REGULATION OF THE RESPONSIVENESS OF VASCULAR SMOOTH MUSCLE

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

by

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November 1994

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ACKNOWLEDGEMENTS

First of all, I must thank Professor Stone for allowing me to undertake my studies in the Department of Pharmacology. I also wish to acknowledge the University of Glasgow for financial support.

I also owe a huge thank-you to my supervisor, David ("The Deep") Pollock, for his tremendous help, encouragement and amazing insight into many aspects of the project, not least the habits of those "monsters".

Thanks also to the others who made my stay in Lab 354 so enjoyable, especially Twirly (Did yez all get that?), Keith and the rest of The Lads (remember Aberfoyle??). I must also mention some blasts from the past, Shonna Moodie, Kevin "Superstud", Jules, Ying, Josie and Definetely not forgetting Annette. There are many others, the names of whom may now be lost in the mists of time, but here's your mention...

A Big thankyou must go to Jane "When's the party?" Nally for fun, driving lessons and last but not least inviting me on all those ski trips!! Also to Carol, Elspeth, Mark, Dunx and everyone else in the Dept. for generally keeping me amused and accompanying me to all those cheese'n wines.

Finally, to my family, John and Pat, and Michael, for reasons too numerous to mention (but they know!) - thank you for everything. And Kelvin - for being there.

Summary

1. The aim of this study was to investigate the roles of hypertension, α_2-adrenoceptors and neurotransmission in the regulation of rat blood vessel responses.

2. Experiments were carried out using the tail artery and aorta of spontaneously hypertensive rats (SHR), and comparisons were made with vessels from the normotensive control strain, Wistar Kyoto (WKY).

3. Rings of aorta and proximal tail artery from SHR were heavier than those from WKY, despite being cut to the same length. This indicated a structural difference, so the histology of the vessels was examined using light microscopy.

4. Both the wall thickness and lumen diameter of the aorta were greater in SHR compared to WKY, but there was no difference in wall thickness to lumen ratio. The area of the medial layer was increased in SHR relative to control.

5. There was no difference in the wall thickness of the proximal part of the tail artery between SHR and WKY rats, but the lumen diameter was reduced in SHR relative to control, giving a greater wall thickness to lumen ratio. The media area was decreased in SHR compared to WKY.

6. Wall thickness was increased and lumen diameter decreased in the distal part of the tail artery from SHR compared to WKY, giving a greater wall thickness to lumen ratio. The media area was increased in SHR compared to WKY.

7. Mechanical responses to drugs were investigated in the aorta and proximal tail artery from SHR and compared to responses obtained in vessels from WKY rats, using both isometric tension and perfusion pressure recording.

8. In the aorta, isometric tension responses to the vasoconstrictor phenylephrine (PE; 10^{-9}-$3x10^{-5}$M) were lower in rings from SHR compared to responses in rings from WKY, with a lower maximum response. There was no difference in the EC_{50} for PE between SHR and WKY. In contrast, responses to the endothelium-dependent vasodilator carbachol (10^{-9}-$3x10^{-5}$M) were greater in rings from SHR compared to relaxations in rings from WKY, with a greater maximum response and a lower EC_{50}. Using the endothelium-independent vasodilator sodium nitroprusside (SNP) there was no difference between hypertensive and control rats at the lower end of the concentration-response curve ($3x10^{-11}$-10^{-8}M). At the higher concentrations ($3x10^{-8}$-$3x10^{-6}$M) however, aortic rings from SHR were more responsive to SNP than were rings from WKY rats, with a greater maximum relaxation, although the EC_{50} was unchanged. There was no difference in the vasorelaxant response to the β-adrenoceptor agonist isoprenaline ($3x10^{-10}$ - 10^{-5}M) between hypertensive and control rats.

9. In tail artery rings, isometric tension responses to PE (10^{-8}-$3x10^{-5}$M), noradrenaline (NA ; 10^{-9} - 10^{-5}M) and UK-14,304 ($3x10^{-10}$ - $3x10^{-5}$M) were greater in SHR compared to WKY with an increased maximum response, but there was no difference in the EC_{50}. Yohimbine (50nM) produced a rightward shift in the concentration-response curve to UK-14,304 in tail artery rings from both SHR and WKY rats.

10. In perfused segments of rat tail artery the response to NA was much greater in vessels from SHR than in vessels from WKY. When expressed as a

percentage of the maximum response achieved however, responses in vessels from SHR were lower than in those from WKY.

11. The relaxations produced by SNP ($3x^{-10}$ - $3x10^{-5}$M) and isoprenaline (10^{-8} - 10^{-4}M) were lower in tail artery rings from SHR compared to those in rings from WKY. In vessels from hypertensive rats, SNP did not fully relax the PE-induced precontraction, whereas isoprenaline did not fully relax the precontraction in either hypertensive or control rats. Carbachol produced little or no response in either group of rat.

12. The biochemical response underlying NA-induced contraction in the rat tail artery was investigated by measuring accumulation of total inositol phosphates in response to NA. The basal phosphoinositide (PI) hydrolysis was higher in vessels from SHR than in those from WKY, and 10^{-5}M NA, which had produced increased contractile responses, gave a greater stimulation of PI hydrolysis in vessels from hypertensive rats as compared to control.

