather than in initiating contraction. Three classes of EDCF appear to be released by the endothelium of blood vessels in response to various stimuli. Rubanyi (1987) has classified them as: 1) Arachidonic-derived EDCF ($EDCF_1$); 2) Polypeptide-derived EDCF ($EDCF_2$) and, 3) Undefined, diffusible EDCF ($EDCF_3$) (cited by Greenberg & Diecke, 1988). Recent studies have indicated that the endothelium is capable of releasing a highly potent vasoconstrictor substance which has been termed endothelin (Yanagisawa et al., 1988). Many substances

including thrombin, adrenaline and the calcium ionophore A23187, cause the release of endothelin from cultured endothelial cells (Yanagisawa et al., 1988). In addition, Vanhoutte and Katusic (1988) has suggested that the superoxide anion (O^{2+}) may be responsible for endothelium-dependent contractions in tissue such as the dog basilar artery.

SECTION 3: SIGNAL TRANSDUCTION IN VASCULAR SMOOTH MUSCLE:

Vascular smooth muscle tone is ultimately controlled by alterations in the cytoplasmic concentration of free intracellular calcium ions $[Ca^{2+}]_i$. In vascular smooth muscle, neurotransmitters, and hormones interact with highly selective sites at the cell membrane. These receptors recognise such extracellular stimuli and discriminate between closely related molecules. Activation of these receptors, generates intracellular signals, that mediate the cellular events. The biochemical machinery of the plasma membrane translates the complex language of extracellular stimuli into a simpler language of ionic, electrical, and chemical signals that control intracellular events. This translation process, which converts external stimuli into internal chemical signals is called stimulus-response coupling (Berridge, 1981; Michel, 1987). Generally, activation of a cell surface receptor stimulates a transducer protein which is a guanine nucleotide-binding regulatory protein (G-protein), that becomes active upon binding of guanosine triphoshate (GTP). The activated G-protein then acts on an amplifier enzyme, which releases a second messenger, which in turn activates

protein kinases, that catalyse a series of biochemical reactions resulting in the cellular response. (Michell, 1987).

The major known transmembrane signalling pathways and their second messengers in vascular smooth muscle are cyclic adenosine 3 ,5 monophosphate (cAMP) and the product of phosphoinositide (PI)-hydrolysis including, 1,2 diacylglycerol (DAG) and inositol 1,4,5-triphosphate ($I_{1,4,5}P_3$ or IP_3), which in turn releases Ca^{2+}, from intracellular stores. Cyclic guanosine 3'5' monophosphate (cGMP) is another second messenger that is involved in vascular smooth muscle tone modulation.

I. INTRACELLULAR CALCIUM HOMEOSTASIS:

The messenger role of calcium is fulfilled in two ways:

1) Ca^{2+}-calmodulin pathway, which is activated by a transient rise in $[Ca^{2+}]_i$ and is responsible for the initial phase of the cellular response; and 2) The protein kinase C (PKC)-Ca^{2+} pathway, which is activated by both the rise in $[Ca^{2+}]_i$ and DAG, which remains in the cell membrane and is responsible for the sustained phase of the cellular response (Rasmussen, 1983). Two integrated membrane systems are involved in the control of smooth muscle $[Ca^{2+}]_i$. The first is the plasmalemma, which is under the control of the membrane potential, and agonists such as neurotransmitters, hormones, and autacoids. The second is, the sarcoplasmic reticulum, which is controlled by second messengers.

Membranes form a barrier to an approximately 10,000-fold concentration gradient of calcium. The extracellular concentration of Ca^{2+} is normally held at

approximately 1000 μM, whereas in the unstimulated cell, $[Ca^{2+}]_i$ is 0.1-0.2 μM (Rasmussen, 1983).

II. ROLE OF PLASMA MEMBRANE:

The plasma membrane controls Ca^{2+} homeostasis by means of Ca^{2+} transporting systems. Typically, these include; A specific ATPase or Ca^{2+} -Mg^{2+} -ATPase, which is a Ca^{2+} extrusion pump, that extrudes Ca^{2+} from the cytosol into the extracelluar space, and Na^+ /Ca^{2+} exchange carrier by which 3 Na^+ are transported for each Ca^{2+} (Van Breemen & Saida, 1989).

These systems maintain the high Ca^{2+} gradient across the plasma membrane, and keep intracellular Ca^{2+} at approximately 0.1μM. Plasmalemmal Ca^{2+} permeability is regulated by Ca^{2+}- channels which include the voltage operated Ca^{2+} -channel (VOCs), that are sensitive to changes in the transmembrane potential, and the receptor operated Ca^{2+} -channel (ROCs) (Bolton, 1979; Van Breemen et al., 1986).

Recently, the role of the second messengers that link the receptor to ROCs have received attention. It was reported that IP_3 releases Ca^{2+} from platelet-derived plasmalemma vesicles, which were Ca^{2+} loaded through the Na^+ /Ca^{2+} exchange (Rengasamy & Feinberg, 1988). Single cation channel activity has been reported in T-lymphocytes, loaded with IP_3 (Kuno & Gardner, 1987). IP_4 has also been proposed as the second messenger linking receptors to ROCs (Irvine & Moor, 1986). Another possibility is that α-subunits of GTP binding protein (G-protein) are involved, as has been shown for muscarinic activation of K^+ channels and Ca^{2+}

channels in other tissues (Brown & Birnbaumer, 1988). A final candidate for indirect activation of at least some cation channels is cytoplasmic Ca^{2+} (Von Tscharner et al., 1986).

A third class of non-selective and stretch-sensitive ion channels, that are permeable to Ca^{2+}, has been reported in endothelial cells (Lansman et al., 1987).

III. INTRACELLULAR MEMBRANE AND $[Ca^{2+}]_i$ STORES:

Other membranes that are involved in Ca^{2+} homeostasis are the endoplasmic or sarcoplasmic reticulum and the mitochondrial membrane, which has a minor role (Carafoli, 1987). It has been shown that the mitochondria and the sarcoplasmic reticulum (SR) are the main intracellular calcium stores (Chiesi & Carafoli, 1986). Other calcium stores have also been suggested. These include plasmalemma-bound calcium (Janis et al., 1987), and Ca^{2+} -buffering proteins (Carafoli, 1987). The sarcoplasmic reticulum contains a specific Ca^{2+}-ATPase, which is different from that of the plasma membrane (Ebashi, 1958; Hasselbach, 1961; cited by Carafoli 1987) for uptake of $[Ca^{2+}]_i$ from the cytoplasm and like plasmalemmal Ca^{2+} -ATPase, it could be stimulated by cyclic nucleotides cAMP and cGMP (Eggermont et al., 1988; Twort & Van Breemen, 1988). Release of Ca^{2+} from the SR membrane occurs as a result of interaction between IP_3 and its specific receptor (Berridge & Irvine, 1984) which opens cation channels, that allow rapid discharge of the SR Ca^{2+} store. A guanosine nucleotide regulatory mechanism also appears to be involved in this receptor-channel coupling system

(Gill et al., 1986; Saida & Van Breemen, 1987). It has been shown that smooth muscle SR contains two separate types of Ca^{2+} release channel: IP_3 - and Ca^{2+}-activated channels (Van Breemen, 1990). This view is supported by the observation that Ca^{2+} enhanced the IP_3 -induced Ca^{2+} release from the SR in skinned smooth muscle (Ilino, 1987). Mitochondria contain an uniporter, which is different from that of the plasma membrane, used for the release of Ca^{2+} from the matrix to the cytosol. Some mitochondria may also contain a Ca^{2+} /H^+ exchange system (Carafoli, 1987). Isolated mitochondria from permeabilized smooth muscle cells do not take up significant quantities of Ca^{2+} unless the Ca^{2+} concentration exceeds 5μM (Somlyo et al., 1982; Yamomoto & Van Breemen, 1986). Mitochondria therefore become Ca^{2+} "sinks" only if locally high concentration of Ca^{2+} are achieved, for example during Ca^{2+} release from the SR or if entry of calcium becomes abnormally high during some pathological state, such as hypertension. (Yamomoto & Van Breemen, 1986).

IV. TRANSDUCTION PROCESS IN CELL MEMBRANE:

The major signalling membrane systems involving adenylate cyclase (AC) and phospholipase C, have unique membrane transduction components that comprise; a specific plasma membrane receptor which is the recognition component, a regulatory protein which is the transducing component and is a guanine-nucleotide dependent (G-protein), and an amplifier component which is a catalytic enzyme (Taylor & Merrit, 1986; Potter, 1990). These two systems are associated and have important roles in $[Ca^{2+}]_i$ homeostasis. The nature of the

receptors and catalytic enzymes are different in these systems but the G-protein is a common mechanism.

A: TRANSDUCING COMPONENT OR G-PROTEINS:

G-proteins function as transducers of receptor signals into effector responses (Fig. 1.3). In the adenylate cyclase (AC) pathway there are two G-proteins, comprising a stimulatory G-protein or (G_s) and an inhibitory G-protein or (G_i). These are structurally similar heterotrimers, composed of highly homologous α subunits, α_s in G_s and α_i in G_i, and a ß and a Υ subunit (Houslay, 1990). The ß and Υ subunits are identical in both G-proteins. The G_s protein is localized on the inner leaflet of the plasma membrane (Levitzki, 1990). The α-subunits are the larger subunits which possess the GTP binding site. The α_s subunits are the target for ADP- ribosylation by NAD^+ which is catalysed by cholera toxin. The α_i subunits are the target for ADP- ribosylation by the toxin of Bordotella pertussis (pertussis toxin) but not by cholera toxin (Gilman, 1987; Casey & Gilman, 1988; Levitzki, 1990). The primary specificity for both the receptor and the target system resides in the α subunit (Litosch, 1990). ß and Υ subunits are smaller subunits that are tightly associated with each other as a ßΥ complex (Casey & Gilman, 1988). These ßΥ subunits have a role in anchoring the α subunit (Sternweis, 1986), and in promoting GDP exchange on the α subunit (Fung, 1983; Correze et al., 1987), and attenuation of α-mediated events (Katada et al., 1984). Agonist binding to the stimulatory receptors (R_s) and GTP binding to G_s at the α_s subunit are both required to induce activation of the catalytic unit (C) of adenyate cyclase (AC) to

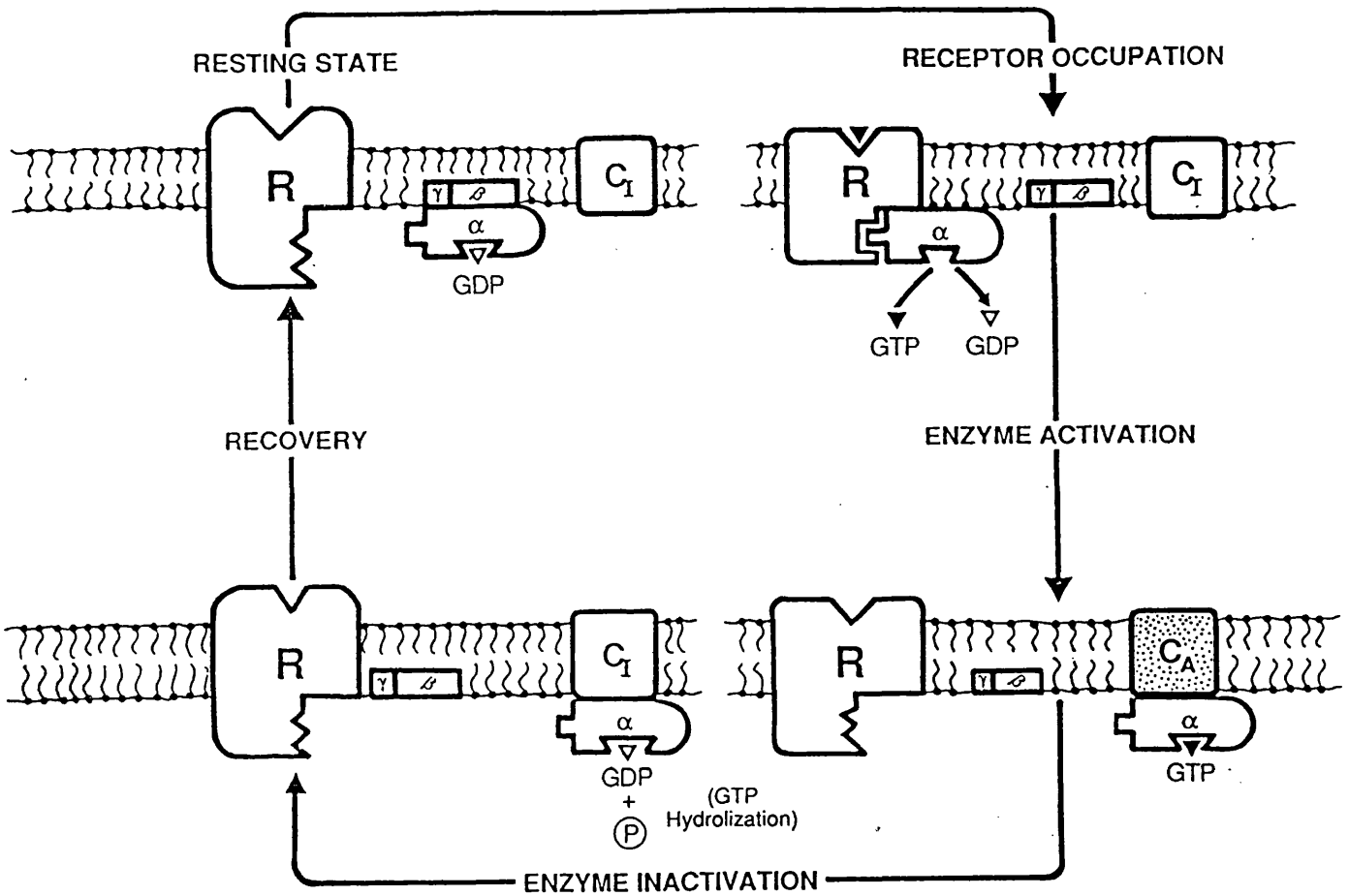

Fig. 1.3.
A schematic diagram of G-protein function. The G-protein consists of three subunits (α, ß, γ). The ßγ complex acts as an anchor for the α-subunit which couples to the receptor-agonist complex and the GDP is replaced by GTP. Then α-subunit dissociate from the receptor and interacts with the catalytic unit (C) of the enzyme and activates it. The enzyme (C) is deactivated by hydrolysis of GTP to GDP and the α-subunit reunites with the ßγ-complex and make a new G-protein.

the cAMP-producing state. The activated state of the system decays concomitantly with the hydrolysis of GTP to GDP and P_i at the G_s regulatory site. Replenishment of G_s with GTP and the continued presence of agonist at the receptor allow the system to regain an active or active/deactivation state. This type of "ON-OFF" cycle accounts for the properties of agonist- dependent AC (Levitzki, 1987; Levitzki, 1990).

In the resting state the G-protein exists as an unattached $\alpha\beta\gamma$ trimer, in which the GDP is attached to the α-subunit. After interaction of an agonist with its receptor, it undergoes a conformational change involving the cytoplasmic domain of the receptor, causing it to develop high affinity for $\alpha\beta\gamma$ trimer (Spiegel, 1990). Receptor association with the G protein occurs with the α subunit, causing bound GDP to dissociate and be replaced with GTP (GTP/GDP exchange). The α subunit with the attached GTP (α-GTP) dissociates from $\beta\gamma$ This state is called "ON state" and α-GTP is in the active form of G-protein and interacts with various enzymes, ion channel causing activation or inactivation. For example, activation of the ß-adrenoceptor leads to the formation of α-GTP and in turn activation of adenylate cyclase, hereby causing production of cAMP. The "ON state " is terminated by hydrolysis of GTP to GDP through the GTPase activity of the α-subunit. The resulting α-GDP dissociates from the enzyme and combines with $\beta\gamma$ to produce the "OFF state" (Levitzki, 1987; Levitzki, 1990; Rang & Dale, 1991). The inhibitory pathway by G_i is similar to G_s. Houslay (1990) indicated that the G_i mediated inhibition of AC is achieved in two distinct ways. First, by releasing the $\beta\gamma$ subunits which are identical to the $\beta\gamma$ subunits of G_i and serve to inhibit the dissociation of G_s, and secondly through the action of GTP bound αG_i which

appears to be capable of interacting with and inhibiting the catalytic unit of AC directly. Activation of phospholipase C is also mediated by a G protein (G_{plc}) (Haslam & Davidson 1984; Blackmore et al., 1985). The identity of this G-protein (G_{plc}) has not yet been established (Litosch, 1990). In 1989 Linden & Delahunty reported that a bidirectional control similar to that which regulates adenylate cyclase, operates on phospholipase C.

Houslay (1990) suggested that at least two distinct G-proteins can couple certain rceptors to the stimulation of inositol phospholipids metabolism. While these have yet to be identified, one is believed to be capable of being ADP-ribosylated and inactivated by pertussis toxin but the other is not.

B: CATALYTIC COMPONENT OF SIGNAL TRANSDUCTION:

1. ADENYLATE CYCLASE AND CYCLIC AMP:

This enzyme has been isolated from the rabbit myocardium, and identified as a glycoprotein which spans the plasma membrane (Pfeuffer et al., 1985). This enzyme produces the intracellular second messenger (cAMP) through interaction with and activation of the catalytic unit with the α-subunit of G protein. The catalytic unit of AC is a hydrophobic protein with a single transmembrane spanning domain carrying its catalytic site at the cytoplasmic side of membrane (Levitzki, 1990). This enzyme is controlled by receptors which can either stimulate (R_s) or inhibit (R_i) its functioning (Houslay, 1990). Activation of this enzyme leads to conversion of ATP into cAMP.

2. PHOSPHOLIPASE-C AND PI-RESPONSE:

A primary receptor-mediated event which occurs when cells are stimulated by a variety of hormones, neurotransmitters and growth factors (Ca^{2+} mobilizing receptors) is the activation of a phosphoinositide specific phospholipase C (PLC). Hydrolysis of PI by PLC results in the generation of the intracellular mediators, IP_3 and diacylglycerol (DAG) (Fig. 1.2), which respectively promote an increase in levels of cytosolic $[Ca^{2+}]_i$ and an activation of protein kinase C.(Berridge & Irvine, 1984; Abdel-Latif, 1986; Berridge, 1987; Litosch, 1990).

Since PLC is involved in the initiating event of the PI-response, several studies have examined the nature of PLC. At least two kinds of PLC including cytosolic and membrane PLC have been reported, and each of these has different subtypes (Abdel-Latif, 1986; Litosch, 1990). However, so far five isoforms of PLC have been demonstrated (Bowman, 1992). The primary receptor mediated event involves stimulation of PLC by the putative G protein (G_{plc}). This activation may occur as a consequence of a conformational change in the enzyme that lowers the Ca^{2+} concentration required for enzyme activation (Litosch, 1990). The elevation of $[Ca^{2+}]_i$ may increase net inositol phosphate (IP) production either through activation of cytosolic PLC or membrane PLC or both. The increase in Ca^{2+} levels, resulting from Ca^{2+} influx may further stimulate PLC activity. PKC may also regulate PLC through phosphorylation (Berridge, 1987; Cobbold et al., 1990; Litosch, 1990).

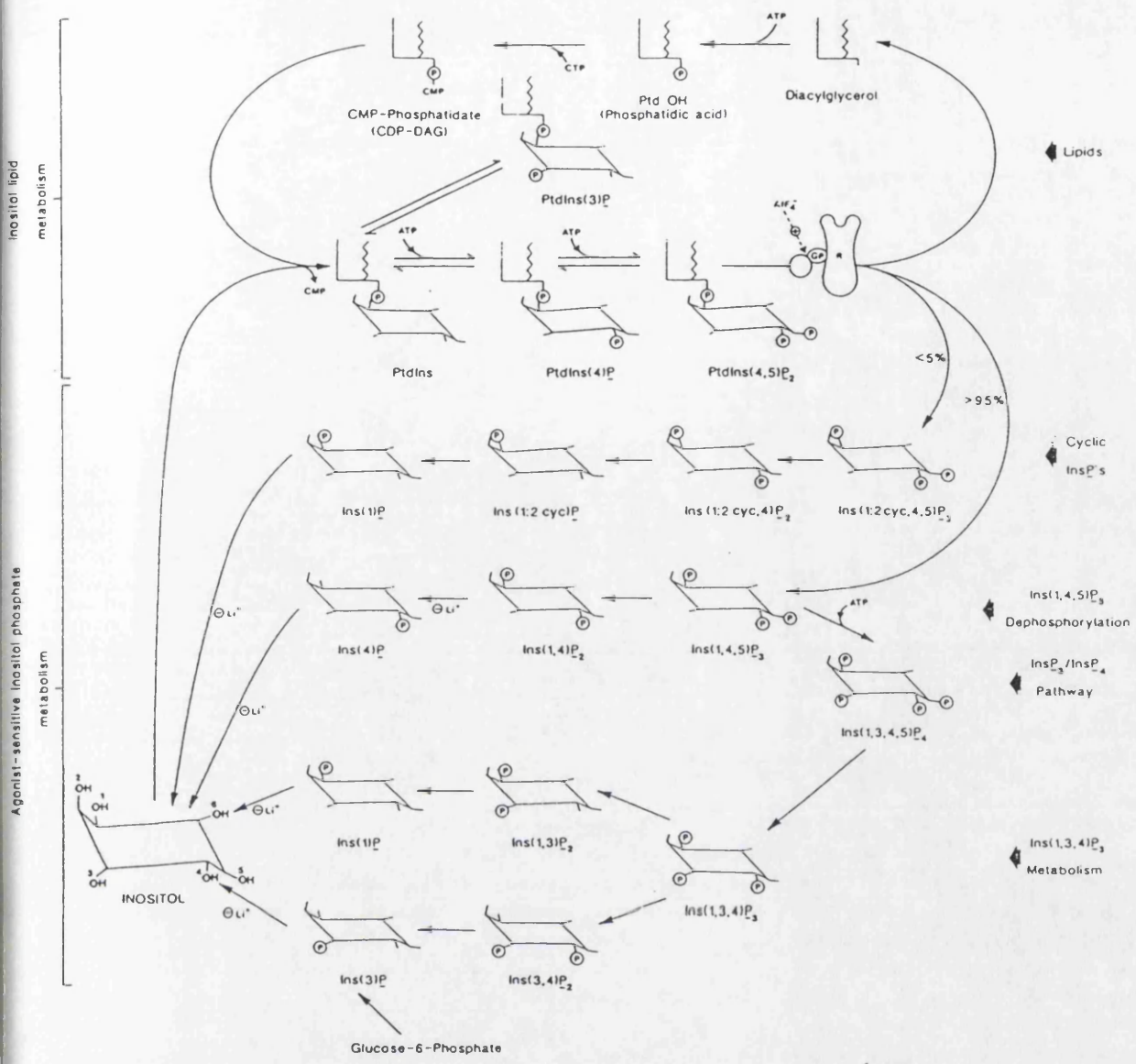

Fig. 1.2.
A schematic digram of the phosphoinositol metabolism (Main routes) in PI-cycle. R= receptor, G = G-protein. (Adapted from Berridge & Irvine, 1989).

Inositol lipids or phosphoinositides constitute less than 10% of total phospholipids. Phosphatidylinositol (PI) is the major compound, and phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4,5 bisphosphate (PIP_2) which are collectively referred to as polyphosphoinositides (PPI) are minor plasma membrane inositol phospholipids (6-8% for PI, 1% for PIP, and 0.5% for PIP_2) (Chuang, 1989). Most of PPI and a minor fraction of PI are plasma-membrane bound, while most of the PI is in intracellular organelles, especially endoplasmic reticulum (ER). Both phosphatidic acid (PA) and myo-inositol, which are precursors for PI synthesis, are synthesized from D-glucose. Briefly, CDP-diacylglycerol (CDP-DG or CMP-PA), formed from PA and CTP in the presence of CTP-PA cytidyltransferase, are key intermediates in PI-biosynthesis (Fig i.2). Biosynthesis of PI from CDP-DG and myo-inositol, via "PI-synthase" takes place at the ER, from which it is transported to the plasma membrane by a specific protein, called "PI-exchange protein". PI can then be sequentially phosphorylated by a specific kinase to generate PIP and PIP_2, this process takes place in the plasma membrane (Abdel-Latif, 1986).

3. GUANYLATE CYCLASE AND CYCLIC GMP:

cGMP is another important intracellular second messenger. Unlike cAMP, which is a second messenger at a wide range of receptors, cGMP is a specific messenger with an established signalling role in only a few cell types. These include intestinal mucosa, and vascular smooth muscle, in which the cGMP-based signal transduction mechanism closely parallels the cAMP-mediated signalling

mechanism (Bourne & Roberts, 1989).

In 1976, cGMP was suggested to be a mediator involved in vascular smooth muscle contraction (Diamond & Blisard, 1976). Synthesis of cGMP is catalysed by the enzyme guanylate cyclase (GC), from GTP. Activation of GC was observed when azide was added to a crude enzyme preparation to inhibit GTPase activity (Kimura et al., 1975). Many of the calcium-mobilizing receptors that trigger an increase in PI- hydrolysis, stimulate GC to elevate the intracellular level of cGMP (Michell, 1975 ; Berridge, 1981; Berridge, 1984).

There are two forms of GC; 1) The soluble (cytosolic) form, and 2) The particulate (membrane) form (Waldman & Murad, 1987). The cellular content of cGMP is about 10 times lower than that of cAMP. Distribution of these forms of GC varies in different tissues. In some tissues, such as platelets (Bohme et al., 1974; Glass et al., 1977), GC is predominantly soluble. In other tissues such as intestinal mucosa (Kimura & Murad, 1975; Quill & Weiser, 1975), the enzyme is predominantly particulate. Activation of soluble GC is of major importance in the modulation of vascular reactivity. However, the atriopeptins, stimulate particulate GC, exclusively in vascular tissue (Waldman et al., 1984; Winquist et al., 1984). Recently it has been reported that GC, like AC is under the control of G-proteins (Rang & Dale, 1991). cGMP like cAMP acts by activating specific protein kinase (PK), in this case cGMP-dependent protein kinase (PKG). Cyclic nucleotides are inactivated by phosphodiesterases (PDEs). Butcher and Sutherland (1962) described cAMP PDEs which catalyse the conversion of cyclic 3' ,5' , AMP to 5' AMP. PDEs are the enzymes that catalyse the hydrolysis of phosphodiesters such as cyclic nucleotides and PIP. Cyclic nucleotide PDEs occur as a number

of isoenzymes, exhibiting distinct kinetic properties, substrate specification and cellular localization. Three groups of cyclic nucleotide PDEs are recognised in mammals. One is membrane-bound with a high afinity for cAMP, the other two forms are cytosolic and are capable of hydrolysing both cAMP and cGMP. After separation by DEAE-cellulose chromatography, they have been classified as:
1) cGMP-PDE which has a higher affinity for cGMP than for cAMP, and hydrolyses principally cGMP, even at low substrate levels. 2) Ca^{2+}- calmodulin PDE since it requires Ca^{2+} and calmodulin and preferably hydrolyses cGMP ; 3) cAMP-PDE which is a specific cAMP PDE (Hidaka et al., 1977; Bowman et al., 1986). In recent years, these PDEs have been further subdivided and classified as: I: Ca^{2+}-calmodulin dependent, II: Cyclic GMP-dependent, III: Cyclic GMP-inhibited, IV: Cyclic AMP-specific, and V: Cyclic GMP-specific (Beavo & Reifsnyder, 1990; Nicholson et al., 1991; Bowman, 1992).

Apart from activation of GC by GTPase inhibitors (Kimura et al., 1975), other nitrogen-containing compounds including nitroprusside, nitroglycerine, sodium nitrate and nitric oxide (NO), activate GC and increase cGMP synthesis in vascular and non-vascular smooth muscle (Katsuki et al., 1977; Gruetter et al., 1981; Axelsson et al., 1982; Axelsson & Anderson 1983). Such substances, which mediate vascular smooth muscle relaxation by activating GC and increasing intracellular synthesis of cGMP are termed "nitrovasodilators". Relaxation of blood vessels by the nitrovasodilators occurs both in the presence and absence of an intact endothelium, hence, these agents have been termed "endothelium-independent". In contrast, agents such as ACh, histamine, bradykinin, the calcium ionophore A23187, ATP, NA, and thrombin require an

intact endothelium in order to relax blood vessels (Furchgott & Zawadski 1980; Rapoport & Murad 1983 a,b; Furchgott, 1981 and 1984). These agents are therefore termed "endothelium-dependent" vasodilators. In fact, in the absence of endothelium some of these agents, become vasoconstrictors, depending on the blood vessel and species being tested. It is known that these agents interact with specific receptors on the endothelium and cause the synthesis and/or release of EDRF (Furchgott & Zawadski 1980; Furchgott, 1984).

SECTION 4. MECHANISMS PRODUCING TONE IN VASCULAR SMOOTH MUSCLE

I. CONTRACTION OF VASCULAR SMOOTH MUSCLE:

Contraction of vascular smooth muscle is mediated by any mechanism that increases $[Ca^{2+}]_i$. These include mechanisms that cause either Ca^{2+} influx, such as occurs with potassium chloride (KCl), or mobilization of intracellular calcium as a result of PI-hydrolysis and generation of second messengers IP_3 and DAG (Berridge, 1981; Michell et al., 1981). This bifurcating pathway leads to muscle contraction by two mechanisms; 1) $IP_3/[Ca^{2+}]_i$- calmodulin pathway; 2) DAG/PKC pathway, both of which cause phosphorylation of the contractile proteins.

A: IP_3 /$[Ca^{2+}]_i$ -CALMODULIN PATHWAY:

The role of inositol lipids in cellular signalling has been known since the initial finding of Hokin and Hokin (1953), who demonstrated the effects of neurotransmitters and hormones on phospholipid turnover. They reported that phospholipids, later identified as phosphatidylinositol (PI) and phosphatidic acid (PA), may play an important part in receptor-mediated cell responses (Hokin & Hokin, 1955; 1958). Michell in 1975, first proposed the term "calcium- mobilising receptors" for receptors that trigger hydrolysis of PI and in turn release $[Ca^{2+}]_i$. Michell also observed that the mobilisation of $[Ca^{2+}]_i$ induced by a number of agonists is prece ded by the breakdown of PI and recognized that it is the breakdown of PIP_2, rather than of PI that is important. The formation of IP_3 and IP_2 in response to the activation of surface membrane receptors was demonstrated by Berridge (1983) and by Streb et al, (1983), who first reported that $I_{1,4,5}$ - P_3 released calcium from a non-mitochondrial store (ER), in permeabilized pancreatic acinar cells. This observation has been confirmed in many other permeabilized cell system (Reviewed by Berridge, 1984 & 1987). In particular, it has been demonstrated in permeabilized vascular smooth muscle by Yamamoto & Van Breemen (1985), in rat aortic smooth muscle, by Hashimoto et al. (1986) in rabbit mesenteric artery. The interaction of IP_3 with its specific receptor on the sarcoplasmic reticulum (SR) opens Ca^{2+} -channels, which allow the rapid release of Ca^{2+} from SR store (Bingham Smith et al., 1985; Baukal et al., 1985). A guanine nucleotide regulatory mechanism also appears to be involved in this receptor- channel coupling system (Saida & Van Breemen 1987; Gill et al., 1986). IP_3 -induced Ca^{2+} release from permeabilized rabbit mesentric smooth muscle is dependent on the presence of GTP or its non-hydrolysable analogue C_1 PP NH_p,

and is inhibited by pertussis toxin (Saida & Van Breemen, 1987). Plasmalemma bound Ca^{2+} has been suggested to be an additional source of Ca^{2+} (Janis et al., 1987), and could be released by PI-hydrolysis during agonist-induced stimulation (Saida & Van Breemen, 1984). The rise in $[Ca^{2+}]_i$ induced by IP_3 from SR can only be transitory, and hence it causes the initial phase of contraction (Berridge & Irvine, 1984), after a short time (in practice, 1-2 min with a high dose of agonist), the plasma membrane Ca^{2+} -pump should drive the Ca^{2+} out of the cell, and with the intracellular stores now empty, the $[Ca^{2+}]_i$ will return to the resting level despite the continuing presence of IP_3. In fact the Ca^{2+} levels and contraction usually remain elevated as a result of influx of extracellular calcium, through VOCs or ROCs but activation of many receptors increases only IP_3 and DAG production and it is by the inositol phosphate that Ca^{2+} entry is increased. This is a controversial issue. Casteels and Droogmans (1981) suggested that the calcium content of an intracellular store may control Ca^{2+} entry. Kuno and Gardner (1987) suggested that IP_3 might act on a Ca^{2+} -channel in the plasma membrane. Another possibility is that the receptor mechanism may generate its own channel in the form of phosphatidic acid (PA) (Putney et al., 1980; Salmon & Honeyman, 1980). The DAG formed by PI-hydrolysis is phosphorylated to PA, which then acts as a calcium ionophore before it is resynthesized back to PI. Another possibility is that the Ca^{2+} enters the intracellular calcium pool directly, by a gap-junction-like structure from outside the cell (Merrit & Rink, 1987). Work by Irvine and Moor (1986) on sea urchin eggs suggested that IP_4 has a role in regulating Ca^{2+} entry. They reported that the Ca^{2+}-releasing action of IP_3 is insufficient to activate the eggs fully, and that Ca^{2+} entry due to IP_4 is necessary as well. Because IP_4 did not

activate the eggs when injected alone, they speculated that the release of Ca^{2+} by IP_3 is a necessary prelude to the action of IP_4 . Increase in $[Ca^{2+}]_i$ leads to formation of the Ca^{2+}-calmodulin complex, which binds to and activates a number of different enzyme systems, including myosin light chain kinase (MLCK), and in turn triggers contraction through MLC phosphorylation. Relaxation would be induced by inactivation of MLCK, following a reduction in $[Ca^{2+}]_i$ and dephosphorylation of MLC by phosphatase (Morgan et al., 1976; Pato & Adelstein 1980; McCall, 1987).

B: DAG/PKC PATHWAY:

The other major product of PIP_2 hydrolysis is DAG, which also has an important second messenger function in that it activates C-kinase or protein kinase C (PKC) to phosphorylate specific proteins (Takai et al., 1979; Kishimoto et al., 1980; Nishizuka, 1983). PKC was first found in 1977, in many tissues, including vascular smooth muscle (Inoue et al., 1977; Kuo et al., 1980). The usual view of PKC is that the enzyme exists in both cytosolic and plasma membrane-bound form but with the equilibrium in favour of the cytosolic form in the resting cell (Burgoyne, 1989). Protein chemistry and molecular cloning techniques have identified at least seven isoforms within a given mammalian species, and it seems likely that this is a minimum estimate (Dreher & Hanley, 1988; Nishizuka, 1988; Bowman, 1992). The activation of PKC by DAG is a complex process that requires Ca^{2+} and phosphatidylserine as cofactors (Berridge, 1984). DAG is normally almost absent from membrane, but is transiently produced from PI-hydrolysis in response

to extracellular signals. Kinetic analysis indicates that a small amount of DAG dramatically increases the affinity of PKC for Ca^{2+}, and fully activates the enzyme without any change in Ca^{2+}levels (Kishimoto et al., 1980; Kaibuchi et al., 1981). Activation of the enzyme appears to cause a physical translocation of the enzyme from the cytosol into the membrane and this might be the role of Ca^{2+} (May et al., 1985; Wolf et al., 1985). PKC exists in vascular smooth muscle (Kuo et al., 1980), and induces contraction of vascular smooth muscle by phosphorylation of MLC at a different site from that of MLCK (Naka et al., 1983; Ikebe et al., 1985; Nishikawa et al., 1984; Itoh, et al., 1986). Moreover, it has been shown that the activation of PKC is responsible for sustained muscle contraction (Defeo & Morgan, 1985; Chatterjee & Tejada, 1986). PKC also could be activated by tumour promoting phorbol esters, such as 12-O-tetradecanoylphorbol -13 acetate (TPA), which has a molecular structure very similar to DAG, and activates the enzyme both in vitro and in vivo (Nishizuka, 1984). Kinetic studies have shown that like DAG, phorbol esters dramatically increase the affinity of PKC for Ca^{2+} so that the requirement for activation of enzyme is less than the $0.1\mu M$. As a result, full activation of PKC occurs without any increase in Ca^{2+} concentration (Kikawa et al., 1986). This enzyme has been shown to have two negative-feedback roles on intracellular Ca^{2+} increase. PKC acts both to inhibit PIP_2 -hydrolysis and to reduce $[Ca^{2+}]_i$ by stimulating the Ca^{2+} -pump (Berridge, 1987).

II. RELAXATION OF VASCULAR SMOOTH MUSCLE (VSM):

Relaxation of VSM is mediated by any mechanism that decreases $[Ca^{2+}]_i$. These mechanisms include: 1) Inhibition of Ca^{2+} influx through Ca^{2+}-channels; 2) Promotion of Ca^{2+} extrusion through the cell membrane; 3) Phosphorylation of MLCK or inhibition of MLCK; 4) Ca^{2+} -sequestration by activation of uptake mechanisms on SR; 5) Inhibition of PI-response, and in turn reduction of IP_3 and DAG production ; 6) Cyclic nucleotide production.

The most important agents that control vascular relaxation are the cyclic nucleotides; cAMP and cGMP, which act through activation of cAMP- dependent protein kinase (PKA), and cGMP-dependent protein kinase (PKG), respectively and through phosphorylation of targets such as MLCK.

In vascular smooth muscle, elevation of cAMP synthesis and thus vasorelaxation occurs in response to ß-adrenoceptor agonists, to PDE-inhibitors and to direct activation of AC (Chiesi & Carafoli 1986).

Cyclic AMP has multiple inhibitory effects on vascular smooth muscle including phosphorylation and inactivation of MLCK and reduced affinity for Ca^{2+}-calmodulin, activation of the Ca^{2+} - ATPase -pump in sarcolemmal membrane, increased Ca^{2+} extrusion, and enhancement of Ca^{2+} uptake by activation of Ca^{2+} -ATPase on the SR membrane. (Conti & Adelstein 1981 ; Eggermont et al., 1988; Van Breemen & Saida 1989). Moreover, the inhibitory effect of cAMP on VOCs has been confirmed by Ousterhout & Sperelakis in 1984.

It has been shown that vascular smooth muscle relaxation induced by both endothelium-dependent and independent vasorelaxants is mediated by elevation of cGMP (Rapoport & Murad 1983). Moreover cGMP analogues directly relax vascular smooth muscle (Lincoln, 1983). Like cAMP, cGMP has multiple

inhibitory effects on vascular smooth muscle including; stimulation of SR Ca^{2+}-pump, (Twort & Van Breemen 1988); stimulation of Ca^{2+}-ATPase in the plasmalemma (Twort & Van Breemen 1988); phosphorylation of MLCK (Chiesi & Carafoli 1986); and inhibition of PI-hydrolysis (Rapoport, 1986). However, despite various suggestions of different mechanisms to explain the effects of cyclic nucleotides on vascular smooth muscle tone, the details of how cyclic nucleotides cause vasorelaxation remain unclear. The complex interacting mechanisms that regulate tone in vascular smooth muscle are illustrated in Figure 1.4.

SECTION 5. ALTERATION IN MECHANISMS MODULATING TONE IN HYPERTENSION:

Essential hypertension has been recognized for 100 years, since Mahomed in 1881 began to measure systemic blood pressure (cited by Heagerty et al., 1988), and define a group of patients whose hypertension was not associated with pre-existing renal disease. Later Volhard and his collaborators in 1918 (cited by Korner 1982), separated what we know as essential hypertension from the hypertension associated with nephritis and other types of parenchymal renal disease. Until then, these disorders had all been known together as complications of Bright's disease (Korner, 1982). The modern classification of hypertension into primary or essential, and secondary hypertension with known pathological causes grew directly from the classification of Vohard and Fahr (cited by Korner 1982).

The elevated arterial pressure in essential hypertension is believed to result

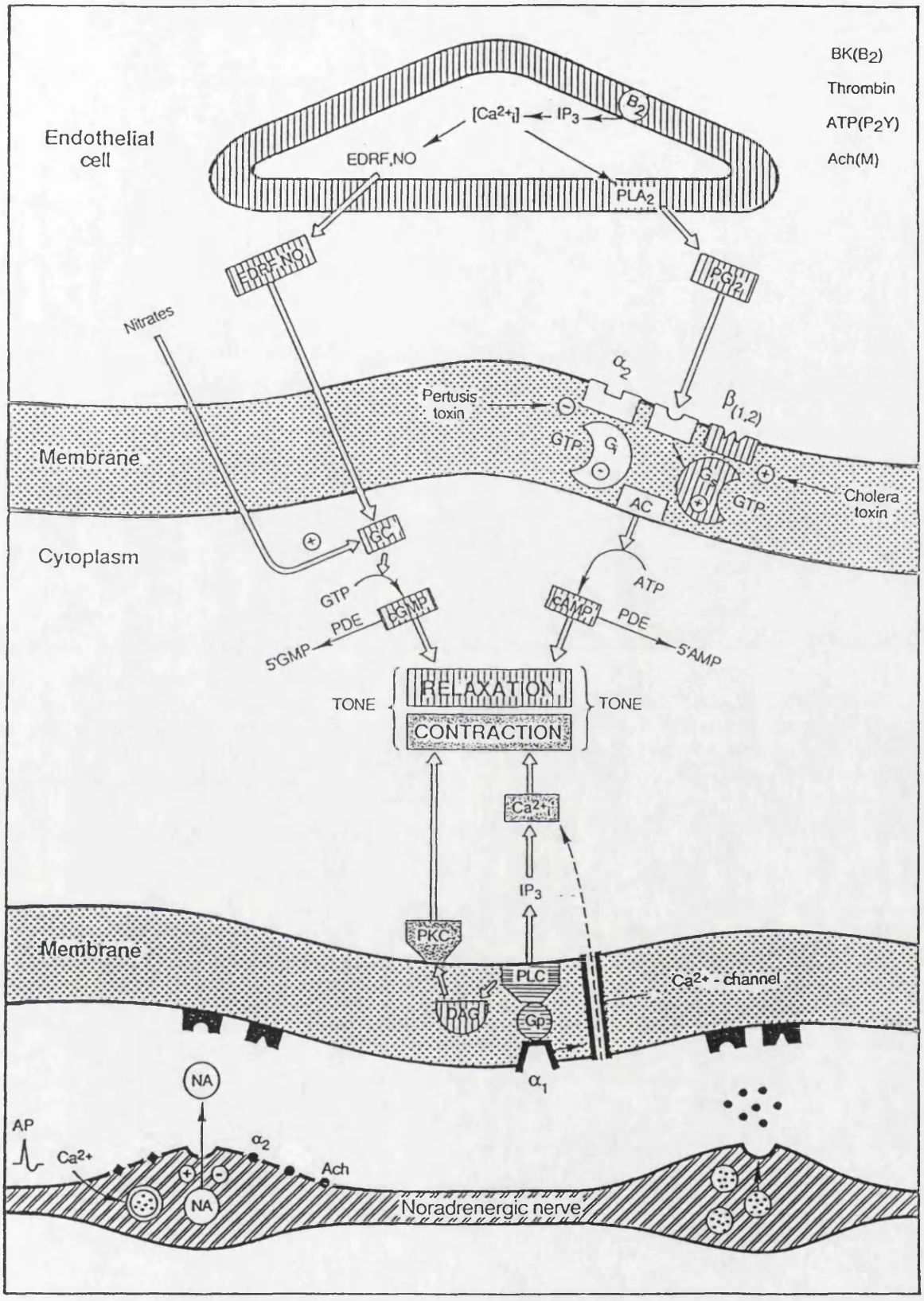

Fig. 1.4.
A schematic diagram showing the principal mechanisms that modulate tone in vascular smooth muscle. The endothelial cell release EDRF (NO) and PGI_2, which respectively activate cytosolic guanylate cyclase (GC) to produce cGMP, and membrane adenylate cyclase (AC) to produce cAMP. AC, unlike GC, is regulated via both excitatory and inhibitory receptors, which control the enzyme by activating G-proteins (G_i and G_s). NA released from noradrenergic nerves acts on α_1-adrenoceptors to cause PI-hydrolysis, which results in the production of IP_3 and DAG which cause contraction. NA release is regulated via various presynaptic receptors. The tone of vascular smooth muscle is the resultant of the opposing mechanisms that cause contraction and relaxation.

from an increase in PVR (Bohr & Webb, 1984). Haemodynamically, hypertension is the result of changes in the vasculature, which undergoes dramatic structural and functional changes.

I. STRUCTURAL CHANGES:

The early work of Bright in 1936 and Johnson in 1868 (Cited by Heagerty et al., 1988) demonstrated that walls of arteries were thickened in hypertension. Pickering (1939) noted that even after maximal vasodilation, resistance to blood flow remained increased in hypertensive pateints, due to the increased wall thickness (Cited by Heagerty 1988). The etiology of wall thickening is unknown and is a controversial issue. Although most studies suggest that structural changes are adaptive responses to the increase in mechanical load, a number of publications suggest that other factors (neural, genetic, humoral) contribute to morphological alteration in the hypertensive vasculature (Nordborg & Johansson 1979; Hart et al., 1980; Bell & Overbeck, 1979; Bevan, 1984; Rorive et al., 1986). In fact, there may be no conflict between these views since some of these factors (neural and humoral) may be the means by which the adaptive response to increased load is achieved. Folkow (1978) established that wall thickness not only increases vascular resistance by structural encroachment on the lumen and changing wall/lumen ratio, but also amplifies all pressor responses caused by vascular smooth muscle contraction. Mulvany in 1978 found that at a given transmural pressure, relaxed blood vessels of spontaneously hypertensive rats (SHR) had 16% smaller lumen diameter and 40% thicker media compared to normotensive vessels. This

may be an adaptive change, in which the vessel wall thickens in response to the increased wall stress, induced by hypertension (Folkow, 1982). The thickness of the media in hypertensive vessels is the result of hypertrophy and/or hyperplasia. Since vascular smooth muscle cell volume and surface area are increased in mineralocorticoid-induced hypertension in rats, it appears that hypertrophy is responsible in this case (Friedman et al., 1971). The DNA content of vascular smooth muscle from hypertensive rabbit aorta is also increased compared to that of control animals (Bevan, 1976). This suggests that the number of smooth muscle cells may be increased in some forms of hypertension.

II. FUNCTIONAL CHANGES:

Functional changes in the vasculature in hypertension are extremely variable. In some types of hypertension, some vascular segments show altered sensitivity or reactivity to certain stimuli, whereas in other types the functional abnormality may be different. The alteration and variability do not appear to be related to the machinery used to produce the contractile force (i.e. actin and myosin). Several studies suggest that the interactions between actin and myosin, and regulatory proteins are unaltered in vascular smooth muscle from hypertensive animals (Seidel, 1979; Moreland et al., 1982; Nghiem & Rapp 1983; Garwitz MacMahon & Paul 1985).

The reported variability is the result of several factors; 1) The primary cause of hypertension; 2) The stage of development and the duration of the elevated pressure; 3) The animal species examined; 4) The age and sex of the animal; 5)

The techniques used to study vascular changes; 6) The stimulus employed and 7) The location of the vascular bed (Bohr & Webb 1984, 1986). However, despite this complexity, it has been suggested that the major abnormality in hypertension is in the altered properties of the vascular smooth muscle membrane (Bohr & Webb, 1988).

A: CHANGES IN SENSITIVITY TO VASOACTIVE AGENTS:

Many studies have examined vascular reactivity in hypertensive blood vessels and have found differences in comparison with the corresponding normotensive vessels, either in maximum contractility or in sensitivity to different contractile and vasorelaxant agonists (for review see Mulvany, 1983; Triggle & Laher 1985).

Numerous reports have provided evidence suggesting that hypertensive vessels in various models of hypertension (including human essential hypertension) are hyporesponsive or subsensitive to various stimuli (Spector et al., 1969; Shibata et al., 1973; Hansen & Bohr 1975; Chaturvedi et al., 1978; Fitzpatrick & Szentivanyi 1980; Moulds 1980, 1981; Pang & Sutter 1980 Swamy & Triggle 1980). In contrast, many reports have shown hypertensive vessels to be supersensitive to various stimuli (Bevan et al., 1975; Hermsmeyer, 1976; Lais & Brody 1978; Cohen & Wiley 1979; Whall et al., 1980; Webb et al., 1981; Jones et al., 1988; Lograno et al., 1989).

Moreover, some investigators including Clineschmidt et al., (1970) and Hallback et al., (1971), reported that aortic strips from SHR and normotensive

rats showed equal sensitivity to vasoactive agents such as NA. Recent observations suggest that some of the variability between studies may be due to the ability of the endothelium to influence vascular reactivity, by releasing vasoactive agents (Vanhoutte et al., 1986).

The majority of observations indicate that hypertension is characterized by increased sensitivity to constrictor agonists, whereas vascular responsiveness to vasodilator agents is decreased. (Bohr & Webb 1986). However, a conclusive answer has not yet been given to the question of the nature of the sensitivity alteration that occurs in vascular smooth muscle in hypertension.

B: ENDOTHELIAL LAYER AND HYPERTENSION:

The vascular endothelium changes morphologically and functionally in hypertension. In normotensive animals the endothelium consist of a thin layer of flat cells. In contrast, in hypertensive animals the cells bulge into the lumen. In addition their permeability is increased and the subendothelial tissue is thickened (Vanhoutte & Lüscher, 1986).

Endothelium-dependent relaxations to ACh, Ca^{2+} ionophore A-23187 and adenosine diphosphate are reduced in SHR rats and Dahl hypertensive rats in comparison with normotensive controls (Winquist et al., 1984 ; Lüscher et al., 1986). Furthermore, Vanhoutte and Lüscher in 1987 have demonstrated that in the SHR, but not in the salt sensitive Dahl rats, ACh caused endothelium- dependent contractions. In this experimental model of hypertension, the reduced relaxation caused by ACh is due not to the abnormal release of EDRF(s), but rather to the

concomitant release of an endothelium-derived constrictor factor, perhaps resulting from cyclo-oxygenase activity. In other animal models of hypertension, the reduced relaxation to ACh could be due to decreased production of EDRF(s), impaired diffusion of the relaxing factors from the endothelium to the smooth muscle (because of subendothelial thickening as a consequence of the hypertensive process), or to a decreased responsiveness of the smooth muscle to EDRF(s) (Collis & Vanhoutte 1977; Cheng & Shibata 1980; Konishi & Su 1983; Lee et al., 1987; Shirasaki et al., 1988). In arteries of the dog, α-adrenoceptor agonists, adrenaline and noradrenaline cause endothelium-dependent relaxation, hence the contractions evoked by non-selective adrenergic agonists, in particular, adrenaline and NA, are augmented, following removal of the endothelium. Although alterations in endothelial cell functioning may be primarily responsible for these changes (Lee et al., 1987; Shirasaki et al., 1988), the exact mechanisms underlying them remain unclear.

C: CELL MEMBRANE ABNORMALITIES AND INTRACELLULAR CALCIUM REGULATION IN HYPERTENSION:

The cell membrane consists of a lipid bilayer with embedded integral proteins that serve as receptors, channels, exchangers or active pumps (Carruthers & Melchior 1986; Rinaldi & Bohr 1988). The intracellular Ca^{2+} concentration, which is the principal determinant of vascular smooth muscle contraction and relaxation is regulated by cell membranes (plasmalemmal and intracellular membranes).

Observations in both clinical and experimental hypertension, suggest that abnormalities may occur in membrane functioning (Doyle & Fraser 1961; Bohr & Webb 1988). Contraction of smooth muscle is mediated by activation of MLCK by Ca^{2+}-calmodulin. This occurs over a Ca^{2+} concentration range of 0.1 to 10 μM as a result of interaction between myosin and actin (Webb & Bohr 1981). In addition, Ca^{2+} -activated, phosholipid-dependent PKC may also regulate smooth muscle contraction, and phorbol esters, by mimicking DAG function in activating PKC, can induce a slowly- developing contraction without altering Ca^{2+} (Itoh et al., 1986; Rasmussen et al., 1987). Nevertheless the major regulator of contraction and relaxation is $[Ca^{2+}]_i$, which is regulated by various mechanisms, including: Ca^{2+} -influx, $[Ca^{2+}]$ mobilization by IP_3, and indirect mechanisms, such as PKC activation, and Ca^{2+} decreasing mechanisms, including: Ca^{2+} extrusion, Ca^{2+} sequestration, cyclic nucleotides elevation. Alterations and abnormality in these regulatory mechanisms has been reported in the etiology of hypertension (Sharma & Bhalla, 1988; Dominiczak & Bohr, 1990).

1. CALCIUM INFLUX:

Calcium can enter the cell through specific Ca^{2+} -channels which are regulated by changes in membrane potential or voltage operated channels (VOC), or by agonist- activated or receptor- operated channel (ROC) (Sharma & Bhalla, 1988; Dominiczak & Bohr, 1990)). In addition to these Ca^{2+}-channels, calcium can enter the cell in the resting state through leak channels. Van Breemen and his co-workers in 1986 measured Ca^{2+} influx in the vascular smooth muscle of resistance vessels,

and reported that in the hypertensive state, Ca^{2+} entry through all of these channels is increased, compared to that of WKY controls. Furthermore Aoki & Asano, 1986 found that the Ca^{2+} -channel activator BAY-K 8644 was more effective in eliciting contractions in femoral arteries from SHR than in those of WKY. These results point to abnormalities in Ca^{2+} -channel functioning in hypertension.

2. INTRACELLULAR CALCIUM RELEASE:

Intracellular Ca^{2+} release is mediated by PLC activation through G-proteins and IP_3 formation and in turn IP_3 -mediated Ca^{2+} release from SR (Abdel-Latif, 1986; Berridge, 1987). Since the turnover rate of PI can be regulated by PLC activation in response to agonists, it is possible that either the quantity or the activation of this enzyme during agonist-receptor interaction may be altered in hypertension. It has been demonstrated that the activity of PLC in rat aorta in SHR is almost twice that found in WKY rats (Uehara et al., 1988), this increase in PLC is evident as early as 4 weeks of age suggesting a causative role in the development and maintenance of hypertension. Moreover, a positive correlation between blood pressure and PLC activity has been observed (Uehara et al., 1988).

It has been suggested that all of the defects in agonist- mediated Ca^{2+} -metabolism in the hypertensive state can be explained on the basis of a single alteration in the plasma membrane at the coupling level, involving G-protein. Although the identity of the G-proteins involved in the PLC activation has not been clearly established, in several systems it appears to be the inhibitory G-protein (G_i or G_i-like protein) that

is involved in inhibiting AC (Berridge, 1987). If indeed Ca^{2+} mobilizing receptors in vascular smooth muscle cells are coupled to G_i then this G-protein may have a bifunctional role of inhibiting AC activity, as well as activating PI-hydrolysis (Meyer & Marche 1988). In hypertension there may be increased activation of PLC by G-protein, leading to IP_3 and DAG production. In addition, activated G-protein during sustained agonist stimulation may interact directly with Ca^{2+} channels (ROC and/or VOC) to allow increased entry of Ca^{2+} (Sharma & Bhalla 1988).

Alteration in the synthesis of inositol phosphates could occur at the different stages in the development of hypertension. In rat aorta from mature SHR, there was no significant difference from normotensive controls in the basal or unstimulated level of inositol phosphates (Heagerty et al., 1986). However, in 5 week-old SHR, IP accumulation was greater than in WKY (Heagerty et al., 1986).

Increased IP-production has been shown in rat aorta from Stroke-prone SHR (Turla & Webb, 1990). These changes occur not only in vasculature but also occur in other systems such as the erythrocyte (Koutouzov et al., 1983) and platelets (Koutouzov et al., 1987). It has been shown that both resting and agonist-stimulated $[Ca^{2+}]_i$ is increased in vascular smooth muscle, in platelets and in lymphocytes from hypertensive patients and from SHR rats (Erne et al., 1984; Bruschi et al., 1984; Losse et al., 1984; Nabika et al., 1985; Cooper et al., 1987; Lechi et al., 1987; Meyer & Marche 1988). The second PLC-mediated product of PI-hydrolysis is DAG, which activates PKC and causes contraction of vascular smooth muscle by a mechanism that is not fully understood (Rasmussen et al.,

1987). DAG production is increased in hypertension, and as a result, activation of PKC is enhanced.

In arteries from genetically hypertensive rats there is increased sensitivity to PKC activators (12-O-tetradecanoyl phorbol- 13-acetate and mezerin) in comparison with arteries from normotensive controls rats (Turla & Webb 1987). Furthermore, a selective inhibitor of PKC [1-(5-isoquin- olinesulfonyl)-2- methylpiperazine] blunts the enhanced sensitivity to serotonin in arteries from SHR (Bohr & Webb 1988). Also, increased PKC activity in caudal artery and mesenteric artery from SHR has been reported (Turla & Webb 1987; Mackay & Cheung 1987; Bendhack et al., 1988).

Recently it has been demonstrated that PKC increases protein phosphorylation of specific proteins during sustained contraction (Rasmussen et al., 1987). PKC can also activate potential-operated Ca^{2+} -channels in smooth muscle, thereby elevating $[Ca^{2+}]_i$ (Fish et al., 1988). It is therefore possible that increased activation of PKC could produce larger contractions of vascular smooth muscle in SHR through direct phosphorylation of the contractile proteins as well as activation of Ca^{2+} influx through VOCs.

3. CALCIUM EXTRUSION AND SEQUESTRATION:

Active Ca^{2+} pumps (ATP-dependent) which are located in the sarcoplasmic reticulum (calmodulin-independent) and plasmalemma (calmodulin-independent) are responsible for maintaining Ca^{2+}- homeostasis and for returning $[Ca^{2+}]_i$ to the resting level after contraction (Van Breemen et al., 1986). Sodium-calcium

exchange has also been proposed as an additional Ca^{2+} extrusion mechanism (Ashida & Blaustein 1987). Any decrease in the activity of these mechanisms would lead to an increase in $[Ca^{2+}]_i$. There is some evidence that this may occur in hypertension. For example, a decrease in the ATP-dependent Ca^{2+} uptake by microsomal and plasma membrane-enriched fractions from vascular smooth muscle and erythrocyte membranes has been demonstrated in SHR (Bhalla et al., 1987; Rinaldi & Bohr, 1988).

Moreover, active calcium extrusion was reduced in mesenteric arteries from SHR and animals with mineralocorticoid-induced hypertension (Kwan et al., 1979). The calmodulin sensitivity of the SHR plasma membrane Ca^{2+} pump is decreased in hypertension. (Rinaldi & Bohr, 1988) and the role of sodium-dependent calcium efflux (Na^+ /Ca^{2+} exchange) was significantly greater in heart sarcolemmal vesicles from SHR than in those from WKY (David-Dulfilho et al., 1986). In mesenteric artery, it has also been shown that calcium uptake by sarcolemmal vesicles from SHR and WKY was dependent upon sodium loading. The level of activity of the Na^+/Ca^{2+} exchange was greater in membrane vesicles from SHR (Matlib et al., 1985).

D. CYCLIC NUCLEOTIDE ALTERATIONS IN HYPERTENSION:

Cyclic nucleotides serve as second messengers, which translate the action of hormones and neurotransmitters at their receptors into biochemical events in target cells. Cyclic nucleotides therefore affect important regulatory mechanisms that modulate vascular smooth muscle tone by regulating $[Ca^{2+}]_i$ homeostasis (Chiesi

& Carafoli 1986; Carafoli, 1987). Abnormalities in cyclic nucleotide metabolism may be involved in hypertension.

Three possible abnormalities of cyclic nucleotide metabolism have been identified in hypertension. These include increased cGMP/cAMP ratio (Amer et al., 1975), depressed basal adenylate cyclase (AC) activity (Ramanathan & Shibata 1974), and a reduced ability of AC to be stimulated (Triner et al., 1975). The first hypothesis was based on the Yin-Yang theory of cyclic nucleotide action. There is evidence that the cGMP/cAMP ratio is elevated in aorta from SHR, and in aorta of rats with mineralocorticoid-induced hypertension (Amer et al., 1975).

The increased cGMP/cAMP ratio was initially thought to support the Yin-Yang theory of cyclic nucleotide action. However, now the significance of these observations is unclear since it is known that cGMP like cAMP, is a vasodilator, whereas previously it was thought to be involved in contraction.

Ramanathan & Shibata in 1974 demonstrated that the levels of cAMP were lower in blood vessels of young SHR before they developed hypertension. Alteration in cAMP metabolism may therefore be a primary event in the development of hypertension. These workers also showed that unstimulated levels of adenylate cyclase were significantly lower in aorta from SHR in comparison with normotensive controls.

Other workers have shown that the basal level of AC and cAMP in aorta of SHR do not differ from controls (Triner et al., 1975; and Klenerova et al., 1975). However, the concentration-response curve to isoprenaline in SHR aorta was shifted to the right of that in normotensive controls, so that higher doses of isoprenaline

were required in aorta from SHR to produce the same increase in cAMP formation as occurred in control aorta.

Most investigators have reported a decrease in cAMP production and a decreased vascular relaxation in response to ß-adrenoceptor agonists in blood vessels from SHR in comparison with normotensive controls (Triner et al., 1975; Cohen & Berkowitz 1976; Godfraind & Dieu 1978; Asano et al., 1982; Silver et al., 1985). This decreased ß-adrenoceptor-mediated relaxation that occurs in hypertension, may reflect diminshed activity of the stimulatory G-protein (G_s) in vasculature from SHR (Asano et al., 1988).

Recently, after the identification of cGMP as a mediator of vasorelaxation, there have been several reports of decreased cGMP levels in hypertension (Lee et al., 1987; and Shirasaki et al., 1988).

The role of the endothelium and its ability to release EDRF have been examined in hypertension. It appears that the spontaneous and/or drug-induced release of EDRF is reduced in hypertension and this is reflected in a decrease in cGMP formation (Otsuka et al, 1988). In contrast, it has been shown that in aorta from SHR, the activity of cGMP-dependent protein kinase (PKG) is elevated (Coquil et al., 1987).

From the available evidence, it is apparent that no consistent pattern emerges to link changes in cyclic nucleotides metabolism with hypertension. The role of cyclic nucleotide in hypertension is complex and remains to be clarified.

AIM OF STUDY:

The aim of this study was to examine the mechanisms that regulate tone in isolated blood vessels from normotensive and hypertensive rats.

In particular, this study sought to determine whether there was any difference in the responsiveness to vasoconstrictors and vasorelaxants or in the corresponding coupling mechanisms in isolated blood vessels from spontaneously hypertensive rats (SHR) and from normotensive Wistar Kyoto (WKY) controls.

This study investigated:

1) The mechanical responses of isolated segments of blood vessels from Wistar, SHR and WKY rats to vasorelaxants, which produced their effects by different mechanisms.

2) The biochemical responses, including changes in the levels of second messengers (IPs, cAMP and cGMP) involved in contraction and relaxation and alteration of these second messengers in vasculature from SHR rats.

3) The interaction between these second messengers, which are involved in regulating the tone of vascular smooth muscle.

CHAPTER TWO

METHODS & MATERIALS

METHODS AND MATERIALS

METHODS:

Experimental animals:

In this study, adult male Wistar rats (200-300g), normotensive Wistar Kyoto (WKY), and Spontaneously Hypertensive Rats (SHR), were used. All animals were housed in a controlled environment, under conditions of a circadian cycle of light and dark (Light: 6:30-18:30 hrs). Rats were allowed tap water and food (Labsure CRM diet) ad libitum. The ambient temperature was maintained between 18-22°C.

Dissection of aorta:

Rats were killed by stunning and exanguination. The chest was opened 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 removed from the thoracic cavity and cleaned of all adhering fat and connective tissue and placed in a petri dish containing cold Krebs physiological buffer, which was originally described by Krebs and Henseleit (1932). This solution had 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.

RECORDING OF MECHANICAL RESPONSES OF TISSUES IN ORGAN BATHS:

I) Preparation of aortic rings:

The aorta were cut into rings (2-3 mm in length), weighed and suspended in 25 ml organ baths between two stainless steel wire hooks and attached to the transducer by a cotton thread under a resting tension of 2g.(Fig.2.1). Care was taken at all times to protect the endothelium from damage. The tissue were bathed in Krebs bicarbonate buffer at 37°C and gassed continuously with a mixture of 95% O_2/5% Co_2. Isometric tension was recorded using a Statham force displacement transducer and was displayed on a Grass polygraph (Model 7 PCPB). Measurement of isometric tension was achieved when the applied tension as the opposing force exceeded the tension developed by the tissue, without over-stretching the muscle fibres. A resting tension of 2g was applied to the aortic rings and this exceeded the tension developed by the tissue. The tissues were allowed to equilibrate for 60 minutes prior to the start of experiments. During this time the resting tension was maintained at 2g and the tissues were washed 2-3 times. This equilibration time was necessary to allow the re-establishment of ion concentration gradients and metabolic processes, following dissection of the tissue from the animal.

II) Preparation of tail artery rings and segments for perfusion:

Rats were killed in the normal manner. The tail with its skin were removed. The

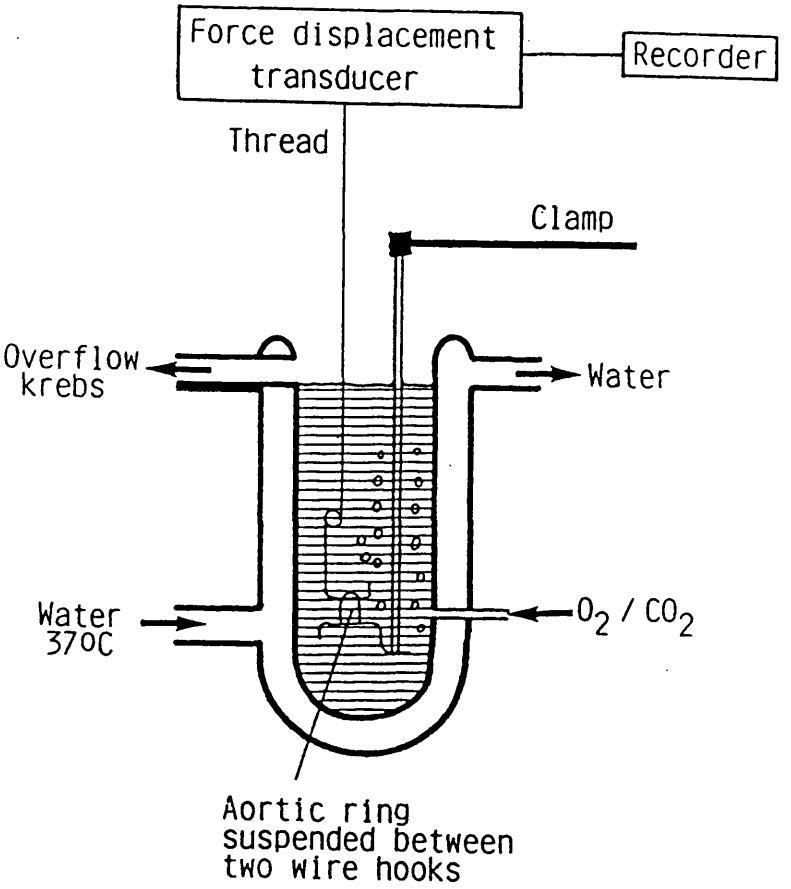

Fig. 2.1.
Set up for recording of mechanical (Tension) responses of aortic rings. A similar apparatus was used for mechanical recording of tail artery rings.

tail artery was located, and dissected out and placed in a petri dish containing oxygenated cold Krebs buffer. From each artery four segments were obtained, two (2-3 cm) for perfusion pressure recording and two (3-4 mm) for isometric tension recording. Segments for perfusion were double cannulated with polythene cannulae. The upper cannula was connected with silicone tubing to a reservoir containing Krebs buffer. The lower cannula was connected to overflow tubing (Fig. 2.2). The cannulated artery segments was then placed in an organ bath (25 ml) containing Krebs buffer (37° C, gassed with 95% O_2/ 5% CO_2). The tail artery segment was perfused with Krebs buffer (or drug solution) at a constant rate of 1 ml/min by means of a Watson-Marlow constant flow pump. The preparation was allowed to equilibrate by being perfused with Krebs buffer for 90 minutes prior to examining the effects of drugs. The resistance of the tail artery to perfusion was measured by a Statham pressure transducer and displayed on a Grass polygraph. Each artery segment used for tension recording was weighed and suspended between stainless steel hooks (0.15 mm diameter) in an organ bath (25 ml) containing Krebs buffer. 1 g of tension was applied to each ring and the preparation was allowed to equilibrate for 90 minutes before any experimental work was carried out.

III) Removal of endothelium:

In some experiments, the endothelium was removed mechanically from aortic rings by gently rubbing the intimal surface with stainless steel forceps. Removal of, or damage to the endothelial cells was confirmed by the inability of acetylcholine to relax precontracted aortic rings treated in this manner (Furchgott

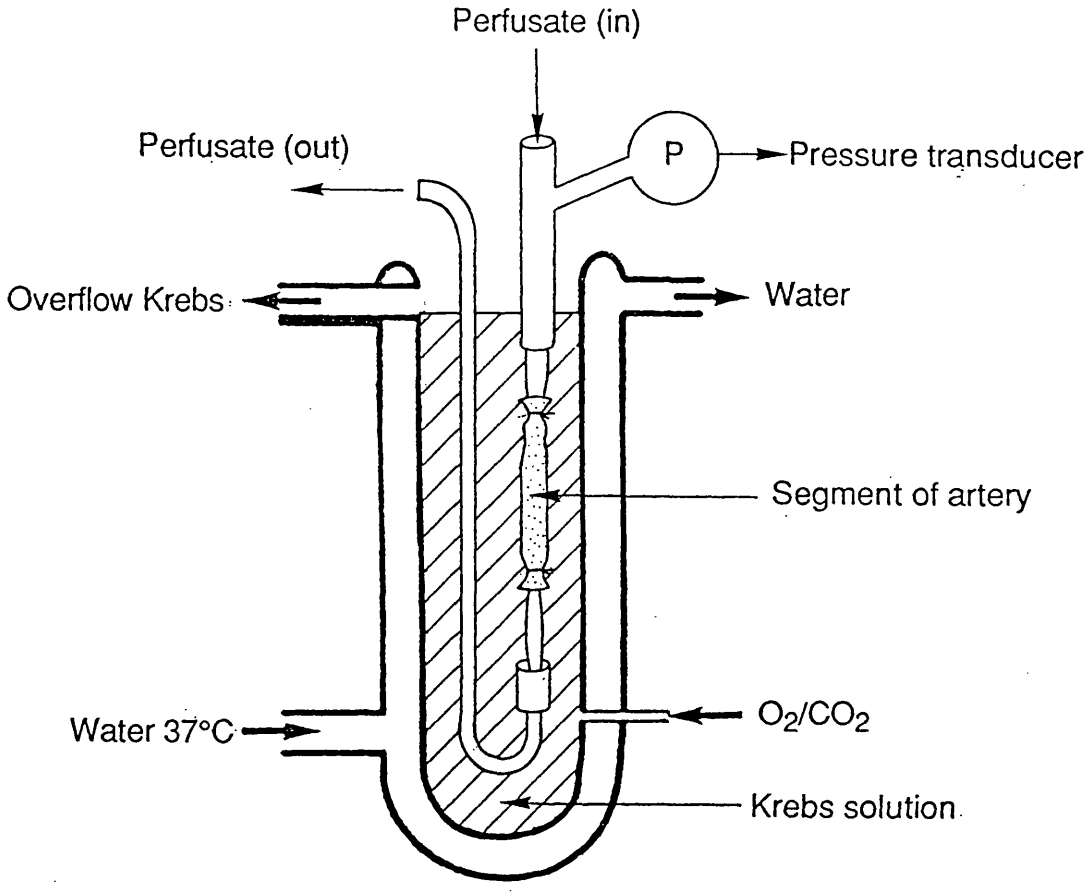

Fig. 2.2.
Set up for recording perfusion pressure responses in perfused tail artery segments.

& Zawadzki, 1980). In preliminary experiments, the successful removal of endothelium was confirmed by light microscopy (Fig. 2.3).

IV) Addition of drugs:

All drugs were added to the bath 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 agonist and (in the presence of a submaximal concentration of a precontracting agonist) for each vasorelaxant. The degree of smooth muscle relaxation induced by each vasodilator was expressed as a percentage (%) of the maximum contraction to the contractile agent (EC_{50} or EC_{75} concentration).

In general, drugs were dissolved in 0.9% of saline to give the required concentration. Forskolin was dissolved in dimethylsulfoxide (DMSO). The concentration of DMSO in the organ bath and in incubation tubes was 0.05% V/V. In these experiments the vehicle was used as the control.

MEASUREMENT OF PHOSPHATIDYL INOSITOL HYDROLYSIS:

Products of PIP hydrolysis were measured using a modified version of the method of Akhtar & Abdel-Latif (1986), by incorporation of myo-(^{3}H)-inositol into the PI-cycle. Inositol phosphates were extracted and separated by ion exchange chromatography.

Plastic pipette tips (5ml) were used as chromatography columns. The tips of these were plugged with a piece of glass wool. Ion exchange resin (Dowex-AG1x8, Formate form, 100-200 mesh), was washed 4-5 times and 1 ml

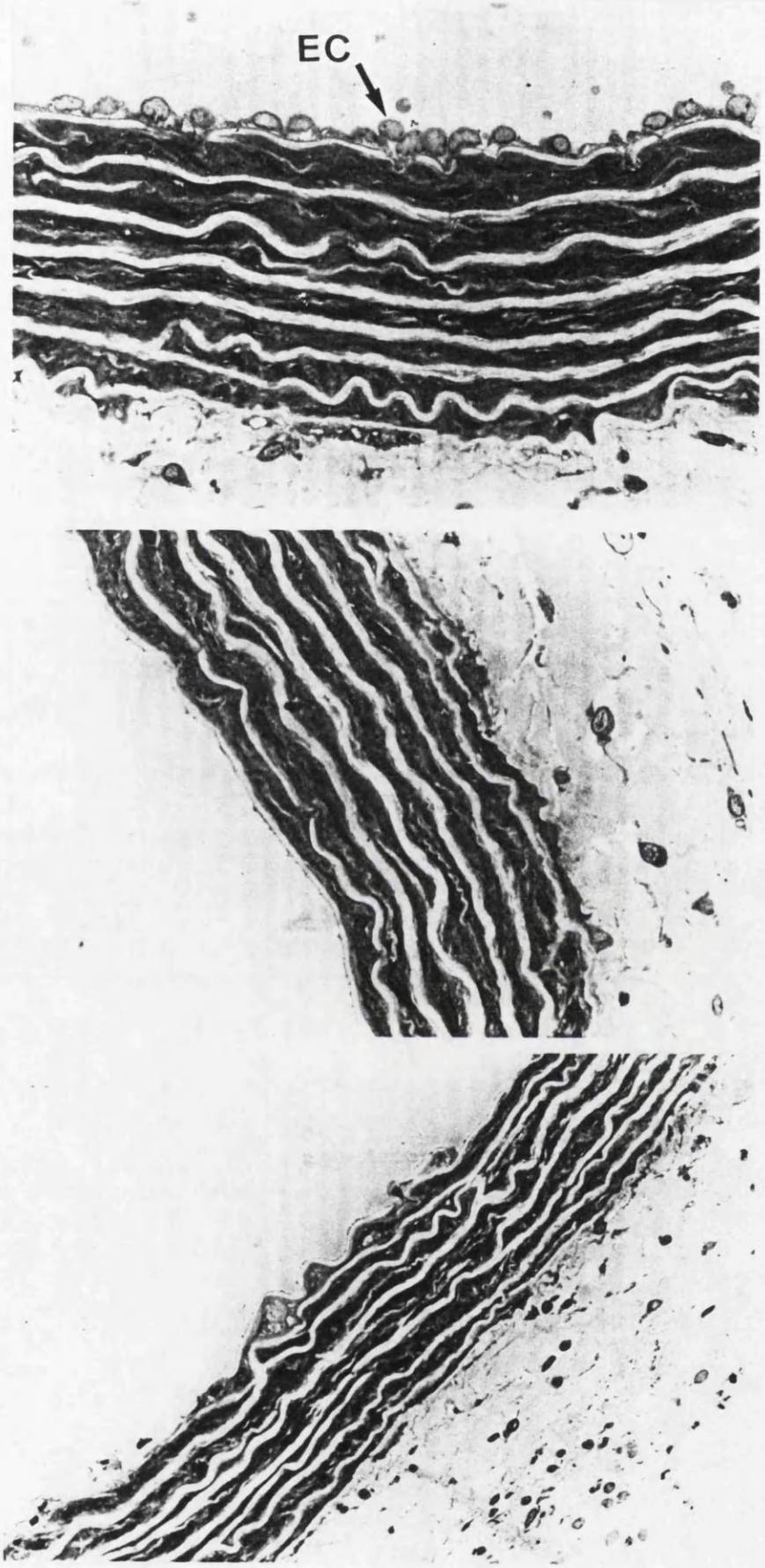

Fig. 2.3.
A light microscopic photograph of a segment of aorta, with intact endothelium, after removal of endothelial cells (EC) mechanically (MER), and chemically (CER).

of resin suspension was added to each column. Columns were prepared by washing with 15 ml of ammonium formate (2.4M), followed by 15-30 ml of distilled water. After using the columns, the resin could be regenerated by washing the columns with ammonium formate (2.4M, 15ml).

Aortic rings were prepared as previously described. The aorta was transferred to ice-cold Krebs buffer, then the aorta was cut into rings (3mm), which were washed with ice-cold Krebs buffer 2-3 times. In each experiment some randomly-selected rings were weighed and the mean weight of these rings was used in each experiment to calculate the results.

The aortic rings were preincubated in Krebs buffer, bubbled with 95%O_2 /5%Co_2 for 15-30 minutes in a shaking bath at 37°C for equilibration. All steps of the experiment before termination were performed in a shaking water bath. The rings were then transferred into 20ml glass vials containing 2ml of Krebs buffer, containing 8μCi/ml of myo-(^{3}H)-inositol in which they were incubated for 2.5-3 hours. After the incubation the excess radioactivity was washed off with oxygenated Krebs buffer (37°C), and each 3mm ring was placed into a plastic insert tube containing 280μl of Krebs buffer (37°C), and 10μl of lithium chloride (LiCl) (10mM), to prevent the metabolism and breakdown of inositol phosphate, to myo-inositol (Berridge et al., 1982). As is shown in Fig. 2.4 the presence of the LiCl significantly increased the amount of labelled IPs in comparison with controls, which were incubated without LiCl. In experiments with vasodilators, the LiCl was added 5-10 minutes after adding the vasodilators and 1 minute before adding the vasoconstrictor (Rapaport, 1986). When LiCl was added prior to the vasorelaxants, the results were not different with controls. This may be due to

Effect of LiCl on PI-hydrolysis in Wistar rat aorta

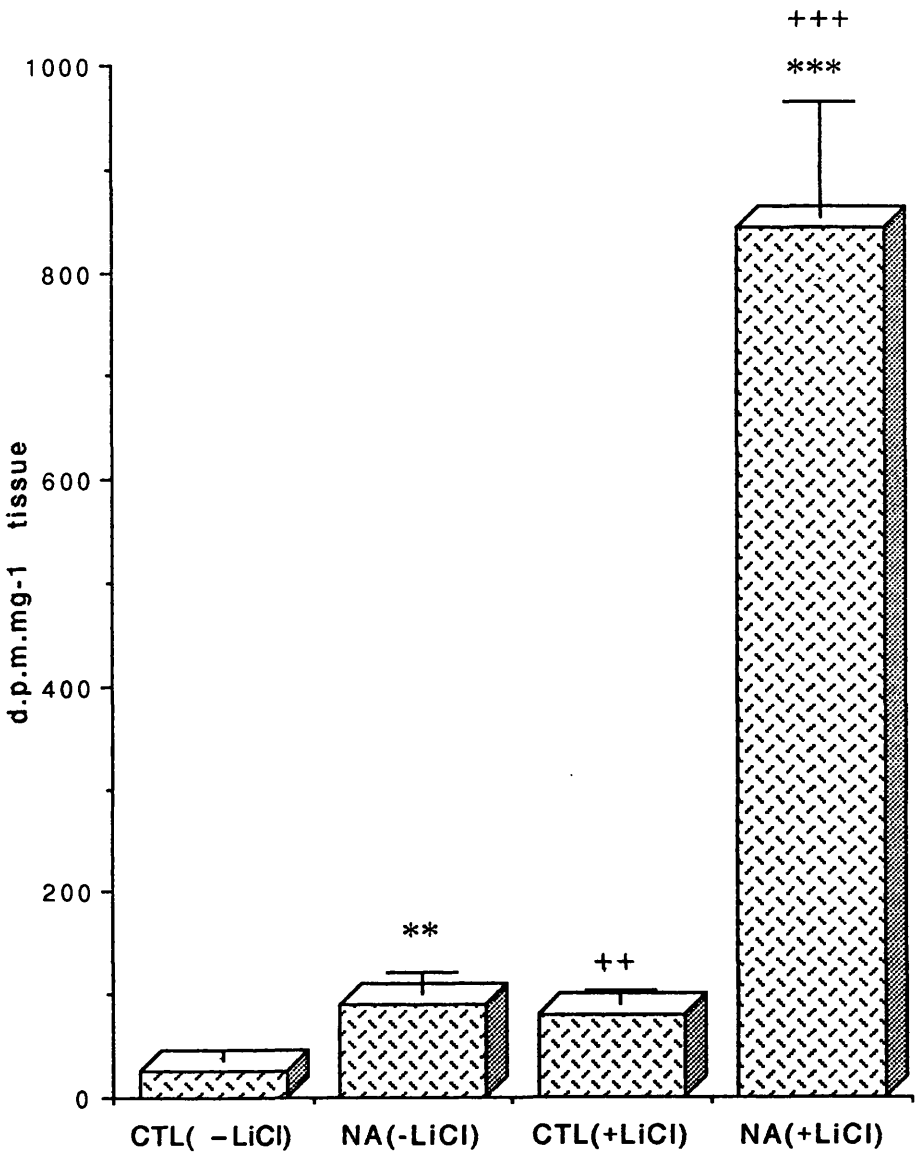

Fig.2.4.
Effect of lithium chloride (LiCl) on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control (CTL) level. (** $p<0.05$, *** $p<0.001$). This effect was significantly greater in the presence of LiCl (10mM), both in control (basal) and in NA-stimulated tissues. (++ $p<0.05$, +++ $p<0.001$). Results are mean $\pm$ s.e.mean. (n=8).

breakdown or inhibition of the PI-cycle which seems to be a prerequisite for synthesis of cGMP. Stimulation of the PI-hydrolysis was started by adding 10μl of agonist to the rings in each tube and incubating the rings for 45-60 minutes at 37°C. During this period the rings were oxygenated. The reaction was then terminated by the procedure of Berridge et al. (1982) and Fox et al. (1987), by adding a mixture of chloroform : methanol (2ml;1:2; V/V). The rings were homogenised using Potter-Elvehjem glass/glass tissue homogenizers and were transferred into centrifuge tubes. Subsequently, more chloroform (0.63ml) and distilled water (1.26ml) were added to form two layers, and the tubes were centrifuged at 2000xg for 5 minutes at 0° C to separate the aqueous and organic phases. The aqueous layer was added to the anion exchange chromatography columns. The columns were eluted sequentially with 20ml of distilled water to remove the excess myo-(^{3}H)-inositol and 15ml of 50mM of ammonium formate to remove glycerophosphoinositides, and eventually total myo-(^{3}H)-inositol phosphates were collected by washing with 8ml of a mixture of ammonium formate (M) and formic acid (0.1 M). 10 ml of scintillation liquid was added to a 2ml aliquot of each sample and the radioactivity was counted in a liquid scintillation counter (Packard 2000 CA Tri-carb). Generally, the total amount of IPs was measured in these experiments. The results were calculated and expressed as disintegrations per minute (d.p.m.) per mg of tissue wet weight.

MEASUREMENT OF CYCLIC GMP:

I) PREPARATION OF TISSUE:

Aortic rings were prepared as previously described for PI-measurement, and placed in a petri dish containing ice-cold Krebs buffer. Each ring was mounted on a stainless steel wire hook, and transferred to a flask containing warm and oxygenated Krebs buffer, and preincubated for 15-20 minutes for equilibration. The rings were then transferred to the vials containing Krebs buffer. Drug was then added to the rings in the vials and incubated for an appropriate amount of time, after which they were immediately frozen in liquid nitrogen this final step was carried out in less than 2 seconds.

II) EXTRACTION OF cGMP:

The frozen aortic rings were deproteinised by extraction in trichloroacetic acid (TCA) (1.6ml; 5% W/V). After a period of 2 hours, the rings were detached from the wire hooks, re-immersed in TCA and hand homogenised using Potter-Elvehjem glass-glass homogenisers. The tissue homogenates were then left for a further 18 hrs. at 4°C. Homogenisation broke down the tissue structure and increased the surface area across which the TCA could permeate. Homogenisation therefore facilitated extraction of cGMP. Morrison (1988), has shown that homogenisation of aortic rings during acid extraction significantly increased the recovery of cGMP. The samples were vortex-mixed and centrifuged (1000xg, 5 mins., 4°C) to remove any non-acid-soluble tissue components. The cGMP 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°C for 2 minutes. The samples (300 μl) were neutralised with sodium acetate buffer (100μl, 200mM, pH 6.2), vortex-mixed and stored at 4°C until required.

III) RADIOIMMUNOASSAY:

Tissue cGMP levels were measured using a commercially available cGMP kit (Amersham International, UK). The assay was based on the competitive binding between unlabelled cGMP and a fixed quantity of the tritium-[^{3}H]-labelled cyclic nucleotide to an antiserum which had a high specificity and affinity for cGMP. Cyclic GMP standard concentrations (0.5-8 pmoles/100μl) were prepared in Tris/EDTA buffer (50mM ; pH 7.5). samples of both standard and unknown tissue concentrations of cGMP (100μl) were acetylated by the addition of a freshly-prepared mixture of acetic anhydride and triethylamine, 1:2 by volume (5μl) (Frandsen and Krishna, 1976). The samples were immediately vortex-mixed. This acetylation process has been shown to increase the sensitivity of the assay (Morrison, 1988). [^{3}H]-labelled cGMP (50μl) was then added to each sample, followed by anti-cGMP antiserum (50μl). Samples were vortex-mixed and incubated for 16h. at 4 C. Following this incubation, 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 and before centrifuging (1000xg, 5 min, 4°C). The supernatant was decanted and the assay tubes were inverted on a paper tissue to drain. Cold distilled water (1.1ml) was then added to each tube, which was vortex-mixed

until the precipitate had dissolved. Samples (1ml) were transferred from the assay tubes to plastic counting vials containing scintillant (Ecosint, 10ml), and antibody bound [^{3}H]- cGMP was measured by counting for 5 minutes in a Packard liquid scintillation counter.

A standard calibration curve was obtained in each experiment and plotted as radioactivity bound (d.p.m.) against concentration of cGMP (Fig. 2.5). The concentrations of cGMP in unknown samples (100μl) were determined by reference to the calibration curve. The concentration of cGMP in the original sample (1.6ml) was expressed in pmol.mg^{-1} of tissue, and was calculated using the following method:

1) Concentration of cGMP in 100μl (from 1.6ml of total volume) multiplied by 16 gives the concentration of cGMP in the sample tube (pmol.tube^{-1}).

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

MEASUREMENT OF CYCLIC AMP:

I) PREPARATION OF TISSUE:

Aortic rings were prepared as for PI and cGMP-measurement, and placed in a petri dish containing ice-cold Krebs buffer. Each ring was mounted on a stainless steel wire hook, and transferred to a flask containig warm, oxygenated Krebs buffer, and preincubated for 15-20 minutes for equilibration. The rings were then transferred to the vials containing Krebs buffer and the drug was added to the

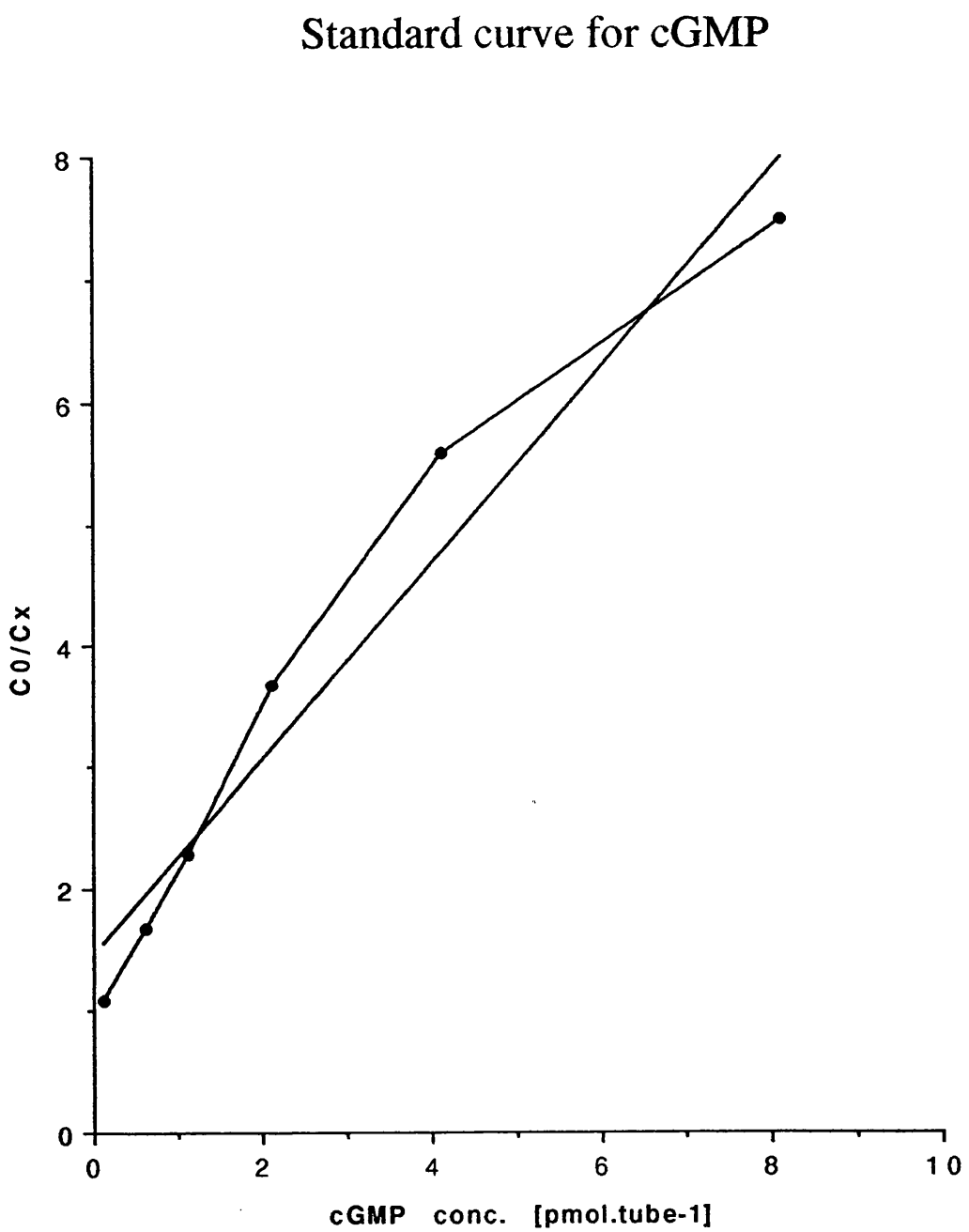

Fig.2.5.
Standard calibration curve for known concentrations of cyclic GMP, measured by radioimmunoassay. The ability of cyclic GMP to compete with (^{3}H)-cyclic GMP for binding to specific anti-cyclic GMP antiserum was measured (see text for details). C_0 is the d.p.m. bound in the absence of unlabelled cGMP, and C_x is d.p.m. bound in the presence of standard unlabelled cGMP. The straight line is a regression line fitted by computer. Points are means. (n=10).

rings in the vials and incubated for an appropriate time and then immediately frozen in liquid nitrogen. This step was carried out in less than 2 seconds.

II) EXTRACTION OF cAMP:

The frozen aortic rings were deproteinised by extraction in TCA (1.6ml; 5% W/V). After a period of 2 hours, the rings were detached from the wire hooks, re-immersed in TCA and as in the cGMP extraction, hand homogenised using Potter-Elvehjem glass-glass homogenisers. The tissue homogenates were then left for a further 18 h at 4°C. Homogenisation broke down the tissue structure and increased the surface area across which TCA could permeate. Homogenisation therefore facilitated extraction of cAMP. The samples were vortex-mixed and centrifuged (1000xg, 5min, 4°C) to remove any non-acid-soluble tissue components. The cAMP 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 samples tubes in a water bath at 80°C for 2 minutes, and stored in 4 C° until required.

III): RADIOIMMUNOASSAY:

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

Cyclic AMP standard concentrations (1-16 pmoles/50μl) were prepared in

Tris/EDTA buffer (50mM , pH 7.5). [^{3}H]-labelled cAMP (50μl) was then added to each (50μl) sample, followed by cAMP binding protein (100μl). Samples were vortex-mixed and incubated for 16h at 4°C. Following this incubation, ice-cold charcoal reagent, was added to each sample and then briefly vortex-mixed. The tubes were replaced in an ice bath or refrigerated centrifuge for 5 minutes. The samples were centrifuged (1000xg, 5 min, 4°C) to sediment the charcoal which was used to separate the protein bound-cAMP from the unbound nucleotides by absorption of the free nucleotide on to it. The tubes were centrifuged for not less than one minute and not more than 6 minutes after addition of the charcoal to the last tube in a batch. Then, without disturbing the sediment, 200μl of supernatant were removed and placed in scintillation vial containing 10ml of scintillant. [^{3}H]-cAMP was measured by counting the radioactivity in each sample for 5 minutes in a Packard liquid scintillation counter. Standard calibration curves were obtained and plotted as C_0 / C_x against concentration of cAMP where C_0 and C_x represent the amount of [^{3}H]-cAMP bound in the absence and presence of unlabelled cAMP respectively (Fig. 2.6).

The concentrations of cAMP in unknown samples (50μl) were determined by reference to the calibration curve. The concentration of cAMP in the original sample (1.6ml) was expressed in pmol.mg^{-1} of tissue and calculated using the following method:

1) Concentration of cAMP in 50μl multiplied by 32, gives concentration of cAMP in sample tube (pmol.tube^{-1}).

2) Concentration of cAMP in sample tube divided by weight of aortic rings (mg), gives concentration of cAMP per weight of tissue (pmol.mg^{-1}).

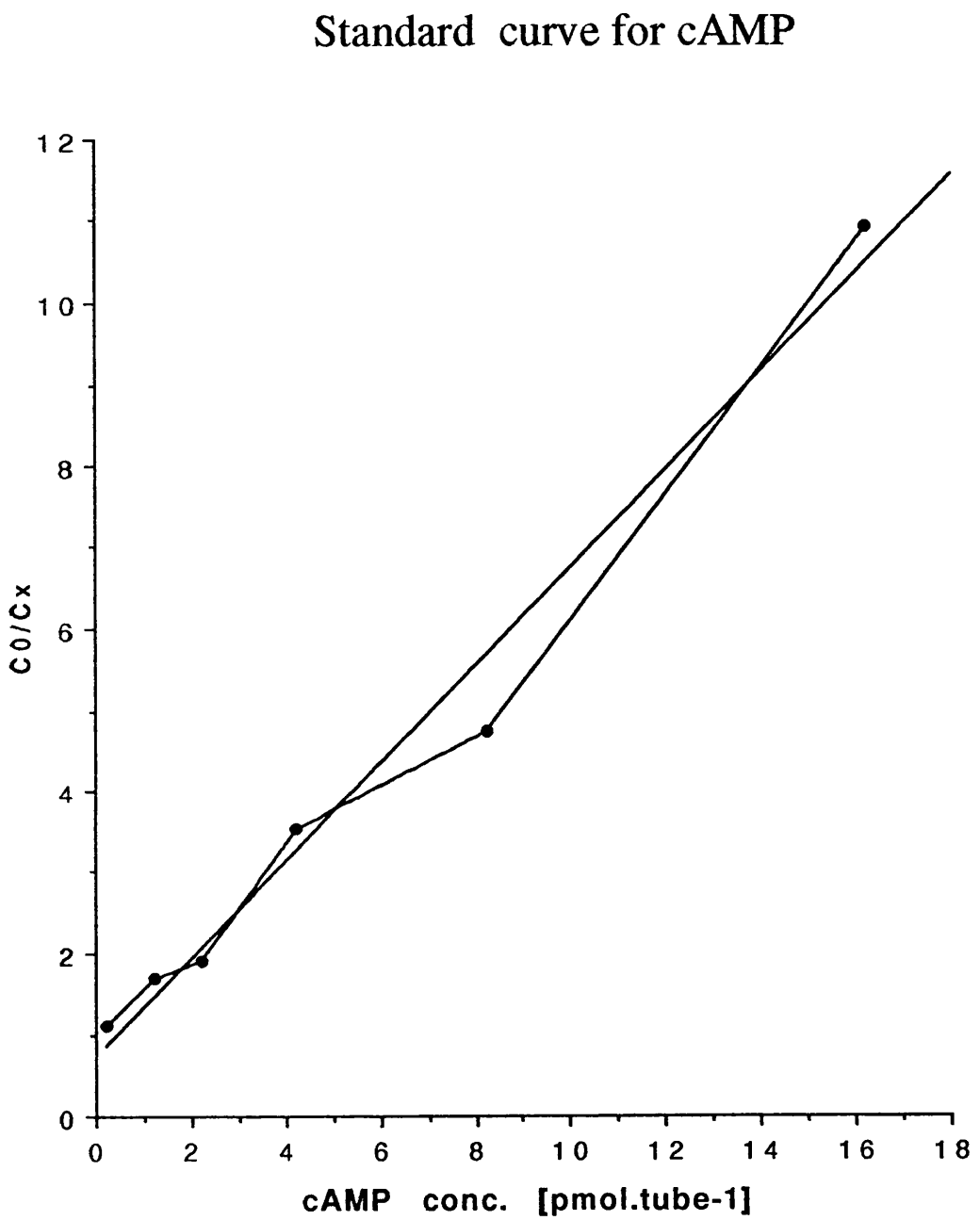

Fig.2.6.
Standard calibration curve for known concentrations of cyclic AMP, measured by radioimmunoassay. The ability of cyclic AMP to compete with (^{3}H)-cyclic AMP for binding to specific anti-cyclic AMP antiserum was measured (see text for details). C_0 is the d.p.m. bound in the absence of unlabelled cAMP, and C_x is dpm bound in the presence of standard unlabelled cAMP. The straight line is a regression line fitted by computer. Points are means. (n=12).

MEASURMENT OF BLOOD PRESSURE:

The systolic blood pressure were measured in conscious animals by the tail-cuff method. The rats were placed in a warming chamber at 37° for 15 minutes prior to measuring the blood pressure. This caused vasodilation of the tail artery and facilitated detection of the pulse. The rats were then restrained in a cloth bag. An inflatable cuff was placed around the base of the tail and a pressure transducer placed distal to the cuff. The signal detected by the pressure transducer was displayed on a calibrated storage oscilloscope. The cuff was inflated by a motor driven piston. The point at which the tail artery pulse stopped, due to occlusion of the vessel, showed the systolic blood pressure.

MATERIALS:

The following drugs and reagents were used in this study:

Noradrenaline bitartrate (Sigma), acetylcholine chloride (Sigma), sodium nitroprusside (Analar), prazosin hydrochloride (Pfizer), myo-[^{3}H]-inositol (Amersham Int.), TCA (B.D.H. Analar), propranolol hydrochloride (Sigma), potassium chloride (Koch-Light), theophylline (Sigma), isobutylmethylxanthine (Sigma), forskolin (Sigma), 8-bromo-cAMP (Sigma), 8-bromo-cGMP (Sigma), isoprenaline (Sigma), salbutamol (Sigma), propranolol hydrochloride (Sigma), nifedipine (Sigma), ammonium formate (B.D.H., Analar), formic acid (B.D.H., Analar), chloroform (B.D.H., Analar), methanol (B.D.H., Analar), cyclic AMP and cyclic GMP assay kit (Amersham, UK).

STATISTICAL ANALYSIS OF RESULTS:

Results were expressed as means $\pm$ standard error of the mean (s.e.mean), and n= number of observation.

Student's t-test was used to test the significance of differences between means. Probability values of less than $p < 0.05$ were taken as being significant.