13. Preliminary experiments were carried out with rats of two different age groups (12-17 weeks and 6-9 weeks).

14. When tail artery segments were cannulated at one end ("single cannulation") and perfused with Krebs solution at a constant rate, the response to NA (10^{-3} - 100μg) was greater in older rats than in younger rats. The response to field stimulation (Supramaximal voltage 80V, 50 pulses, 0.4ms duration, 0.5-80Hz) tended to be greater in older than in younger rats.

15. When tail artery segments were cannulated at both ends ("double cannulation") and perfused with drug solution at a constant rate, there was no

difference in the response to either NA (10^{-8} - 10^{-5}M) or to KCl (0.01-0.05M) between older and younger rats.

16. The single cannulation experiment was repeated, but with the addition of a heating coil between the reservoir and the cannula. This time there was no difference in the response to NA between older and younger rats.

17. Part of the study investigated the contractions mediated via post-junctional α_2-adrenoceptors in rat tail artery rings.

18. In the proximal tail artery, concentration-response curves (CRC's) to clonidine (10^{-8} - 10^{-5}M) and to UK-14,304 (10^{-10} - $3x10^{-5}$M) were obtained. Yohimbine (30nM) produced a rightward shift in the clonidine CRC, with a greater effect at the lower end of the curve. In the distal tail artery, mounted as ring preparations on a myograph, the CRC to UK-14,304 was biphasic. This curve became monophasic in the presence of yohimbine (50nM), which produced a rightward shift in the first phase but did not affect the second phase.

19. Low concentrations of clonidine (10nM) and UK-14,304 (50nM) produced little or no response in rat tail artery rings. When the vessels were precontracted with arginine-vasopressin (AVP; 0.4mU ml^{-1}) or PE (0.5μM) (the "precontracting agonists") and then relaxed with 3-isobutyl-1-methylxanthine (IBMX; 10μM), the responses to clonidine and to UK-14,304 in the presence of these drugs were markedly enhanced. The response to clonidine was also enhanced in the presence of KCl (0.03M) and IBMX, indicating that activation of a receptor by the precontracting agonist was not necessary in producing this enhancement.

20. The response to clonidine (10nM) was also enhanced when IBMX was added to the organ bath before AVP, indicating that the order of drug addition was not important for the enhancement to be observed.

21. The effects of vasodilators other than IBMX were also investigated. The response to clonidine was increased in the presence of AVP and diltiazem (2μM). The response to UK-14,304 was increased in the presence of AVP and either forskolin (36nM), dibutyryl cyclic AMP (0.2mM) or SNP (0.1μM). The response to UK-14,304 was also increased in the presence of PE and SNP.

22. The responses to clonidine and to UK-14,304 enhanced under the described conditions were markedly inhibited or abolished by yohimbine (50nM). Unlike clonidine or UK-14,304, the response to a low concentration (0.2μM) of the α_1-adrenoceptor agonist PE was not potentiated in the presence of AVP and IBMX. When the protocol using AVP and IBMX was repeated in the presence of prazosin (50nM), the enhanced response to clonidine was unaffected. These results are consistent with the hypothesis that the enhanced responses to clonidine and to UK-14,304 were mediated via α_2-adrenoceptors.

23. There was a positive correlation between the size of the potentiated response to clonidine or to UK-14,304 and the size of the response to the precontracting agonist. In addition, when the time between AVP/IBMX and clonidine addition increased, there was a corresponding decrease in the size of the enhanced response to clonidine, which matched the decline in the response to AVP alone which occurred over time.

24. When vessels were precontracted with AVP, PE or 5-hydroxytryptamine (5-HT; 50nM) then relaxed with IBMX, the enhanced response to UK-14,304 was abolished by the AVP antagonist (β-mercapto-β,β-

cyclopenta-methylenepropionyl[1], O-Me-Tyr[2], Arg[8])-vasopressin (MCVP; 35nM), prazosin (0.1μM) or ketanserin (50nM) respectively. The enhanced response was unaffected by an antagonist not specific for the precontracting agonist, but was abolished in all cases by yohimbine (50nM).

25. Part of this study investigated the nature of the neurotransmission in the rat tail artery, including the possibility of NA/ATP (adenosine 5'-triphosphate) co-transmission. The concentration-response curve to exogenous NA was shifted to the right by prazosin (0.1μM). Exogenous ATP ($3x10^{-7}$ - $3x10^{-3}$M) produced a rapid response which was not maintained but declined sharply, and was inhibited by α,β-methylene ATP (α,β -MeATP; 0.5μM).

26. Field stimulation-induced contractions (supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms) were abolished by tetrodotoxin (TTX; 0.1μM). The frequency-response curve (FRC; 0.1-40Hz) was biphasic, peaking first at 5-10Hz and then at 30-40Hz. Responses at all frequencies were abolished by prazosin (0.1μM) but unaffected by α,β−MeATP (0.5μM). A lower concentration of prazosin (5nM) reduced the contraction to trains of stimuli at 10Hz and the residual response was abolished by yohimbine (50nM).

27. Field stimulation-induced contractions (supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms) were inhibited by yohimbine (50nM), clonidine (50nM) and UK-14,304 (50nM). Yohimbine curtailed the duration of the response, but clonidine and UK-14,304 prolonged the response.

28. The response to field stimulation was tested following precontraction with AVP and subsequent relaxation by IBMX. Under these conditions, previously shown to enhance contractions mediated via α_2-adrenoceptors, there

was no change in the amplitude of the field stimulation-induced contraction, but the response was prolonged.

29. Following the abolition of the response to 10Hz with prazosin, a biphasic response was revealed in the presence of AVP and IBMX. The second, more prolonged component of this response was abolished by yohimbine (50nM), leaving the first component, a spike. This initial spike was almost completely abolished by α,β –MeATP (0.5μM).

30. Field stimulation (Supramaximal voltage 50V, 10 pulses, 0.1ms, 0.1-40Hz) was examined in tail artery rings from SHR and WKY rats. In both groups of rat the FRC was biphasic, reaching a plateau at 2-10Hz, with responses again increasing from 10-40Hz.

31. Responses at the lower frequencies (0.1Hz, 0.2Hz) were greater in SHR compared to WKY. Following two consecutive repetitions of the FRC, responses at 0.1-2Hz diminished in vessels from SHR, while responses from WKY were well-maintained at each frequency. In SHR, responses at 0.1-0.2Hz increased in the presence of α,β –MeATP (1μM) but returned to control in the FRC following a second addition of α,β -MeATP. In WKY, α,β– MeATP had no effect on the response to field stimulation at any of the frequencies examined.

32. In rats pretreated with reserpine or 6-hydroxydopamine (6-OHDA), field stimulation (supramaximal voltage 70V; 0.5Hz, 10Hz or 20Hz, pulse width 0.4ms, 10 pulses) produced a prolonged, monophasic contraction which was not frequency-dependent. This response did not diminish with repetition, and was unaffected by TTX (0.1μM), prazosin (0.1μM), yohimbine (50nM) or α,β–MeATP (1μM).

PUBLICATIONS

Much of the work in this thesis has been presented at scientific meetings both in the U.K. and abroad, and the abstracts of these presentations are listed below.

1. AIDULIS, D.M., EL BISHTI, W., SHAW, A.M., MCGRATH, J.C. & POLLOCK, D. (1993). A comparison of the responsiveness of isolated pulmonary artery and tail artery rings from hypertensive rats to vasoconstrictors and vasorelaxants. *Br.J.Pharmacol.*, **108**, 141P.

2. AIDULIS, D.M., FUGLSANG, A. & POLLOCK, D. (1992). Investigation of the mechanism underlying drug-induced uncovering of enhanced contractions to UK-14,304 in rat tail artery rings in vitro. *Br.J.Pharmacol.*, **105**, 299P.

3. AIDULIS, D.M., FUGLSANG, A., SAVAGE, S.A.H. & POLLOCK, D. (1991). Drug induced enhancement of contractions to clonidine in rat tail artery rings in vitro. *Br.J.Pharmacol.*, **104**, 51P.

4. AIDULIS, D.M., MACKENZIE, F.R., MORRISON, K.J. & POLLOCK, D. (1990). Alteration in the responsiveness of isolated blood vessels from spontaneously hypertensive rats to vasoconstrictors. *J. Physiol.*, **426**, 88P.

5. AIDULIS, D.M., MACKENZIE, F.J., MORRISON, K.J. & POLLOCK, D. (1990). Subsensitivity to agonists in blood vessels from spontaneously hypertensive rats . *Eur.J. Pharmacol.*, **183 (3)**, 834.

6. AIDULIS, D.M. & POLLOCK, D. (1992). Inhibition of enhanced contractions to UK-14,304 by yohimbine, prazosin or a vasopressin antagonist in rat tail artery rings in vitro. *Br.J.Pharmacol*, .**107**, 183P.

7. DOWELL, F.J., AIDULIS, D.M., SHARIFI, A.M. & POLLOCK, D. (1990). Increased sensitivity to noradrenaline and noradrenaline-induced phosphoinositide hydrolysis in tail artery from spontaneously hypertensive rats. *J. Physiol.*, **429**, 76P.

8. FUGLSANG, A., AIDULIS, D.M. & POLLOCK, D. (1991). "Uncovering" enhanced responses to clonidine in rat tail artery. *Blood Vessels*, **28 (4)**, 289.

9. YOUNG, D.A., AIDULIS, D.M. & POLLOCK, D. (1992). A comparison of the effects of vasorelaxants in vascular and non-vascular smooth muscle from spontaneously hypertensive rats. *Br.J.Pharmacol.*, **107**, 189P.

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Introduction

GENERAL

The main function of the blood vessels is to transport oxygen and nutrients to the cells and tissues of the body, and to deliver waste products to their sites of excretion. Blood vessels also serve as a communication system whereby substances such as hormones are transported from sites of release to target tissues, and constitute part of the body's defences against invading pathogens, the immune system. In addition, the thermoregulatory properties of blood vessels serve as an important homeostatic mechanism for maintaining a constant body temperature (Vander, Sherman & Luciano, 1990; Rang & Dale, 1991).