CHAPTER THREE

RESULTS

RESULTS:

The experimental results are shown in Figures 3.1 to 3.60, which follow page 70.

SECTION 1: MECHANICAL RESPONSES TO VASOCONSTRICTORS AND VASORELAXANTS IN AORTIC RINGS FROM WISTAR RATS.

The aim of this series of experiments was to examine the effects of vasoconstrictors, whose mechanismsof action were either receptor-mediated, such as noradrenaline (NA) or the result of depolarisation (KCl). These experiments also examined the effects of vasorelaxants, whose mechanisms of action were either endothelium-dependent, such as acetylcholine (ACh) or endothelium-independent, such as sodium nitroprusside (SNP), isoprenaline (ISO), forskolin (FOR) and isobutylmethylxanthine (IBMX). Two parameters were measured in these experiments. These were (i) the isometric tension developed in isolated aortic rings in response to vasoconstrictors; and (ii) the degree of relaxation produced by vasorelaxants in aortic rings that had been precontracted with a vasoconstrictor.

PART 1: **Preliminary experiments in isolated aortic rings from Wistar rats.**

NA (10^{-10}-$3x10^{-7}$ M) caused concentration-dependent contractions in aortic rings (Fig. 3.1). At concentrations higher than 10^{-7} M, responses to NA decreased with increasing concentration. KCl (10^{-2}-10^{-1}M) also caused concentration-dependent

contractions but these occurred over a narrow concentration range (Fig. 3.3), which was higher than that required to produce contractions to NA (Fig. 3.4).

The endothelium-dependent vasorelaxants ACh ($3x10^{-9}$ -$3x10^{-6}$ M) relaxed aortic rings that had been precontracted with NA (EC_{75}= $3x10^{-8}$ M) (Fig. 3.5). The endothelium- independent vasorelaxant, SNP (10^{-10} -$3x10^{-6}$ M) also relaxed aortic rings that had been precontracted with NA ($3x10^{-8}$ M) (Fig. 3.6). SNP was more potent than ACh and the maximum inhibition (100%) produced by SNP ($3x10^{-8}$ M) was greater than that (80%) produced by ACh ($3x10^{-6}$ M) (Fig. 3.7).

ISO ($3x10^{-10}$ -10^{-7} M) caused concentration-dependent vasorelaxation of aortic rings that had been precontracted with NA ($3x10^{-8}$ M) (Fig. 3.8). The direct activator of adenylate cyclase, forskolin (10^{-10}-10^{-7} M) (Fig. 3.9) and the phosphodiesterase inhibitor, IBMX ($3x10^{-6}$-10^{-4} M) (Fig. 3.10), also relaxed aortic rings that had been precontracted with NA ($3x10^{-8}$ M). The relaxation produced by IBMX suggests that there was a basal synthesis of cyclic nucleotides in the isolated aortic rings that had been precontracted with NA.

PART 2: **Mechanical responses to vasoconstrictors and vasorelaxants in aortic rings from SHR and WKY control rats:**

NA (10^{-10}-10^{-5} M) caused concentration-dependent contractions in aortic rings from SHR and WKY control rats. Over the entire concentration range, responses to NA were smaller in rings from SHR than those obtained in rings from WKY control rats (Fig. 3.14). KCl ($3x10^{-3}$-$8x10^{-2}$ M) also caused concentration-dependent contractions in aortic rings from SHR and WKY rats.

Again, over the whole concentration range, responses to KCl were smaller in rings from SHR than were those obtained in rings from WKY controls (Fig. 3.15). These results indicate that vasculature from SHR was less sensitive to vasoconstrictors than was that from WKY controls.

ACh ($3x10^{-9}$-10^{-6} M) relaxed isolated rings that had been precontracted with NA ($5.6x10^{-9}$ M) (Fig. 3.16). The capacity of ACh to relax aortic rings was similar in rings from SHR and WKY rats (Fig. 3.16). In contrast, responses to SNP were different in rings from WKY control rats. At low concentrations, inhibitory responses to SNP were smaller in rings from SHR than those obtained in rings from WKY control rats, but responses to high concentrations of SNP, were larger in rings from SHR than were those obtained in rings from WKY controls (Fig. 3.17).

ISO ($3x10^{-10}$ - $3x10^{-7}$ M) produced concentration- dependent inhibitions of NA-induced tone in aortic rings from SHR and WKY control rats. At low concentrations, ISO produced smaller inhibitions in aortic rings from SHR than in rings from WKY controls (Fig. 3.18). However, at higher concentrations there was no significant difference between ISO-induced relaxations in SHR and WKY rats (Fig. 3.18).

The Ca^{2+} -channel blocker, nifedipine (NIF, 10^{-9}-$3x10^{-6}$ M) caused concentration-dependent relaxation of aortic rings from both SHR and WKY rats (Fig. 3.19, 20). In rings precontracted with NA ($5.6x10^{-9}$), NIF at low concentrations, produced smaller relaxations in rings from SHR than in rings from WKY but, at higher concentrations, there was a greater relaxation of rings from SHR than in those from WKY control (Fig. 3.19). At all concentrations exept the highest, NIF produced

smaller relaxations of KCl ($1.3x10^{-2}$M)-induced tone in rings from SHR than in rings from SHR than in those from WKY (Fig. 3.20).

SECTION 2: BIOCHEMICAL RESPONSES TO VASOCONSTRICTORS AND VASORELAXANTS IN ISOLATED AORTIC RINGS FROM WISTAR RATS.

The aims of these experiments were first, to examine the effects of vasoconstrictors on phosphoinositide (PI) hydrolysis and secondly, to determine the effects of vasorelaxants on the levels of the inhibitory second messengers, cAMP and cGMP. A third objective was to determine the effects of vasorelaxants on NA-induced PI-hydrolysis in the isolated vasculature.

PART 1: **The effects of vasoconstrictors alone and in combination with vasorelaxants on PI-hydrolysis in aortic rings from Wistar rats.**

The time-course of the effect of NA on PI-hydrolysis in aortic rings from Wistar rats is shown in Fig. 3.29. NA (10^{-4} M) increased the formation of (^{3}H)-inositol phosphates, which continued to accumulate over the period of 1 hour, during which the experiment was carried out. NA (10^{-10}-10^{-5} M) caused a concentration-dependent accumulation of ^{3}H-inositol phosphates [(^{3}H)-IPs] in isolated aortic rings from Wistar rats (Fig. 3.30). This ability of NA ($6.2x10^{-6}$ M) to increase the formation of [(^{3}H)-IPs] in aortic rings was reduced to control levels by the α_1- adrenoceptor antagonist, prazosin (10^{-6} M) (Fig. 3.31).

Surprisingly, KCl ($3x10^{-2}$ M) also increased the accumulation of [(^{3}H)-IPs] in rat aortic rings (Fig. 3.32). This effect of KCl was inhibited by NIF (10^{-6} M) (Fig. 3.33).

The vasorelaxants, ACh (10^{-5} M) and SNP (10^{-6} M) inhibited the NA-induced PI-response in aortic rings from Wistar rats (Fig. 3.34, 3.36). Removal of the endothelium either mechanically, by rubbing, or chemically with Triton-X 100 (0.1% V/V) abolished the inhibitory action of ACh on NA-induced PI-hydrolysis (Fig. 3.35). The permeant cyclic GMP analogue, 8-bromo-cGMP ($3x10^{-4}$ M) also inhibited the NA-induced PI-response (Fig. 3.37). The ß-adrenoceptor agonists, isoprenaline (ISO, 10^{-6} M) (Fig. 3.38) and salbutamol (SAL, 10^{-6} M) (Fig. 3.39) also inhibited the NA-induced PI-response. This effect of the ß-adrenoceptor agonists was antagonized by propranolol (PROP, $5x10^{-6}$ M).

The phosphodiesterase inhibitors, IBMX (10^{-6} M) (Fig. 3.40) and theophylline ($5x10^{-6}$ M) (Fig. 3.41) also inhibited the NA-induced PI-response. The directly-acting activator of adenylate cyclase, forskolin (FOR, 10^{-6} M), inhibited the NA-induced PI-response (Fig. 3.42). The combination of SNP (10^{-6} M) and FOR (10^{-6} M) produced a greater inhibition than was produced by either drug alone (Fig. 3.43). The permeant cAMP analogue, 8-bromo-cAMP ($3x10^{-4}$ M), also inhibited the NA-induced PI-response (Fig. 3.44). NIF (10^{-6} M) inhibited the NA-induced PI-response in aortic rings (Fig. 3.45).

It was notable that the vasorelaxants, ACh (10 μM), SNP (1 μM), FOR (1 μM), IBMX (1 μM), 8-bromo-cGMP (0.3mM) and 8-bromo-cAMP (0.3mM) all inhibited the NA-induced PI-response by less than 50% although the relaxations they produced were greater than 50%.

These results suggest that drugs which either increase the synthesis of cAMP or cGMP or prevent the breakdown of these cyclic nucleotides in vascular smooth muscle, can inhibit the NA-induced PI-response, which underlies these contractions.

PART 2: **The effect of vasorelaxants on cyclic nucleotide levels in aortic rings from Wistar rats.**

In the presence of NA ($3x10^{-8}$ M), ACh (10^{-5} M) and SNP (10^{-6} M) increased the levels of cGMP in isolated aortic rings from Wistar rats. At a concentration which maximally relaxed vascular smooth muscle that had been precontracted with NA ($3x10^{-8}$ M), ACh produced a 4-fold increase in cGMP levels (Fig. 3.53). In contrast, SNP (10^{-6} M) also abolished the NA-induced tone, produced a 35-fold increase in cGMP levels (Fig. 3.54).

ISO (10^{-8} M) and FOR (10^{-6} M) increased the cAMP levels in aortic rings (in the presence of NA, $3x10^{-8}$ M) to 2.5 times and 4 times control values (Fig. 3.55, 3.56).

PART 3: **The effects of vasoconstrictors on NA-induced PI-hydrolysis in aortic rings from SHR and WKY rats.**

NA (10^{-8}-10^{-3} M) induced PI-hydrolysis in aortic rings from SHR and WKY rats in a concentration-dependent manner (Fig. 3.46). The results obtained in these experiments were influenced by the age of the animals used. In experiments with rats

that were between 10 and 12 weeks old, NA (10^{-8}-10^{-6} M) produced a larger PI-response in aortic rings from SHR than in rings from WKY. In these rings, the higher concentrations of NA (10^{-5}-10^{-3} M) produced a similar PI-response in rings from SHR and WKY rats. In contrast, in experiments with older rats (18-20 weeks), NA (10^{-8}-10^{-3} M) produced a smaller PI-response in rings from SHR than in rings from WKY rats (Fig. 3.47).

PART 4: **The inhibitory effects of vasorelaxants that increase the synthesis of cyclic nucleotides on the NA-induced PI- response and on cAMP and cGMP synthesis in aortic rings from SHR and WKY rats.**

ACh (10^{-5} M) and SNP (10^{-6} M) inhibited the NA-induced PI-response in rings from both SHR and in WKY control rats (Fig. 3.48, 3.49) but the inhibitions produced by both ACh and SNP were larger in rings from SHR than in those from WKY (Fig. 3.48, 3.49). In contrast, ISO (10^{-8} M) and FOR (10^{-6} M) also inhibited the NA-induced PI-response but the inhibitions produced by both drugs were smaller in rings from SHR than in those from WKY rats (Fig. 3.50, 3.51).

ACh (10^{-5} M) and SNP (10^{-6} M) increased the levels of cGMP in isolated aortic rings from SHR and WKY rats but the increase was greater in rings from SHR than in those from WKY rats (Fig. 3.57, 3.58). ISO (10^{-8} M) and FOR (10^{-6} M) increased the levels of cAMP in aortic rings from SHR and WKY but the increase in cAMP levels was smaller in rings from SHR than in those from WKY control rats (Fig. 3.59, 3.60).

SECTION 3: MECHANICAL AND BIOCHEMICAL EXPERIMENTS IN RAT ISOLATED TAIL ARTERY.

The aims of these experiments were, first to examine mechanical responses, including tension recording and also perfusion pressure responses to vasoconstrictors and secondly, to investigate vasoconstrictor-induced PI-responses in rat tail artery.

PART 1: **Mechanical responses to vasoconstrictors in isolated tail artery rings from SHR and WKY control rats.**

NA ($3x10^{-9}$-10^{-5} M) produced concentration-dependent contractions in isolated tail artery rings from WKY and SHR rats. NA at low concentrations ($3x10^{-9}$-10^{-6} M) produced responses that were not significantly different in rings from SHR and WKY rats, but at higher concentrations ($3x10^{-6}$ -10^{-5} M) tail artery rings from SHR were significantly more responsive to NA than those from WKY control rats (Fig. 3.21).

These results can also be expressed as percentages of the maximum response. (Fig. 3.22). There was no significant difference in the responses of rings from SHR and WKY when expressed in this manner.

KCl (10^{-2}-10^{-1} M) produced concentration-dependent contractions in isolated tail artery rings from WKY and SHR rats. Except at one concentration, responses to KCl were not significantly different in rings from SHR and from WKY controls. (Fig. 3.25)

When these results were expressed as percentages of the maximum response.

(Fig. 3.26), there was no significant difference in the responses of rings from SHR and from WKY controls.

PART 2: **Perfusion pressure responses to vasoconstrictors in perfused tail artery segments from SHR and WKY control rats.**

NA ($3x10^{-9}$-10^{-5} M) produced concentration-dependent contractions in isolated perfused tail artery segments from WKY and SHR rats. Lower concentrations of NA ($3x10^{-9}$-$3x10^{-6}$ M) produced responses that were not significantly different in segments from SHR and WKY rats. However at the highest concentration (10^{-5} M), tail artery segments from SHR were significantly more responsive to NA compared to those from WKY control rats. (Fig. 3.23).

When these results were expressed as percentages of the maximum response (Fig. 3.24), except in highest and lowest concentrations, the responses were significantly smaller in segments from SHR than those of WKY controls.

KCl (10^{-2}-$5x10^{-1}$ M) produced concentration-dependent contractions in isolated perfused tail artery segments from WKY and SHR rats. There was no significant difference between the responses to KCl in rings from SHR and WKY control. (Fig. 3.27).

PART 3: **Experiments on PI-hydrolysis in rat tail artery segments from WKY and SHR.**

NA (10^{-6} M, 10^{-5} M) produced concentration-dependent PI-hydrolysis in tail

artery segments from SHR and WKY control rats. The basal (control) level of PI-hydrolysis in segments from the SHR was significantly greater than segments from the WKY rats (Fig. 3.51).

NA (10^{-6} M and 10^{-5} M) significantly increased PI-hydrolysis in tail artery segments from both SHR and WKY rats. There was no significant difference in response to NA (10^{-6} M) in segments from SHR and WKY, but a higher concentration of NA (10^{-5} M) induced significantly greater PI-hydrolysis in segments from SHR than in WKY controls (Fig. 3.52).

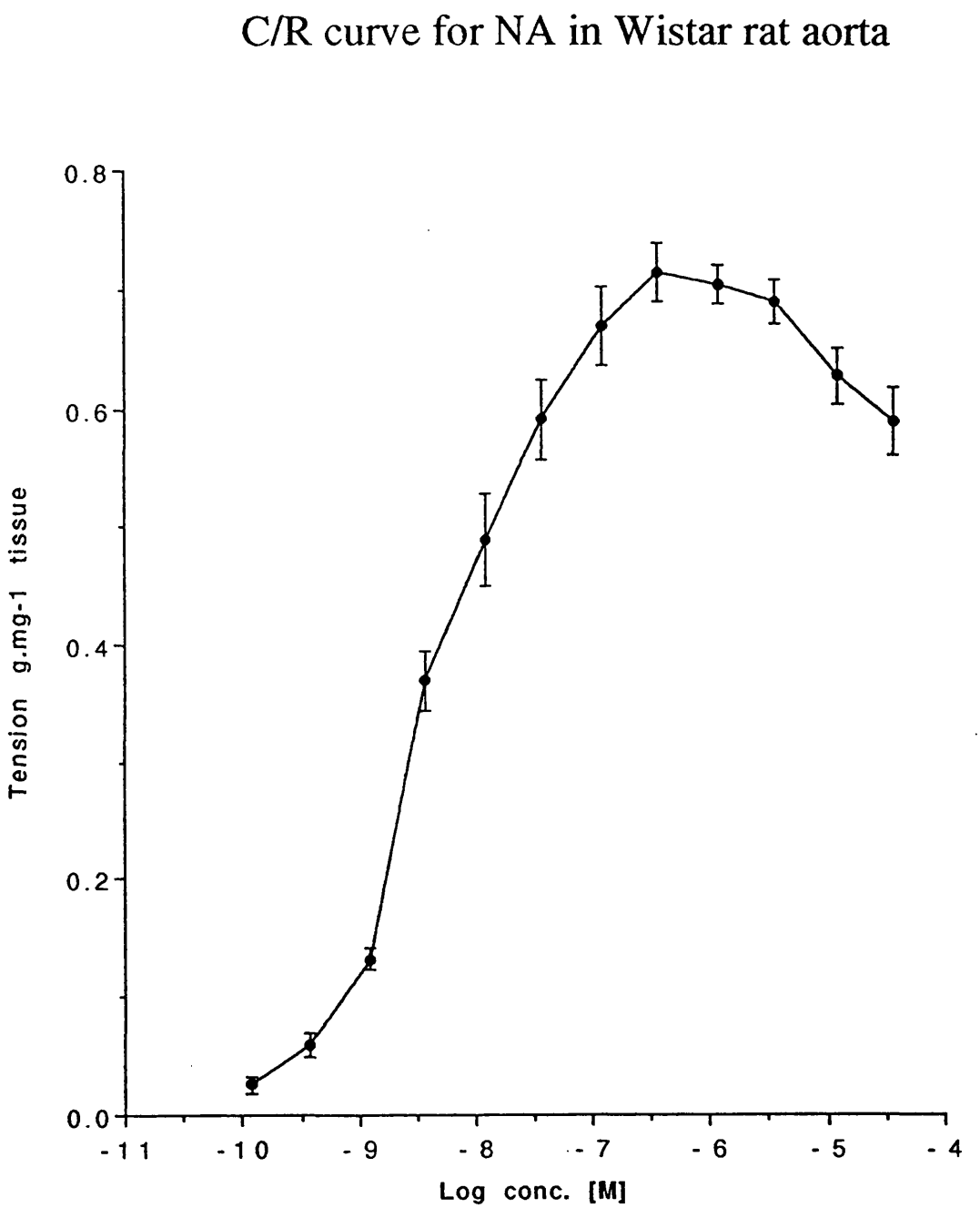

Fig.3.1.
Concentration-response curve for NA in aortic rings from Wistar rats. Tension was recorded. NA (10^{-10} - $3x10^{-7}$M) induced concentration-dependent contractions of aortic rings. At higher concentrations (10^{-7}-$3x10^{-5}$M) responses to NA decreased with increasing concentration. Points are means ± s.e.mean. (n=10).

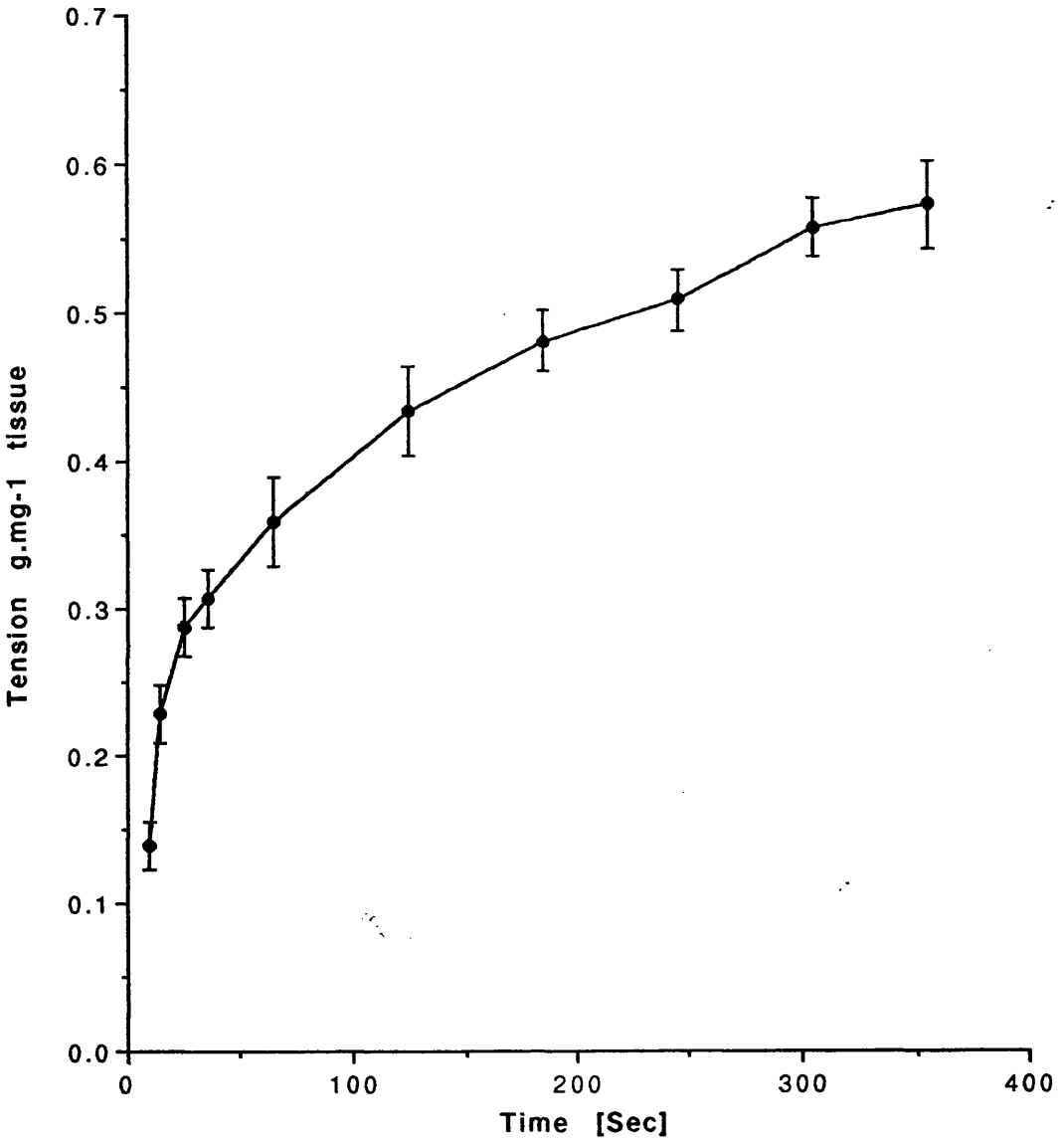

Fig.3.2.
Time-course for NA-induced contraction in aortic rings from Wistar rats. Tension was recorded. NA (10^{-6}M) induced time-dependent contraction of aortic rings. Muscle tone stabilised after 5 mins. Points are means $\pm$ s.e.mean. (n=8).

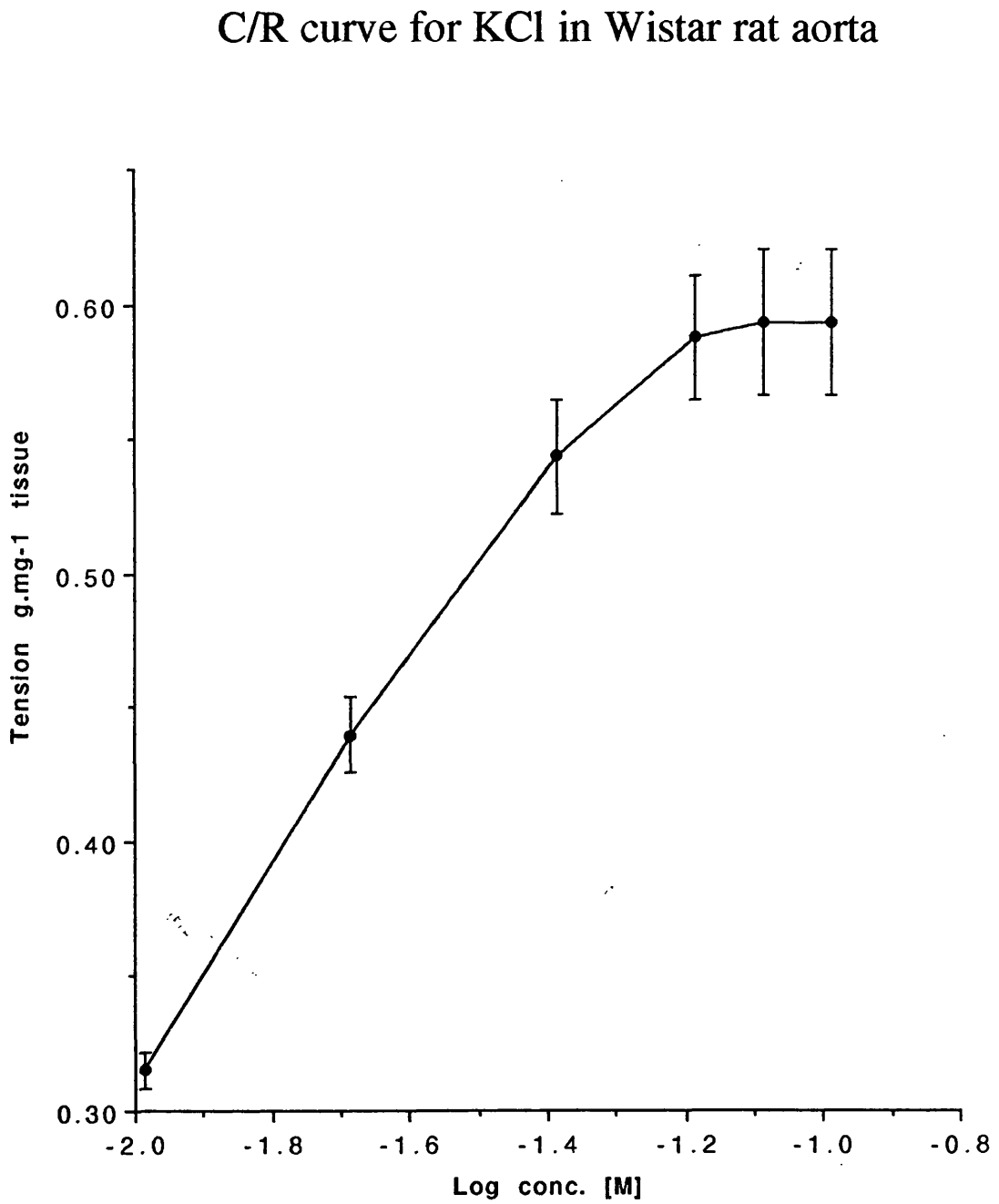

Fig. 3.3.
Concentration-response curve for KCl in aortic rings from Wistar rats. Tension was recorded. KCl induced concentration-dependent contractions over a narrow concentration range. Points are means ± s.e.mean. (n=8).

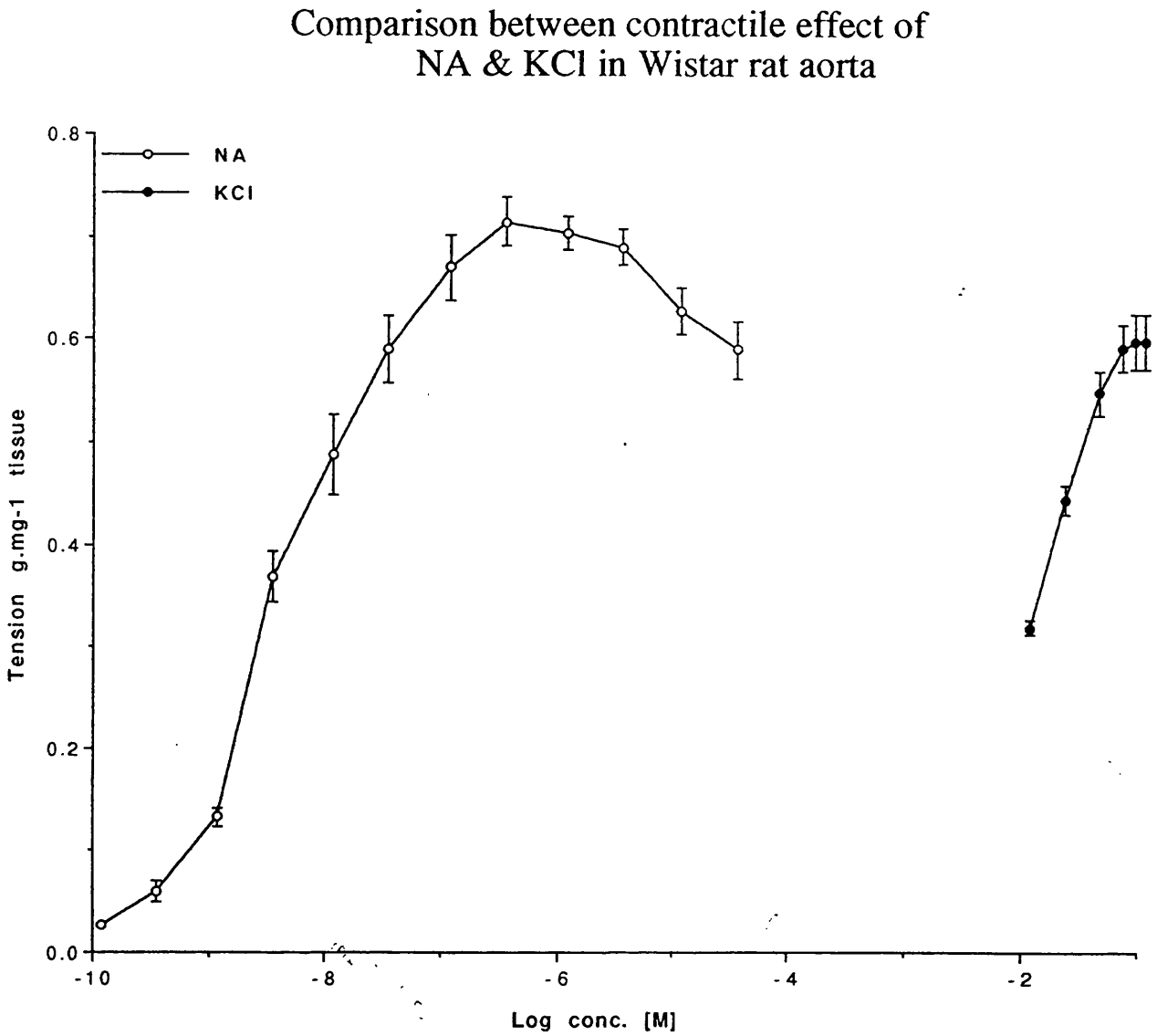

Fig. 3.4.
Comparison between the contractile effects of NA and KCl in aortic rings from Wistar rats. Muscle tension was recorded. NA was more potent than KCl. Points are means ± s.e.mean. (n=8)

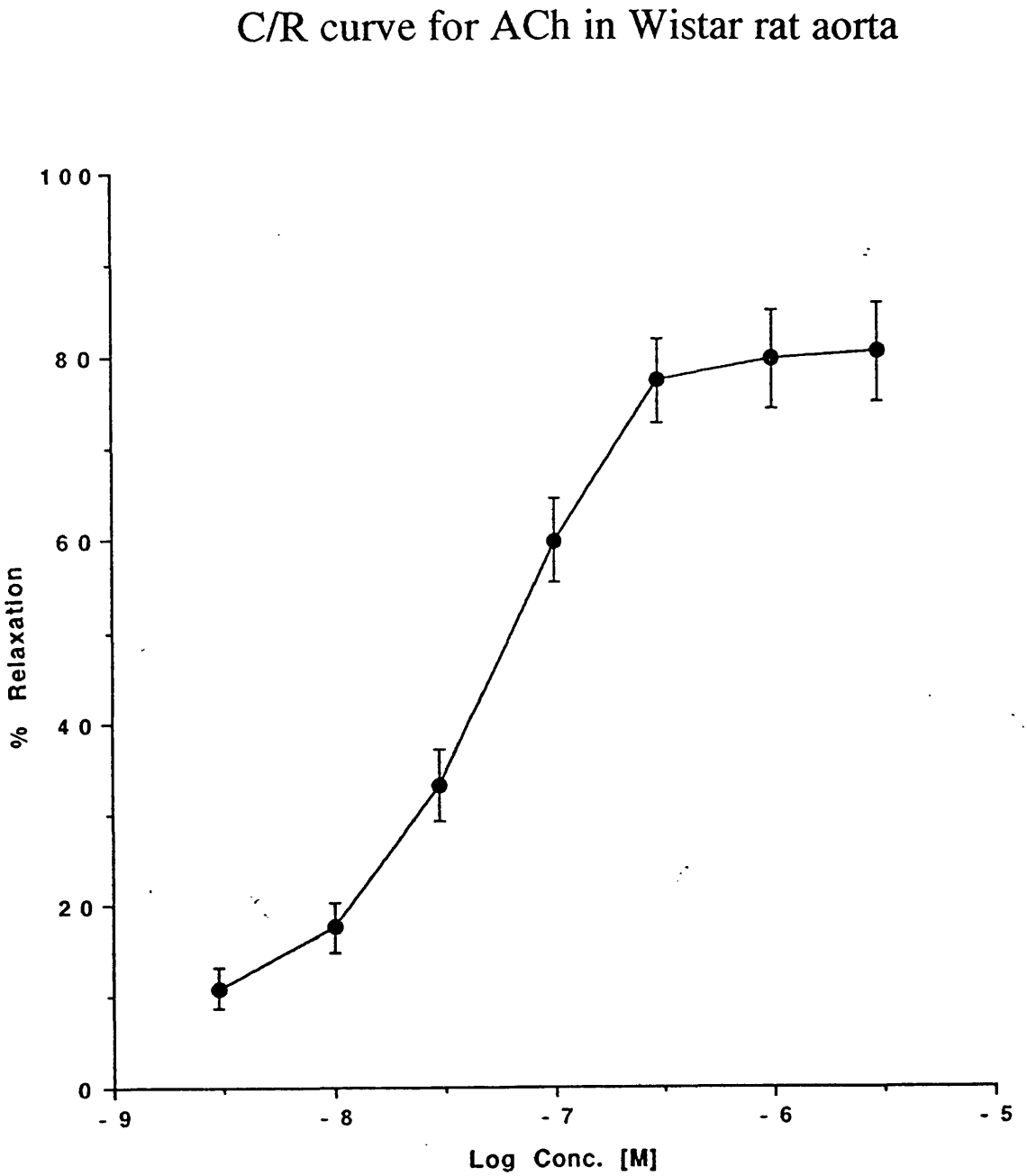

Fig. 3.5.
Concentration-response curve for ACh in aortic rings from Wistar rats. The degree of muscle relaxation produced by ACh was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}$M). Points are means ± s.e.mean. (n=8).

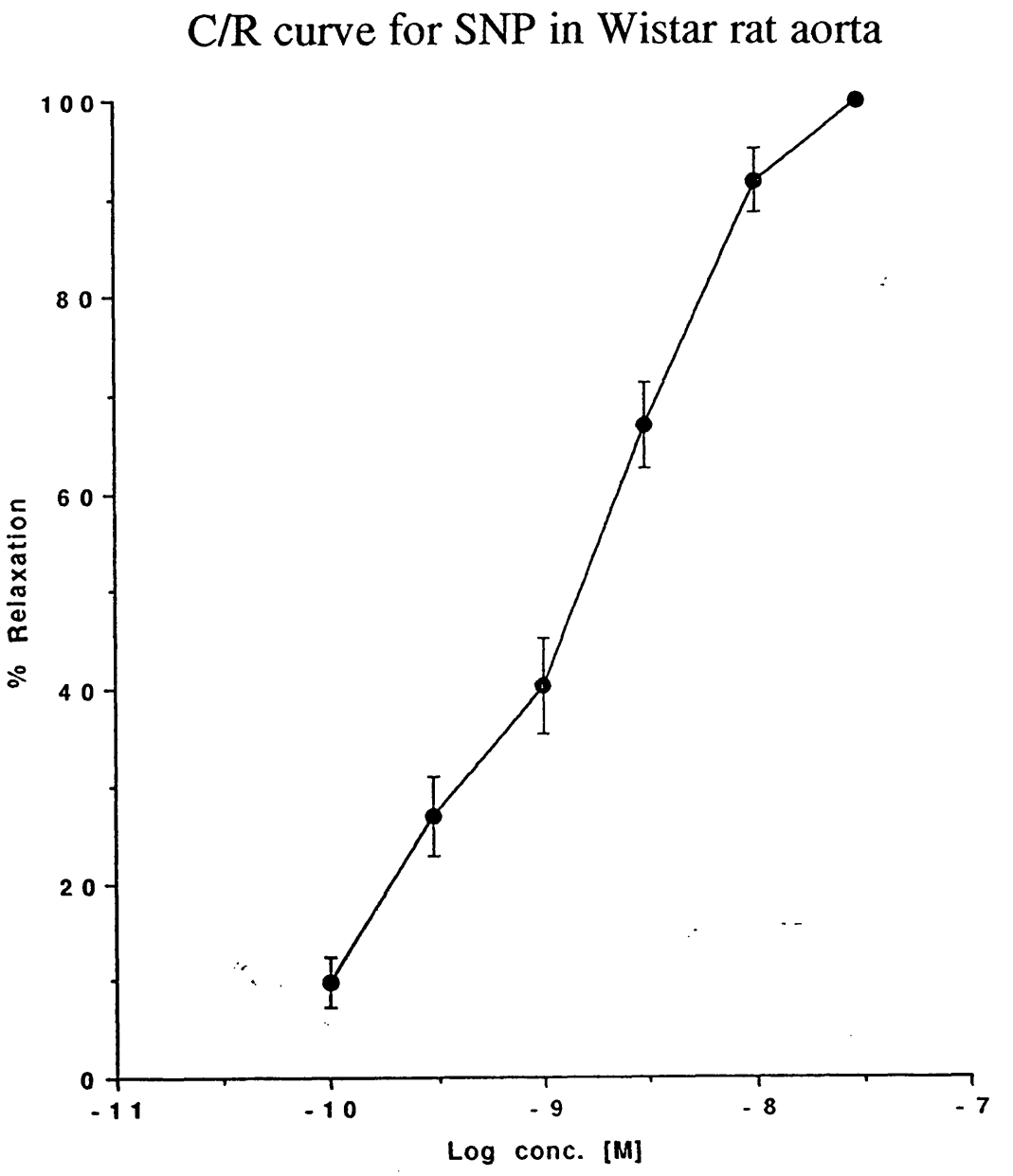

Fig. 3.6.
Concentration-response curve for SNP in aortic rings from Wistar rats. The degree of muscle relaxation produced by SNP was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}$M). Points are means $\pm$ s.e.mean. (n=8).

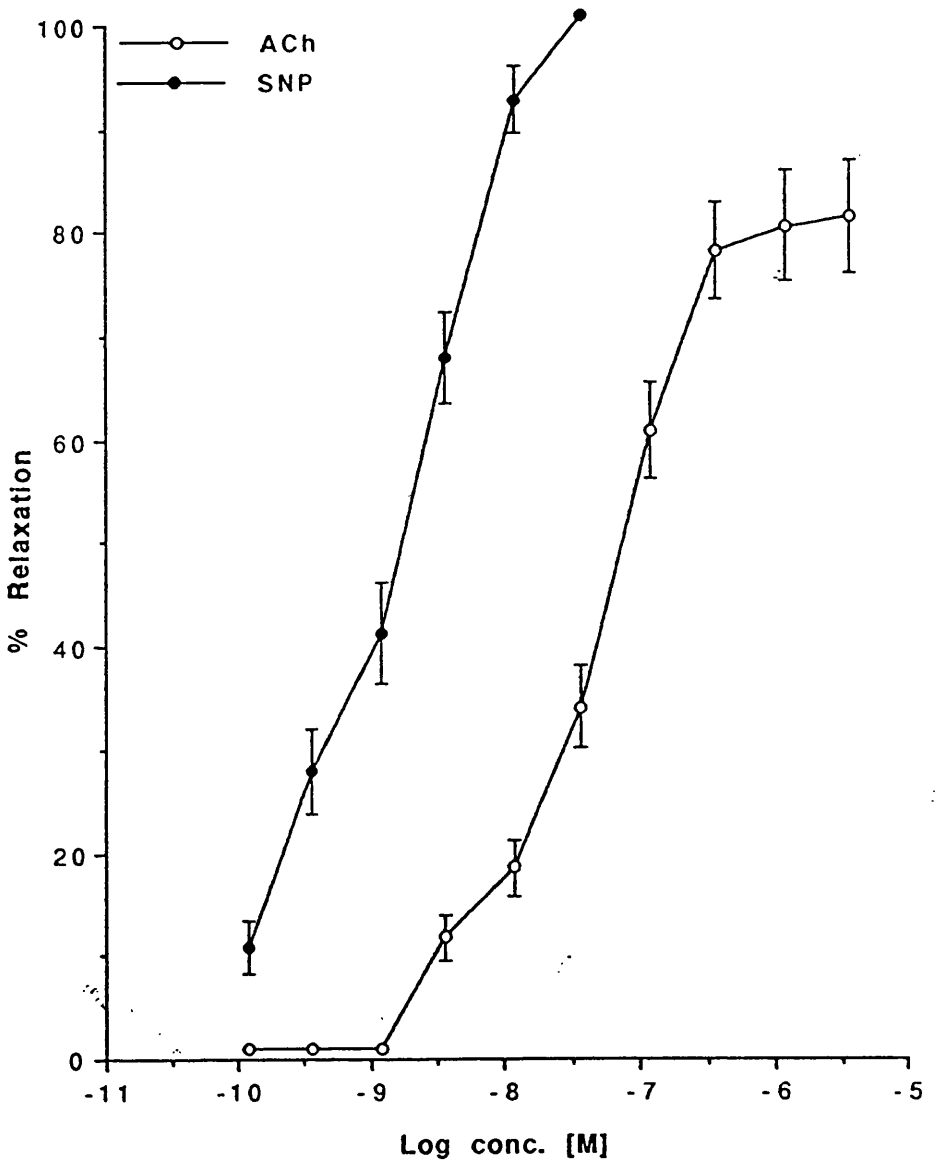

Fig. 3.7.
Comparison between the relaxant effects of ACh and SNP in aortic rings from Wistar rats. The degree of muscle relaxation produced by ACh and SNP was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}$M). Points are means ± s.e.mean. (n=8).

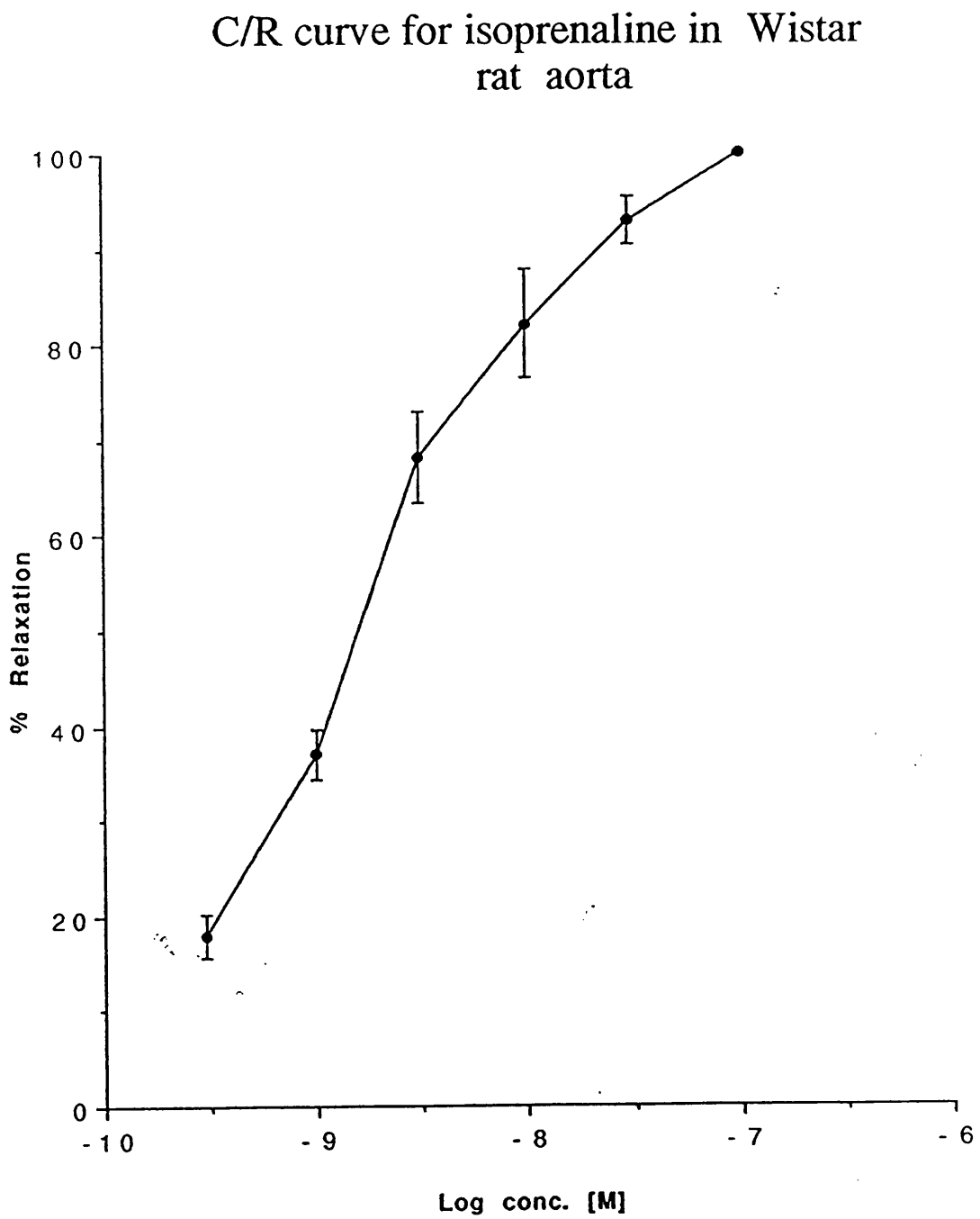

Fig. 3.8.
Concentration-response curve for isoprenaline in aortic rings from Wistar rats. The muscle relaxation produced by isoprenaline was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}M$). Points are means ± s.e.mean. (n=8).

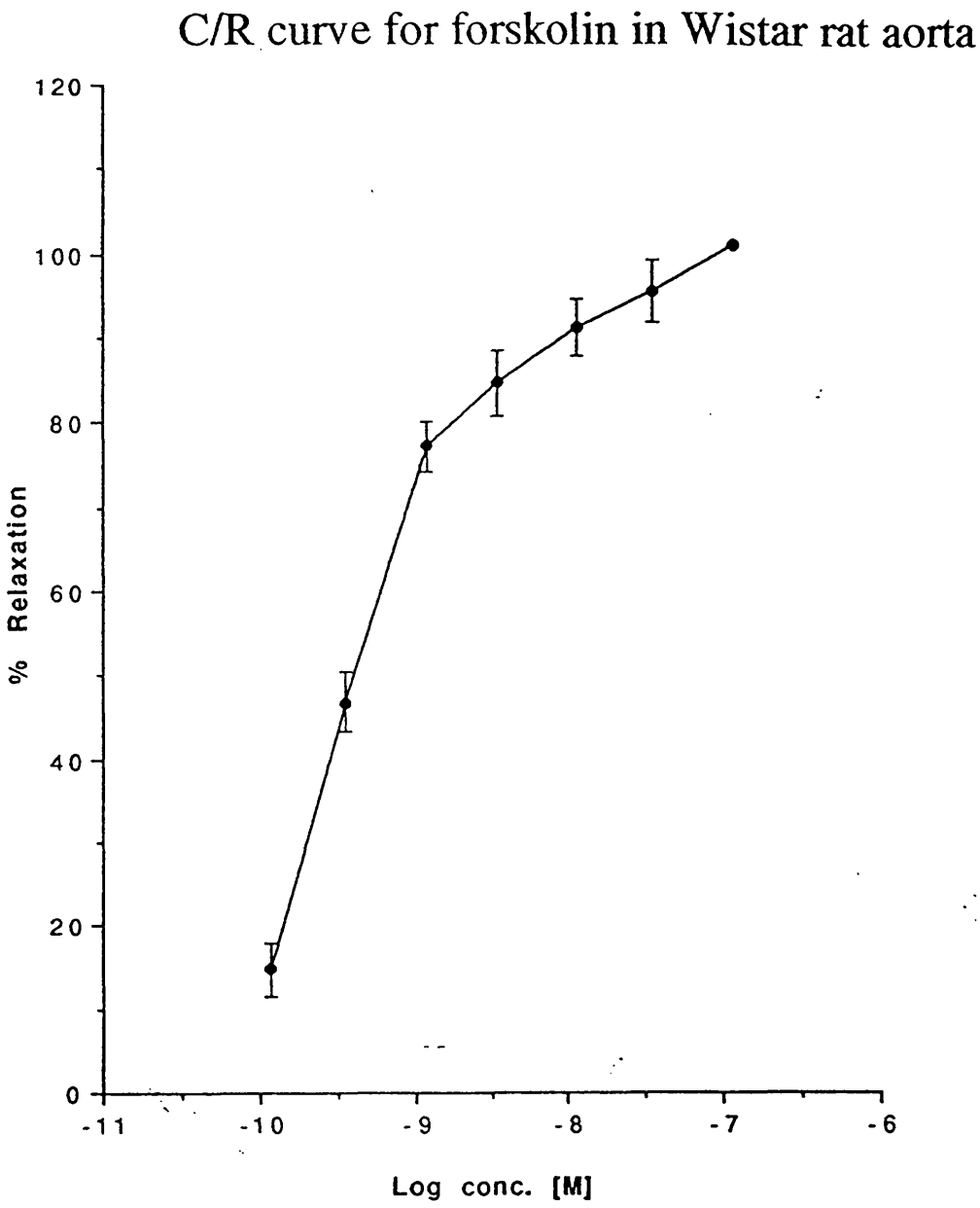

Fig. 3.9.
Concentration-response curve for forskolin in rat aortic rings from Wistar rats. The muscle relaxation produced by forskolin was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}$ M). Points are means $\pm$ s.e.mean. (n=8).

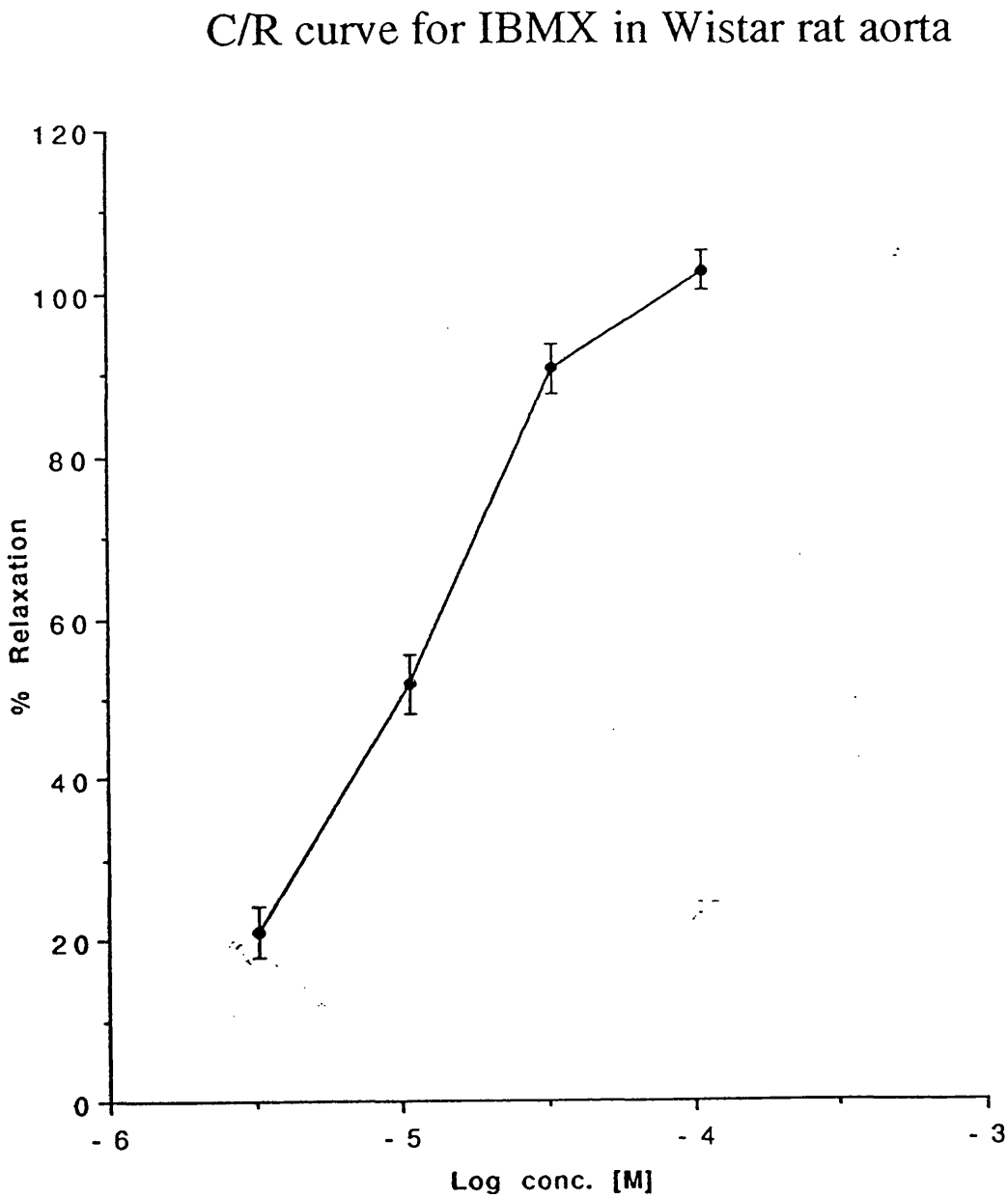

Fig. 3.10.
Concentration-response curve for isobutylmethylxanthine (IBMX) in aortic rings from Wistar rats. The muscle relaxation produced by IBMX was recorded and expressed as a % of the contraction elicited by NA ($3x10^{-8}$M). Points are means ± s.e.mean. (n=8).

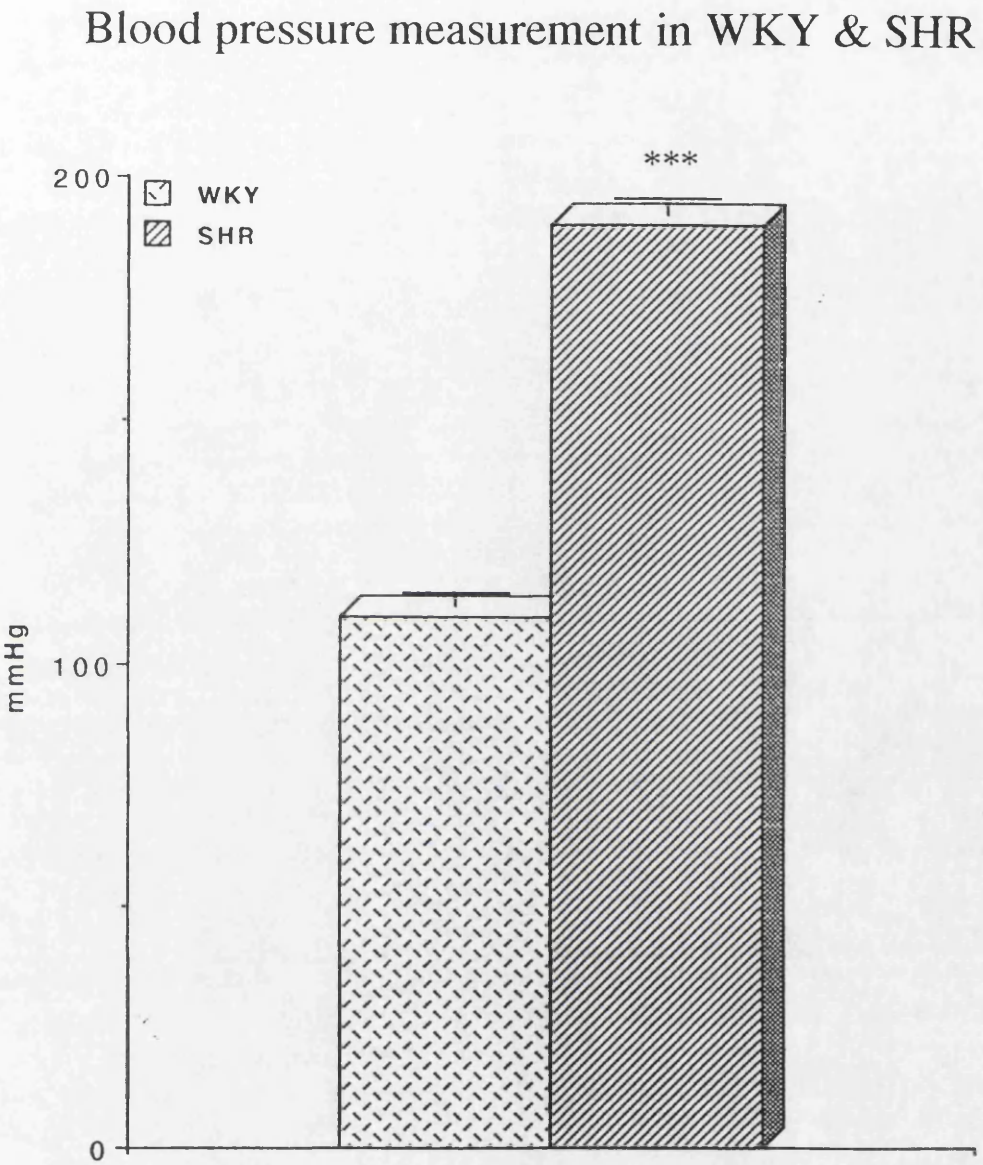

Fig. 3.11.
Measurement of blood pressure in conscious SHR and WKY rats. The blood pressure measured in SHR was significantly higher than that in WKY controls. (*** $p<0.001$). Results are means $\pm$ s.e.mean. ($n=12$).

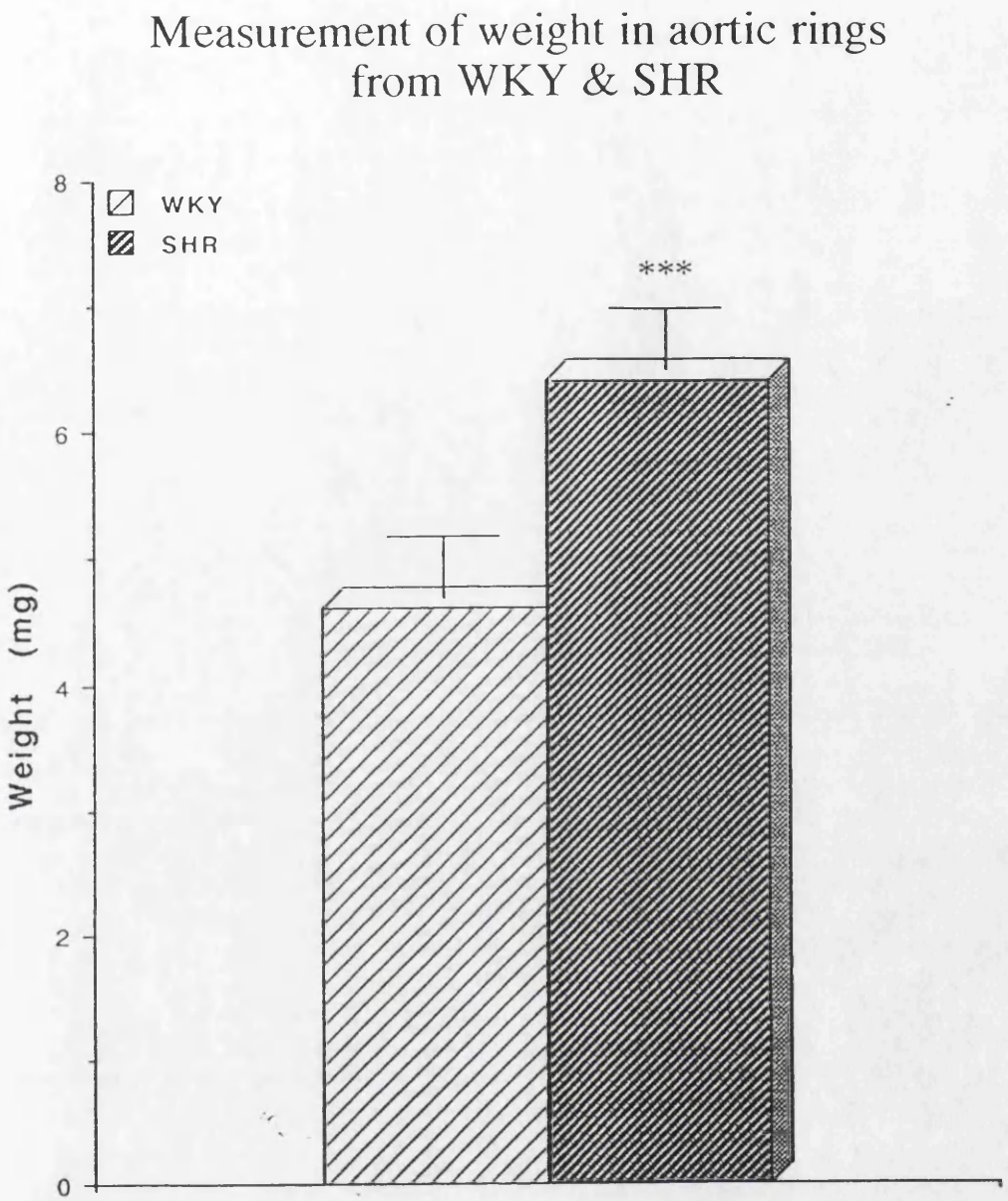

Fig. 3.12.
Measurement of weight of aortic rings from SHR and WKY rats. Aortic rings from SHR were significantly heavier than those from WKY control rats. (*** $p < 0.001$). Results are means ± s.e.mean. (n=20).

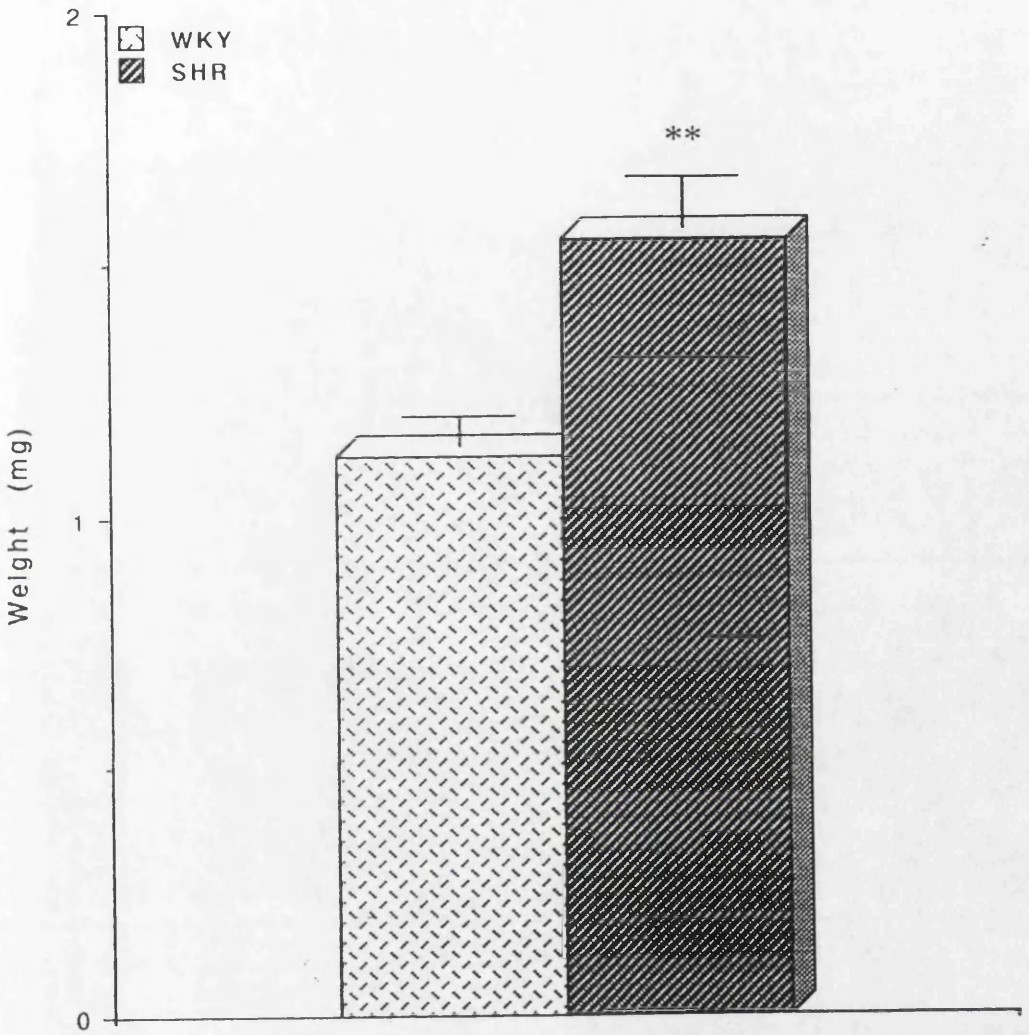

Fig. 3.13.
Measurement of weight of tail artery rings from SHR and WKY rats. Rings from SHR were significantly heavier than those from WKY control rats. (** $p<0.01$). Results are means $\pm$ s.e.mean. (n=32).

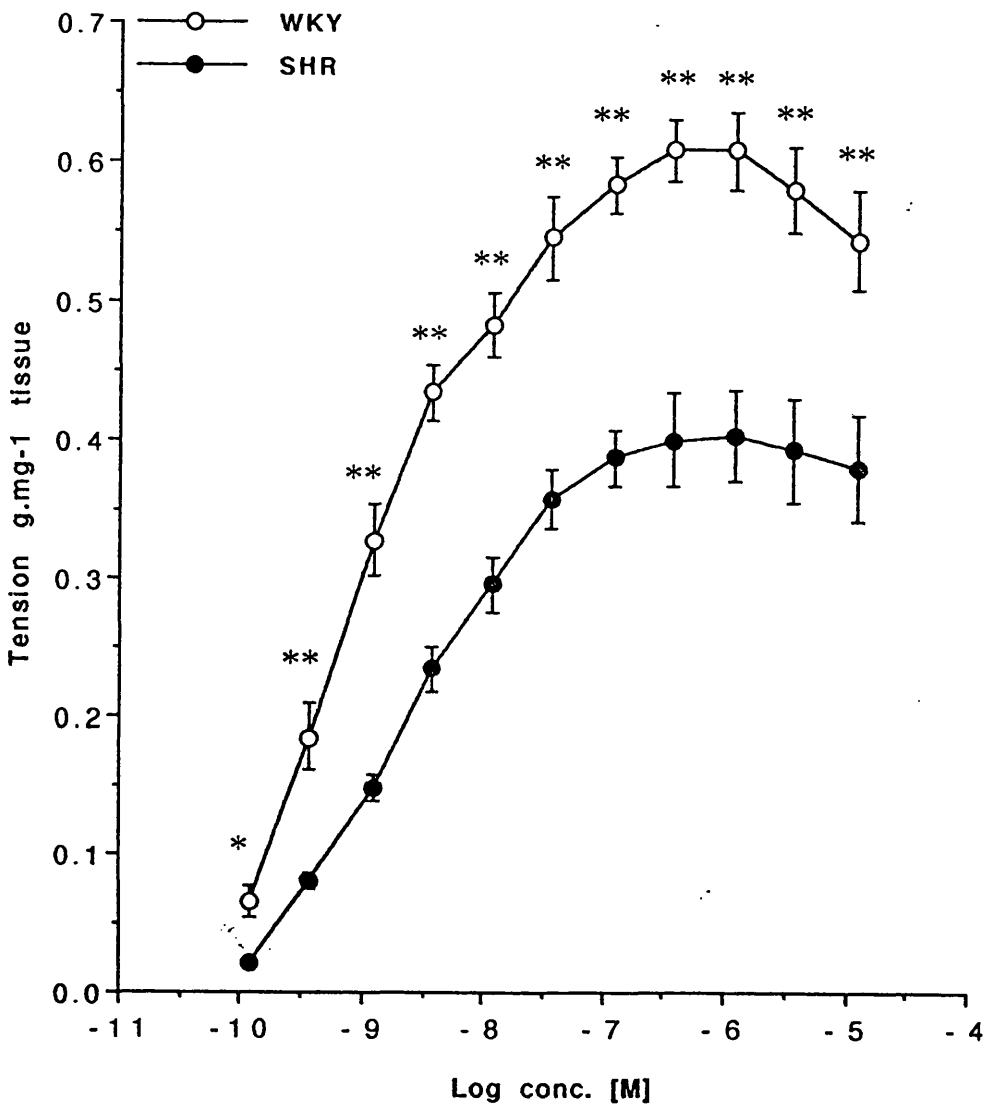

Fig. 3.14.
Concentration-response curves for NA in aortic rings from SHR and WKY control rats. Muscle tension was recorded. NA induced significantly smaller contractions in rings from SHR than in rings from WKY control rats. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=10).

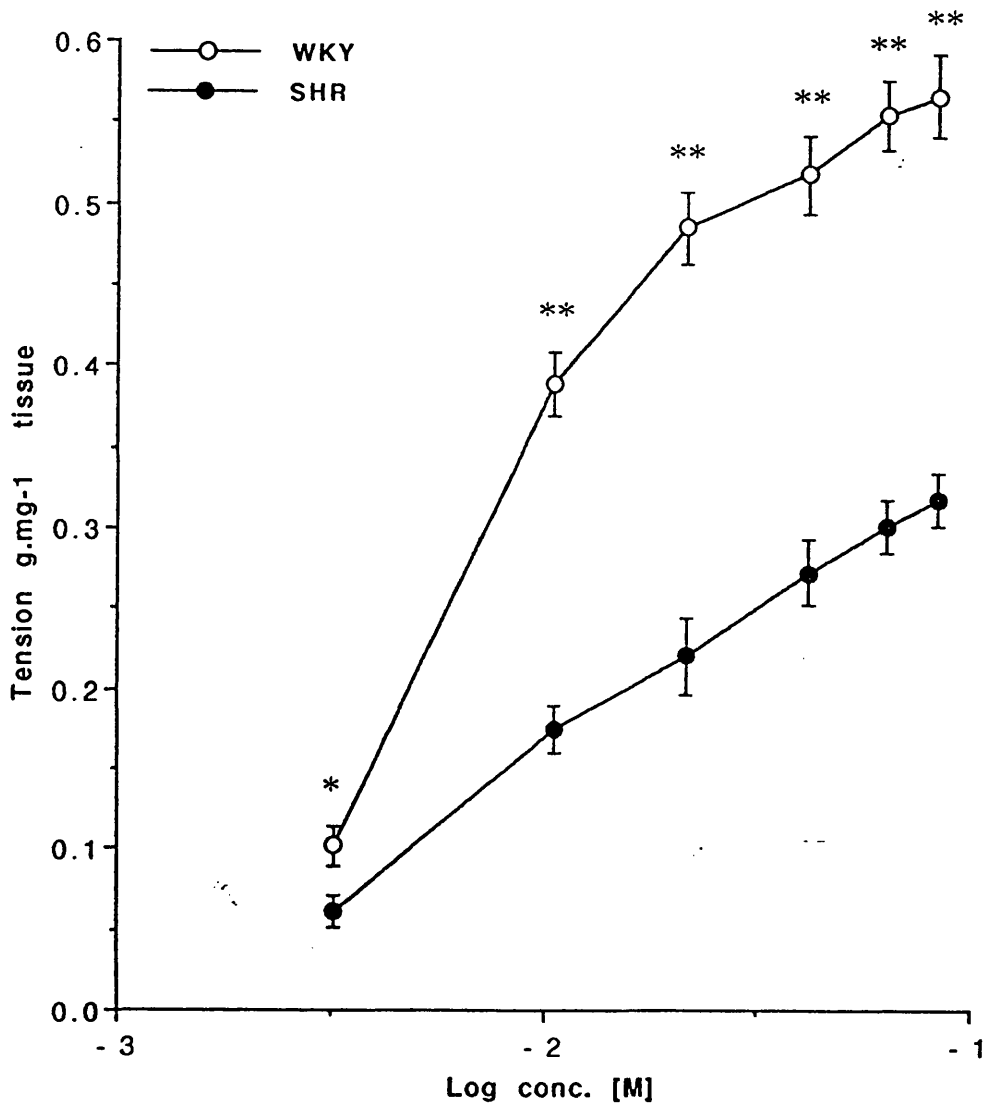

Fig. 3.15.
Concentration-response curves for KCl in aortic rings from SHR and WKY control rats. Muscle tension was recorded. KCl induced significantly smaller contractions in tissues from SHR than in tissues from WKY control rats. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=10).

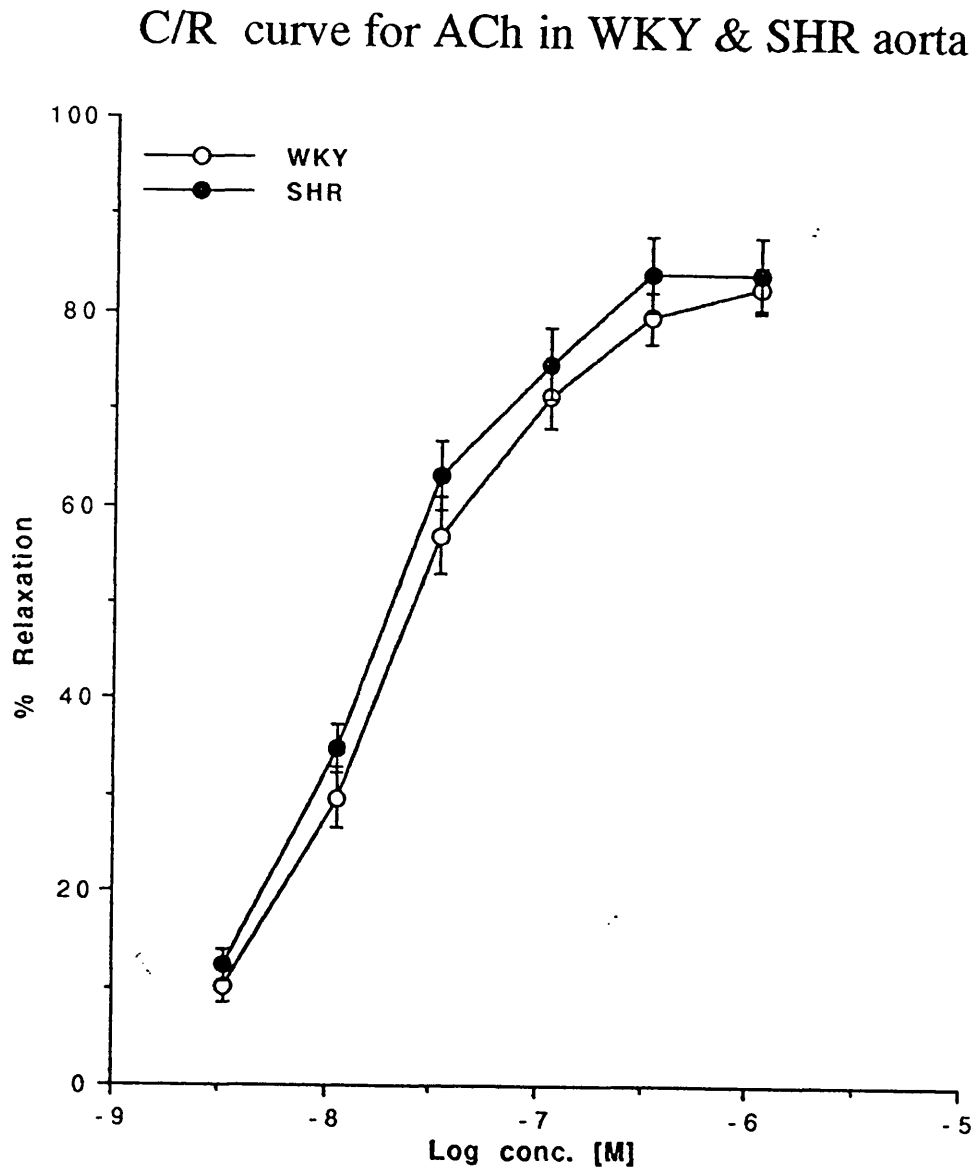

Fig. 3.16.
Concentration-response curves for ACh in aortic rings from SHR and WKY control rats. Muscle relaxation was recorded and expressed as a % of contraction elicited by NA (5.6×10^{-9}M). ACh-induced relaxation was not significantly different in rings from SHR and WKY controls. Points are means $\pm$ s.e.means. (n=8).

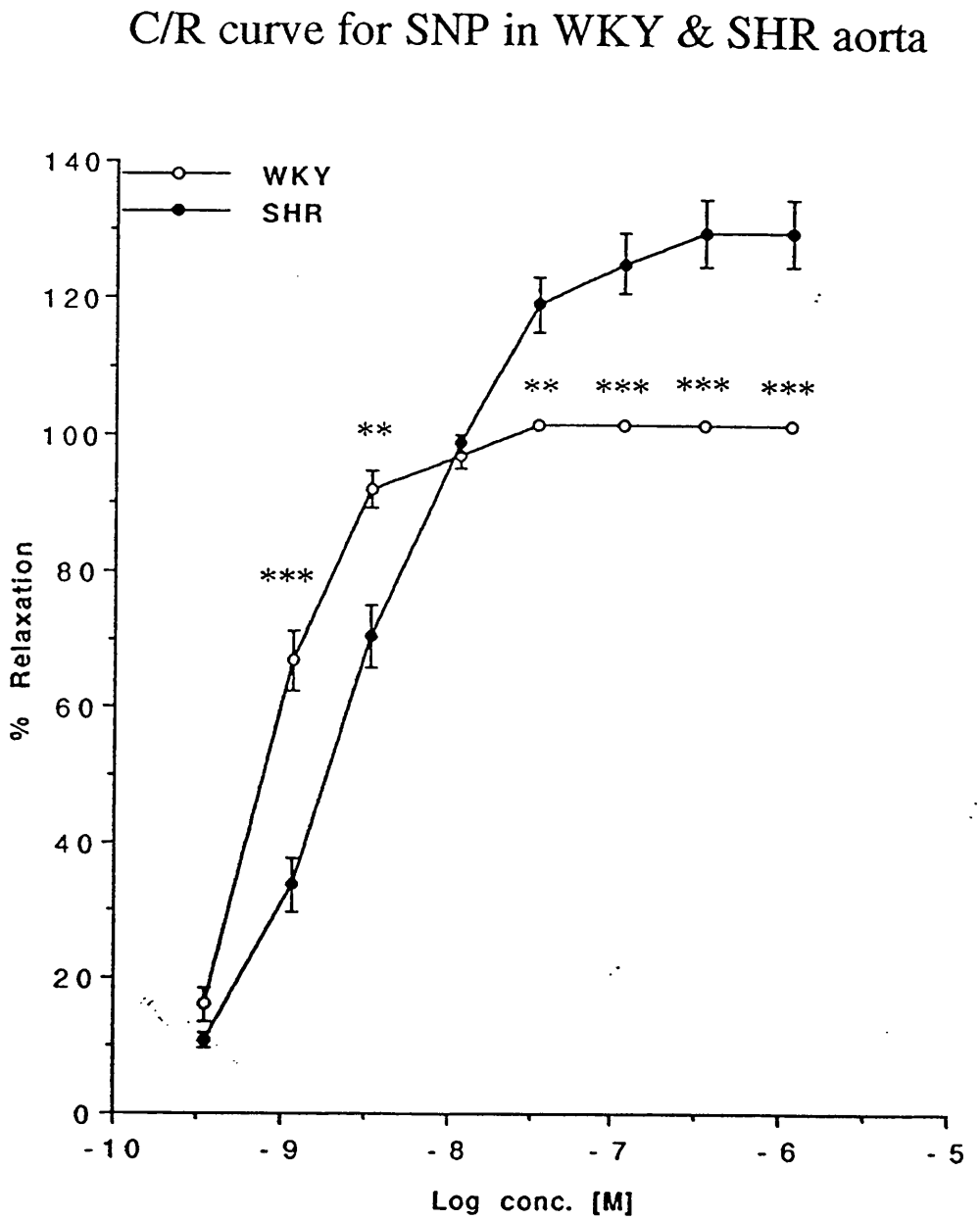

Fig. 3.17.
Concentration-response curves for SNP in aortic rings from SHR and WKY control rats. Muscle relaxation was recorded and expressed as a % of contraction elicited by NA (5.6×10^{-9}M). SNP at low concentrations induced significantly smaller relaxations in rings from SHR than in rings from WKY controls. In contrast, at higher concentrations SNP induced significantly greater relaxations in rings from SHR than in WKY controls. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=8).

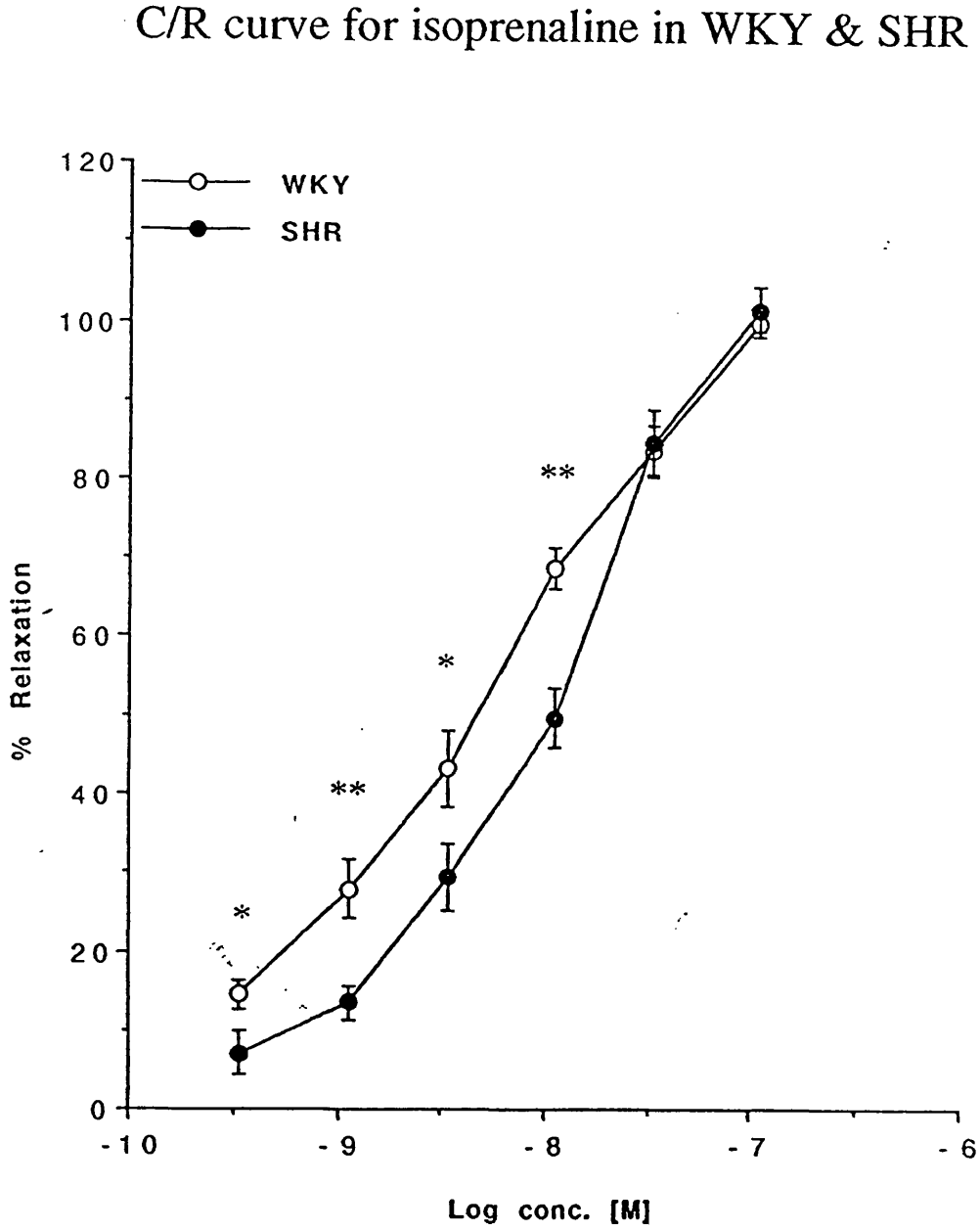

Fig. 3.18.
Concentration-response curves for isoprenaline in aortic rings from SHR and WKY control rats. Muscle relaxation was recorded and expressed as a % of the contraction elicited by NA (5.6×10^{-9} M). Isoprenaline at low concentrations induced significantly smaller relaxations in rings from SHR than in WKY controls, but at higher concentrations there was no significant difference between the relaxation of rings from SHR and WKY controls. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. ($n=8$).

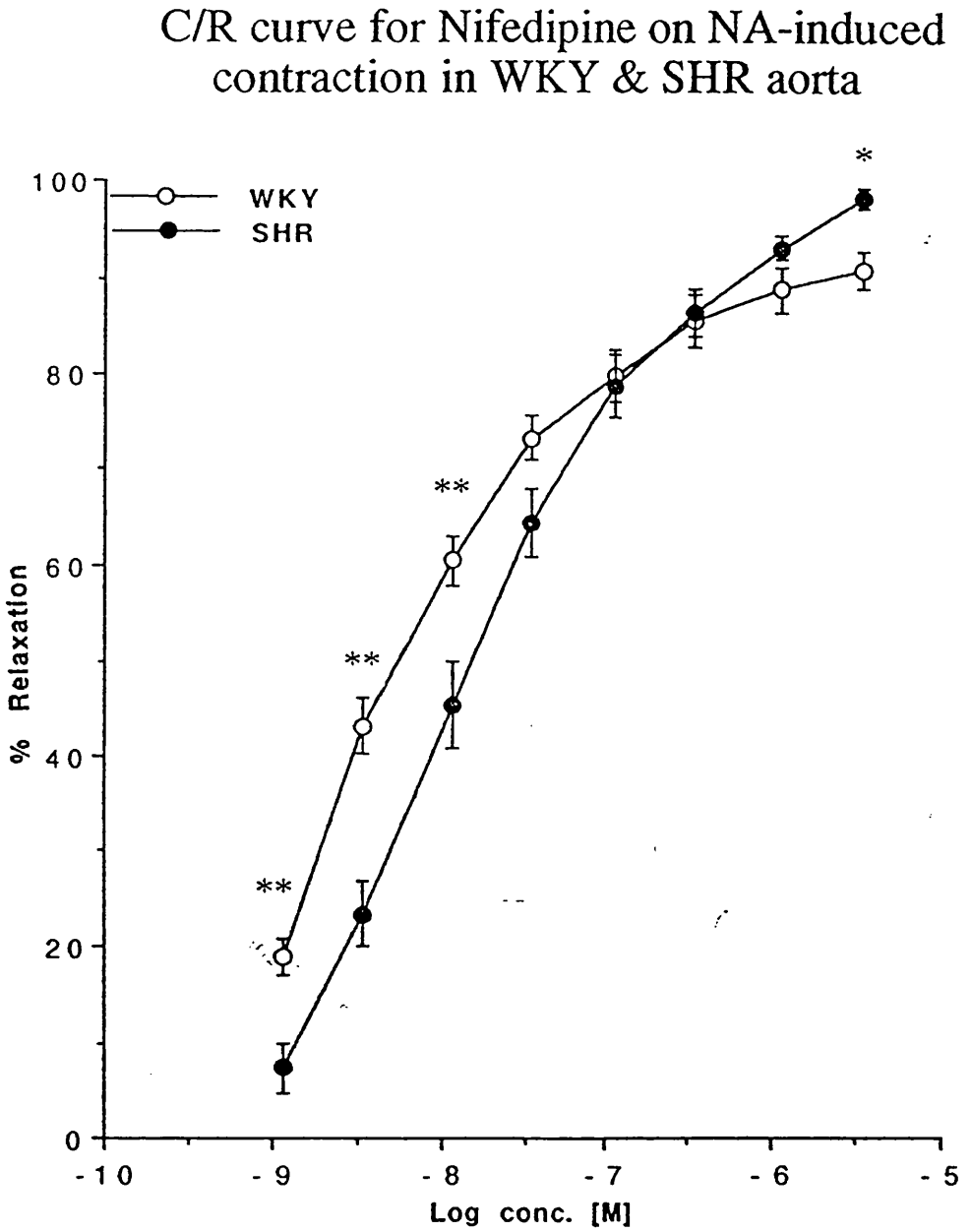

Fig. 3.19.
Concentration-response curves for nifedipine in aortic rings from SHR and WKY control rats. Muscle relaxation was recorded and expressed as a % of the contraction elicited by NA ($5.6x10^{-9}$ M). Nifedipine at low concentrations induced significantly weaker relaxations in rings from SHR than in those from WKY controls. In contrast, at higher concentrations nifedipine induced significantly greater relaxation in rings from SHR than in those from WKY rats (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=8).

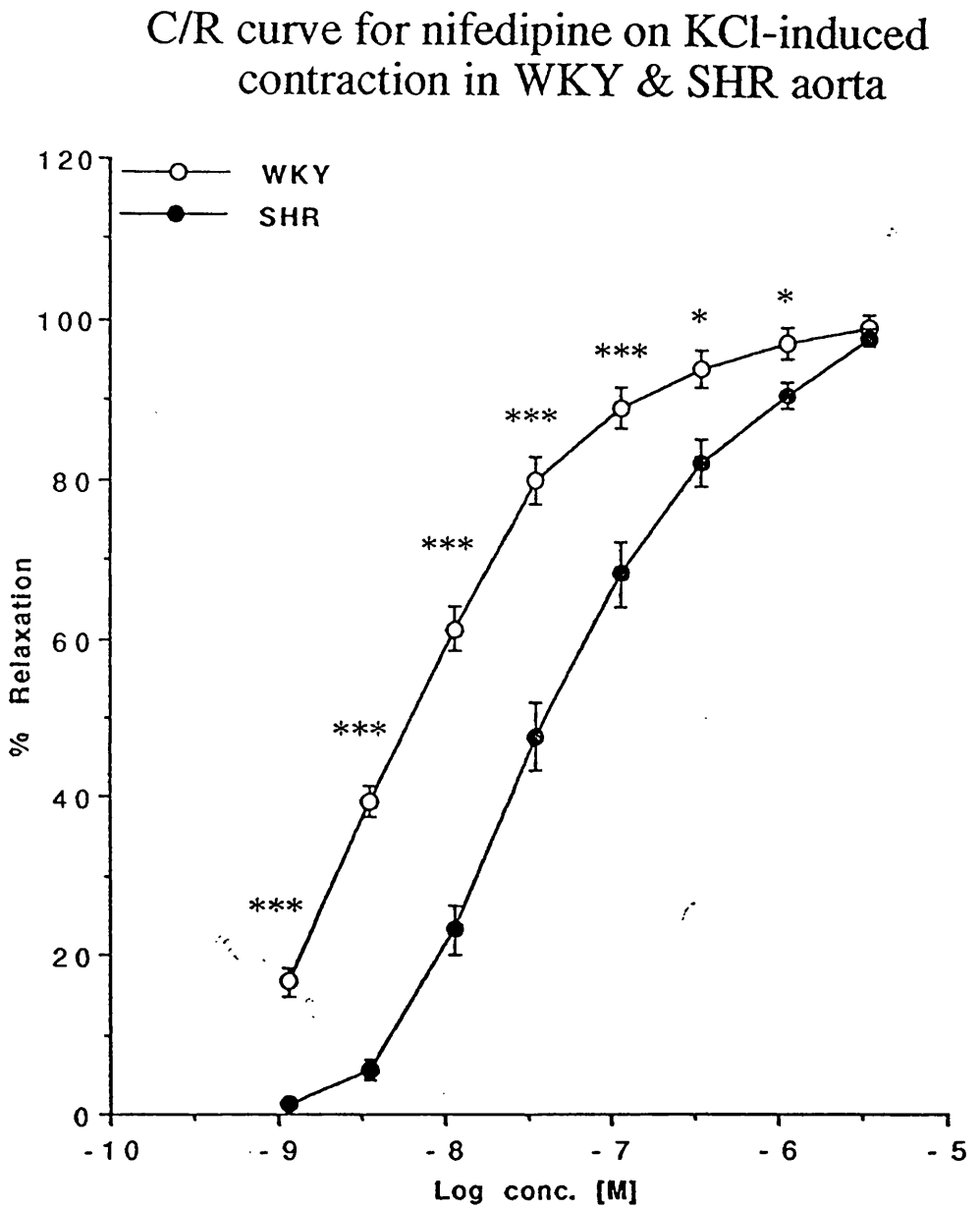

Fig.3.20.
Concentration-response curve for nifedipine in aortic rings from SHR and WKY control rats. Muscle relaxation was recorded and expressed as a % of the contraction elicited by KCl ($1.3x10^{-2}$ M). nifedipine at all concentrations except the highest concentration induced significantly smaller relaxations in rings from SHR than in those from WKY controls. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=8).

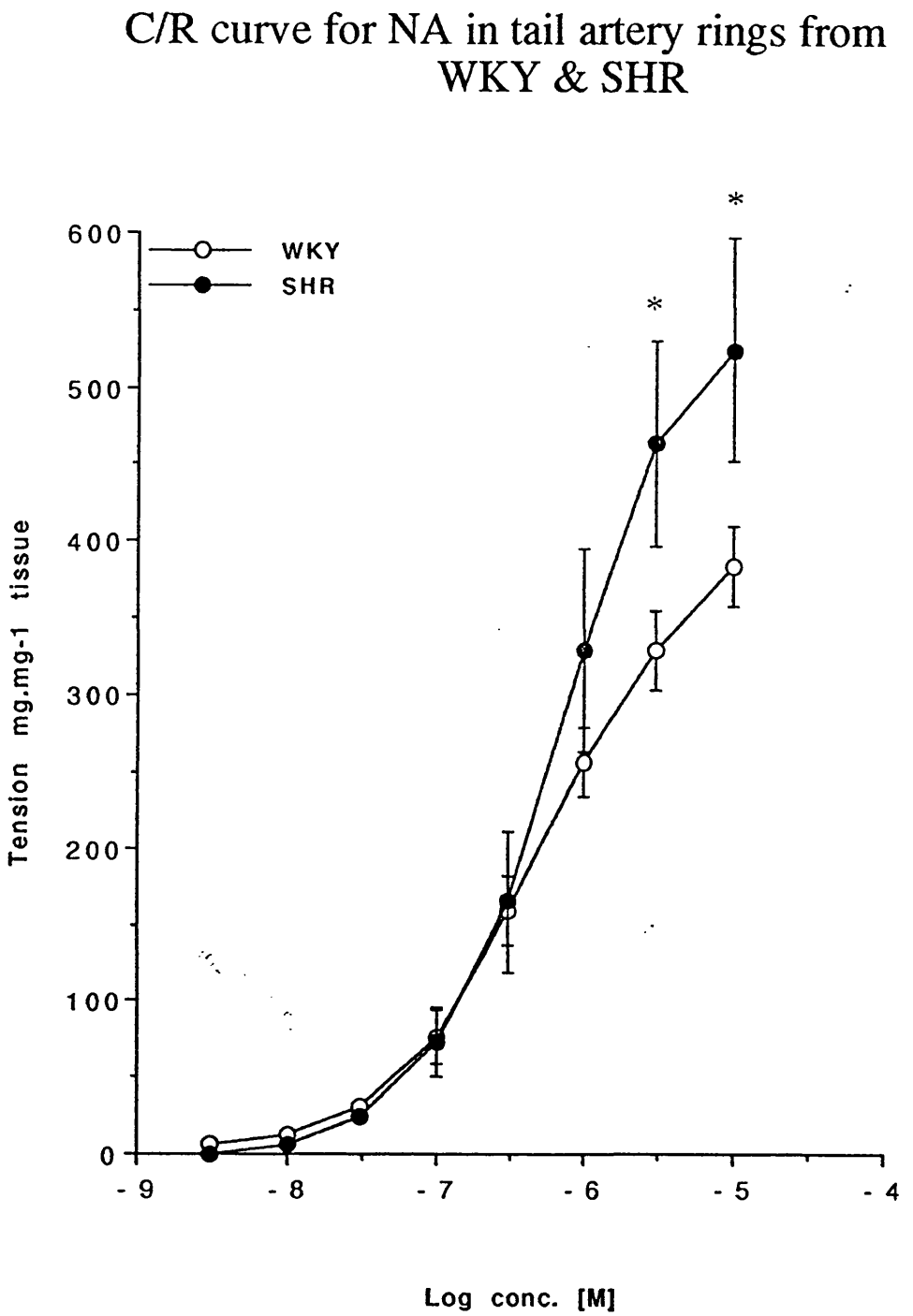

Fig. 3.21.
Concentration-response curves for NA in tail artery rings from SHR and WKY control rats. Muscle tension was recorded. Low concentrations of NA caused contractions that were not significantly different in rings from SHR and WKY controls. Higher concentrations of NA induced significantly greater contractions in rings from SHR than in those from WKY controls. (* $p<0.05$). Points shown are means $\pm$ s.e.mean. (n=12),

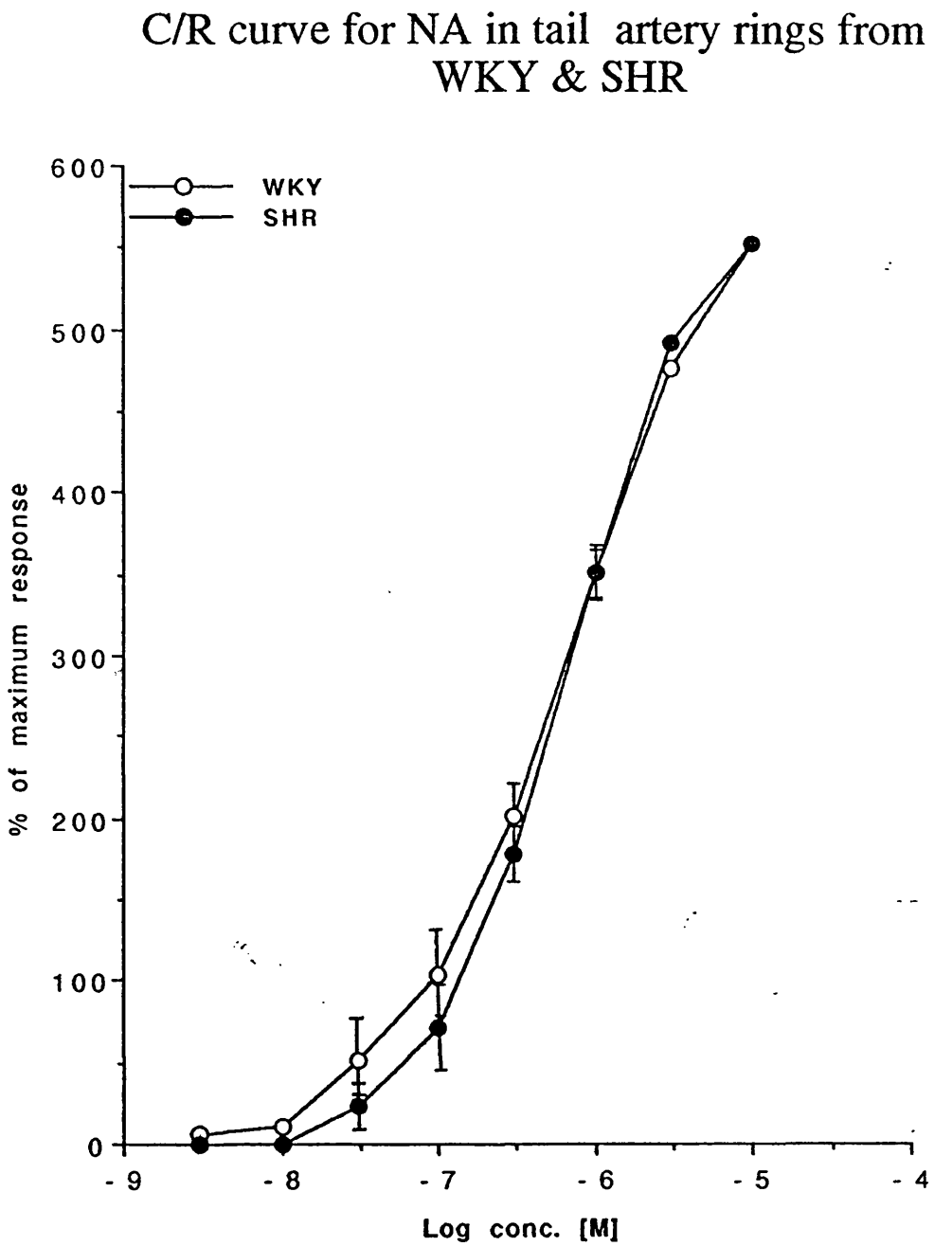

Fig. 3.22.
Concentration-response curves for NA in tail artery rings from SHR and WKY control rats. Muscle tension was recorded. Results are expressed as percentages of the maximum response. There was no significant difference between the NA-induced responses obtained in rings from SHR and from WKY controls. Points are means $\pm$ s.e.mean. (n=12).