Blood vessels are regulated by various mechanisms, which act in concert to ensure that they respond appropriately. These mechanisms involve neurotransmitters, hormones and locally-released substances, whose effects are complex and may be interrelated. For example, the sensitivity of blood vessels to the neurotransmitter, noradrenaline (NA), is regulated by corticosteroids and tri-iodothyronine (Gibson, 1981). Some regulatory mechanisms, such as those concerned with neurotransmission, are quickly activated and of transient duration, whilst others, such as hormone-induced sensitivity changes (Gibson & Pollock, 1975) are activated more slowly and exert a more persistent influence. Because of their nature and complexity, there is considerable scope for alteration or derangement of vascular regulatory mechanisms. Moreover, it appears that explanation of the phenomenon of essential hypertension, a major pathological condition affecting the vasculature, will depend on clarification of how these various mechanisms act and interact.

BLOOD VESSEL STRUCTURE

Blood vessel walls consist of three layers, the intima, the media, and the adventitia. The inner layer, the intima, is composed of a single layer of endothelial cells lining the entire circulatory system, and may also contain some smooth muscle cells. Vascular smooth muscle cells are mainly located in the media, together with connective tissue such as elastin and collagen. The outermost layer, the adventitia, contains sympathetic nerve endings and connective tissue (Figure 1).

The proportion of these components varies according to the type of blood vessel, whose structure is related to its function. In the systemic circulation for example, the aorta has thick walls with a high proportion of elastic tissue and a large radius, and serves as a low-resistance conduit conducting blood to the various organs. In contrast, the thick-walled muscular arterioles with smaller radii are the major site of resistance to flow in the vascular tree, and are involved in the maintenance of systemic blood pressure (Vander, Sherman & Luciano, 1990).

There is evidence that blood vessel structure is altered in hypertension (Short, D., 1966; Folkow, 1982; Jespersen et al., 1985). In addition, changes in the properties of the endothelium (Vanhoutte & Luscher, 1987; Yin, Chu & Beilin, 1992) and smooth muscle (Mulvany et al., 1980; Bendhack, Sharma & Bhalla, 1992) and in the innervation of blood vessels (Ferrone et al., 1979; Kawasaki, Cline & Su, 1984) have been reported in vascular diseases such as hypertension. The precise nature of these changes however is far from clear.

Blood vessels often exhibit altered responses to stimuli in hypertension (for reviews, see Winquist, Webb & Bohr, 1982; Marin, 1993). Many factors have been implicated, for example alterations in the release of, or response to, neurotransmitters (Ekas & Lokhandwala, 1981; Marin, 1993), or changes in receptors on the smooth muscle cell membrane, such as the α-adrenoceptors