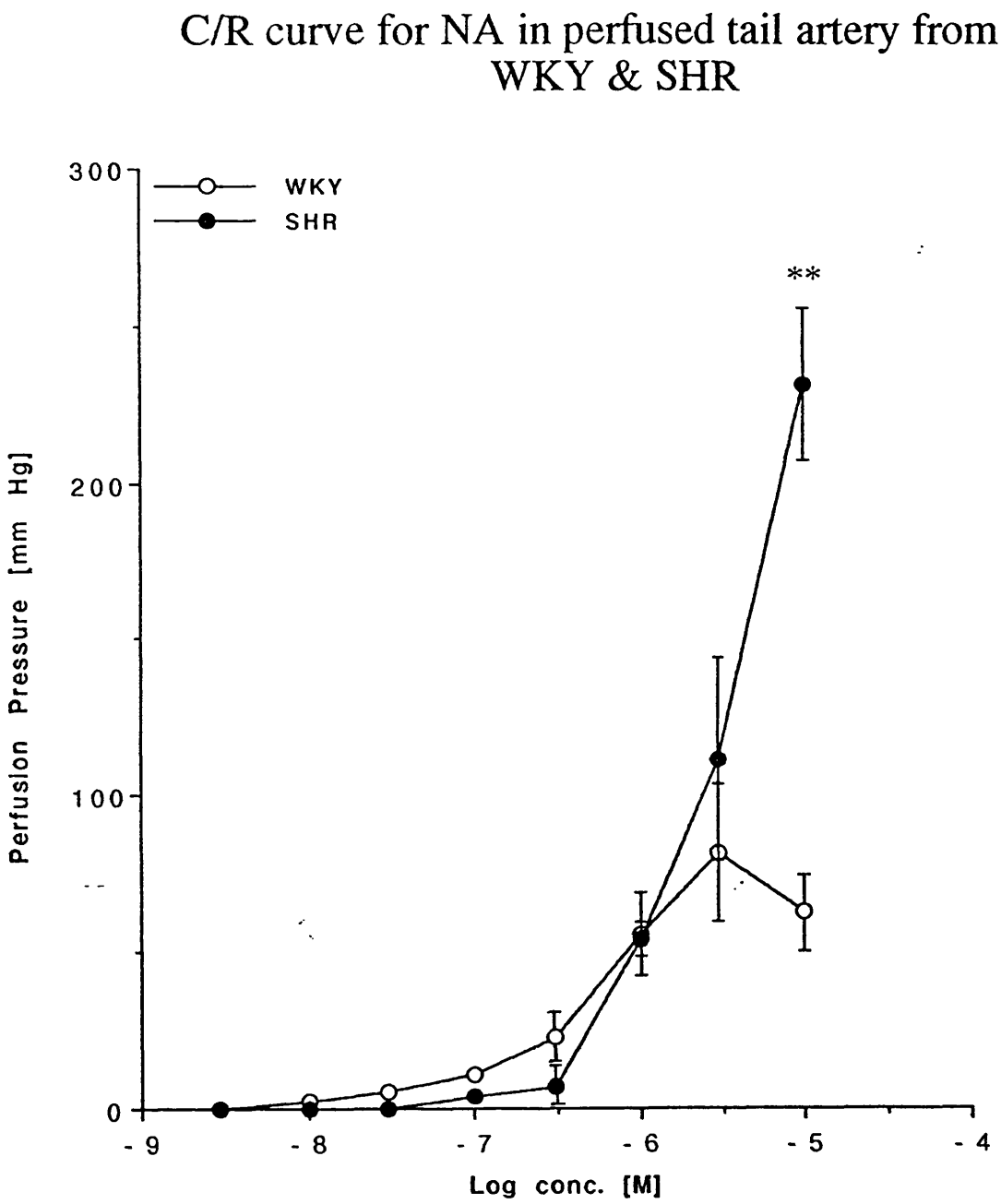

Fig. 3.23.
Concentration-response curves for NA in perfused tail artery segments from SHR and WKY control rats. Perfusion pressure was recorded and expressed in mm Hg. NA at low concentrations produced similar responses in arteries from SHR and WKY controls, but at higher concentrations NA produced significantly greater responses in segments from SHR than in those from WKY controls. (** $p<0.01$). Points are means $\pm$ s.e.mean. (n=8).

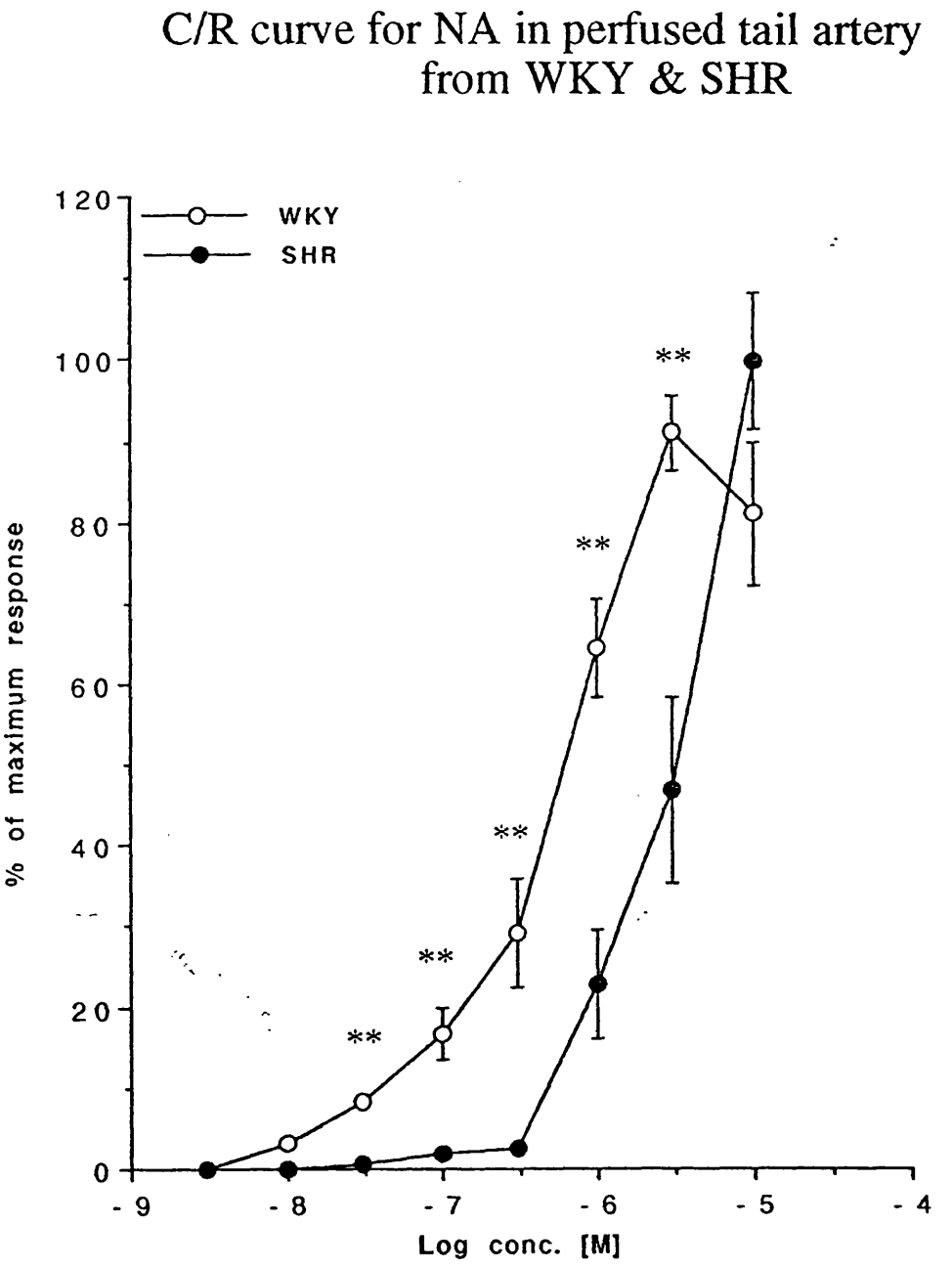

Fig. 3.24.
Concentration-response curves for NA in perfused tail artery segments from SHR and WKY control rats. Perfusion pressure was recorded and expressed as percentages of the maximum response. NA at all concentrations except the highest, produced significantly smaller responses in segments from SHR than in those WKY controls. (** $p<0.01$). Points are means $\pm$ s.e.mean. (n=8).

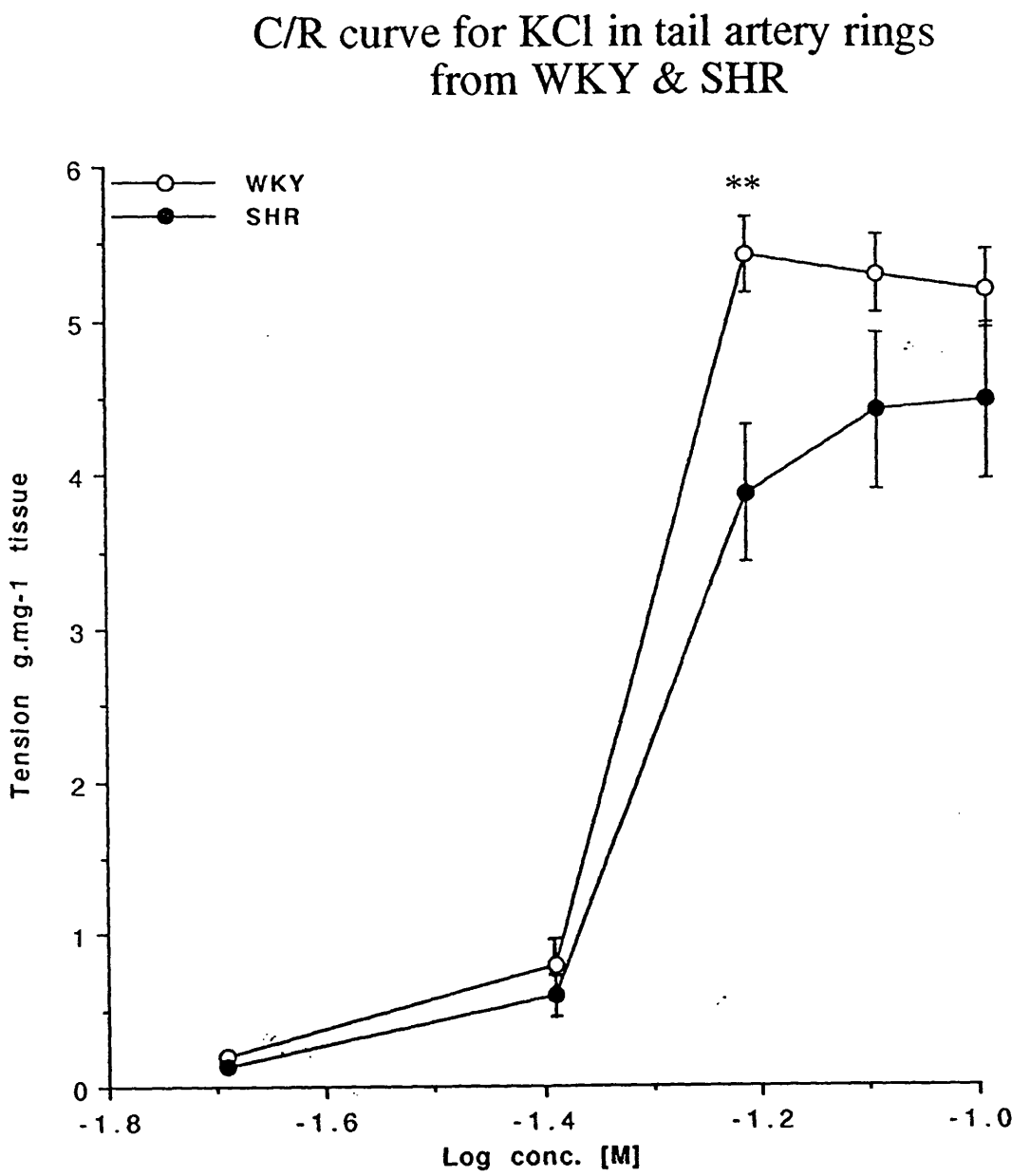

Fig. 3.25.
Concentration-response curves for KCl in tail artery rings from SHR and WKY control rats. Muscle tension was recorded. Apart from the responses obtained at one concentration, responses to KCl, were not significantly different in rings from SHR and WKY controls. (** $p<0.05$). Points are means $\pm$ s.e.mean. ($n=12$).

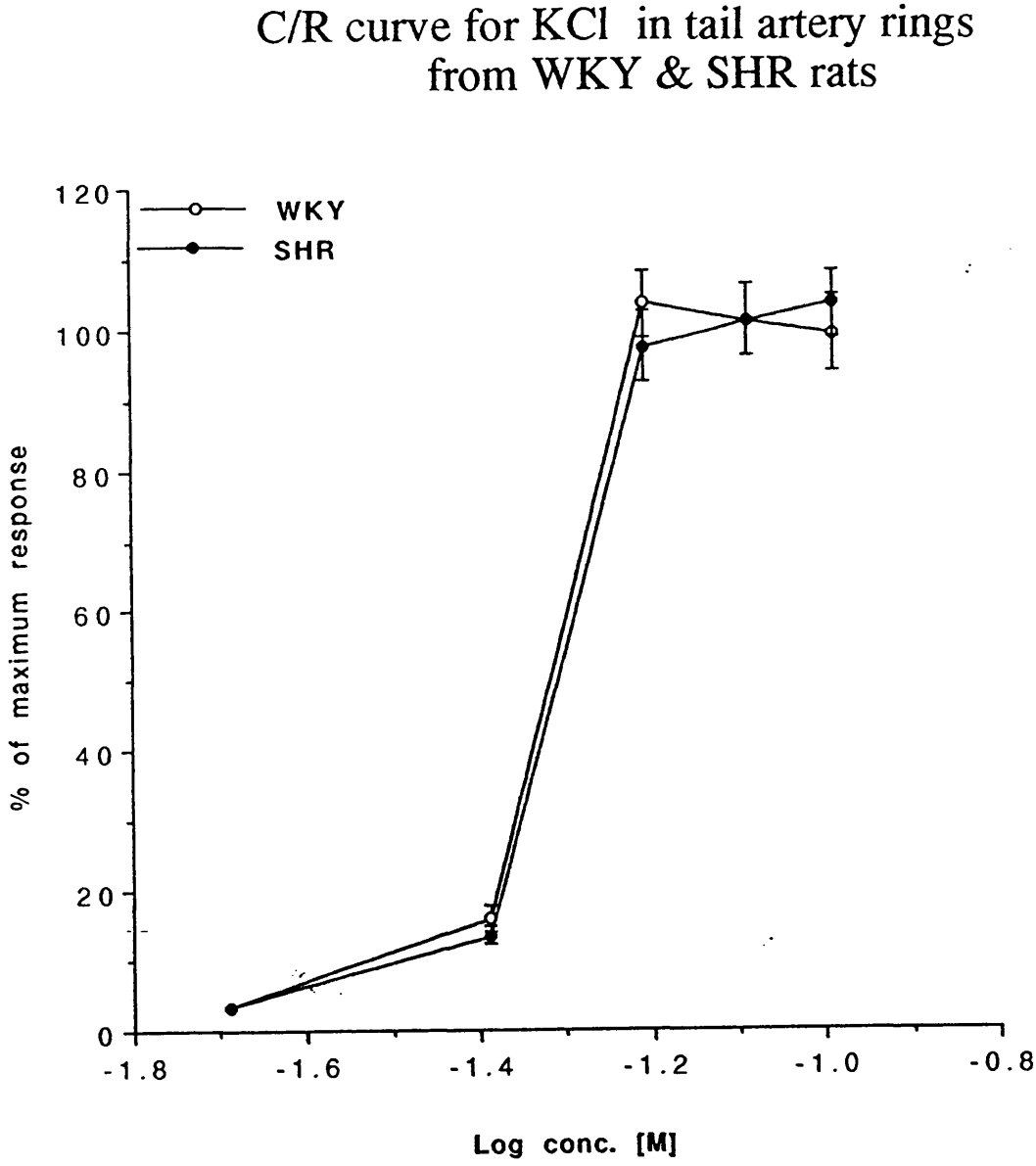

Fig. 3.26.
Concentration-response curves for KCl in tail artery rings from SHR and WKY control rats. Muscle tension was recorded. Results were expressed as percentages of the maximum response. Responses to NA were not significantly different in rings from SHR and WKY controls. Points are means ± s.e.mean. (n=12).

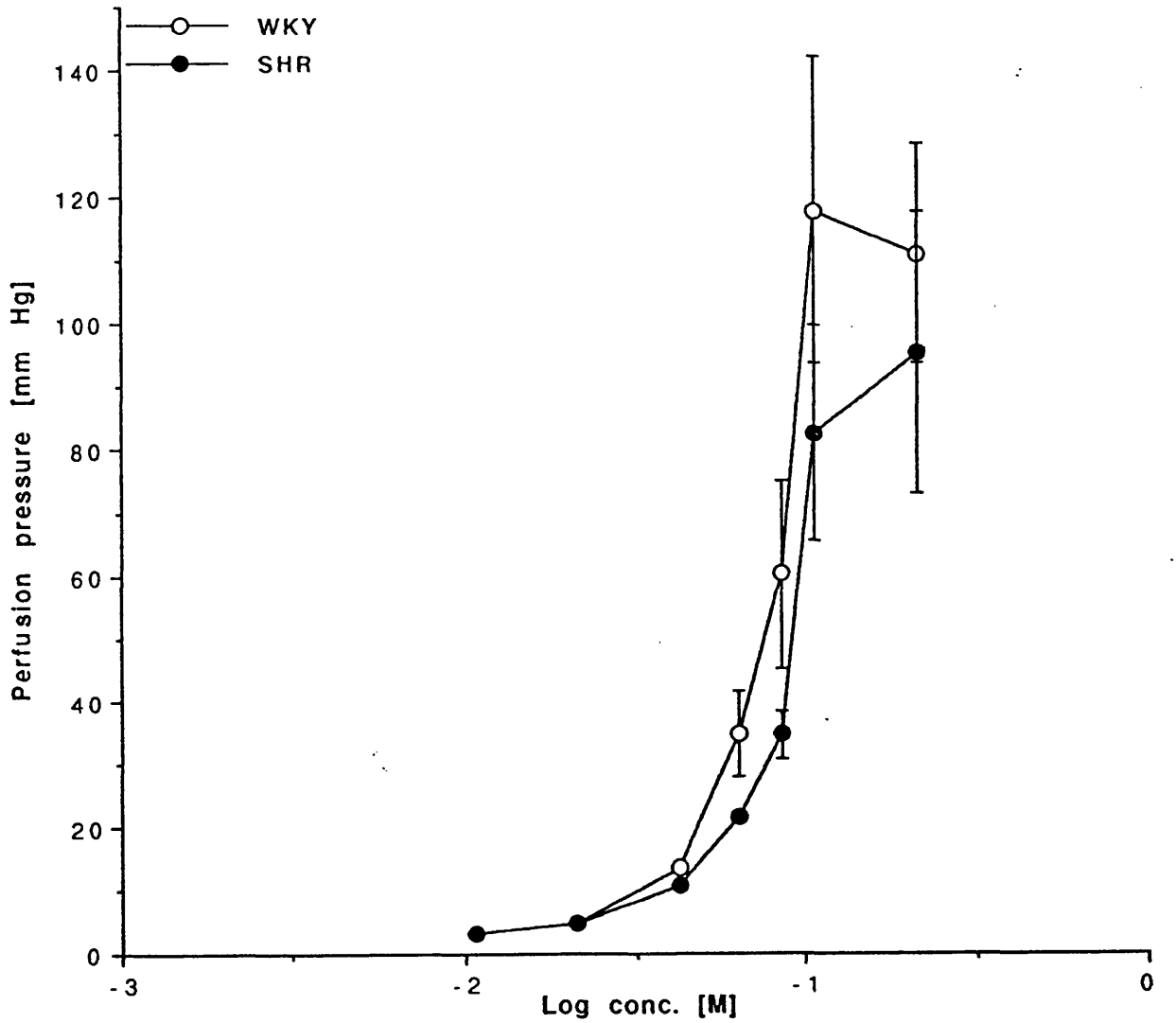

Fig. 3.27.
Concentration-response curves for KCl in perfused tail artery segments from SHR and WKY control rats. Perfusion pressure was recorded and responses to KCl were expressed in mm Hg. Responses to NA were not different in rings from SHR and WKY controls. Points are means ± s.e.mean. (n=8).

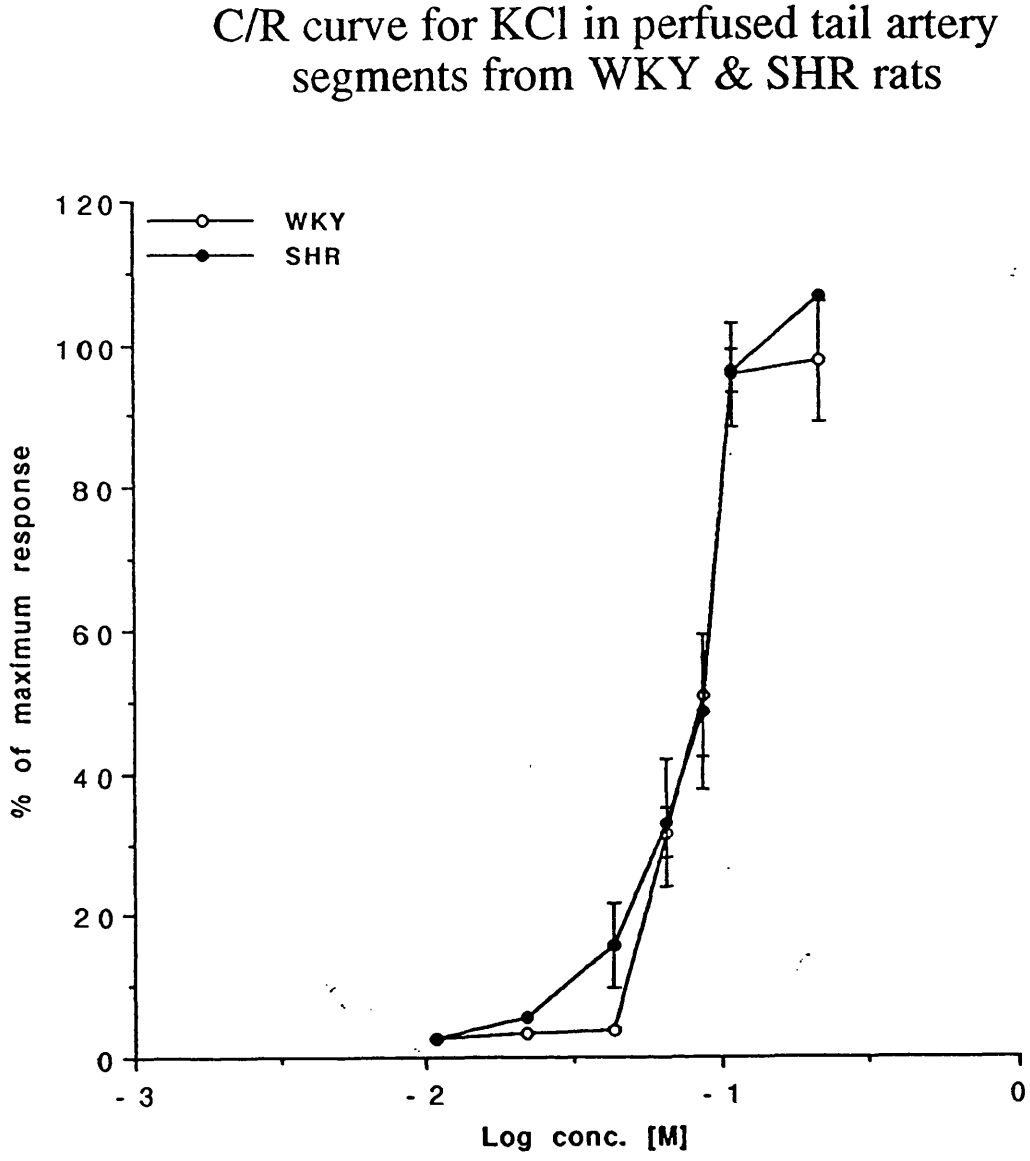

Fig. 3.28.
Concentration-response curves for KCl in perfused tail artery segments from SHR and WKY control rats. Perfusion pressure was recorded and responses were expressed as percentages of the maximum response. KCl-induced responses were not significantly different in arteries from SHR than in WKY controls. Points shown are means $\pm$ s.e.mean. (n=8).

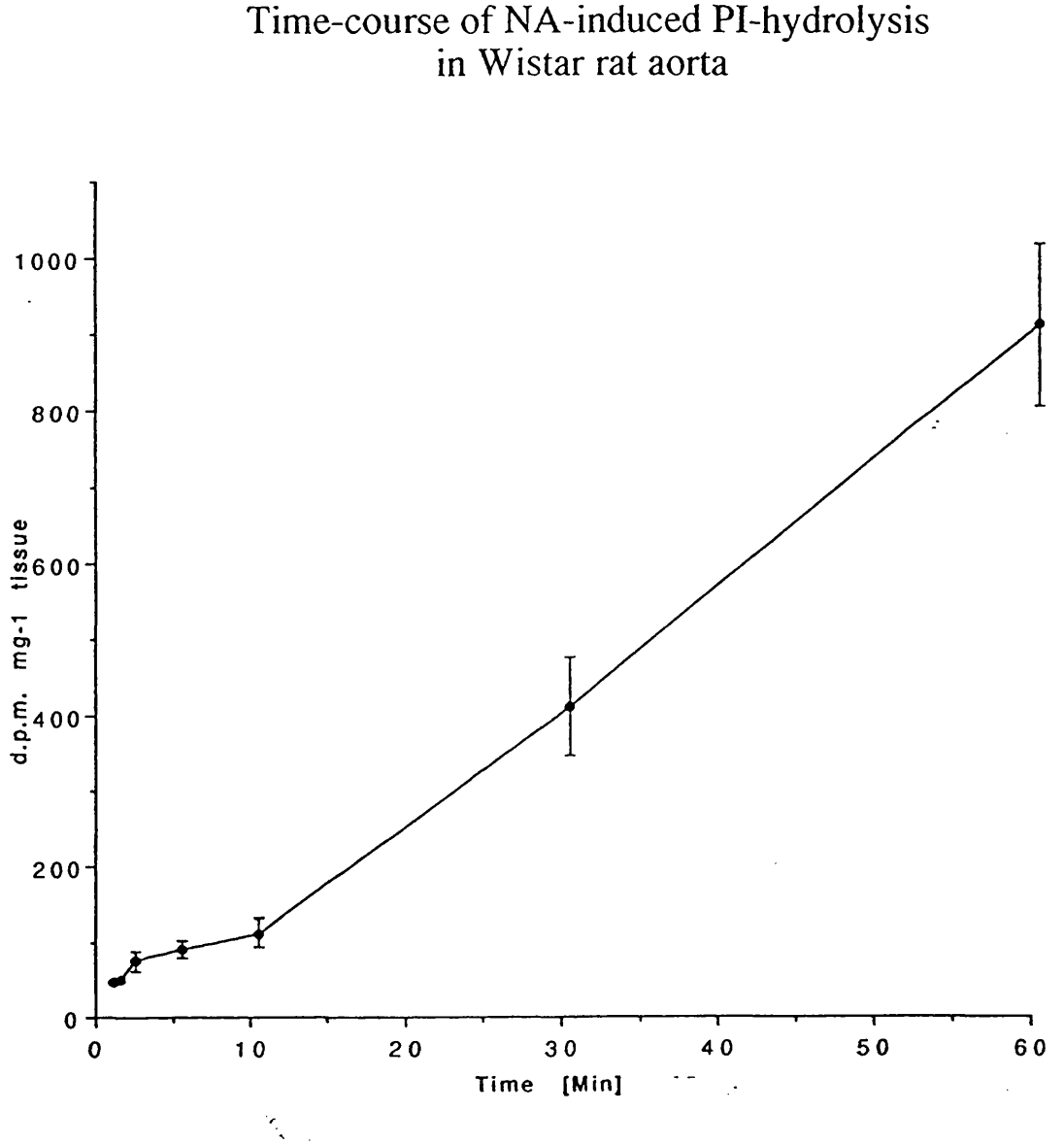

Fig.3.29.
Time-course of NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored and expressed as disintegrations per minute (d.p.m.) mg weight of tissue. The time-course of the effect of NA (10^{-4}M) is shown. Points are means $\pm$ s.e.mean. (n=8).

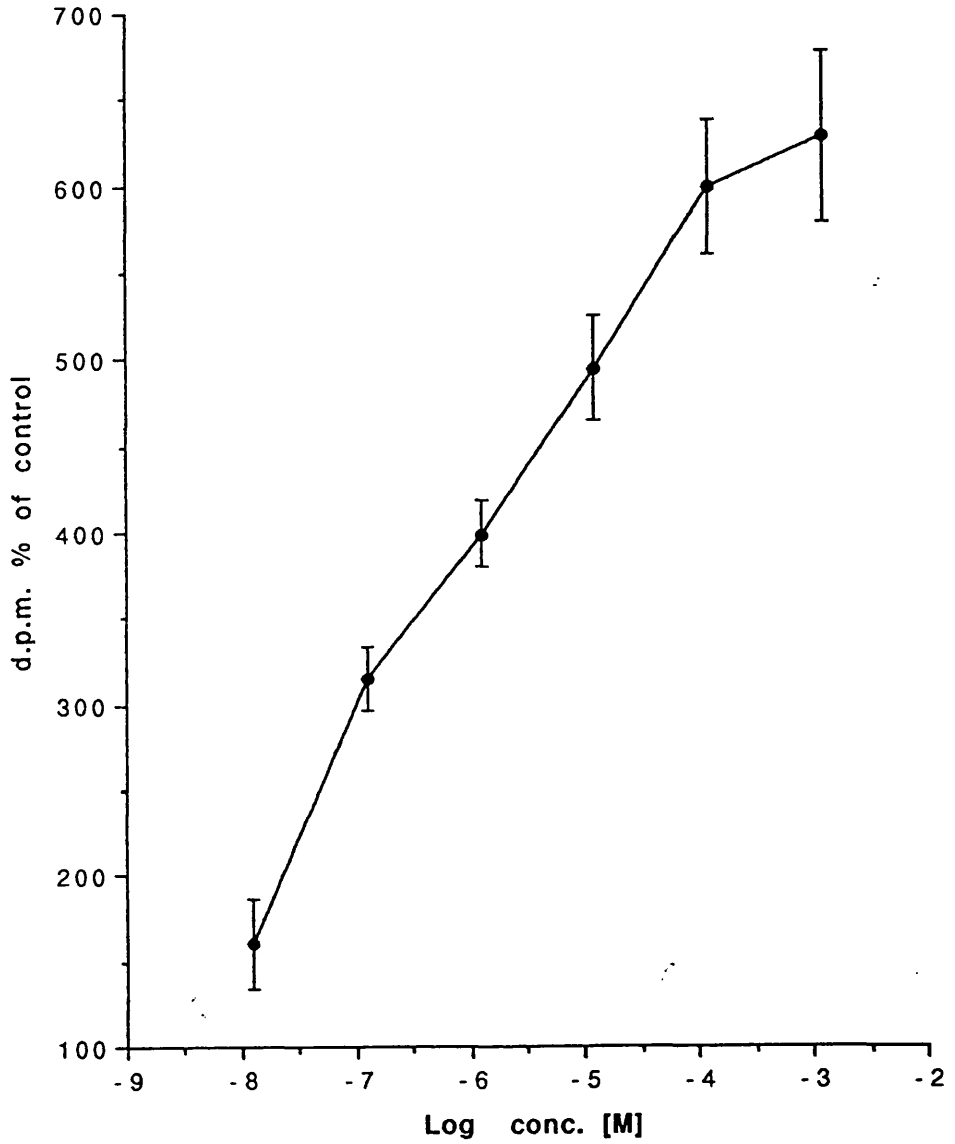

Fig. 3.30.
Concentration-response curve for NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates and expressed as percentages of the control (basal) PI-response. Points are means ± s.e.mean. (n=8).

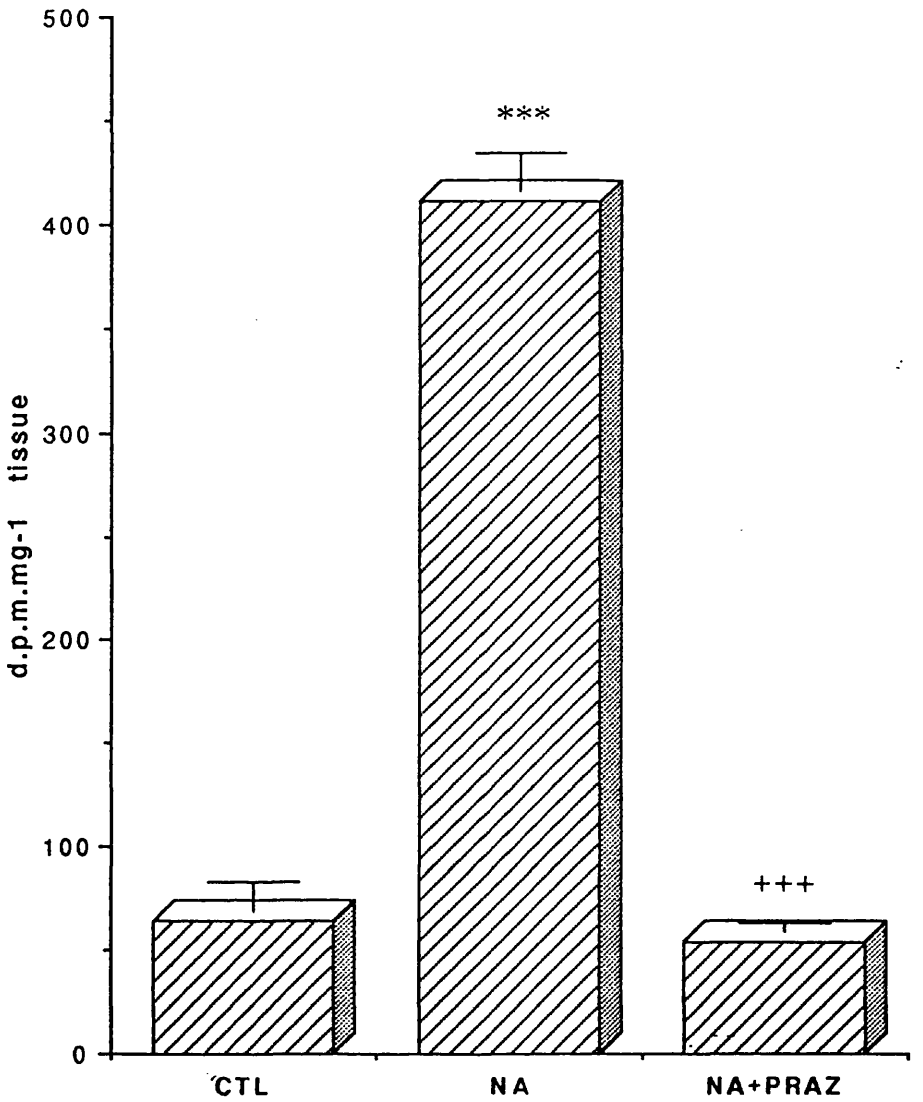

Fig. 3.31.
Effect of prazosin on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control (CTL) level. (*** $p<0.001$). Prazosin (PRAZ, 1×10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis to the control level.(+++ $p<0.001$). Results are means $\pm$ s.e.mean. ($n=8$).

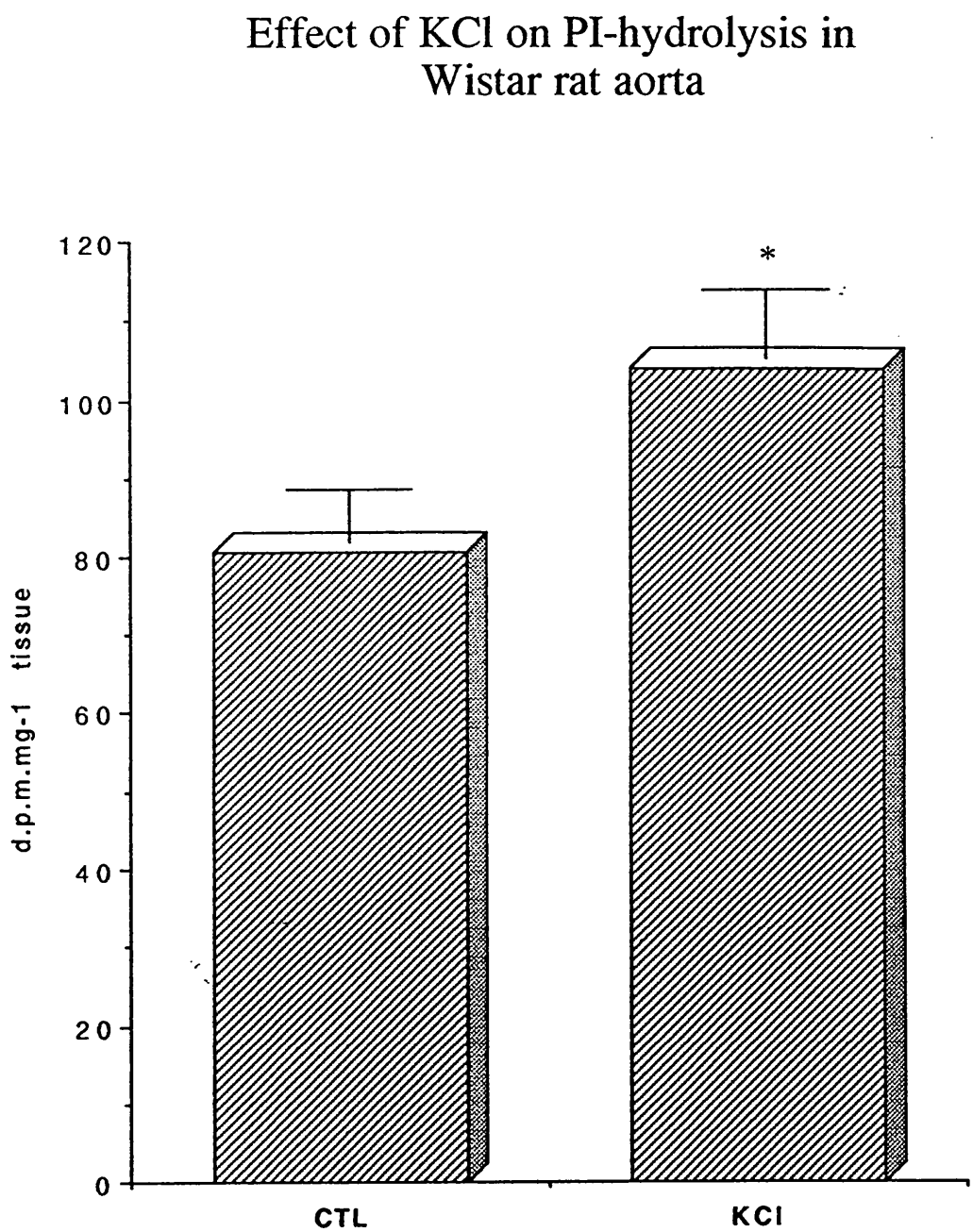

Fig. 3.32.
Effect of KCl on PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. KCl (30mM) significantly increased the PI-hydrolysis above the control level. (* $p<0.05$). Results are means $\pm$ s.e.mean. ($n=12$).

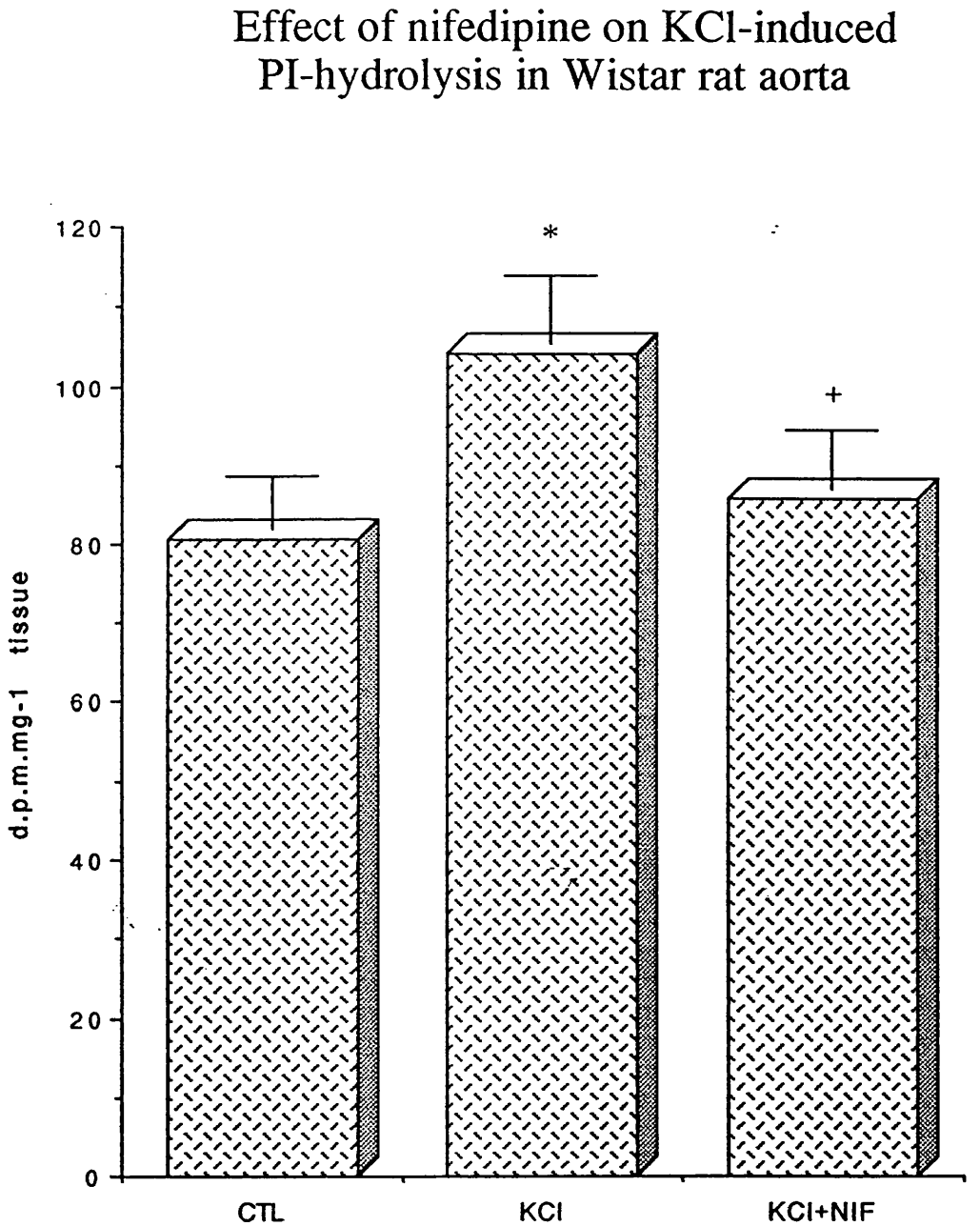

Fig. 3.33.
Effect of nifedipine on KCl-induced PI-hydrolysis in aortic rings from Wistar rats. The PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. KCl (30mM) significantly increased PI-hydrolysis above control levels. (* $p<0.05$). In the presence of nifedipine (NIF, 10^{-6} M), the effect of KCl was significantly inhibited. (+ $p<0.05$). Results are means $\pm$ s.e.m. (n=10).

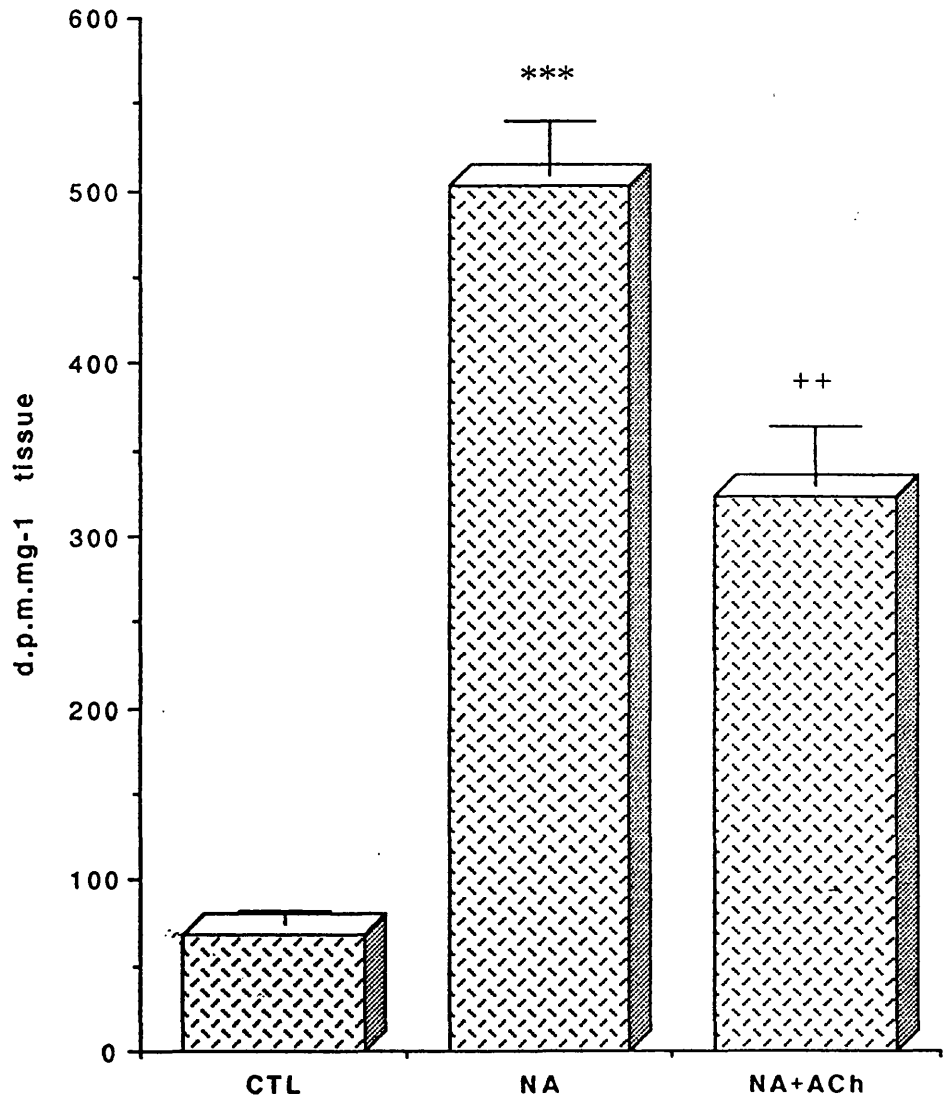

Fig. 3.34.
Inhibitory effect of ACh on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control level. (*** $p<0.001$). ACh significantly inhibited the NA-induced PI-hydrolysis. (++ $p<0.01$). The endothelium was intact in these experiments. Results are means $\pm$ s.e.m. ($n=8$).

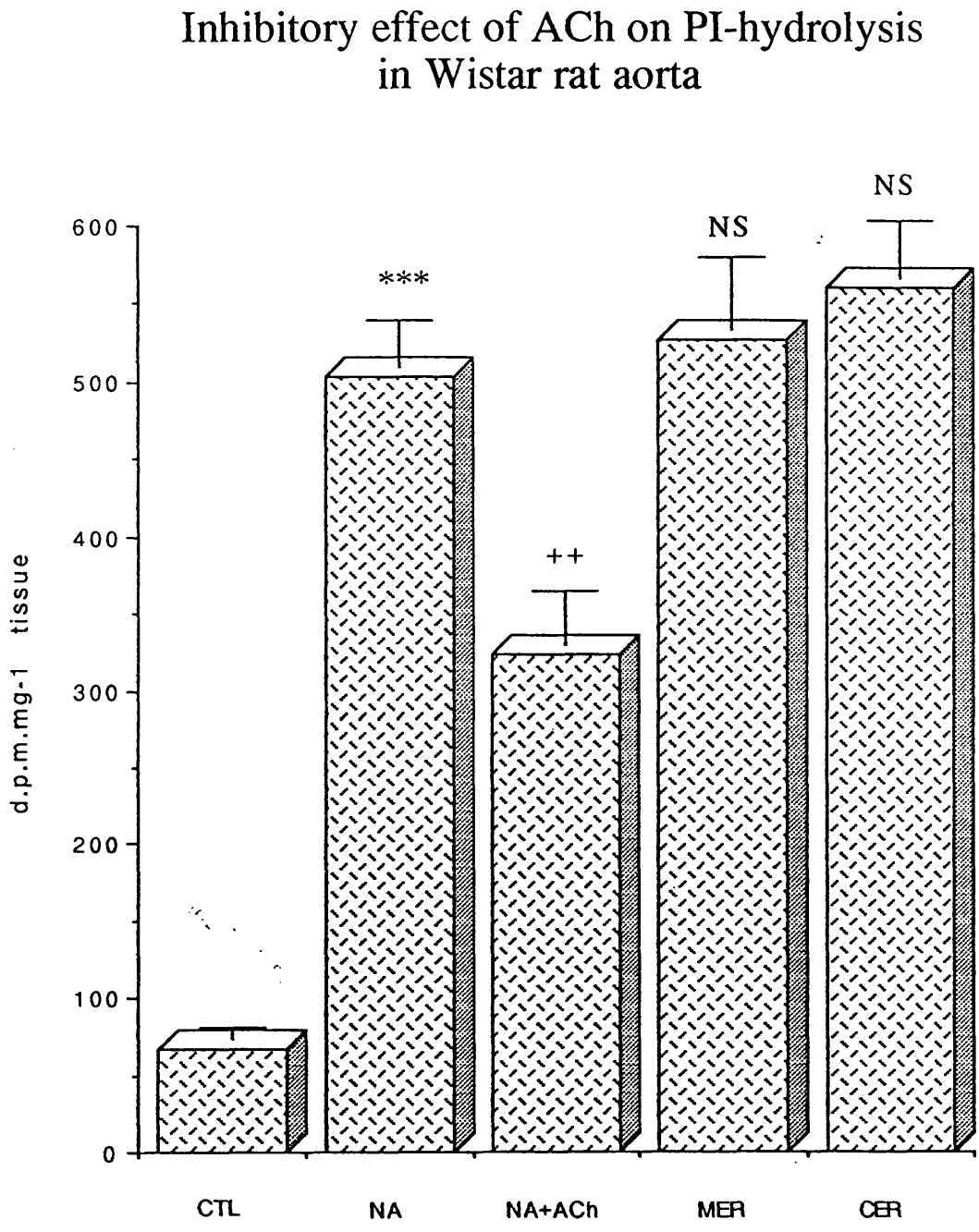

Fig. 3.35.
Inhibitory effect of ACh on NA-induced PI-hydrolysis in the presence and absence of endothelium in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. NA ($6.2x10^{-6}$M) significantly increased PI-hydrolysis above control levels. (*** $p<0.001$). ACh (10^{-5}M) significantly inhibited NA-induced PI-hydrolysis. (++ $p<0.01$). When the endothelium was removed either mechanically (MER) by rubbing or chemically (CER) with Triton X-100 (0.1% V/V), the inhibitory effect of ACh (10^{-5}M) was completely abolished. (NS). Results are means $\pm$ s.e.mean. (n=8).

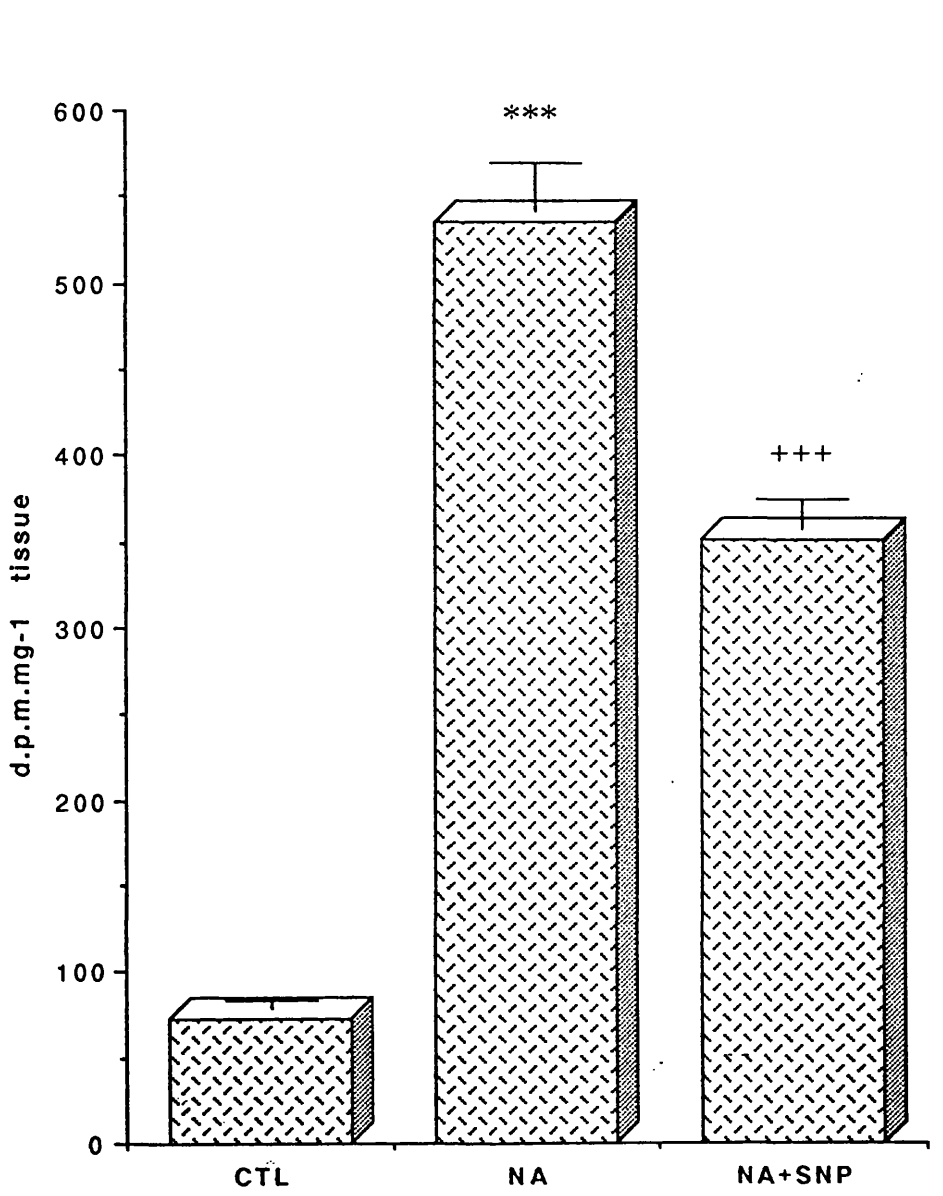

Fig. 3.36.
Inhibitory effect of SNP on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA ($6.2x10^{-6}$M) significantly increased PI-hydrolysis above the control levels. (*** $p<0.001$). SNP (10^{-6}M) significantly inhibited NA-indued PI-hydrolysis (+++ $p<0.001$). Results are means $\pm$ s.e.mean. (n=10)

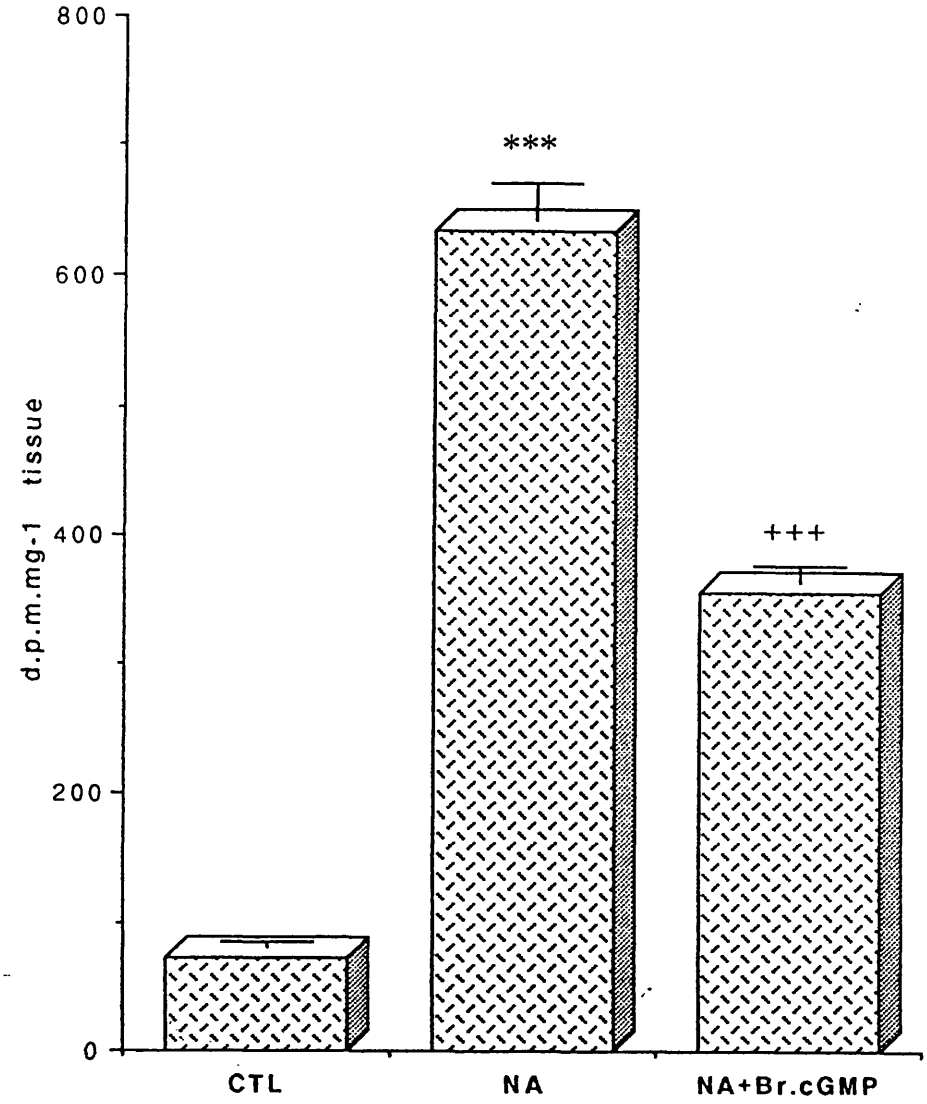

Fig. 3.37.
Inhibitory effect of 8-Bromo-cGMP on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p<0$ 001). 8-Bromo-cGMP (0.3mM) significantly inhibited the NA-induced PI-hydrolysis (+++ $p<0.001$). Results are means $\pm$ s.e.mean. (n=8).

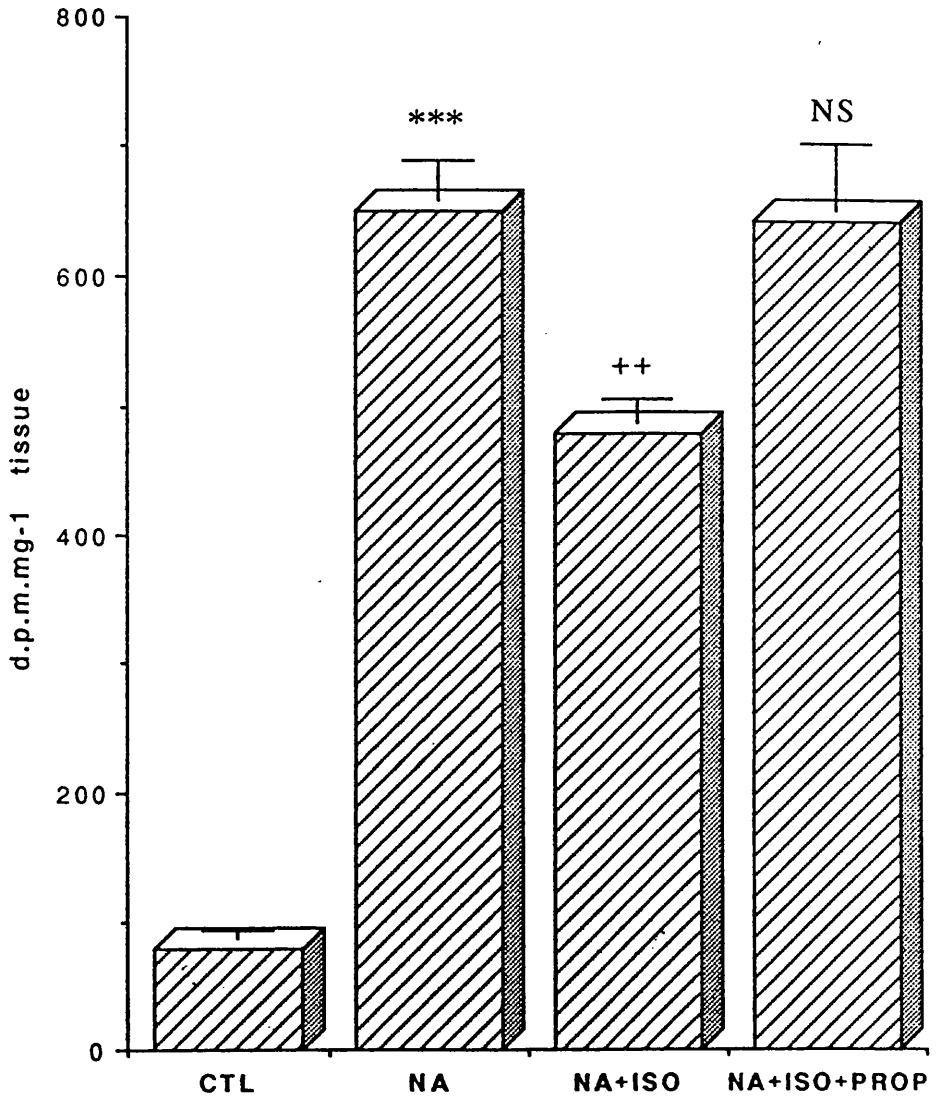

Fig. 3.38.
Inhibitory effect of isoprenaline on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $P < 0.001$) Isoprenaline (ISO, 10^{-8}M) significantly inhibited the NA-induced PI-hydrolysis (++ $p < 0.01$). This inhibitory effect was abolished by propranolol (5×10^{-6}M). (NS). Results are means $\pm$ s.e.mean. (n=8).

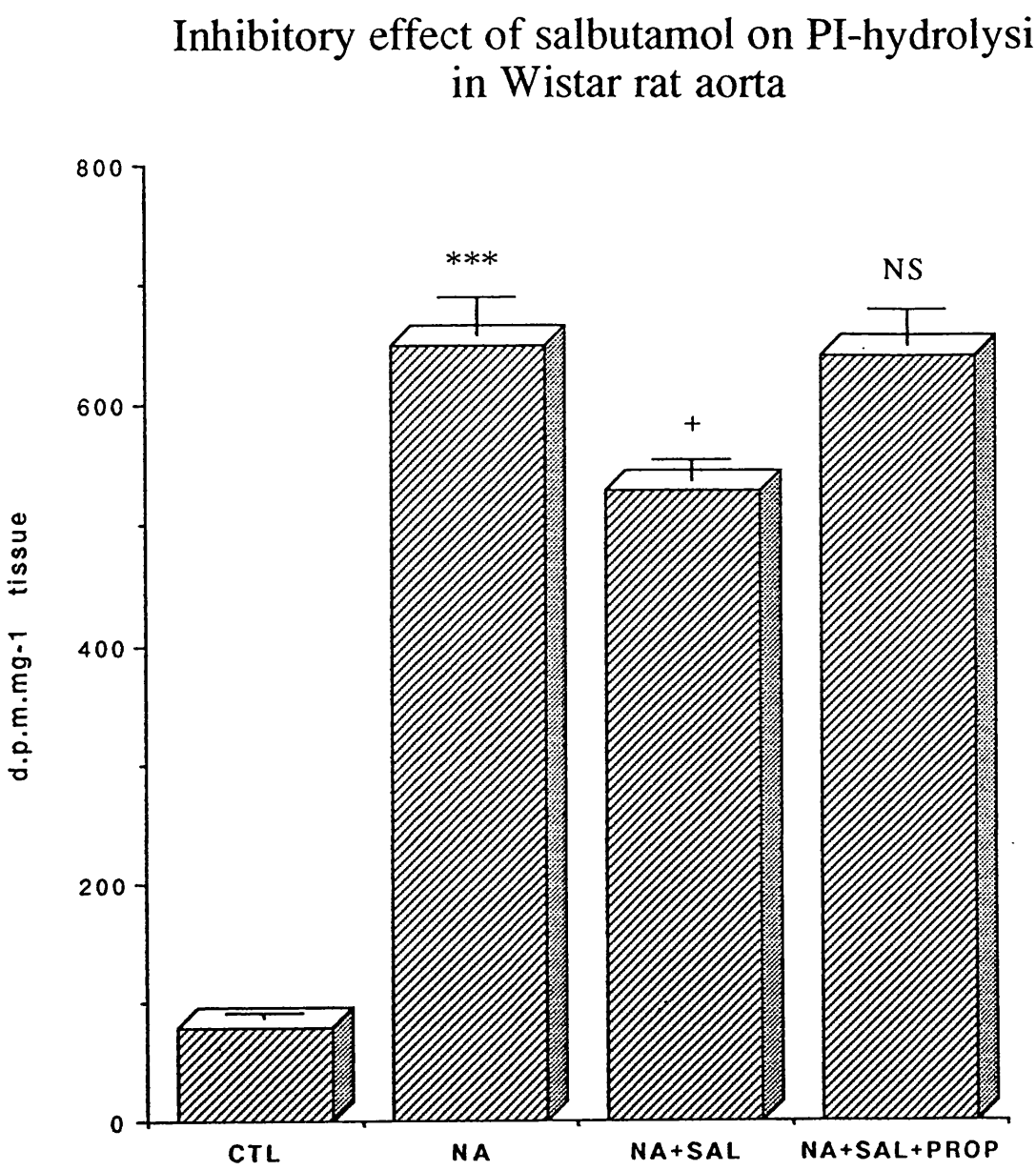

Fig. 3.39.
Inhibitory effect of salbutamol on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p<0.001$). Salbutamol (SAL, 10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis. (+ $p<0.05$). This inhibitory effect of SAL was completely abolished by propranolol (PROP, 5×10^{-6}M). Results are means $\pm$ s.e.mean. (n=8).

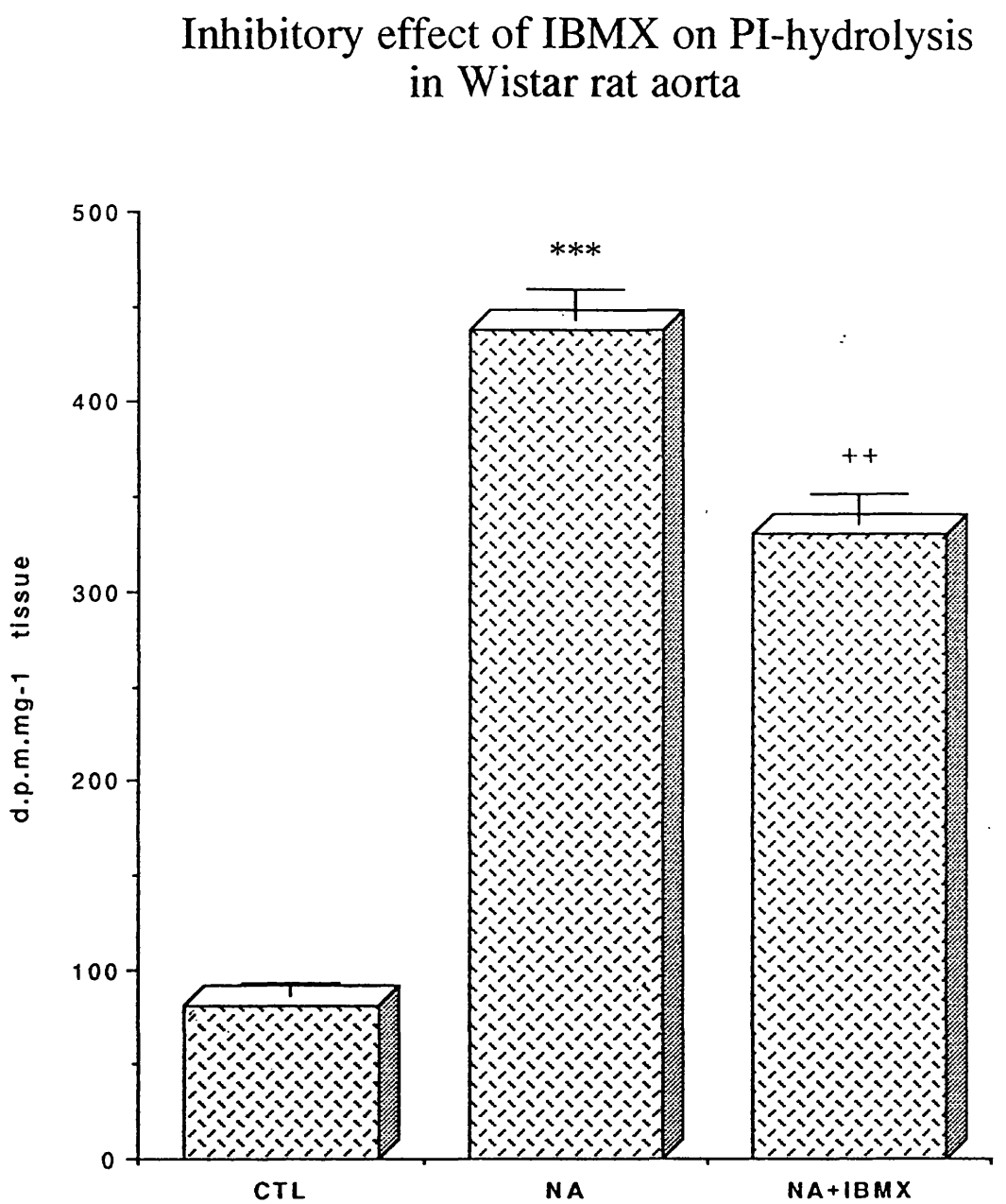

Fig. 3.40.
Inhibitory effect of isobutylmethylxanthine (IBMX) on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels (*** $p < 0.001$). IBMX (10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis (++ $p < 0.01$). Results are means ± s.e.mean. (n=8).

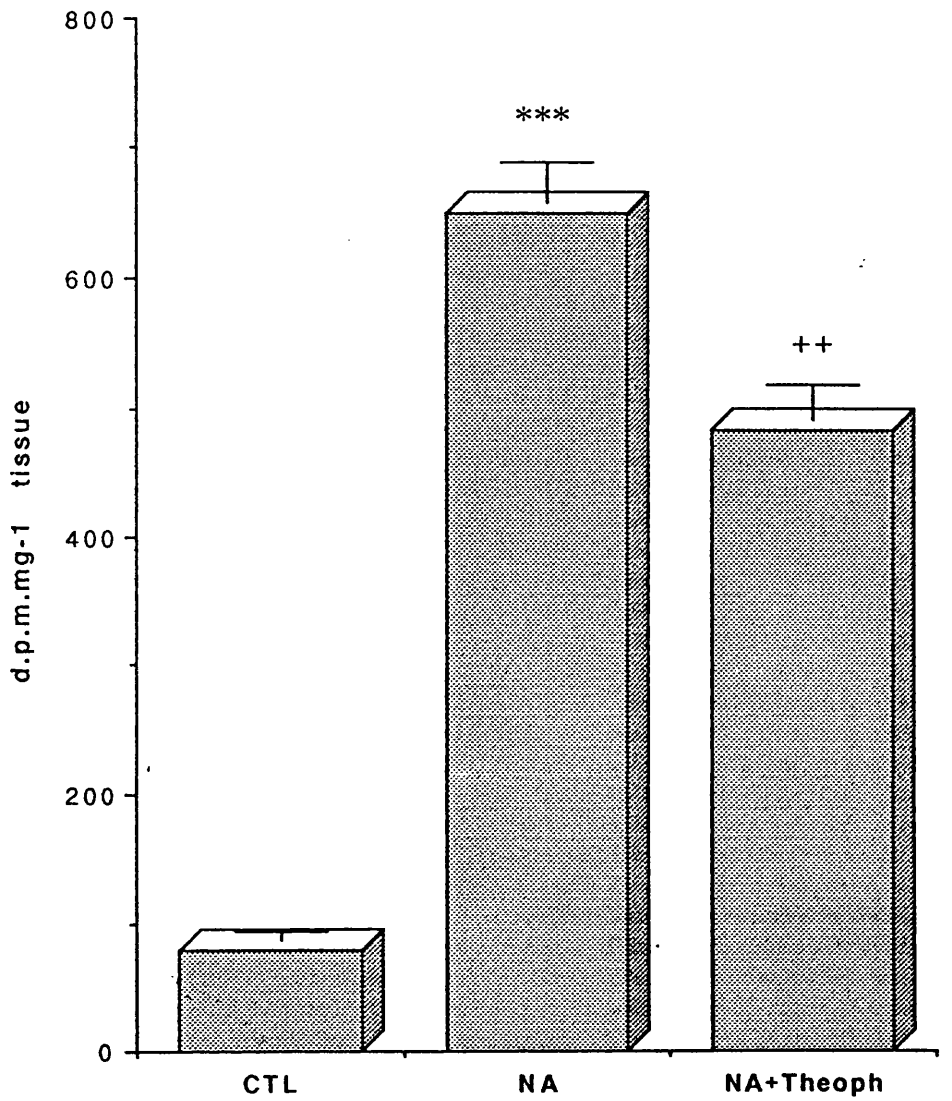

Fig. 3.41.
Inhibitory effect of theophylline on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p<0.001$). Theophylline (5×10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis (++ $p<0.01$, *** $p<0.001$). Results are means $\pm$ s.e.mean. (n=8).

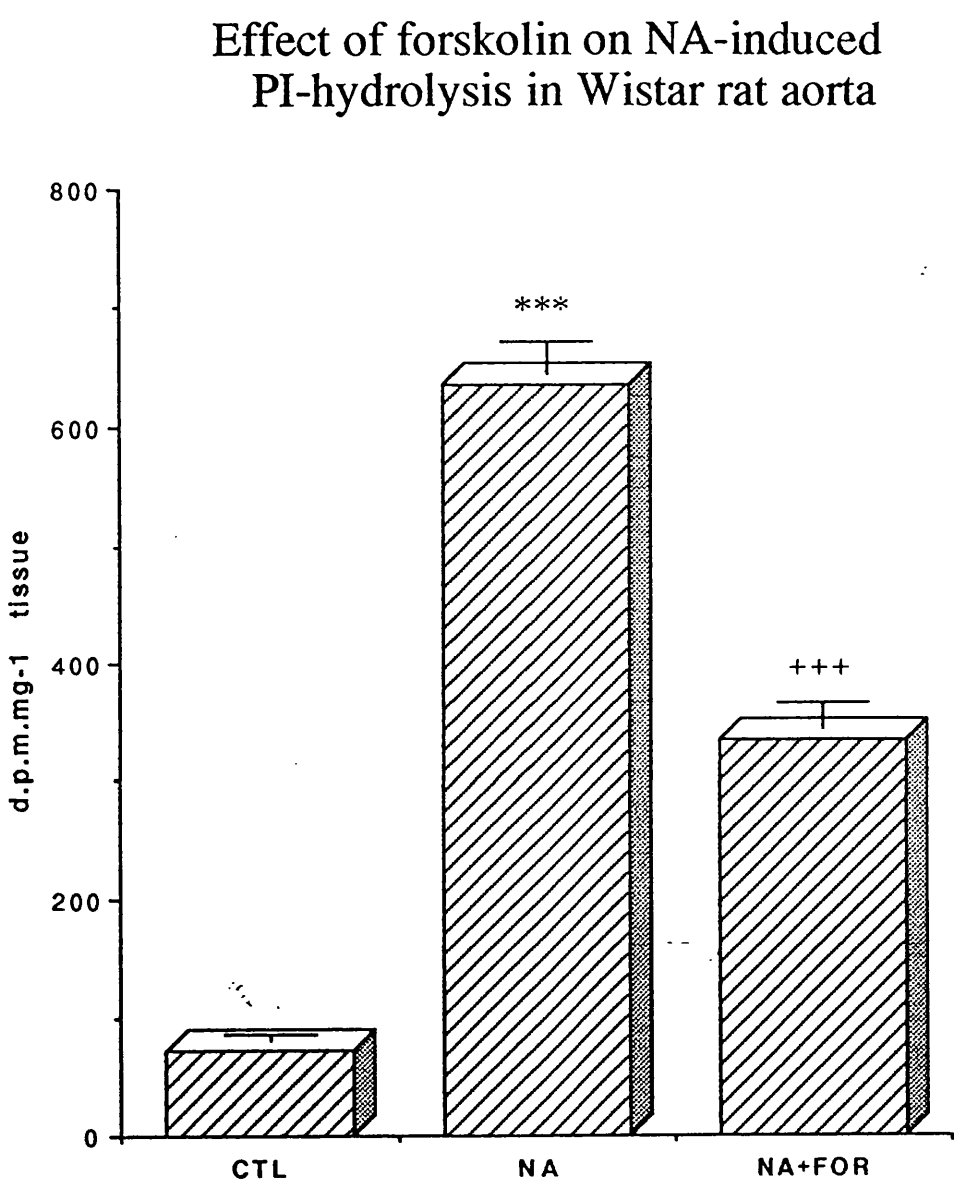

Fig. 3.42.
Inhibitory effect of forskolin on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p<0.001$). Forskolin (FOR, 10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis (+++ $p<0.001$). Results are means $\pm$ s.e.mean. (n=8).

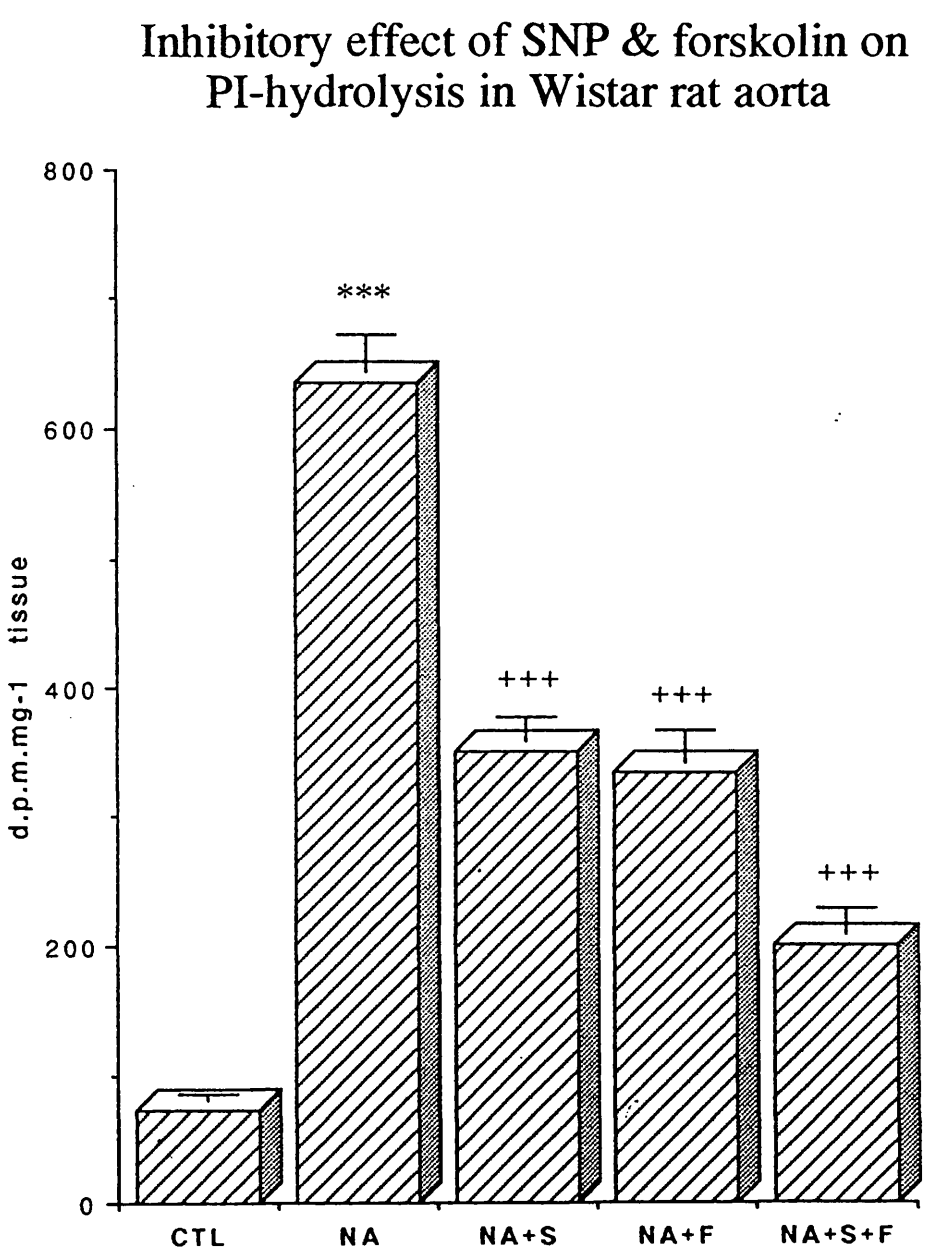

Fig. 3.43.
The separate and combined inhibitory effects of SNP (S) and forskolin (F) on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p < 0.001$). SNP (S, 10^{-6}M) and forskolin (F, 10^{-6}M) individually inhibited the NA-induced PI-hydrolysis. Combination of these drugs produced a significantly larger inhibition of NA-induced PI-hydrolysis. (+++ $p < 0.001$). Results are means $\pm$ s.e.mean. (n=8).

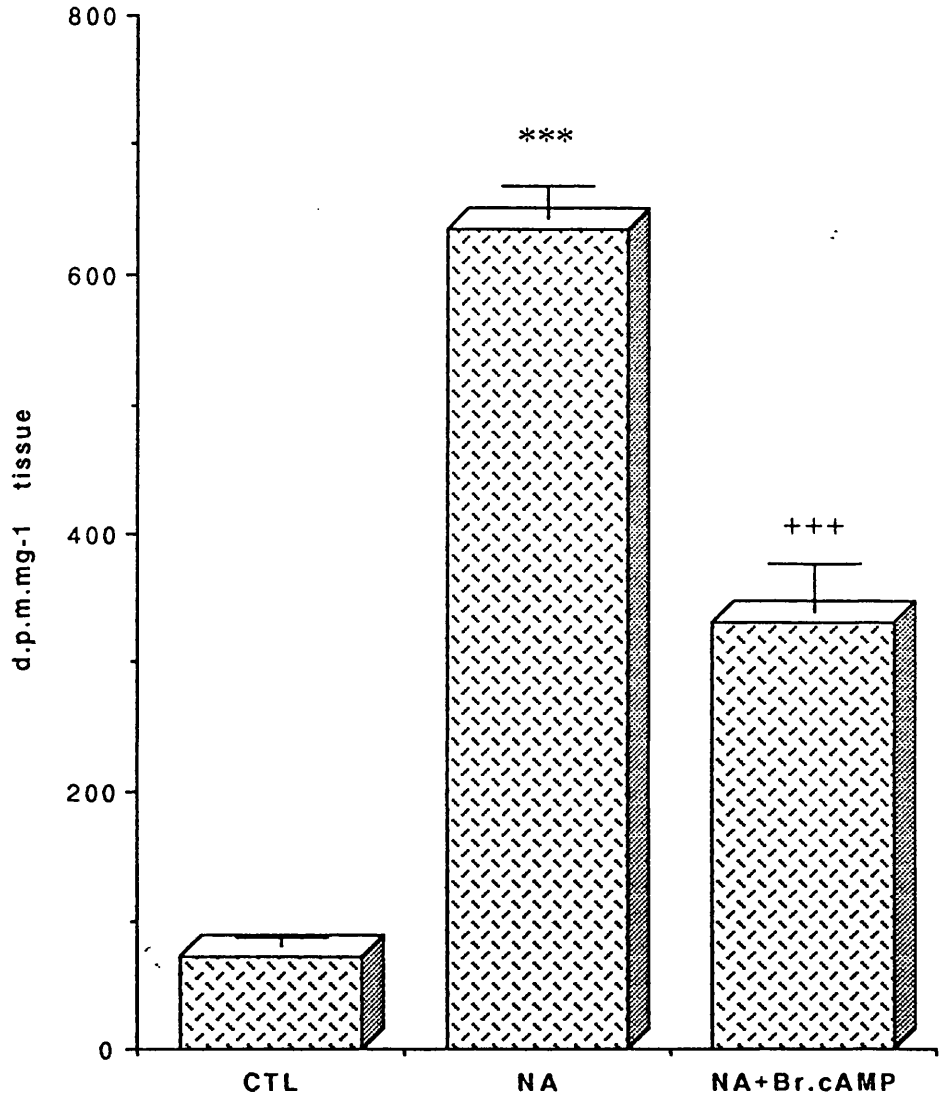

Fig. 3.44.
Inhibitory effect of 8-Bromo-cAMP on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p < 0.001$). 8-Bromo-cAMP (0.3mM) significantly inhibited the NA-induced PI-hydrolysis (+++ $p < 0.001$). Results are means $\pm$ s.e.mean. (n=8).

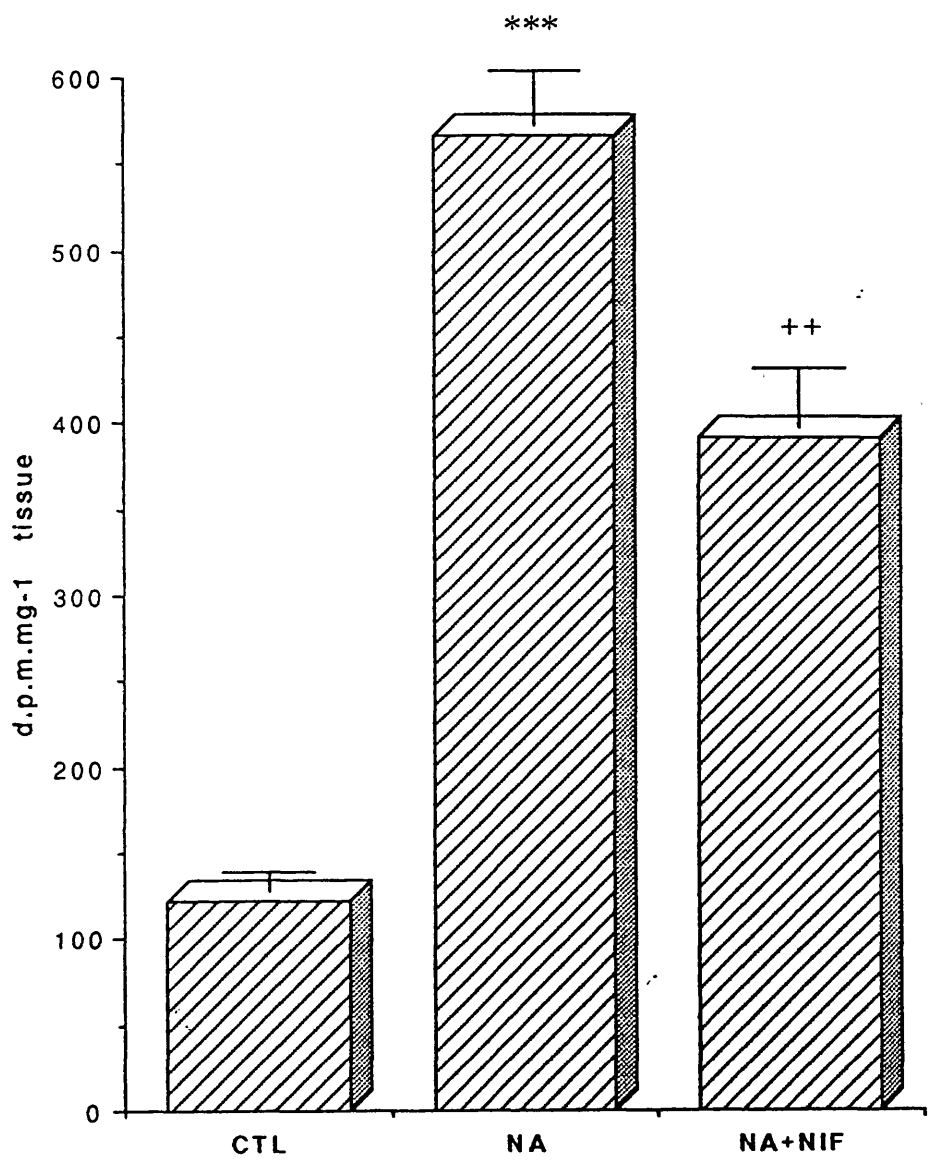

Fig. 3.45.
Inhibitory effect of nifedipine (NIF) on NA-induced PI-hydrolysis in aortic rings from Wistar rats. PI-hydrolysis was monitored by measuring (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above control levels. (*** $p<0.001$). NIF (10^{-6}M) significantly inhibited the NA-induced PI-hydrolysis (++ $p<0.01$). Results are means $\pm$ s.e.mean. (n=8)

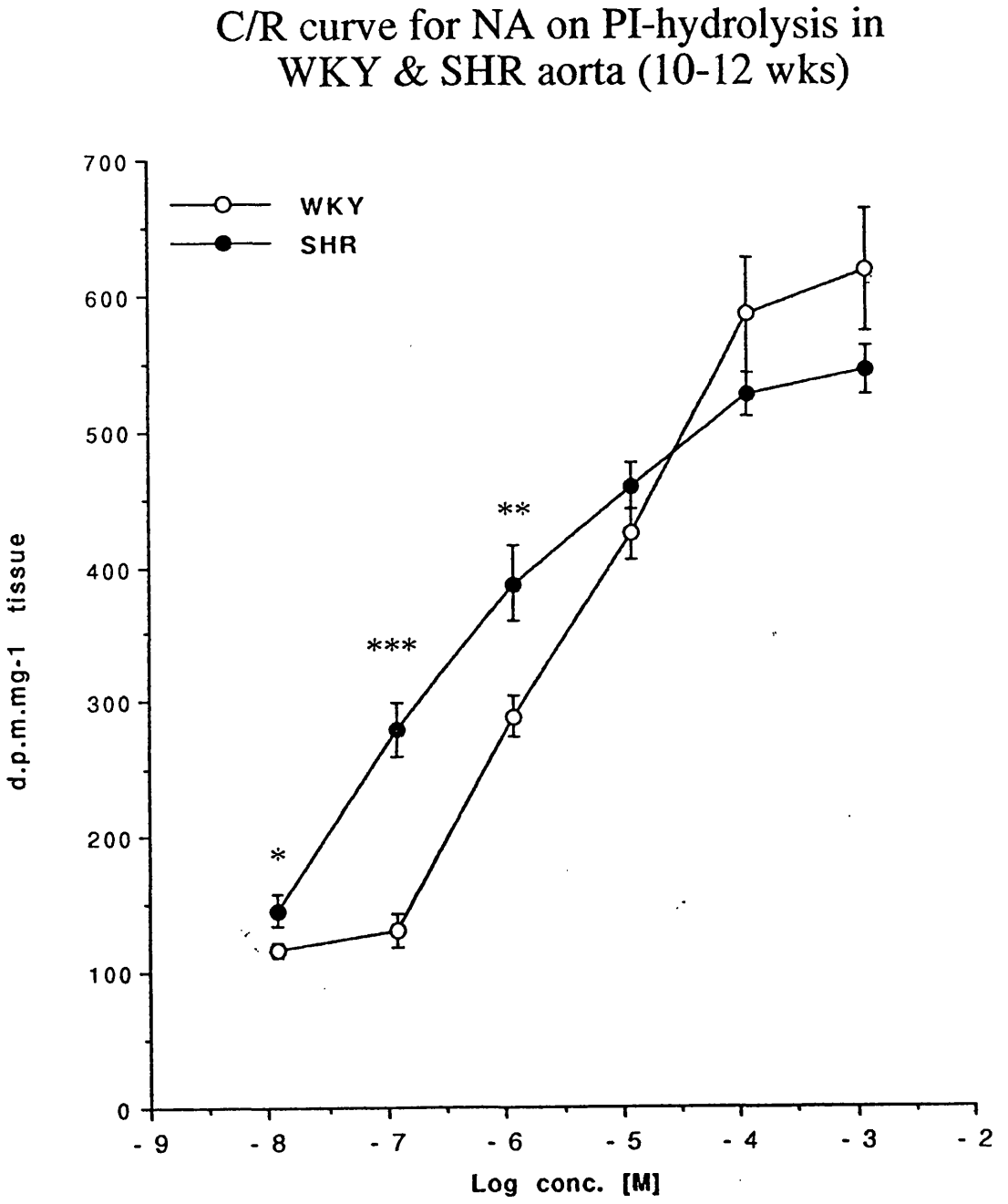

Fig. 3.46.
Concentration-response curves for NA-induced PI-hydrolysis in aortic rings from SHR and WKY control rats (10-12 weeks old). PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. Low concentrations of NA induced significantly higher levels of PI-hydrolysis in rings from SHR than in those from WKY controls, but at higher concentrations there was no significant difference between responses obtained in rings from SHR and WKY. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=8).

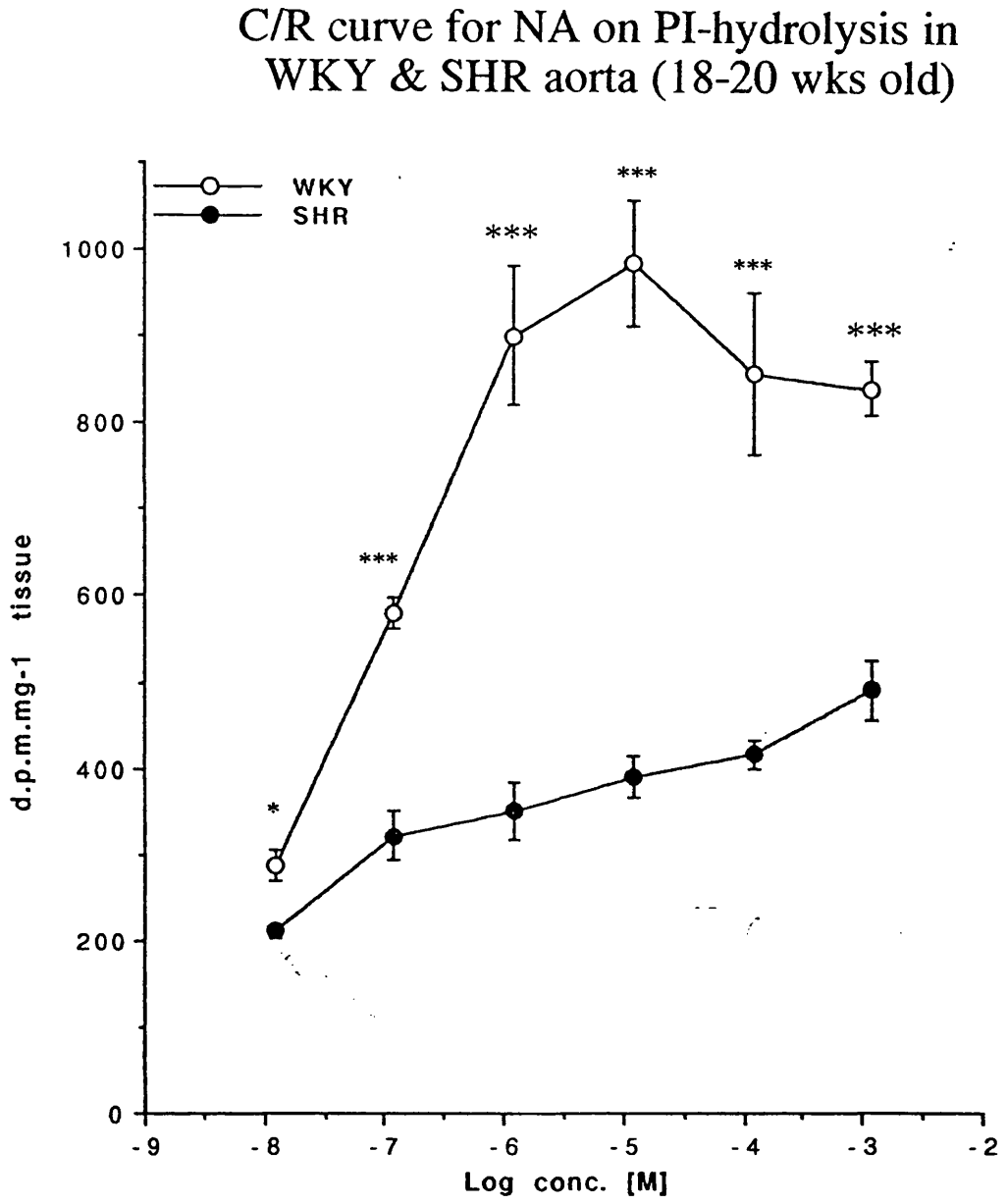

Fig. 3.47.
Concentration-response curves for NA-induced PI-hydrolysis in aortic rings from SHR and WKY control rats (18-20 weeks old). PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA at all concentrations induced significantly less PI-hydrolysis in rings from SHR than in those from WKY controls. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Points are means $\pm$ s.e.mean. (n=8).