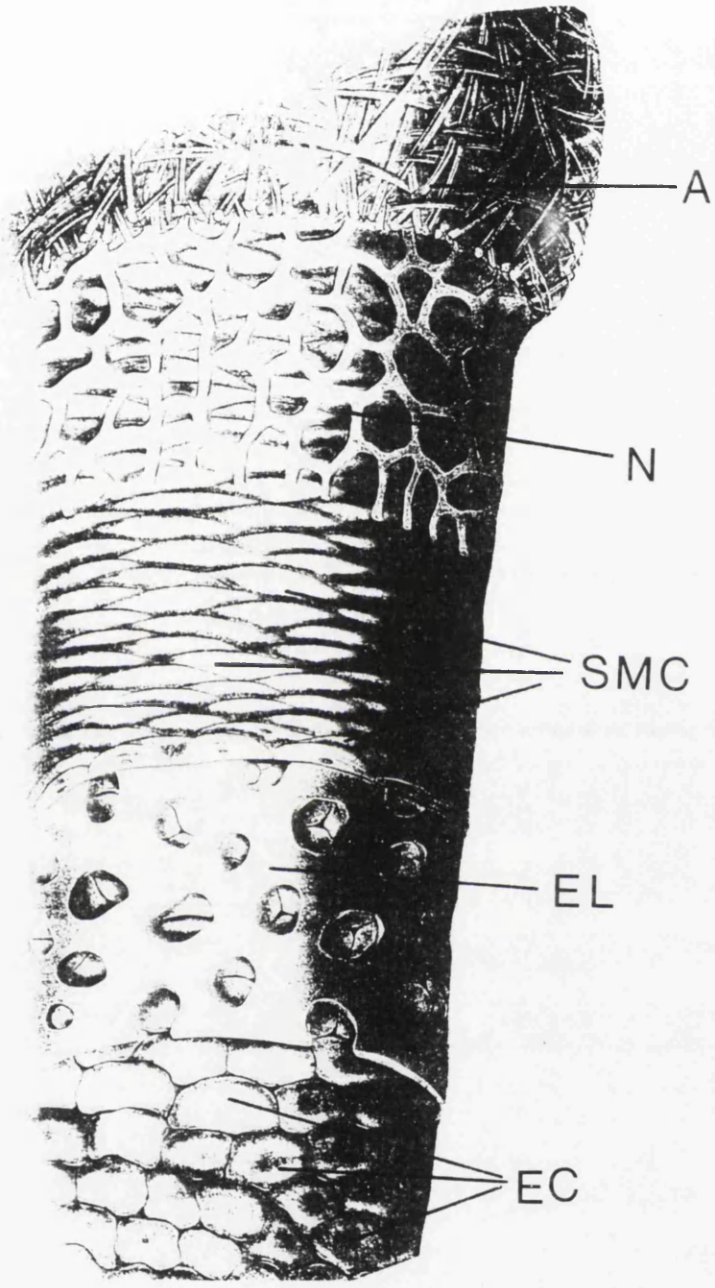


Figure 1. **Schematic representation of a blood vessel wall.**

A=Adventitia; **N**=Nerve net; **SMC**= Smooth muscle cells; **EL**=Internal elastic lamina; **EC**=endothelial cells.
(Adapted from Lee, 1993).

(Fukuda et al., 1983; Sanchez et al., 1986), the latter perhaps being determined by the former. In order to gain insight into how the responsiveness of vascular smooth muscle can be regulated by such factors, in hypertension or otherwise, it is important to understand the processes underlying vascular smooth muscle contraction and relaxation.

BLOOD VESSEL CONTROL MECHANISMS

The vascular smooth muscle cell is the main site of action for vasoactive substances, for example circulating hormones, and agents liberated from the endothelium and sympathetic nerves. Bundles of smooth muscle fibres are arranged in a circular or helical fashion around the blood vessel wall (Vander, Sherman & Luciano, 1990) and respond to stimuli by contracting or relaxing, thus producing vasoconstriction or vasodilatation respectively. An important concept is that, as with virtually all physiological systems, blood vessel calibre is the resultant of a variety of excitatory and inhibitory influences, which are dynamic in nature and subject to moment to moment, and long term, regulation.

VASOCONSTRICTION

Contraction of smooth muscle is less well characterised than that of striated muscle (Kamm & Stull, 1985), but there are common features such as the dependence on calcium ions (Ca^{2+}; Filo, Bohr & Ruegg, 1965), the presence of actin and myosin, overlapping thick and thin filaments with crossbridges (Somlyo & Somlyo, 1975; Johansson, 1978; Murphy, 1982; van Breemen et al., 1986), and a length-tension relationship (Vander, Sherman & Luciano, 1990). These features suggest that smooth muscle contraction occurs by a sliding-filament mechanism similar to that in skeletal and cardiac muscle.