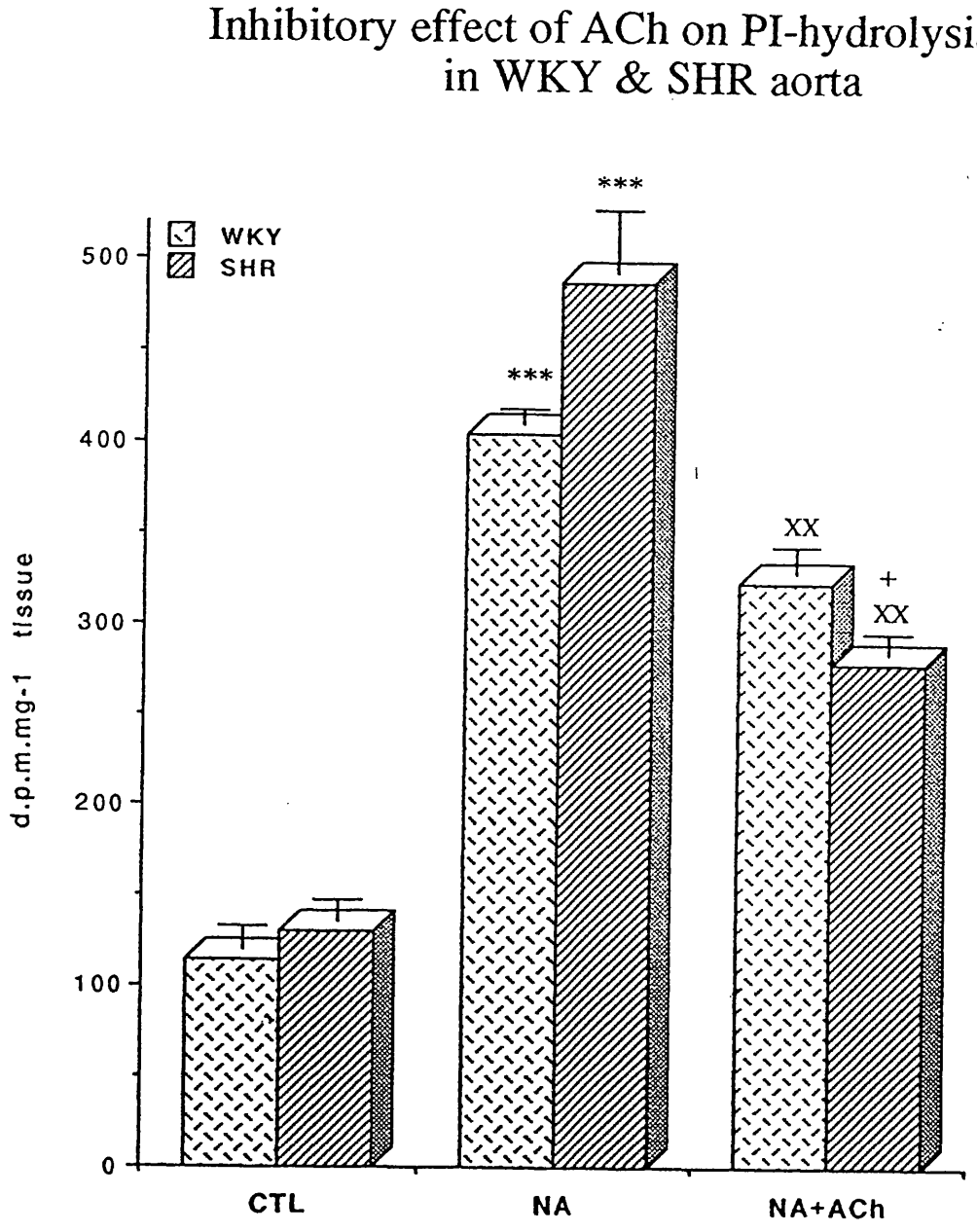

Fig 3.48.
Inhibitory effect of ACh on NA-induced PI-hydrolysis in aortic rings from SHR and WKY control rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control level. (*** $p < 0.001$). ACh (10^{-5}M) significantly inhibited this NA-induced PI-hydrolysis in both SHR and WKY controls. (xx $p < 0.01$). The inhibitory effect of ACh was significantly greater in rings from SHR than in rings from WKY rats (+ $p < 0.05$). Results are means $\pm$ s.e.mean. (n=8).

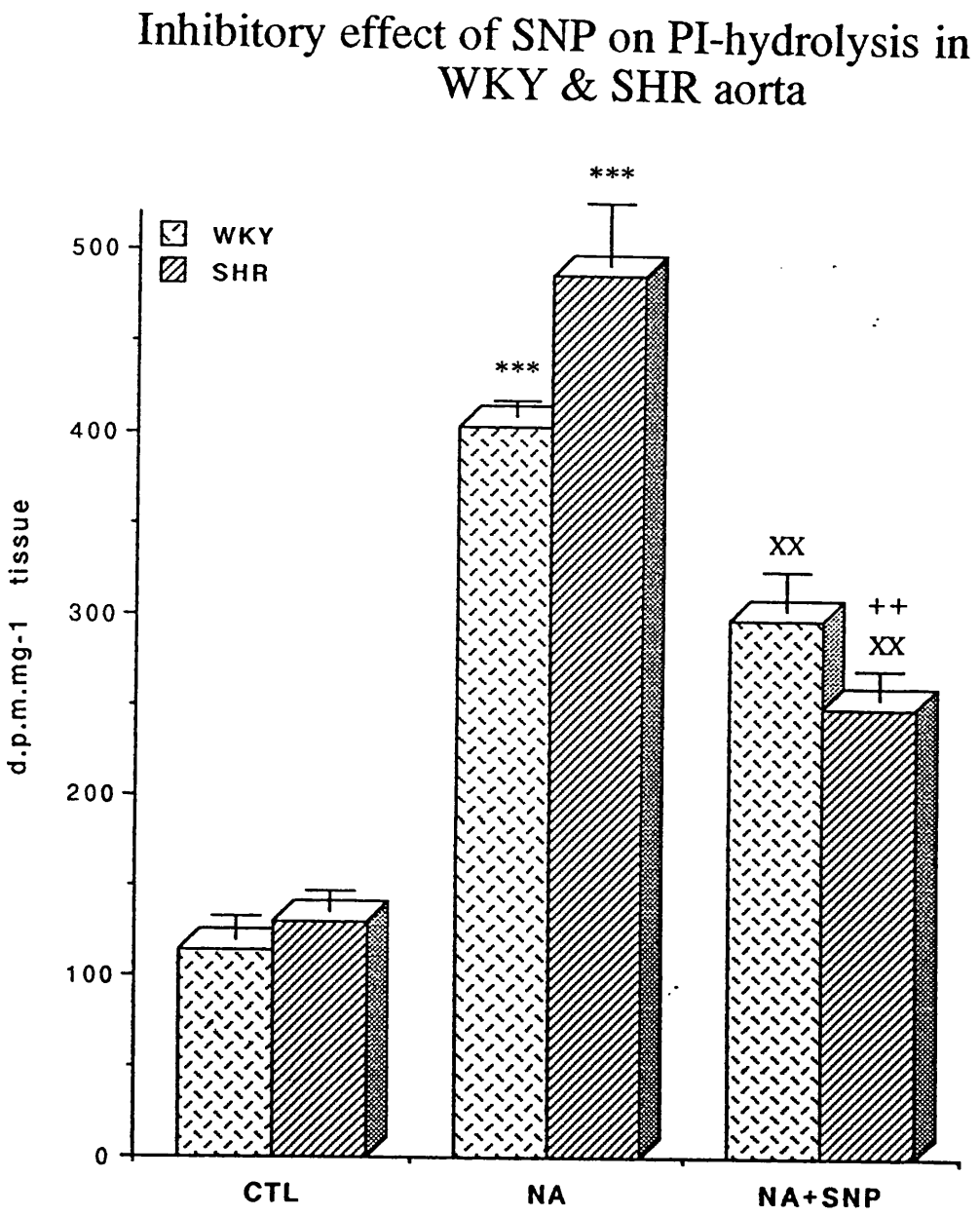

Fig 3.49.
Inhibitory effect of SNP on NA-induced PI-hydrolysis in aortic rings from SHR and WKY control rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control levels. (*** $p<0.001$). SNP (10^{-6}M) significantly inhibited NA-induced PI-hydrolysis in rings from SHR and in those from WKY controls. (xx $p<0.01$). The inhibitory effect of SNP was significantly greater in rings from SHR than in WKY rings (++ $p<0.01$). Results are means $\pm$ s.e.mean. (n=8).

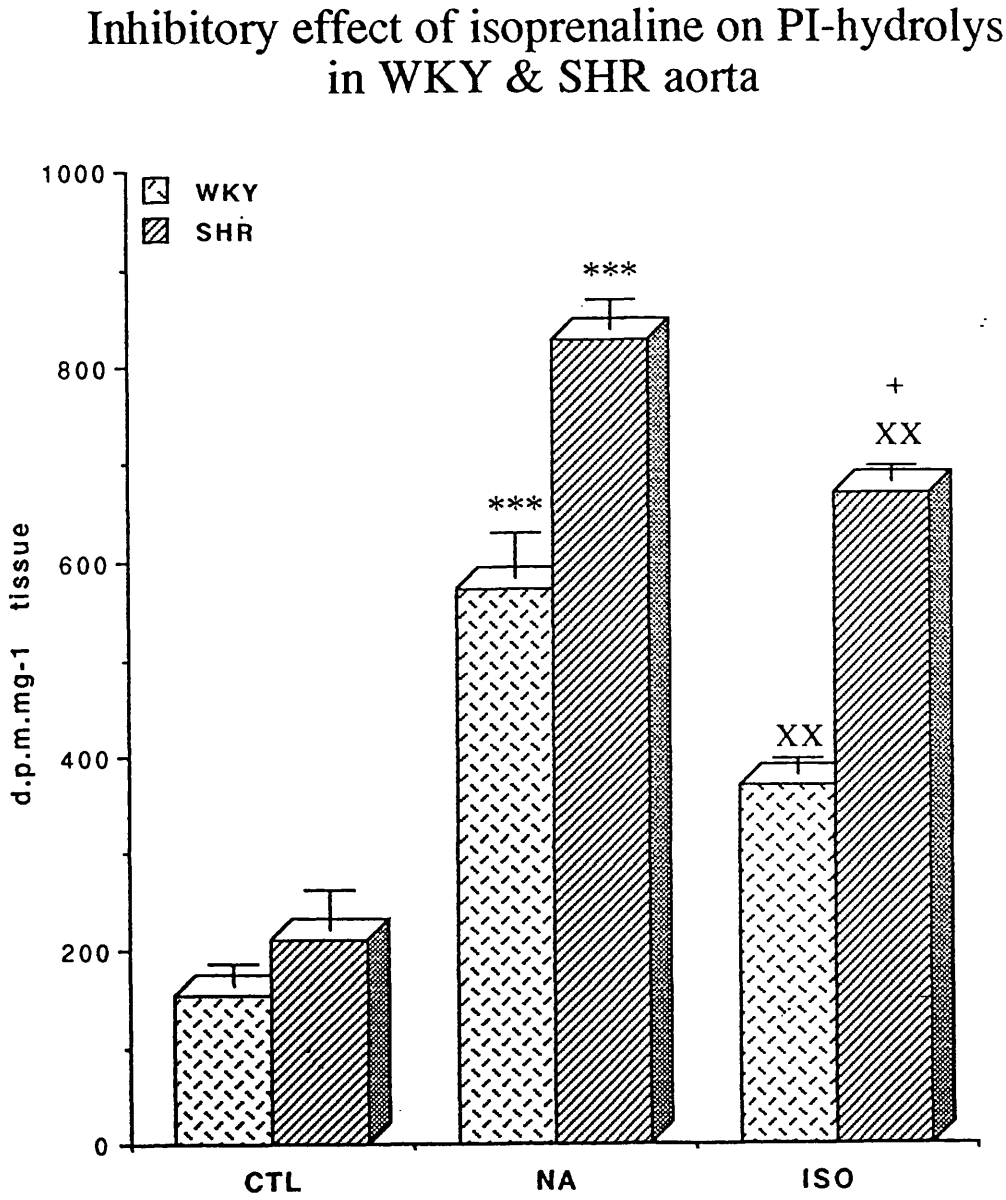

Fig 3.50.
Inhibitory effect of isoprenaline (ISO) on NA-induced PI-hydrolysis in aortic rings from SHR and WKY control rats. PI-hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control levels. (*** $p < 0.001$). ISO (10^{-8} M) significantly inhibited NA-induced PI-hydrolysis in rings from SHR and in those from WKY controls. (xx $p < 0.01$). The inhibitory effect of ISO was significantly smaller in rings from SHR than in those from WKY controls. (+ $p < 0.05$). Results are means $\pm$ s.e.mean. (n=8).

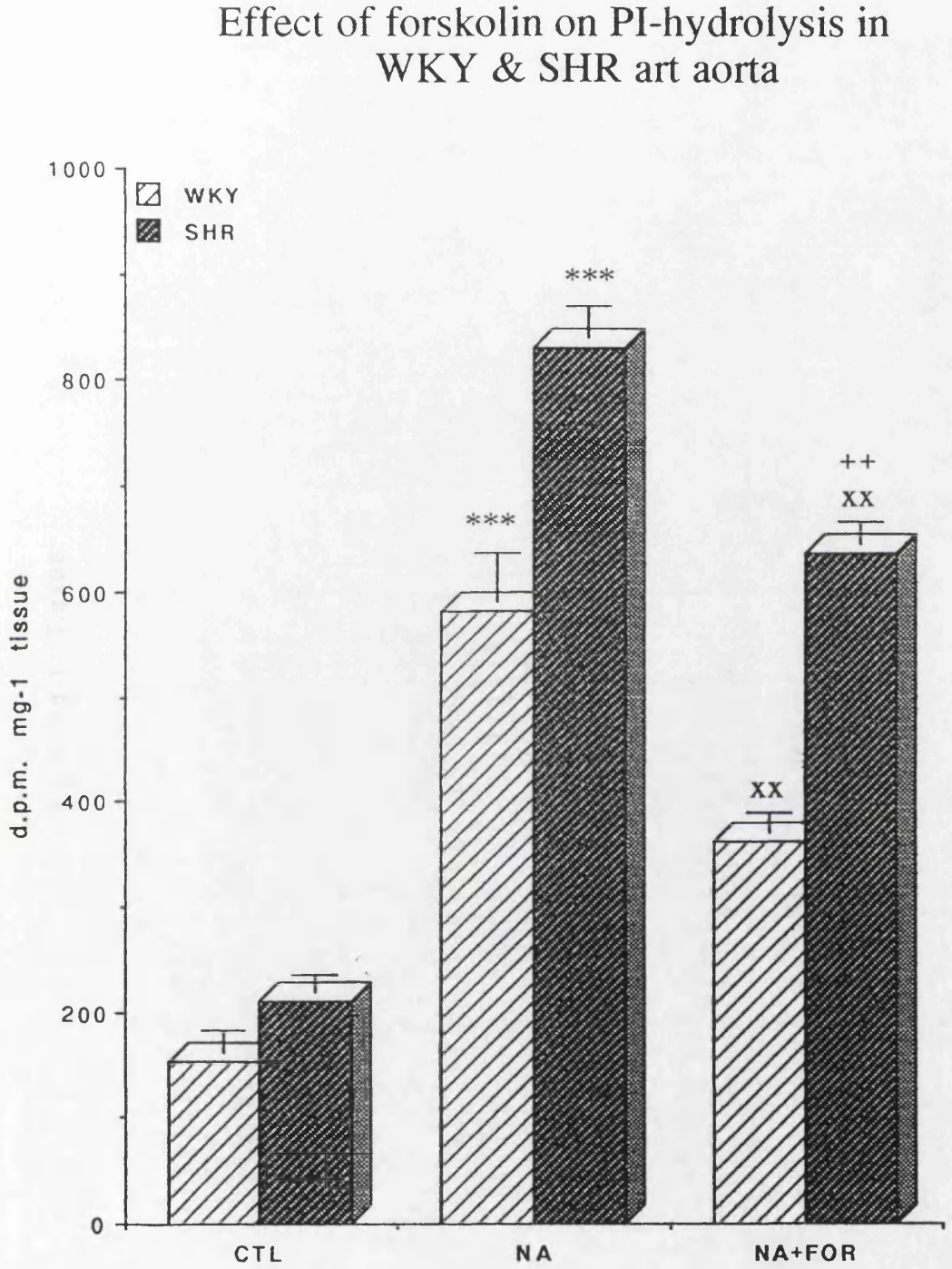

Fig 3.51.
Inhibitory effect of forskolin (FOR, 10^{-6}M) on NA-induced PI- hydrolysis in aortic rings from SHR and WKY control rats. PI- hydrolysis was monitored by measuring the levels of (^{3}H)-inositol phosphates. NA (6.2×10^{-6}M) significantly increased PI-hydrolysis above the control levels. (*** $p < 0.001$). FOR (10^{-6}M) significantly inhibited NA-induced PI-hydrolysis in rings from SHR and those from WKY controls. (xx $p < 0.01$). The inhibitory effect of ISO was significantly smaller in rings from SHR than in those from WKY controls. (++ $p < 0.05$). Results are means $\pm$ s.e.mean. (n=8).

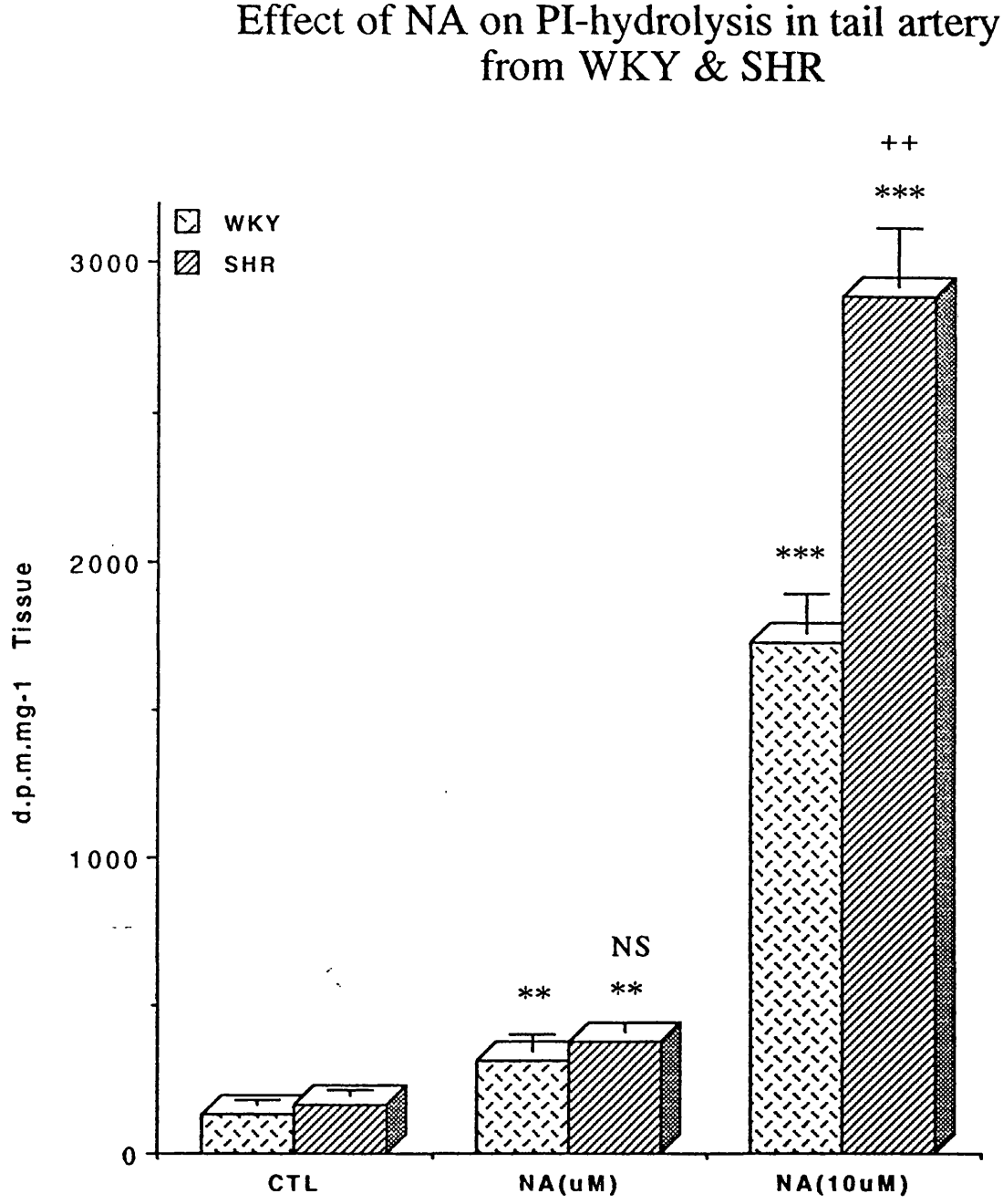

Fig. 3.52.
Effect of NA on PI-hydrolysis in tail artery rings from SHR and WKY control rats. PI-hydrolysis was monitored by measuring the level of (^{3}H)-inositol phosphates. NA increased PI-hydrolysis in rings from both SHR and WKY rats. (** $p<0.01$, *** $p<0.001$). The responses to NA ($1x10^{-6}$M) was not significantly different in rings from SHR and in those from WKY controls. The response to NA ($1x10^{-5}$M) was significantly greater in rings from SHR than in those from WKY controls. (++ $p<0.01$). Results are means ± s.e.mean. (n=8).

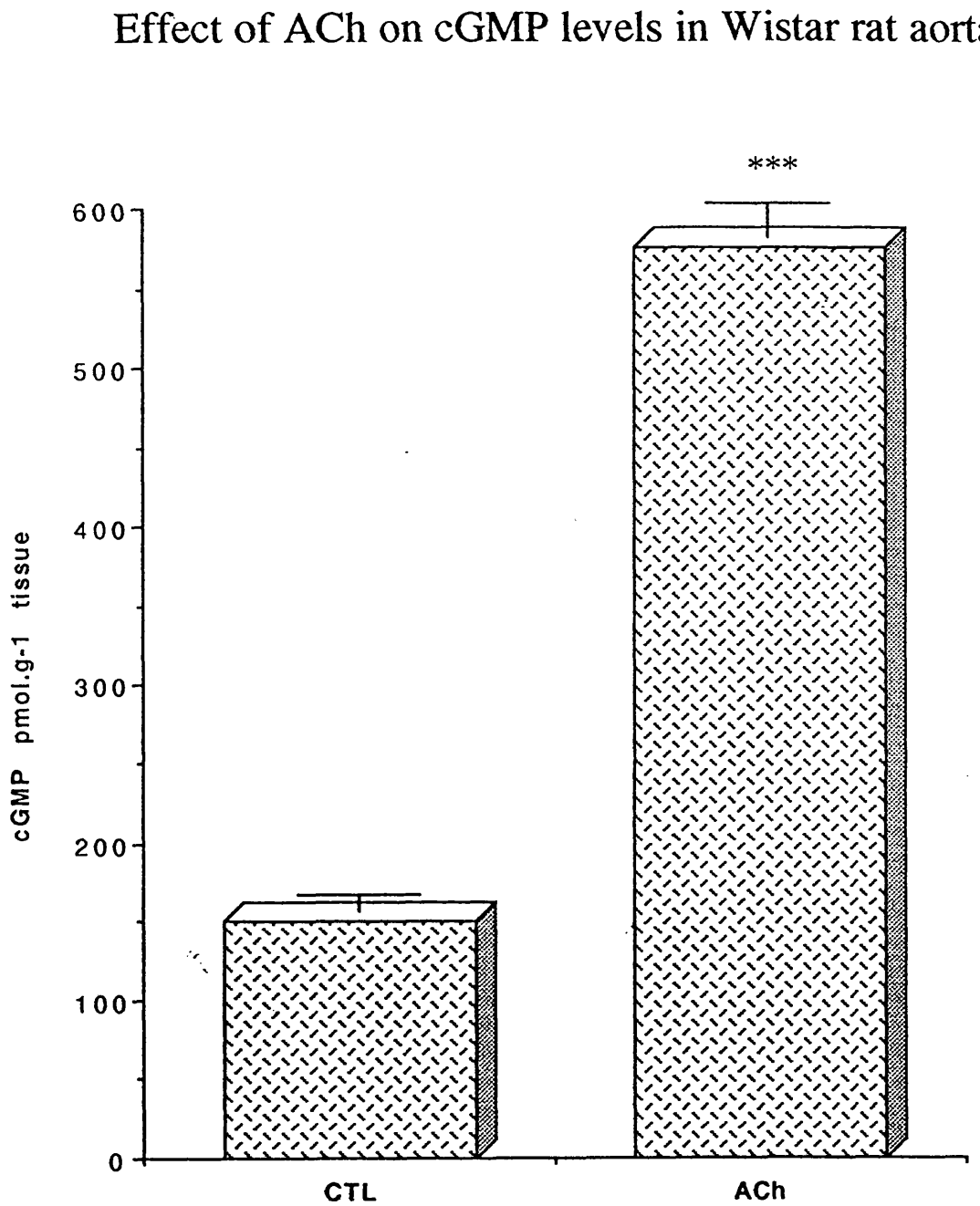

Fig. 3.53.
Effect of ACh on cGMP levels in aortic rings from Wistar rats. In rings pretreated with NA ($3x10^{-8}$ M), ACh (10^{-5}M) significantly increased cGMP levels above the control (basal) level (*** $p<0.001$). Results are means ± s.e.mean. ($n=8$).

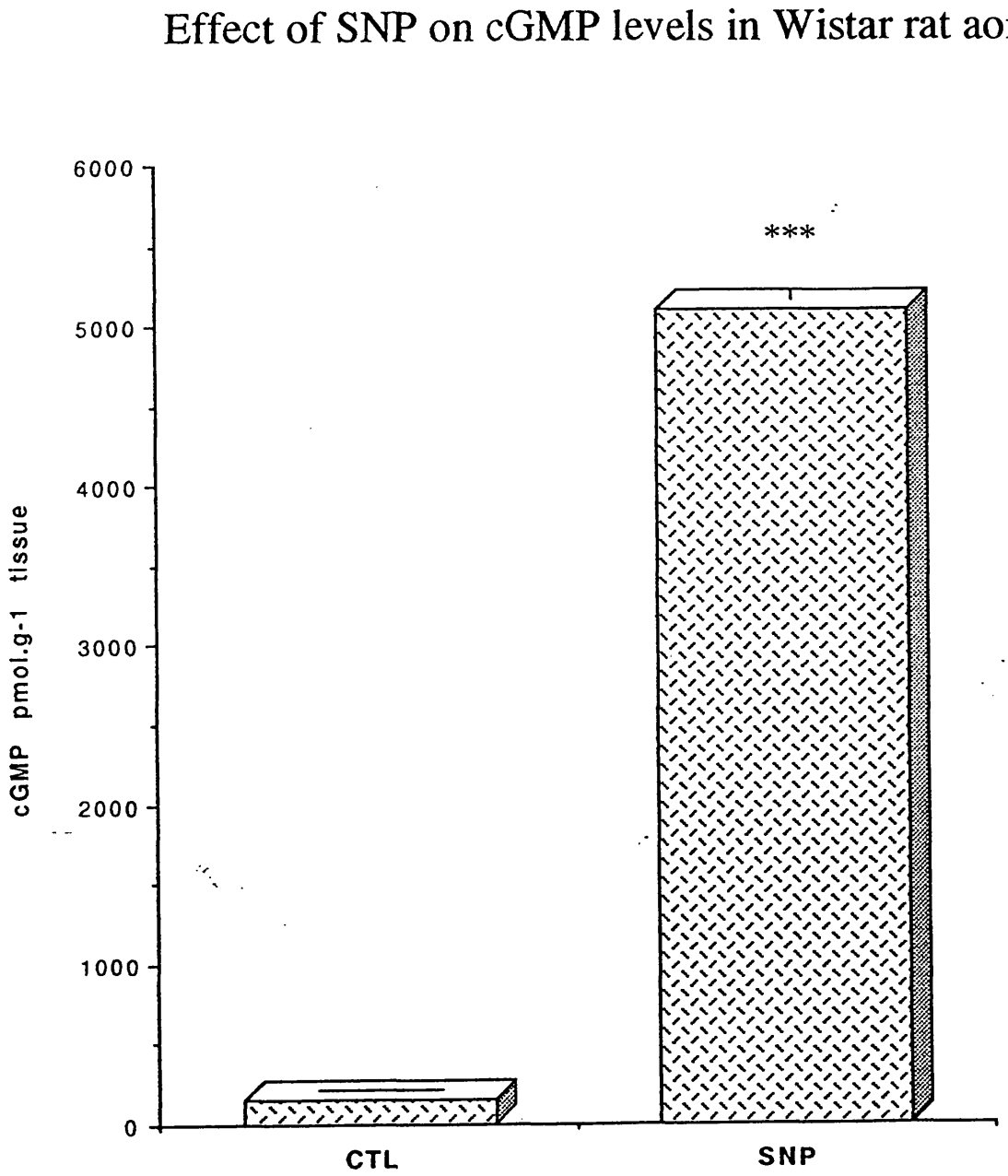

Fig. 3.54.
Effect of SNP on cGMP levels in aortic rings from Wistar rats. In aortic rings pretreated with NA ($3x10^{-8}$M), SNP (10^{-6}M) significantly increased cGMP levels above the control (basal) level (***$p<0.001$). Results are means ± s.e.mean. (n=8).

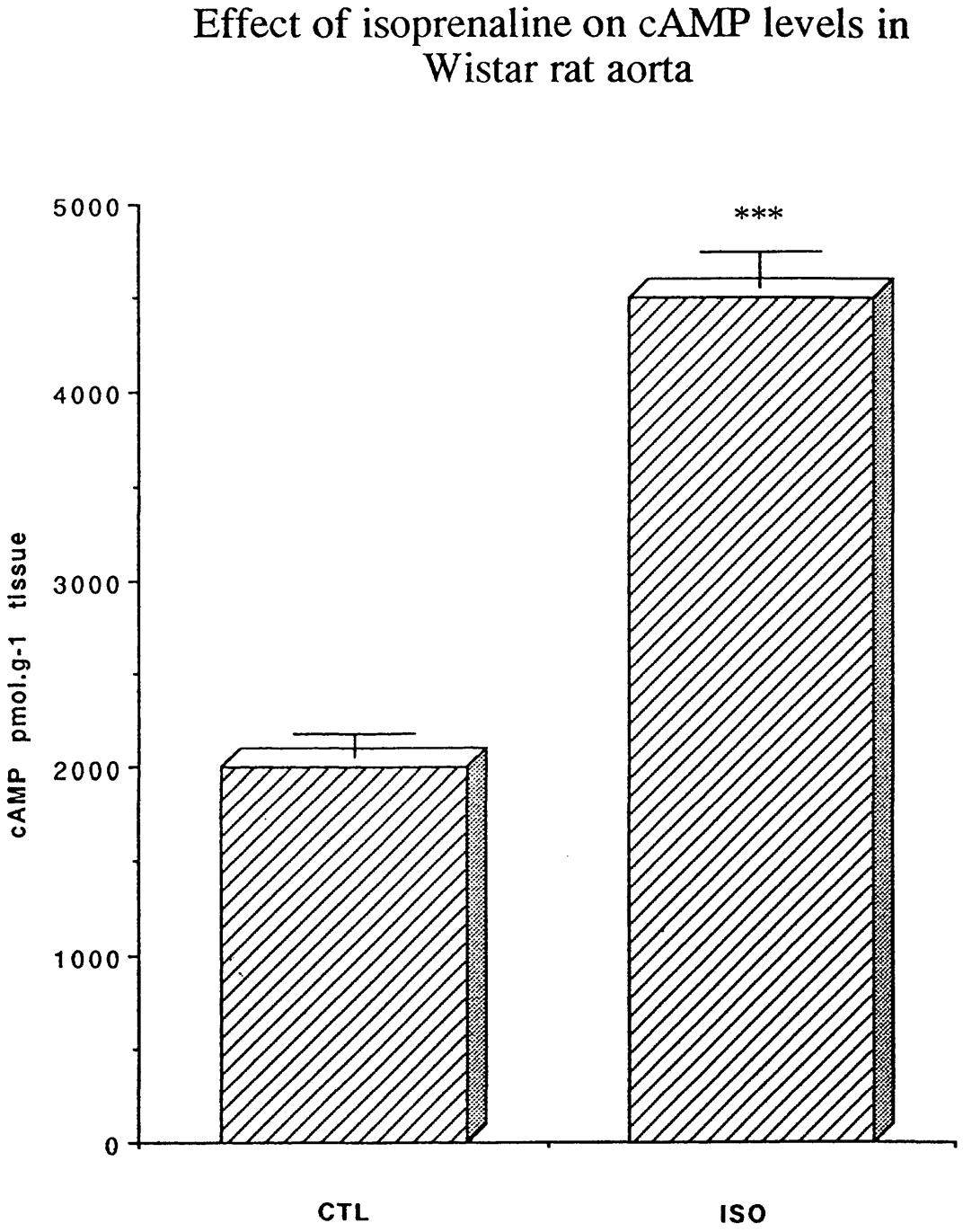

Fig. 3.55.
Effect of isoprenaline (ISO) on cAMP levels in aortic rings from Wistar rats. In rings pretreated with NA ($3x10^{-8}$M), ISO (10^{-8}M) significantly increased cAMP levels above the control (basal) level. (*** $p<0.001$). Results are means ± s.e.mean. (n=8).

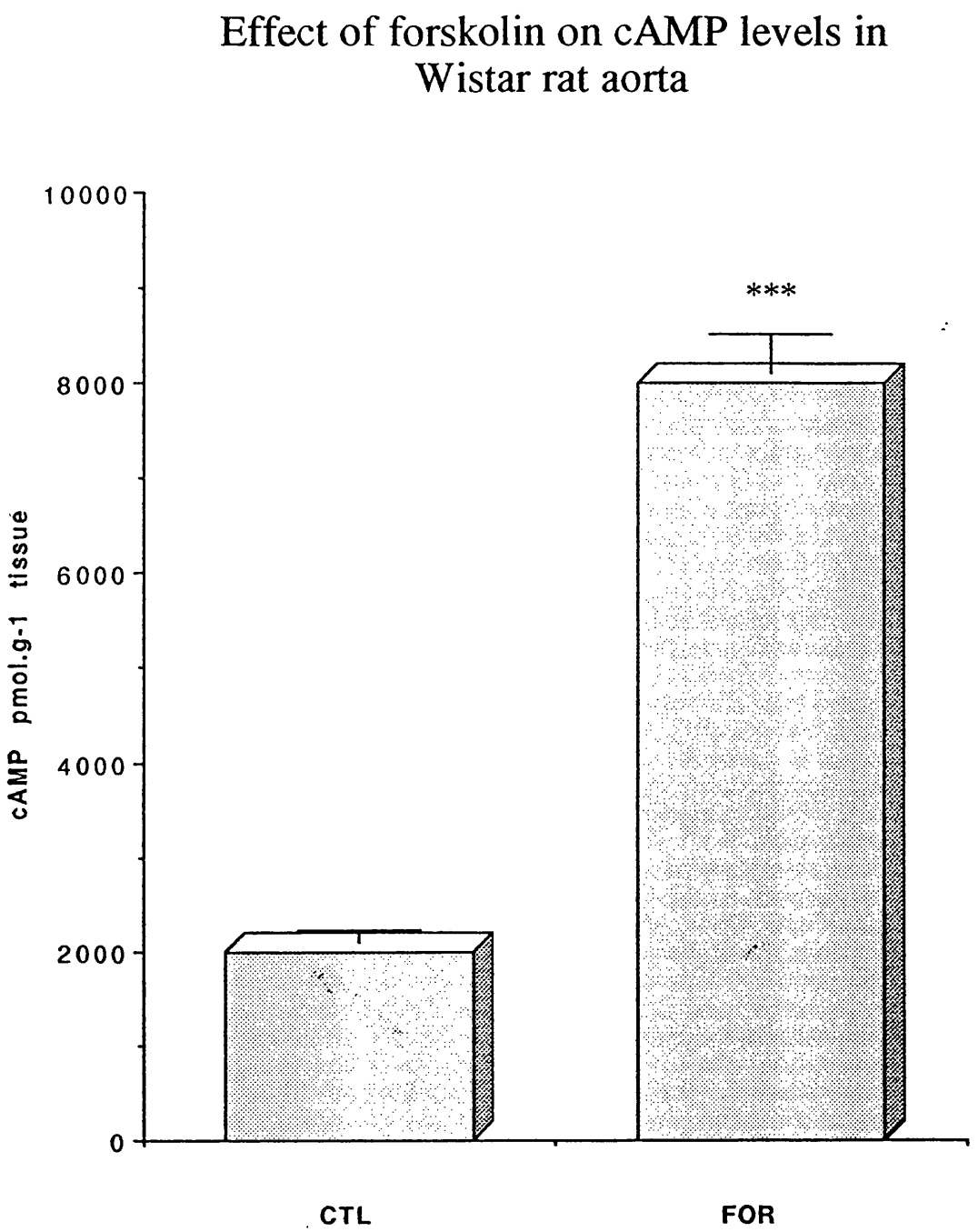

Fig. 3.56.
Effect of forskolin (FOR) on cAMP levels in aortic rings from Wistar rat aorta. In rings pretreated with NA ($3x10^{-8}$M), FOR (10^{-6}M) significantly increased cAMP levels above the control level (basal). (*** $p<0.001$). Results are means $\pm$ s.e.mean. (n=8).

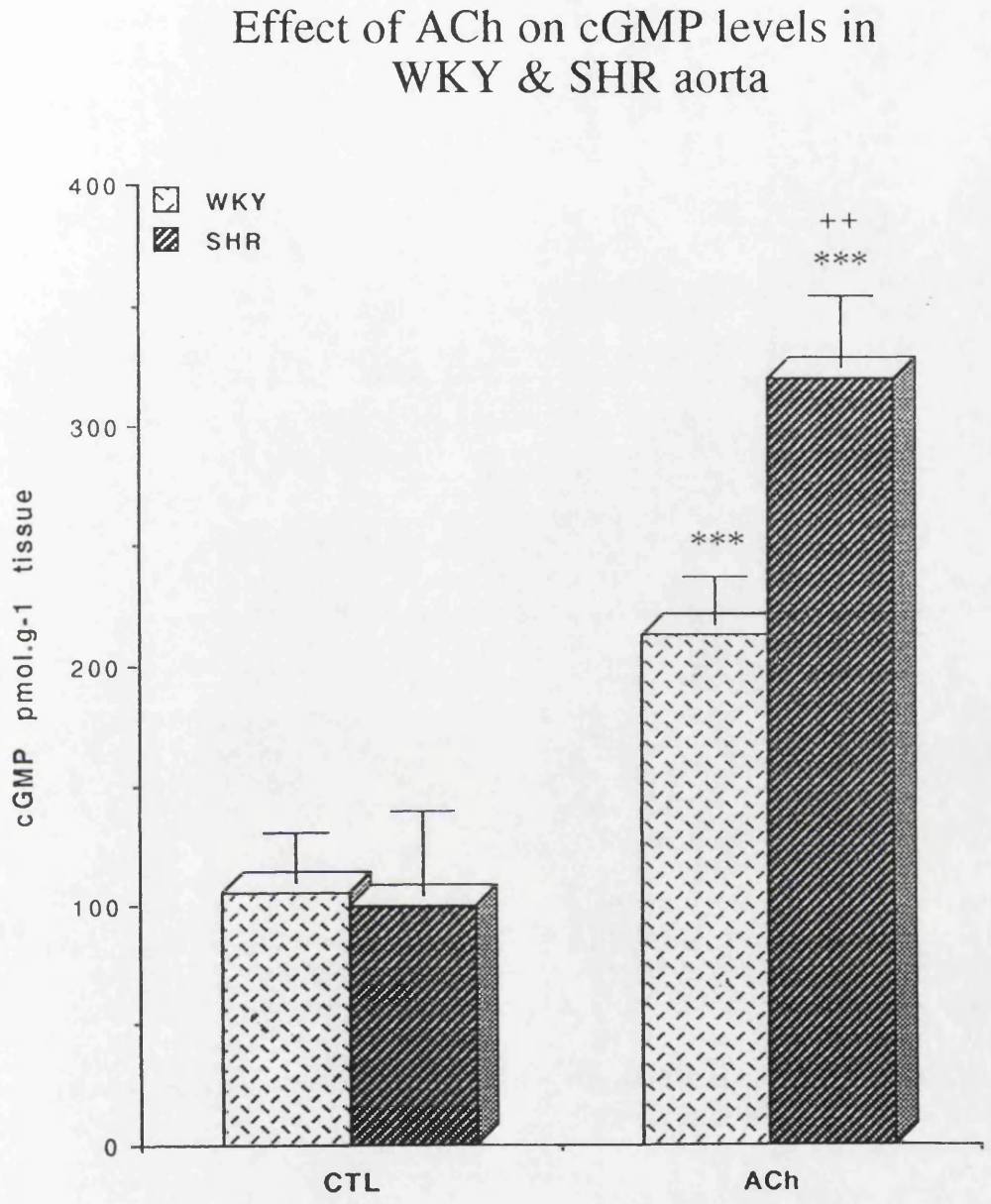

Fig. 3.57.
Effect of ACh on cGMP levels in aortic rings from SHR and WKY control rats. In rings pretreated with NA (5.6×10^{-9}M), ACh (10^{-5}M) significantly increased cGMP levels above the control (basal) level. (*** $p < 0.001$). This effect was significantly greater in rings from SHR than in those from WKY controls. (++ $p < 0.01$). Results are means $\pm$ s.e.mean. (n=8).

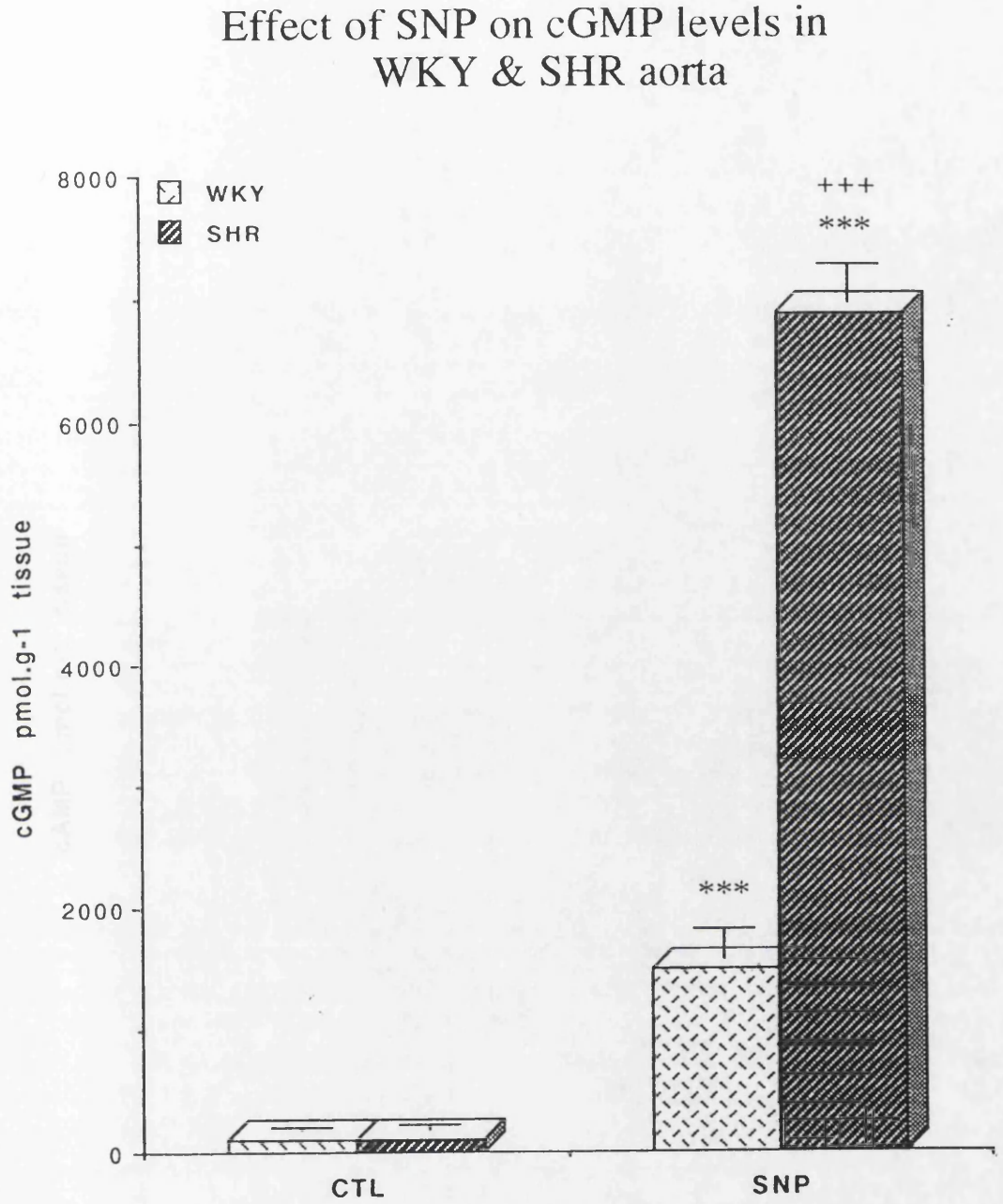

Fig. 3.58.
Effect of SNP on cGMP levels in aortic rings from SHR and WKY control rats. In rings pretreated with NA ($5.6X10^{-9}M$), SNP ($10^{-6}M$) significantly increased cGMP levels above the control (basal) levels. (*** $p<0.001$). The responses to SNP was significantly higher in rings from SHR than in those from WKY controls. (+++ $p<0.001$). Results are means $\pm$ s.e.mean. ($n=8$).

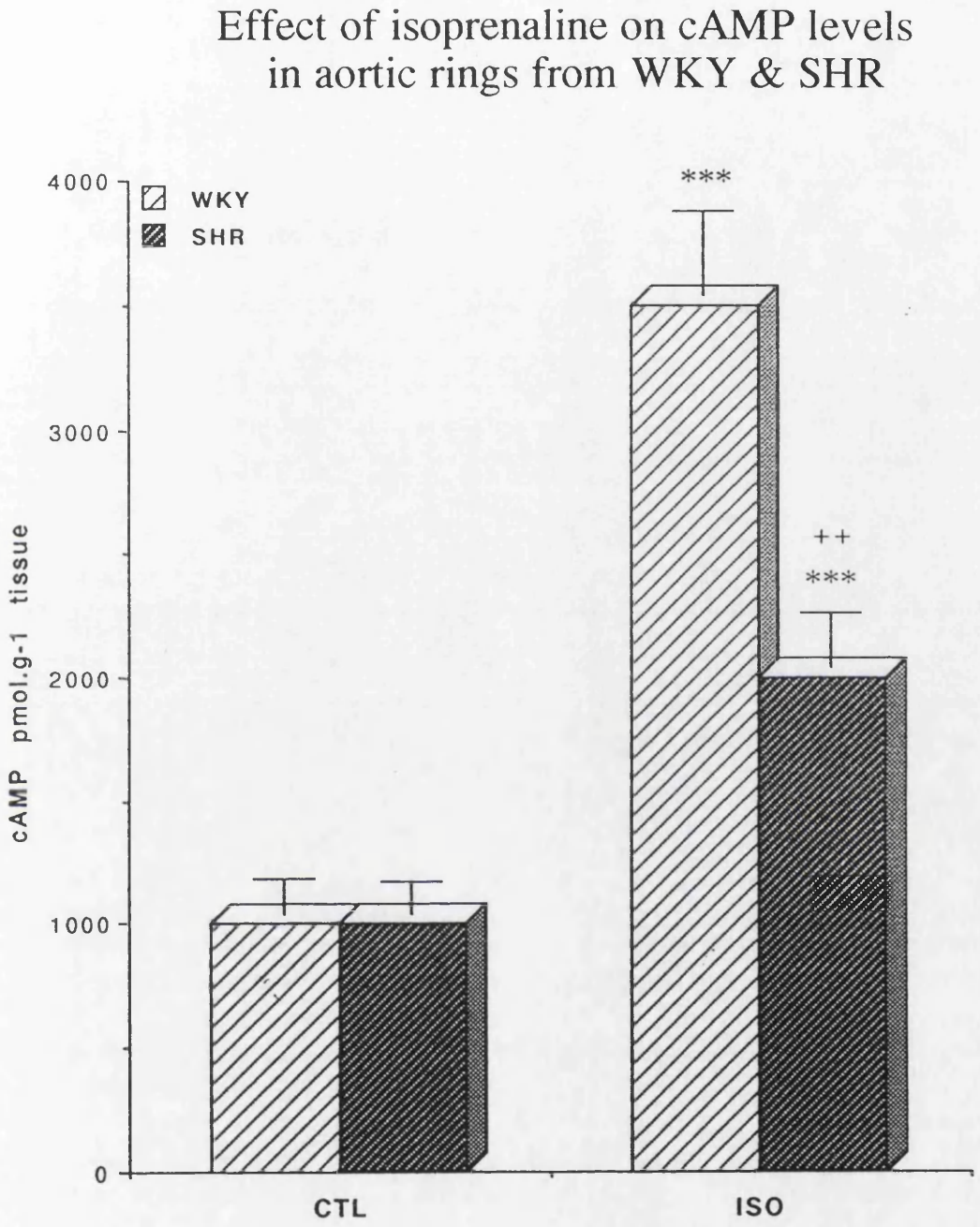

Fig. 3.59.
Effect of isoprenaline (ISO) on cAMP levels in aortic rings from SHR and WKY control rats. In rings pretreated with NA ($5.6x10^{-9}$M), ISOP (10^{-8}M) significantly increased cAMP levels above the control (basal) level. (*** $p<0.001$). This effect of ISO was significantly smaller in rings from SHR than in those from WKY controls. (++ $p<0.01$). Result are means ± s.e.mean. (n=8).

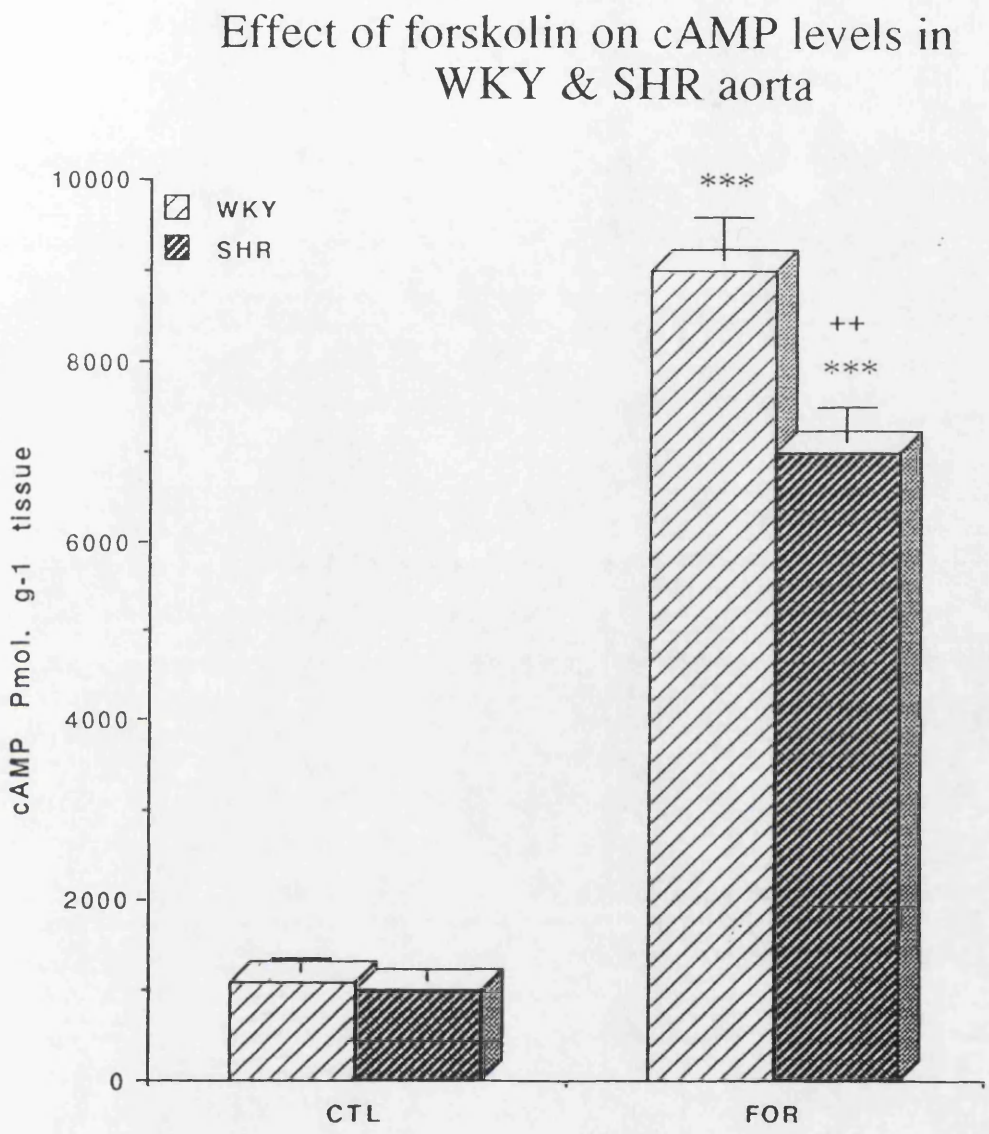

Fig. 3.60.
Effect of forskolin (FOR) on cAMP levels in aortic rings from SHR and WKY control rats. In rings pretreated with NA (5.6×10^{-9}M), FOR (10^{-6}M) significantly increased cAMP levels. (*** $p < 0.001$). This effect of FOR was significantly smaller in rings from SHR than in those from WKY controls. (++ $p < 0.01$). Results are means $\pm$ s.e.mean. (n=8).