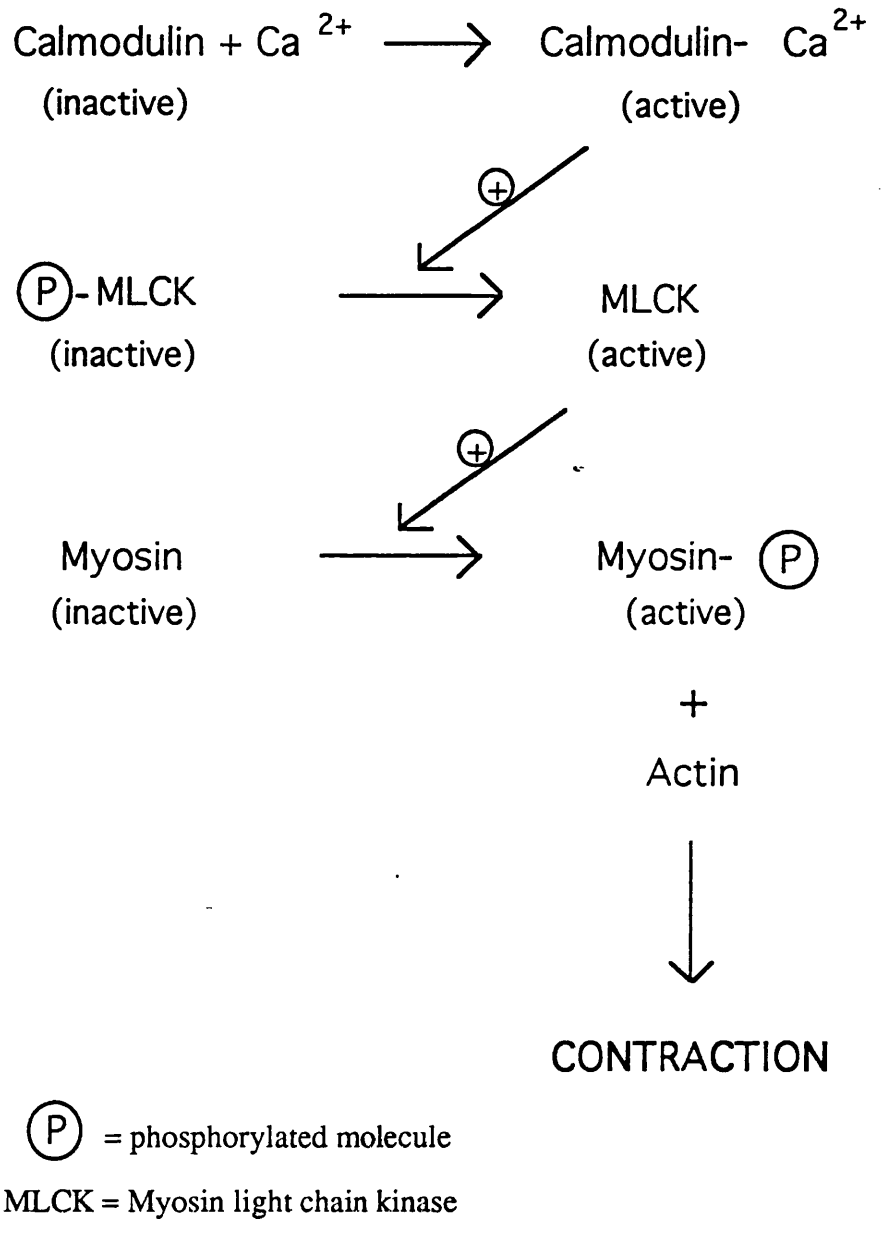


Figure 2. **Schematic representation of the enzyme cascade leading to vascular smooth muscle contraction.** Cytosolic calcium (Ca^{2+}) complexes with calmodulin to dephosphorylate and thus activate myosin light chain kinase (MLCK). This enzyme phosphorylates and thus activates myosin, which combines with actin to initiate cellular contraction.

It is now widely accepted that the major messenger of contractile activation is calcium (Somlyo & Himpens, 1989), which initiates the enzyme cascade leading to smooth muscle contraction (Figure 2; Sobieszek, 1977; Adelstein & Einsenberg, 1980; Somlyo & Himpens, 1989). Changes in vascular smooth muscle tone occur as a result of changes in the level of intracellular free calcium ($[Ca^{2+}]_i$) or in the sensitivity of the contractile apparatus to a constant level of $[Ca^{2+}]_i$ (Murphy, 1982; van Breemen et al., 1986; Somlyo & Himpens, 1989; Somlyo et al, 1990; Kobayashi et al., 1991). $[Ca^{2+}]_i$ may be increased by entry from the extracellular compartment via voltage-operated calcium channels (VOC's; activated by a change in membrane potential) or receptor-operated channels (ROC's; activated by receptor occupation by agonists) (Bolton, 1979; van Breemen, Aaronson & Loutzenhiser, 1979). In addition, a Ca^{2+} influx in the absence of excitation exists, termed the "calcium leak" (van Breemen et al., 1986).

As well as these extracellular sources, Ca^{2+} is released from intracellular stores such as the sarcoplasmic reticulum by inositol 1,4,5-trisphosphate ($InsP_3$). This second messenger is a product of the phosphoinositide (PI) signalling pathway, which was discovered after the initial chance finding that hormones have effects on PI metabolism (Hokin & Hokin, 1953; 1955). Since then it has become apparent that a large number of agonists can stimulate an increase in the metabolism of membrane phosphoinositides (Michell, 1975; Berridge, 1984), including NA (Uchida et al., 1982), 5-hydroxytryptamine (5-HT; Fain & Beridge, 1979), arginine-vasopressin (AVP; Creba et al., 1983; Rhodes et al, 1983), angiotensin II (AII; Billah & Michell, 1979) and platelet-derived growth factor (PDGF; Habenicht et al., 1981).

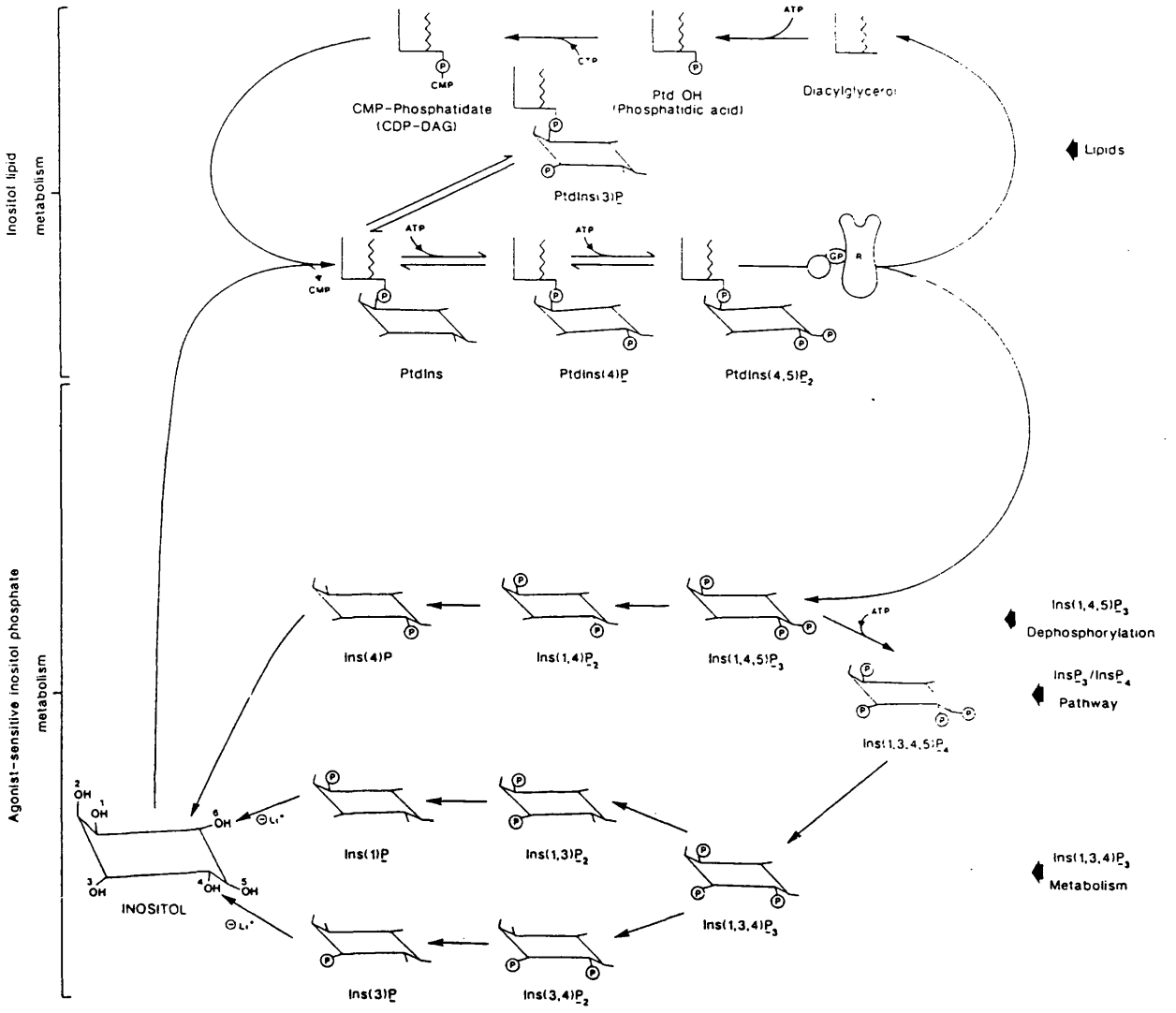


Figure 3. **The phosphoinositide cycle.**

The main routes of metabolism in the phosphoinositide cycle are represented. **R**=receptor; **Gp**=G=protein. (Adapted from Berridge & Irvine, 1989).

THE PHOSPHOINOSITIDE (PI) PATHWAY

The phosphatidylinositols are a class of membrane phospholipids which comprise less than 10% of the total phospholipid content in animal cells, but are the most metabolically active (Williamson, 1986), and are now recognised to be very important in signal transduction.

Initially, it was believed that the predominant inositol-containing lipid, phosphatidylinositol (PtdIns), was split by phospholipase C (PLC) to form diacylglycerol (DAG) and inositol 1-phosphate. However, it has since been shown that the initial reaction is hydrolysis of the PtdIns derivative, phosphatidylinositol-4,5-bisphosphate ($PtdInsP_2$), by the G-protein-linked enzyme PLC. This yields the products DAG and $InsP_3$ (Michell et al, 1981; Berridge, 1983), both of which have second messenger functions.

$InsP_3$ is very effective in releasing Ca^{2+} from intracellular stores (Berridge, 1983), probably from a Ca^{2+} pool in the endoplasmic reticulum (Burgess et al., 1983; Streb et al., 1984), and this Ca^{2+} contributes to the contraction produced by an agonist. $InsP_3$ is then rapidly dephosphorylated to inositol and re-incorporated to PtdIns in the membrane, allowing the cycle to continue (Figure 3).

Extensive study of the metabolic pathway for $InsP_3$ has revealed the formation of highly phosphorylated, cyclic derivatives of the inositol phosphates (Batty et al., 1985), and of another possible second messenger, inositol 1,3,4,5-tetrakisphosphate ($InsP_4$). $InsP_4$, either alone or in combination with $InsP_3$, may have a role in promoting slow entry of extracellular Ca^{2+}, and has been shown to control the transfer of Ca^{2+} between intracellular pools (Irvine & Moore, 1986).

DAG, the other "arm" of this bifurcating signalling pathway, stimulates the enzyme protein kinase C (PKC; Nishizuka, 1984), thus initiating various protein phosphorylation reactions. The role of PKC in vascular smooth muscle

contraction is as yet unclear, but it may be linked to phosphorylation, and subsequent activation of the contractile proteins (Kamm et al., 1989).

The whole cascade and the physiological functions of all the compounds released are far from elucidated, but it is clear that the phosphoinositide signalling system may be important in the regulation of vascular smooth muscle by virtue of its effects on calcium mobilisation and PKC activation. Accordingly, the PI pathway may be important in conditions where blood vessel control is altered, such as hypertension (Heagerty & Ollerenshaw, 1987).