CHAPTER FOUR

DISCUSSION

DISCUSSION:

In the cardiovascular system, the total peripheral resistance (TPR) to the flow of blood is dependent on the level of vascular tone, which is the resultant of vascular smooth muscle contraction and relaxation. The elevated arterial pressure that occurs in hypertension is the result of increased TPR, probably caused by changes in the structure and functioning of the blood vessels (Webb & Bohr, 1987). Since vascular smooth muscle tone has an essential role in regulating TPR and in turn, in regulating blood pressure, this study examined the mechanisms that modulate tone in normal vasculature and in vasculature from hypertensive rats and considered the possibility that in hypertension, changes may occur in the capacity of the vascular smooth muscle to contract or relax. The mechanical responses of isolated arteries were examined together with the corresponding biochemical responses asssociated with contraction and relaxation. In particular, the effects of vasoconstrictors and vasorelaxants on the coupling mechanisms and second messengers that regulate vascular tone were investigated.

In this study, rat isolated aortic rings and tail artery rings were compared, since these vessels were representative respectively of conducting and resistance vessels and also since they allowed mechanical and biochemical responses to be readily investigated.

This study was divided into three sections, which were:

1. Preliminary mechanical and biochemical experiments to examine vascular contraction and relaxation in the isolated aorta from control Wistar rats.

2. Investigation of the mechanical and biochemical responses that occur in aortic rings from SHR and WKY control rats in response to vasoconstrictors and vasorelaxants.

3. Investigation of the mechanical and biochemical responses that occur in isolated tail artery rings from SHR and WKY control rats.

SECTION 1: **Study of pharmacological and biochemical mechanisms modulating tone in Wistar rat aorta.**

Throughout this study the responses of rat aortic rings were recorded isometrically (Fig. 2.1) and standard methods commonly cited in the literature were employed to prepare the aortic rings and record their responses (Cauvin & Malik, 1984; Rapoport, 1987). True isometric recording occurs in tissues that contract without any shortening of the muscle fibres. This is achieved when the applied tension, which is the "opposing force", exceeds the tension developed by the tissue, without overstretching the muscle fibre. A tension of 2g was applied to aortic rings and this exceeded the tension developed by the tissue. In all experiments in which contractile responses to vasoconsrictors were examined, the tissues were weighed so that the results could be expressed as $g.mg^{-1}$ tissue. This was necessary since blood vessels from hypertensive rats were generally heavier and thicker-walled than those from controls, presumably because the smooth muscle had undergone hypertrophy and hyperplasia.

Since each aortic ring was suspended in the organ bath between hooks, inserted into the lumen, another factor that had to be considered was the condition of the endothelium. Clearly, there was a possibility that the wire hooks used to suspend the rings would damage the endothelium. Some damage may have occurred but it was insufficient to prevent endothelium-dependent vasorelaxants inhibiting tone. Moreover, throughout this study, a standard procedure was used and so if there was any damage, it would be likely to have been similar in all experiments. It has been reported that only a small fraction of the endothelial layer needs to be intact for complete relaxation of aortic rings (Peach et.al., 1985). It appears that normally only minor damage to the endothelium occurred and did not significantly influence the results obtained.

When it was necessary to remove the endothelium, this was achieved either mechanically by rubbing with forceps or chemically with Triton X-100 (0.1% V/V). These methods proved to be reliable and this was confirmed by light microscopy and by the absence of ACh-induced relaxations. During the rubbing of aortic rings, care was taken to avoid damaging the smooth muscle, whose capacity to contract and relax and ability to generate second messengers could have been impaired. There was no evidence that any such damage occurred.

Tissues were equilibrated for 60 minutes prior to the start of the experiments. This is a common procedure in such experiments, although the reason for it is not clear. A possible justification for this preincubation is that it was necessary to allow the re-establishment of the ionic gradients and the recovery of the coupling mechanisms. In the absence of such an equilibration period, responses to vasoconstrictors were variable.

The method used to investigate agonist-induced PI- hydrolysis in this study measured myo-[^{3}H]-inositol incorporation into membrane phosphoinositides and in turn, into the water soluble metabolites of PI-hydrolysis (Fig. 1.2) This method has been widely used before by, among others, Fox and Friedman (1987), Hashimoto et al, (1986) and Chiu et al., (1987). This method was reliable and more specific than earlier techniques that measured ^{32}P incorporation into phosphatidic acid as an index of PI-hydrolysis.

In this study, lithium chloride was used to inhibit the phosphatase enzyme that hydrolyses the inositol phosphates (IPs) to release inositol (Fig. 2.4). Since IPs are generated during activation of, for example, α_1-adrenoceptors, lithium chloride, by inhibiting the phosphatase enzyme, amplifies the observed PI response to vasoconstrictors such as NA. This effect of lithium chloride was confirmed (Fig. 2.3).

In the preliminary experiments, mechanical and biochemical responses to vasoconstrictors and vasorelaxants that act by different mechanisms were examined. The results obtained in these experiments with NA, which causes PI-hydrolysis and KCl, which depolarizes the cell membrane, largely confirmed previous findings. The results obtained with the endothelium- dependent vasorelaxant, acetylcholine (ACh) and the endothelium-independent vasorelaxant, sodium nitroprusside (SNP) and with the ß-adrenoceptor agonist, isoprenaline (ISO), with the adenylate cyclase activator, forskolin (FOR) and also with the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) also confirmed previous findings. The justification of these experiments, which repeated the work and confirmed the results of others, was that it was necessary to demonstrate the

suitability of the methods before looking for changes in the responsiveness or coupling mechanisms in blood vessel from hypertensive rats.

In this study, NA caused concentration-dependent contractions of rat isolated aortic rings (Fig. 3.1) and increased PI-hydrolysis. These effects of NA were probably mediated via α_1-adrenoceptors, since both the mechanical and biochemical responses to NA were inhibited by prazosin (Fig. 3.31). These results agree with previous observations (Villalobos-molina et al., 1982; Cauvin & Malik, 1984; Beckering et al., 1984a; Chiu et al., 1987).

There is a possibility that the contractile response to NA may have been mediated in part via post-junctional α_2-adrenoceptors, which do not cause PI-hydrolysis (Ruffolo et al., 1982, 1987; Ruegg, 1988). However, more recent work suggests that the postjunctional α-adrenoceptors in the rat aorta should be classified as a homogenous population of α_1-adreoceptors (Beckering et al., 1984b, Chiu et al., 1986). In order to minimize any contribution from α-adrenoceptors other than α_1-adrenoceptors, a more selective α_1-adrenoceptor agonist, such as phenylephrine could have been used. Instead, NA was used in this study since it is the endogenous α_1-adrenoceptor agonist, which also has the capacity to activate α_2- and ß-adrenoceptors, whose fuctioning may be modified in hypertension. By using NA any such changes could be identified but might have been overlooked if a more specific α_1-adrenoceptor agonist had been used.

An interesting feature of the concentration-response curve for NA was the decline in the response that occurred at higher concentrations (Fig. 3.1). This did not occur when the endothelium was removed and so may have been due

to the release of a vasodilator from the endothelium. A similar result was obtained by Cocks and Angus (1983), who attributed this phenomenon to the release of EDRF (NO), as a result of activation of endothelial α_2-adrenoceptors. Similar results have been obtained by Miller and Vanhoutte in canine pulmonary and systemic vessels (1985). It is possible that the release of an endothelial vasorelaxant may also modify responses to lower concentrations of NA too. This possibility was confirmed by Carrier and White (1985) who showed that removing the endothelium in rat aorta enhanced both α_1- and α_2-adrenoceptor-mediated responses. Another possibility is that at high concentrations, $ß_2$-adrenoceptors are also activated, so that the α_1-adrenoceptor-mediated contraction is attenuated. One possibility that can probably be excluded is that the NA was oxidised. This was avoided since the bathing medium contained, the anti-oxidant, ascorbic acid (0.23 mM) and EDTA (0.06 mM) (Gillespie & Sheng, 1988).

The results obtained with KCl were interesting (Fig. 3.3), since KCl was not only less potent than NA but produced a smaller maximum contraction. Although both agonists increase intracellular free calcium $[Ca^{2+}]_i$ levels, albeit by different mechanisms, the difference in the maximum responses suggests that there are likely to be differences in the ability of vasorelaxants to inhibit NA and KCl-induced tone. In this study KCl ($3x10^{-2}$ M), which depolarizes the cell and causes vascular smooth muscle to contract by the influx of Ca^{2+}, increased the accumulation of [^{3}H]-IPs. This result is consistent with previous observations, which indicated that KCl-induced depolarization increased the accumulation of IPs (Novotny et al.,

1983; Bone et al., 1984; Berridge, 1987; Sasaguri & Watson 1988; ; Delahunty et al., 1988; Lai et al., 1989; Watson et al., 1990). This result is however, contradicted by the work of Chiu et al., (1986) in vascular smooth muscle, Rebecchi and Gershengorn (1983) in GH_3 pituitary tumour cells, and by Akhtar and Abdel- Latif (1984) in iris smooth muscle.

There are a number of possible explanations of this discrepancy. First, high concentrations of K^+ can depolarize nerves and release NA. It has also been reported that the Ca^{2+} -ionophore A23187, can cause release of NA from adrenergic nerve terminals in smooth muscle (Cohen et al., 1981). Moreover, in the iris smooth muscle, the ionophore, A23187 has also been shown to cause accumulation of IP_3, an effect which is secondary to the release of neurotransmitters by the ionophore, since this effect of A23187 was inhibited by prazosin (Akhtar & Abdel-Latif, 1984). However, since the rat thoracic aorta has a sparce adrenergic innervation (Kuchii et al., 1973), KCl-induced release of NA would not contribute to or only have a minor effect in causing accumulation of IPs. The inability of the indirect sympathomimetic, tyramine to contract the aorta supports the view that neuronal NA plays little part in the response to KCl in this tissue.

The second, more likely possibility is that Ca^{2+} influx caused activation of PLC and in turn caused accumulation of IPs. It has been suggested by Litosch, (1990) that the elevation of $[Ca^{2+}]_i$ may increase net production of IPs, either through activation of cytosolic or membrane PLC or both. In this study, KCl-induced activation of PI-hydrolysis was abolished in the presence of the voltage-dependent Ca^{2+}- channel blocker, nifedipine (10^{-6} M). In conclusion, these results suggest that

Ca^{2+}-influx may play a part in promoting PI-hydrolysis, since when Ca^{2+}-influx, was blocked, this effect no longer occurred.

Since the detailed mechanism of vascular relaxation is still unclear, the effect of different vasorelaxant agents were examined in aortic rings from Wistar rats. In this study the endothelium-dependent vasorelaxants, ACh ($3x10^{-9}$-$3x10^{-6}$ M) (Fig. 3.5), and the endothelium- independent vasorelaxant, SNP (10^{-10}-$3x10^{-6}$ M) (Fig. 3.6), relaxed isolated aortic rings that had been precontracted with NA (EC_{75}, $3x10^{-8}$ M), in a concentration-dependent manner. The maximal inhibition of NA-induced tone produced by ACh (1 μM), was 80% but that produced by SNP (30 nM) was 100% (Fig. 3.7). It is possible that the ability of ACh to produce only 80% inhibition is due to the fact that ACh is an endothelium-dependent vasorelaxant and some endothelial cells may have been damaged during the dissection and mounting of the rings. This seems unlikely since only a small fraction of the endothelium needs to be intact for complete relaxation of isolated vasculature to occur (Peach et al., 1985). In this study SNP was also more potent than ACh (Fig. 3.7). It is now well established that vascular smooth muscle relaxation induced both by endothelium-dependent vasorelaxants and the endothelium-independent nitro-vasodilators, is mediated by cGMP (Rapoport and Murad 1983b).

In this study the vasorelaxant activities of the endothelium-dependent and independent vasodilators were shown to be associated with increased levels of cGMP (Fig. 3.53, 3.54), measured by radioimmunoassay. The cGMP levels were measured in tissues mounted on hooks but in which no tension was applied. Assaying cGMP in aortic rings in this way had some advantages and some disadvantages. I): by not mounting the rings in an organ bath and applying tension

to them, this method would be likely to cause less damage to the endothelial cells, and obviously this would be reflected in cGMP production in response to endothelium - dependent agents. II): since mounting tissues in an organ bath takes some time, especially when there are many rings, this method was less time consuming, especially since the incubation time was also saved. III): since cGMP phosphodiesterase (PDE) hydrolyses the cGMP very quickly, the time required to remove the tissue from the hooks in an organ bath and terminate PDE activity is important. Incubating the aortic rings on hooks in test tubes rather than in organ bath simplified the experiment and allowed the tissues to be transferred quickly to liquid nitrogen to inactivate PDE and minimise hydrolysis of cGMP. IV): measuring cGMP in aortic rings in which the mechanical responses had also been recorded would have been desirable, since the conditions under which both responses were measured would have been similar.

The major disadvantage of the method used in this study to incubate the rings prior to measuring cGMP was that the levels of this nucleotide and the mechanical responses to drugs were not measured in the same tissues under identical conditions. However, this potential disadvantage was counterbalanced by the speed with which PDE could be inactivated by transferring the rings to liquid nitrogen and by the reduced risk of damage to the endothelium since the rings were not mounted on hooks and no tension was applied. However in spite of the disadvantages, the method used in this study was a standard procedure and the limitations applied to all experiments, so that a comparison between different relaxants is therefore valid.

It has been shown, by a previous study in our labaratory (Morrison, 1988) that extraction of cGMP from aortic rings was more efficient following tissue homogenisation, which was thought to increase the surface area across which the acid could permeate and therefore increase the yield of cGMP. However, this result contrasts with the finding of Bowman and Drummond, (1984), who reported that in the bovine retractor penis muscle, tissue homogenisation during acid extraction did not significantly increase the yield of cGMP. This discrepancy may be due to differences in the tissues. Since the aorta is a tough tissue, it may need to be homogenised to enable the acid to penetrate the tissue and increase the efficiency of extraction of cGMP. It is possible that the tissue cGMP content is located in distinct intracellular compartments (Waldman & Murad, 1987). Furthermore, tissue homogenisation may facilitate extraction of concentrations of cGMP that originate from different pools. Even if homogenisation had little or no effect on the total amount of cGMP extracted, by facilitating access of the acid throughout the tissue, it would increase the likelihood that samples of cGMP would be obtained from all the compartments within the tissue. Then, if changes in cGMP levels occurred in only one or a limited number of compartments within the tissue, this change could be detected. Throughout this study, tissue levels of cGMP were measured by a radioimmuoassay kit, based on the competitive binding between unlabelled cGMP and [^{3}H]-labelled cGMP to an antiserum with high affinity and specificity for cGMP. By using [^{3}H], a beta emitter, as the isotope label, the radioimmunoassay provided a convenient, reproducible and safe means of measuring cGMP.

Acetylcholine (ACh, 10^{-5} M) and sodium nitroprusside (SNP, 10^{-6} M), increased the levels cGMP in aortic rings in the presence of NA (EC_{75}, $3x10^{-8}$ M) (Fig. 3.53.

3.54). Although SNP (10^{-6} M) and ACh (10^{-5} M) relaxed the muscle completely, the level of cGMP induced by SNP was 9-fold higher than that induced by ACh. (Fig. 3.53, 3.54). It therefore appears that SNP increased the levels of cGMP above the level required for complete muscle relaxation. The differences in the levels of cGMP induced by SNP and ACh may be accounted for by differences in the mechanisms leading to stimulation of guanylate cyclase activity. The relaxant effects of ACh are receptor-mediated and require the synthesis and release of EDRF (NO) (Furchgott & Zawadzki, 1980). This is probably an ordered process that is regulated at various sites by other intracellular mechanisms. In contrast, SNP-induced relaxation involves the diffusion of the relaxant across the plasma membrane, after which nitric oxide is thought to be generated via an S-nitrosothiol intermediate (Ignarro & Kadowitz, 1985). This process appears to be a more rapid but crude means of stimulating guanylate cyclase activity and may partially explain the different potencies of ACh and SNP in increasing levels of cGMP.

The vasorelaxant agents (ACh and SNP) which increased cGMP synthesis not only relaxed NA-induced tone but also inhibited NA-induced PI-hydrolysis in aortic rings. Inhibition of NA-induced muscle tone by cGMP, may occur via several pathways. It has been proposed that cGMP may inhibit α_1- adrenoceptor mediated vascular contraction by Ca^{2+}- independent mechanisms, involving phosphorylation and inhibition of myosin light chain kinase (MLCK) via cGMP-dependent protein kinase (Nishikawa et al., 1984; Chiesi and Carafoli, 1986). Ca^{2+}-dependent mechanisms may also be involved and could include; 1): reducing $[Ca^{2+}]_i$ by Ca^{2+}-extrusion through the sarcolemma (Popescu et al., 1985; Twort & Van Breemen, 1988), 2): inhibition of Ca^{2+}-influx into the cells (Taylor & Meisheri,

1986), 3): inhibition of Ca^{2+}- release from intracellular Ca^{2+} stores (Rapoport, 1986); 4): stimulation of SR-Ca^{2+}-pump (Twort & Van Breemen, 1988).

In addition to these mechanisms for cGMP, Rapoport (1986), suggested that inhibition of α_1-adrenoceptor-induced contraction by agents that increase cGMP production, may be partly mediated by inhibition of PI-hydrolysis. In intact vascular tissues, ACh and SNP may inhibit NA-induced contraction by an initial activation of guanylate cyclase, with a secondary reduction of IP_3 synthesis. This mechanism is consistent with that proposed by Takai, (1984), and Watson et al (1984), who suggested that cGMP inhibits PIP breakdown in platelets through activation of protein kinase G.

In this study, in order to confirm the effect of cGMP, the permeant analogue of cGMP, 8-Bromo cGMP, was used and this analogue also inhibited NA-induced PI-hdrolysis in aortic rings (Fig. 3.37, 4.2). The endothelium- dependent vasorelaxant ACh, did not inhibit the PI-response in aortic rings (Fig.3.35), in which the endothelium had been removed either mechanically by rubbing or, chemically, by Triton X-100.

This result shows that the endothelium has an essential role both in the production of cGMP and in ACh-induced inhibition of the PI-response (Fig.4.1). However, these results suggested that cGMP only partially inhibits the PI-response, suggesting that cGMP-mediated vasorelaxation is only partly due to this effect of cGMP on PI-response.

One possible explanation of this mechanism is based upon the fact that, PLC, which is the enzyme that hydrolyses PI, is Ca^{2+}-dependent. Since cGMP lowers $[Ca^{2+}]_i$ by multiple mechanisms it may be that cGMP inhibits PI- hydrolysis by

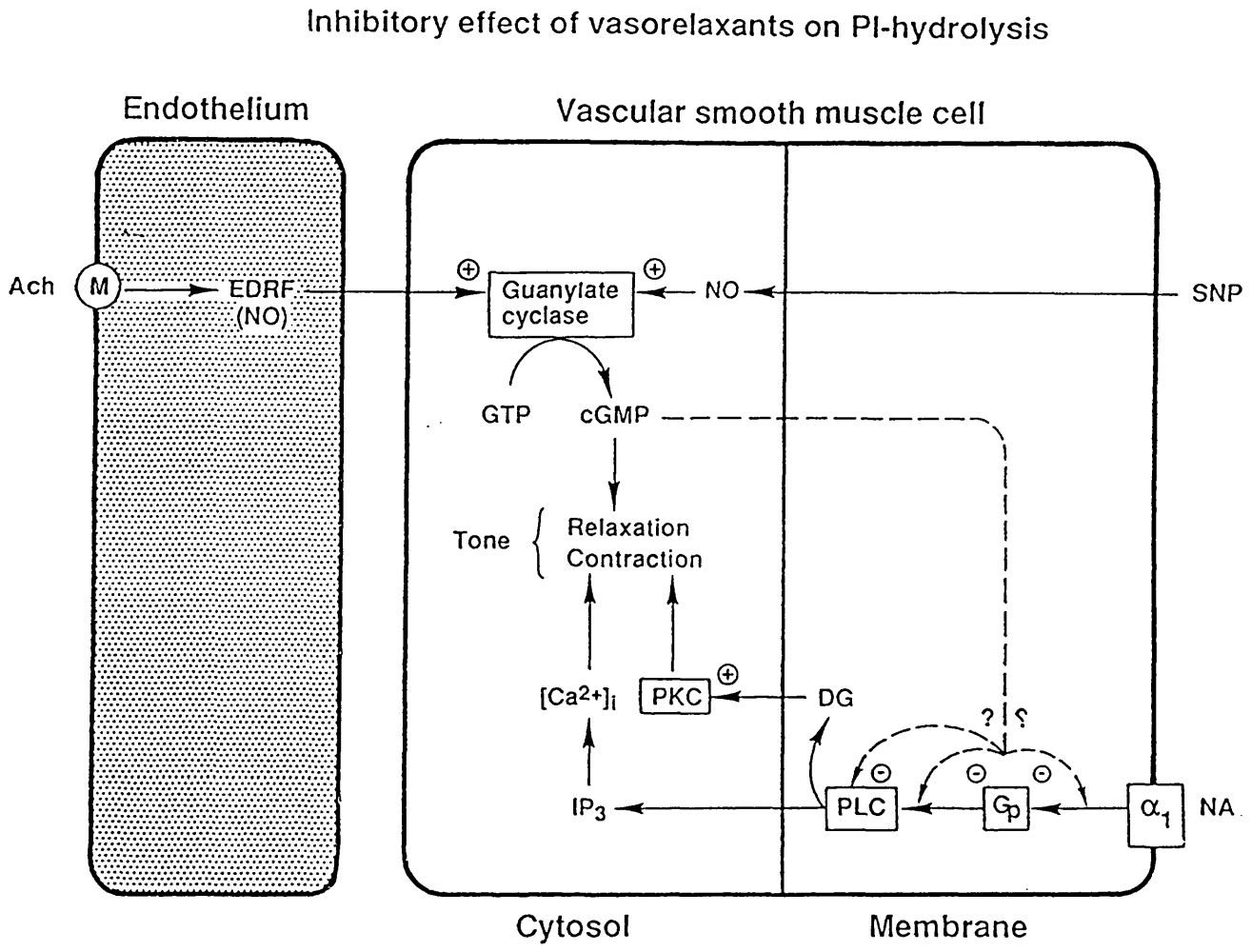

Fig. 4.1.
A schematic diagram showing possible sites of action for cGMP invascular smooth muscle. Both the endothelium-dependent vasorelaxant, ACh, and the endothelium-independent vasorelaxant, SNP, release NO, which activates guanylate cyclase to produce cGMP, which causes relaxation and inhibits the PI mechanism. cGMP inhibits the PI-hydrolysis but the site of action is still unknown.

attenuating Ca^{2+}-dependent PLC activity. cGMP may also have a direct inhibitory effect on one of the stages in the PI signal transduction mechanism, such as the G-proteins, which are the important components of the PI-pathway. It is not clear where the inhibitory action of cGMP occurs. The precise mechanism by which cGMP acts needs further investigation.

Since in vascular smooth muscle there is a second inhibitory mechanism that involves cyclic nucleotides, this study investigated the role of cAMP, which like cGMP has a vasorelaxant effect. The effects of the ß-adrenoceptor agonist, ISO, and directly-acting activator of adenylate cyclase, forskolin were examined. These drugs caused vasorelaxation and accumulation of cAMP in aortic rings (Fig. 3.8, 3.9, 3.55, 3.56).

In this study the ß-adrenoceptor agonists, ISO (10^{-6} M) (Fig. 3.38) and salbutamol (10^{-6} M) (Fig. 3.39), inhibited NA- induced PI-hydrolysis. Since these effects were antagonised by propranolol ($5x10^{-6}$ M), it appears that these effects were mediated via ß-adrenoceptors. Similar inhibitory effects were obtained with other agents such as the directly-acting adenylate cyclase activator, forskolin (10^{-6} M) (Fig. 3.42), and the phosphodiesterase inhibitors, isobutylmethylxanthine (IBMX, $1x10^{-6}$ M) (Fig.3.40), and theophylline ($5x10^{-6}$ M) (Fig. 3.41).

The effect of cAMP was confirmed using the permeant analogue of cAMP, 8-Bromo cAMP ($3x10^{-4}$ M) (Fig. 3.44), which also inhibited the PI-response.

This is the first time that it has been shown in vascular smooth muscle, that cAMP and cAMP-increasing agents, inhibit the NA-induced PI-response (fig.4.2).

These results are consistent with the previous results obtained in other tissues,

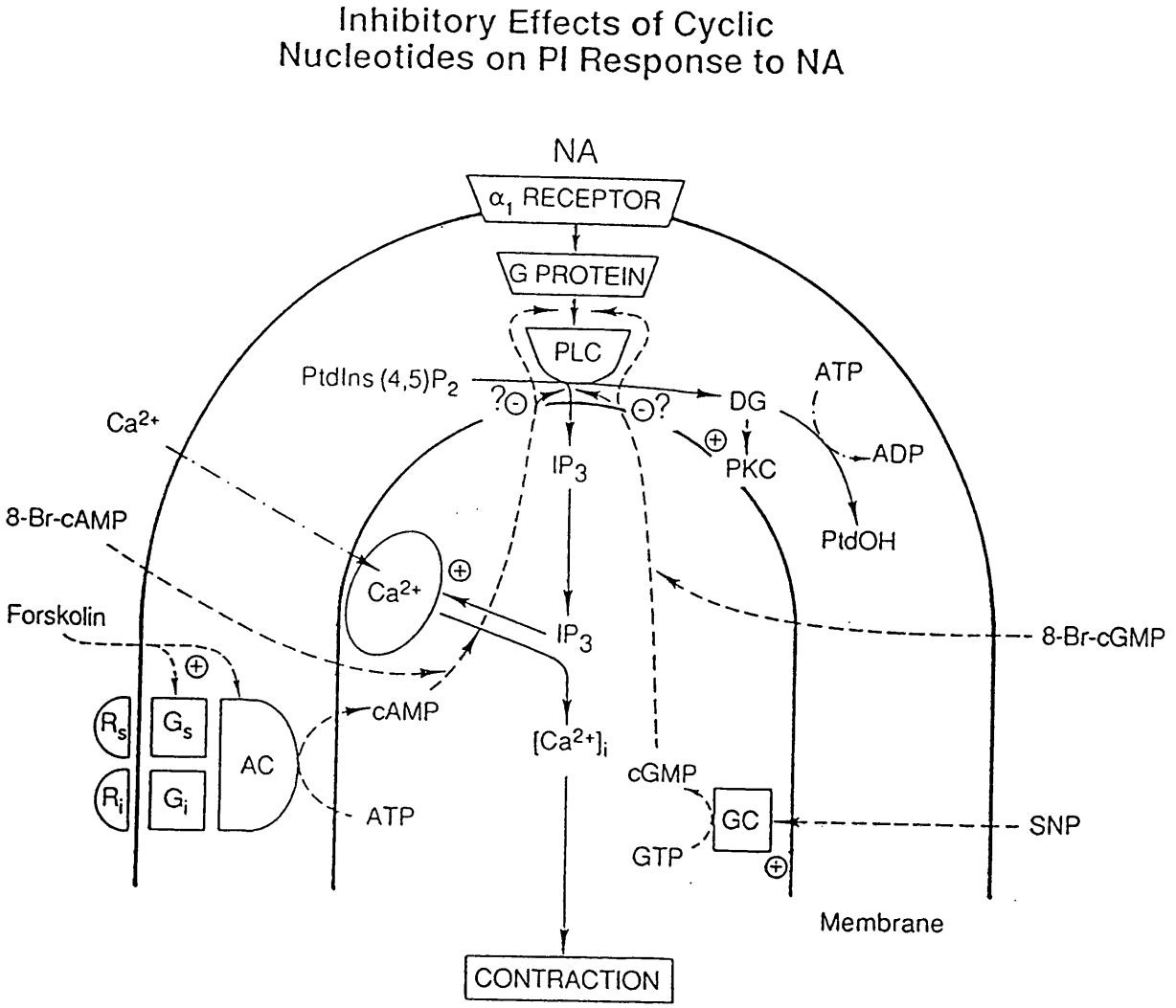

Fig. 4.2.
A schematic diagram showing possible sites of action for the cyclic nucleotides (cAMP and cGMP) in vascular smooth muscle. These nucleotides, whether synthesised within the cell or administered as stable, permeant derivatives, may inhibit the PI mechanism by an action on G-protein or on PLC.

including platelets (Takai et al., 1982; Watson et al., 1984), slices of rat kidney (Neylon & Summers, 1988) and in tracheal smooth muscle (Hall & Hill, 1989). The site of action of the cAMP is at present unknown but G-proteins, PLC and phospholipid kinases (which converts PI to PIP and then to PIP_2), are the most likely sites of action, but the exact sites of action of cAMP in vascular smooth remain to be established.

Like cGMP, inhibition of NA-induced muscle tone by cAMP may take place via multiple pathways, including; 1): Phosphorylation of MLCK via protein kinase A (Chiesi & Carafoli, 1986); 2): Activation of Ca^{2+}-ATPase in sarcolemmal membrane (Van Breemen & Saida, 1989); 3): Activation of Ca^{2+}-ATPase in SR membrane (Conti & Adelstein, 1981); 4): Inhibition of voltage-dependent Ca^{2+}-channels and in turn, inhibition of Ca^{2+}-influx (Ousterhout & Sperelakis, 1984).

cAMP may also influence G-protein functioning and PLC. It is generally accepted that when the G-proteins are activated, the heterotrimer ($\alpha ß \gamma$) dissociatesinto subunits (α and $ß\gamma$). In the adenylase cyclase (AC) system it has been suggested that, activation of G_i (AC inhibitor), opposes the action of the G_s protein (AC activator) directly and also indirectly by generating excess $ß\gamma$-subunits, which bind to and inactivate $G_s\alpha$ (Katada et al., 1984). By analogy, it is possible that if the PLC activator $G_{p(s)}\alpha$ interacts with $ß\gamma$-subunits, then generation of sufficient $ß\gamma$-subunits as a result of activation of another G-protein (in this case G_s, by ß-adrenoceptor agonists), will inhibit the activated $G_{p(s)}\alpha$.

Another possibility is that $ß\gamma$ has a directly inhibitory effect on PLC. This is consistent with a role of $ß\gamma$ in the regulation of PLC in Xenopus oocytes (Moriarty et al., 1988), in which injection of purified $ß\gamma$ (but not α) inhibited a muscarinic

response known to be mediated by activation of PLC. This explanation may account for receptor-mediated inhibition of PI, but a direct effect of cyclic nucleotides on PI-metabolism can not be excluded.

Another possibility for cAMP-induced inhibition is that lowering of $[Ca^{2+}]_i$ might indirectly inhibit PI-hydrolysis. This hypothesis has been supported by findings, which suggest that in whole cells (Delahunty et al., 1988; Vallar et al., 1988), or broken cells (Irvine, 1982), lowering $[Ca^{2+}]_i$ inhibits PLC activity, by attenuating Ca^{2+} -dependent PLC activity. However, the precise sites and mechanisms of action by which cyclic nucleotides cause inhibition of PI-hydrolysis, is unclear and needs further investigation.

In this study the Ca^{2+}-channel blocker, nifedipine (10^{-6} M), inhibited the NA-induced PI-hydrolysis in aortic rings (Fig. 3.45). This result suggests that extracellular Ca^{2+} may be required for the maintenance of the PI-response.

The contraction of vascular smooth muscle induced by α_1- adrenoceptor activation in rat aorta is believed to be caused either by Ca^{2+} release from the intracellular stores, or Ca^{2+} influx through Ca^{2+}-channels (Cauvin & Malik, 1984). Experimentally, α_1-adrenoceptor-mediated contraction, can be separated into phasic and tonic components; the phasic component seems to be dependent on intracellular Ca^{2+}, due to PI-hydrolysis (IP_3) (Rapoport, 1987), while the tonic component depends on influx of extracellular Ca^{2+} (Cauvin & Malik, 1984). Recently Nishimura and Ito (1989) and Nishimura et al. (1991), have reported that the tonic contraction caused by α_1-adrenoceptor activation in rat aorta is composed of two components, one of which is clearly associated with the cascade of PI- hydrolysis and probably involves protein kinase C. The other component is due to Ca^{2+} influx through

nifedipine-sensitive Ca^{2+}-channels.

In NA-induced PI-hydrolysis, the first part which corresponds to the phasic contraction is due to production of IP_3, which releases $[Ca^{2+}]_i$ from intracellular stores and the maintained or tonic contraction, requires influx of Ca^{2+}. This influx of Ca^{2+} has been shown to be caused via one or more metabolites of PI-hydrolysis such as IP_3, IP_4 or both (Irvine and Moor, 1986). On the other hand, this present study has shown that KCl-induced Ca^{2+}-influx can induce PI-hydrolysis. It has been suggested that lowering $[Ca^{2+}]_i$ inhibits PLC activity (Delahunty et al., 1988) and therefore the most likely explanation is that α_1-adrenoceptor activation causes an immediate release of intracellular Ca^{2+} by IP_3, from stores which are maintained by Ca^{2+} influx caused by IP_3, IP_4 or both. It appears that the influx of Ca^{2+} itself helps to activate the PI-hydrolysis since nifedipine, which inhibits Ca^{2+} -influx, partly inhibited PI-hydrolysis. This result is consistent with those of Rapoport, 1987; Vallar et al., 1988; & Delahunty et al., 1988).

SECTION 2: **Study of alterations in pharmacological and second messengers mechanisms in hypertensive rat aorta and tail artery.**

Essential hypertension is associated with elevated systemic arterial blood pressure and is caused by increased peripheral resistance. The mechanisms mediating this increase in peripheral resistance, are not clearly understood, and have given rise to conflicting reports on the mechanisms of hypertension. Many studies have examined the responsiveness of isolated blood vessels from hypertensive animals to

determine whether any changes in the sensitivity of these vessels occurred and whether these might explain the hypertension.

In isolated blood vessels from hypertensive animals both supersensitivity and subsensitivity to vasoactive agents have been recorded. (Reviewed by Winquist et al., 1982; Mulvany, 1983; Triggle & Laher, 1985). In most of these studies only mechanical responses were measured and the related intracellular events were not recorded. It is possible that the variation in the results obtained in these studies may be due to differences in the model of hypertension chosen and in the time-course of the development of the phenomenon, as well as differences in the blood vessels and techniques used. The mechanisms of action of the agonists investigated may also have affected the results obtained.

The current series of experiments examined the mechanisms of hypertension in only one model of hypertension, and sought to relate the mechanical responses of tissues to the underlying biochemical changes. Using this approach, it was hoped to gain a better understanding of how vascular responsiveness in hypertension may change. The model of hypertension chosen for study was that observed in spontaneously hypertensive rats (SHR), which are genetically predisposed to this condition (Trippodo & Frohlich, 1981). These animals were confirmed to be hypertensive in comparison with Wistar Kyoto (WKY) control rats by measuring the blood pressure (Fig. 3.11).

Surprisingly, NA produced much weaker contractions in aortic rings from SHR than in aortic rings from normotensive WKY control rats (Fig. 3.14). This finding is consistent with the work of some workers (Shibata et al., 1973; Swamy & Triggle 1980 a,b,; Pang & Sutter, 1980; Templeton et al., 1987). However, other

reports have shown that blood vessels from hypertensive rats are supersensitive to NA, (Bevan et al., 1975); Webb et al., 1981). In contrast, other investigators (Clineschmidt et al., 1970; Hallback et al., 1971) have reported that the sensitivities of blood vessels from normotensive and hypertensive rats are similar.

In this study the reduced responsiveness of aortic rings from SHR to NA may be related to the higher resting tension developed by these tissues compared with that recorded ina ortic rings from WKY control rats. It is possible, that SHR aortic rings were incapable of developing further substantial tension in response to NA since these tissues were already partially contracted.

KCl-induced contractions were smaller in aortic rings from SHR than in those from WKY controls (Fig. 3.15). These smaller contractions to KCl in aortic rings from SHR may also be explained by the high resting tension seen in these rings. If the contractile proteins in these rings were partially phosphorylated, then KCl, acting via potential-operated Ca^{2+}-channels, would only activate the contractile apparatus to a limited extent. It is possible that high resting tension in tissues from SHR might be due to abnormally high levels of intracellular Ca^{2+} (Bohr & Webb, 1988; Dominizak and Bohr, 1989, 1990) in the resting condition due to impairment of the mechanisms responsible for reducing $[Ca^{2+}]_i$ in hypertension. Furthermore, it might be speculated that high levels of $[Ca^{2+}]_i$ in hypertension would already have bound to the calmodulin and therefore increasing of $[Ca^{2+}]_i$ either by activation of receptors or depolarisation would be ineffective since there would not be enough calmodulin to bind the newly-entered Ca^{2+}. On the other hand, there may have been some previously-bound Ca^{2+}- calmodulin, which caused phosphorylation of the enzyme and contractile proteins, and prevented normal contraction. Another

possibility is that because of the high availability of $[Ca^{2+}]_i$ in hypertension, the affinity of calmodulin for Ca^{2+} might be reduced. In addition, either Ca^{2+}-calmodulin binding, or the consequent phosphorylation event or perhaps even the IP_3 receptors on the sarcoplasmic reticulum (SR) may undergo desensitization so that the aorta is less responsive to contractile agonists in hypertension. This observation requires further investigation.

The fact that NA produced smaller contractions in aortic rings from SHR was inconsistent with the ability of NA to cause PI-hydrolysis, in aortic rings from age-matched (10-12 weeks) animals (Fig. 3.46), but they were consistent with the ability of NA to cause smaller PI-hydrolysis in older (18-20 weeks) rats (Fig. 3.47). In rings from 10-12 weeks rats, low concentrations of NA (10^{-8}-10^{-6} M), produced a PI-response that was larger in rings from SHR than in rings from WKY. In this study, it has been shown that in aorta from Wistar rats, vasorelaxant agents agents such as ACh, SNP, and isoprenaline, at the concentrations in which they can maximally inhibit the NA-induced tone, inhibit the PI-response by less than 50%. These results suggest that even in a situation in which 50% of the agonist-induced increase in PI-hydrolysis remains, the tone is completely inhibited. It can therefore be concluded that, although the PI-hydrolysis pathway is important and has a crucial role in triggering Ca^{2+}-release and in the initiation of contraction, it may not be a precise or reliable indicator of a maintained mechanical response. These results also show that even when the coupling mechanism that causes contraction is active, agents which increase the synthesis of cGMP or cAMP can have potent vasorelaxant effects so that they overcome the contractile mechanisms and as a result, the net effect is

vasorelaxation. This explanation might be applicable for the results obtained with tissues from hypertensive animals.

In aortic rings from SHR, in which the NA-induced PI-response is high but the NA-induced contractions are smaller it is possible that the inhibitory mechanisms are more active in aortic rings from SHR than in those from WKY. Such a change may be due to an adaptive process in hypertensive tissue. Since, it is known that cGMP is produced in response to PI-hydrolysis (Berridge, 1984), increased PI-hydrolysis might be responsible for producing higher levels of cGMP. Thus a more powerful inhibitory mechanism may develop in tissues from SHR, resulting in weaker contractions to vasoconstrictors such as NA which causes PI hydrolysis. These results are consistent with the finding of others (Heagerty & Ollerenshaw, 1987), who have shown that agonist-induced PI-hydrolysis is enhanced at a time when the hypertension has not been established. However, with age and after the hypertension has been established, the PI-response is no longer enhanced and may even be less than in controls. It is possible that the increased PI-response may have a role in inducing and promoting the development of the hypertension, but once it is established, the role of PI would become less obvious because of the enhanced cGMP-mediated vasorelaxation.

Sodium nitroprusside-(SNP) induced concentration- dependent relaxations of both SHR and WKY aortic rings that had been precontracted with NA (Fig. 3.17). At low concentrations ($3x10^{-10}$- $3x10^{-9}$ M) (Fig. 3.17), SNP induced weaker relaxations of aortic rings from SHR than of WKY. This result is consistent with that of Otsuka et al., (1988), and it is possible that the relaxant effect of SNP at low concentrations was attenuated by highly active cGMP phosphodiesterases in aortic

rings from SHR. This possibility needs to be further investigated.

At higher concentration (10^{-6} M), SNP induced more powerful relaxations of aortic rings from SHR than of rings from WKY (Fig. 3.17). This effect was associated with a significantly greater increase in the level of cGMP in aortic rings from SHR than in those from WKY tissues (Fig. 3.58). Moreover, this effect was also reflected in a greater inhibitory effect of SNP (10^{-6} M) on PI-hydrolysis in SHR (Fig. 3.49). It is likely that this greater increase in cGMP level may account for the enhanced relaxation and bigger inhibition of NA-induced PI- hydrolysis in SHR aortic rings.

Acetylcholine (ACh, 10^{-6} M) (Fig. 3.57), also caused greater increases in cGMP levels and larger inhibitions of NA-induced PI-hydrolysis (Fig. 3.48) in aortic rings from SHR, and this may also be explained by the same mechanism. This increased capacity to elevate cGMP levels and relax aortic rings from SHR might be due to an adaptation or a compensatory mechanism that is activated in response to the high blood pressure. This finding is consistent with the results reported by Coquil et al., (1987), who showed that in aorta from SHR, the activity of cGMP-dependent protein kinase (PKG) was elevated. Webb et al. (1987), also have shown enhancement of vasodilator responses to ACh in hypertensive mice aorta, and the same compensatory response in SHR aorta has also been reported by Konishi & Su, (1983). However, the results obtained in this study are inconsistent with those of other workers (Lee et al., 1987; Shirasaki et al., 1988; Otsuka et al., 1988). Elevation of cGMP might be due to higher PI-hydrolysis in SHR rings and in turn, increased production of cGMP, and also decreased activity of cGMP-PDE. The ß-adrenoceptor agonist, isoprenaline (ISO, $3x10^{-10}$-$3x10^{-7}$ M)

caused concentration-dependent relaxations of NA-induced precontractions in aortic rings from both SHR and WKY rats but the inhibitory effect of ISO ($3x10^{-10}$ -$3x10^{-8}$ M) was less marked in rings from SHR that in those from WKY controls (Fig.3.18).

This effect of ISO, (10^{-8} M) was associated with a reduced ability to increase the levels of cAMP in aortic rings from SHR in comparison with rings from WKY controls. (Fig. 3.59). This difference was also reflected in smaller inhibitions of NA-induced PI-hydrolysis by ISO (10^{-6} M) (Fig. 3.50). It is likely that this smaller increase in cAMP level accounted for the weaker relaxations and smaller inhibitions of NA-induced PI-hydrolysis in SHR rings. Forskolin (FOR, 10^{-6} M) (Fig. 3.56) also, induced smaller increases in cAMP and smaller inhibitions of NA-induced PI-hydrolysis in rings from SHR (Fig. 3.51), probably for the same reason. These results are consistent with those of majority of the investigators (Triner et al., 1975; Cohen and Berkowitz 1976; Godfraind and Dieu 1978; Asano etal., 1982; Silver et al., 1985; Asano et al., 1988).

Since forskolin was less effective in aortic rings from SHR, it appears that in SHR vasculature, the alteration in the cAMP-mediated mechanism occurs at the level of the signal transduction mechanism rather than at the ß-adrenoceptor. Depressed basal and agonist-stimulated adenylate cyclase activity has been reported by Ramathan and Shibata (1974), who demonstrated lower levels of cAMP in blood vessels of young SHR, in which the level of adenylate cyclase activity was significantly lower than in WKY vessels. Others (Triner et al., 1975; Klenerova et al., 1975; Fukuda et al., 1990), have also shown this reduced ability to stimulate adenylate cyclase. In addition, reduced G-protein function has been shown

by Asano (1988) in vascular smooth muscle from SHR, in which the cAMP level is less than in control, suggesting that reduced G-protein activity is involved in this abnormality of the ß-adrenoceptor adenylate cyclase system.

It is possible that the reduced cAMP level occurs as a result of increased cAMP-PDE activity, which hydrolyses more cAMP. This also needs to be further studied. It may also be possible that cGMP-activated PDE hydrolyses the cAMP (Beavo & Reifsnyder 1990). The results obtained with nifedipine were also complex. Nifedipine (NIF, 10^{-9}-$3x10^{-6}$ M) caused smaller inhibitions of KCl-induced tone in aortic rings from SHR than in rings from WKY. In addition low concentrations of NIF (10^{-9}-$3x10^{-8}$ M) caused smaller inhibitions of NA-induced tone in rings from SHR than in those WKY, but higher concentration of NIF (10^{-6}-$3x10^{-6}$ M) produced larger inhibition of rings from SHR than WKY. A possible explanation for these results is that aortic rings from SHR are more dependent on intracellular Ca^{2+} and so are less dependent on extracellular Ca^{2+} than those from WKY controls.

The difference between these two results may reflect the different mechanisms by which the precontracting agents mobilise Ca^{2+}. Since, KCl depolarizes the cell membrane and causes an influx of Ca^{2+} through VOCs, NIF is an appropriate drug for studying the effect of KCl. However, NA uses both intracellular and extracellular Ca^{2+}. In the case of NA-induced tone, with a low concentration of NIF, the results were the same as with KCl-induced tone. However, with high concentrations of nifedipine, the results were different. A possible explanation of this observation may be that while intracellular $[Ca^{2+}]_i$ is available, vascular smooth from SHR is more dependent on it, than that from WKY

rats. However, once these stores have been depleted and are no longer available, no refilling or replacement of Ca^{2+} occurs so that, the hypertensive muscle then would become more dependent on extracellular Ca^{2+} than normotensive muscle. These results indicate that changes do occur in the responsiveness of isolated vasculature from hypertensive rats. Surprisingly, these changes in responsiveness are in the opposite direction to what might have been expected. Reduced responsiveness to contractile agonists and increased responsiveness to vasodilators is unexpected and certainly could not explain the marked hypertension present in the SHR. It is more likely that such changes reflect an adaptation to the hypertension rather than an explanation of it. The aorta is a conducting vessel which does not contribute to the peripheral resistance but it could be influenced by prolonged elevation of the peripheral vascular tone.

The results obtained with the tail artery were notably, different from those obtained in aortic rings. In this case, the changes in responsiveness may well be associated directly with the hypertension. These studies not only showed that the tail artery rings from SHR were heavier than those from WKY controls (Fig. 3.13) but were also more responsive to NA. When the responses to NA in isolated tail artery rings were measured as changes in isometric tension, the maximum response in rings from SHR was greater than that obtained in rings from WKY controls (Fig. 3.21). However, when these results were expressed as a percentage of the maximum response (Fig. 3.22) there was no shift in the curve, indicating that there was no changes in the sensitivity in the conventional sense but there certainly was an enhanced maximum respone to NA in the tail artery rings from SHR. This enhanced responsiveness was also seen in the ability of NA to cause

PI-hydrolysis in tail artery rings from SHR (Fig. 3.52). Thus, the enhanced mechanical response was consistent with the enhanced biochemical response. It was notable that in both cases the increased responsiveness was only seen at the highest concentration of NA.

When the responsiveness of the isolated tail artery was examined in isolated perfused segments rather than in isolated rings (Fig. 3.23), similar results were obtained but the magnitude of the enhancement of the maximum response to NA was much greater in perfused segments than in isolated rings. As with the isometric tension recording the concentration-response curves were superimposable at the lower concentration ranges. Only at higher concentrations was any difference seen. The increase in maximum response in tail artery segments from SHR compared to WKY controls was approximately 300%, whereas it was approximately 40% when the response are measured as changes in isometric tension. This enhancement of the response probably reflects the vascular wall thickening and the Laplace effect i.e. a small increase in tension is manifested as a large increase in perfusion pressure (Guyton, 1986). When these results were expressed as a percentage of the maximum response (Fig. 3.24) there was a rightward shift of the curve for perfused segments from SHR compared to the curve for perfused segments from WKY rats. This could be interpreted as subsensitivity but is more likely to have arisen because the maximum response was exaggerated as a result of the vascular wall thickening. No significant change in sensitivity or responsiveness to KCl was seen in tail artery segments (Fig. 3.25, 26, 27, 28). However, with both tension recording and perfusion studies there was a tendency toward a decreased responsiveness of tail artery segments

from SHR to KCl. No shift of the curve was seen when the results were expressed as a percentage of the maximum response. It is possible that in the tail artery rings any decreased responsiveness to KCl in the segments of the SHR might have been masked by KCl-induced NA release from nerve terminals.

These results indicate that the results obtained in a particular blood vessel depend upon the method used to record the response. A small increase in responsiveness, measured as tension in isolated rings, was amplified when the blood vessel was perfused and the response was resistance to the perfusion. It seems likely that the tail artery, which is a resistance vessel and is unlike the aorta, there is an increased capacity to respond to NA. This may be related to the hypertension that occurs in SHR but further work is required to confirm this association.

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