Many of the studies on PI metabolism in hypertension have used the erythrocyte as a model system (Kiselev et al., 1981; Koutouzov et al., 1982; Riozzi et al., 1987), following evidence that in essential hypertension there are abnormalities in the erythrocyte membrane handling of uni- and divalent cations (Postnov et al., 1977; Postnov, 1990; Swales, 1991). One model of human essential hypertension is the spontaneously hypertensive rat (SHR), which is genetically predisposed to the condition (Okamoto & Aoki, 1963). Several pieces of evidence indicate that erythrocyte membranes from SHR have an altered turnover of membrane phosphoinositides, as measured by the rate of [^{32}P] incorporation into phosphatidylinositol 4-phosphate (PtdInsP) and PtdInsP_2 (Kiselev et al., 1981; Koutouzov et al., 1982). Interestingly, studies in erythrocyte membranes from hypertensive patients showed no difference in phosphoinositide metabolism from control subjects, as measured by the rate of [^{32}P] incorporation into PtdInsP and PtdInsP_2. However, in normotensive offspring of hypertensive patients, erythrocyte membrane phosphoinositide activity was greatly enhanced (Riozzi et al., 1987). These authors concluded that PI metabolism was increased in subjects genetically at risk of hypertension, before the blood pressure rises, and that once the blood pressure was established, PI metabolism was normal. This would suggest that the

phosphoinositide system may have a role in the genesis of hypertension, but the picture as yet is far from clear.

VASORELAXATION

The ability of NA and other vasoconstrictors to contract blood vessels is opposed by the action of vasorelaxants, either by an endothelium-dependent, or an endothelium-independent, mechanism. Endothelium-dependent relaxation was first demonstrated in 1980, when Furchgott & Zawadzki showed that the endothelial lining was essential for the vasodilator action of acetylcholine (ACh) in isolated arterial strips or rings from rabbit. ACh acts on muscarinic receptors on the endothelial cells to release a compound which Furchgott & Zawadzki termed "endothelium-derived relaxing factor" (EDRF). This compound then diffuses to the underlying vascular smooth muscle cells, possibly via a carrier molecule such as a sulphur-containing amino acid (Ignarro et al., 1981), where it induces vascular smooth muscle relaxation by activating guanylate cyclase, producing a rise in guanosine 3': 5'-cyclic monophosphate (cyclic GMP) levels (Rapoport & Murad, 1983; Vanhoutte et al., 1986; Waldman & Murad, 1987; Garthwaite, Charles & Chess-Williams, 1988).

It is now generally accepted that EDRF is nitric oxide (NO) or a related compound (Ignarro et al,. 1987; Kahn & Furchgott, 1987; Palmer, Ferridge & Moncada, 1987; Moncada, Radomski & Palmer, 1988), and several substances acting on the appropriate receptors on the endothelial cell membrane can stimulate its release, including 5-HT, histamine, thrombin, AVP, substance P, bradykinin and the calcium ionophore A23187 (Vane, Gryglewski & Botting, 1987).

Exogenous vasorelaxants which do not require the presence of a functional endothelium act directly on the vascular smooth muscle itself. Nitrovasodilators such as glyceryl trinitrate (GTN) and sodium nitroprusside

(SNP) diffuse into the muscle cells where they are metabolised to NO (Bennet & Marks, 1984), thus stimulating guanylate cyclase and elevating cyclic GMP levels within the cell. In contrast, β-adrenergic agonists such as salbutamol act on β_2-adrenoceptors on the cell membrane, which are coupled to adenylate cyclase via the stimulatory G-protein, G_S (Gilman, 1987), raising cellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels.

CYCLIC NUCLEOTIDES AND VASCULAR RELAXATION

Originally, it was believed that cyclic AMP promoted smooth muscle relaxation, while cyclic GMP promoted smooth muscle contraction (Goldberg, O'Dea & Haddox, 1973). It is now known that both of these cyclic nucleotides are involved in vascular smooth muscle relaxation (Ignarro & Kadowitz, 1985; Waldman & Murad, 1987).

The role of cyclic AMP as a second messenger was first revealed by the work of Sutherland and his colleagues in the late 1950's. They demonstrated that a heat-stable factor was generated in liver membranes following hormone treatment, and that this factor, cyclic AMP, led to a marked glycogen phosphorylase activity in the previously unresponsive supernatant fraction. Since then, it has been shown that cyclic AMP regulates many different kinds of cellular function.

In vascular smooth muscle, cyclic AMP produces relaxation by reducing intracellular Ca^{2+} levels which have been raised by contractile agonists (Lincoln & Cornwell, 1991). Possible mechanisms of Ca^{2+} reduction include inhibition of Ca^{2+} influx and activation of Ca^{2+} sequestration (Meisheri & van Breemen, 1982; Hwang & van Breemen, 1987; Abe & Karaki, 1988, 1989). In addition, cyclic AMP may relax vascular smooth muscle by reducing the sensitivity of the contractile elements to Ca^{2+} (Abe & Karaki, 1989). Furthermore, cyclic AMP has been shown to inhibit the action of the contractile proteins by

phosphorylating myosin light chain kinase, thus converting the enzyme to its inactive form (Adelstein & Einsenberg, 1978).

The cyclic GMP system has been less extensively studied than the cyclic AMP pathway, which was the first second messenger system to be discovered. Like cyclic AMP however, it is thought that cyclic GMP relaxes vascular smooth muscle by lowering intracellular calcium levels (Cornwell & Lincoln, 1989). This may be achieved by various mechanisms including stimulation of Ca^{2+} uptake into stores (Twort & van Breemen, 1988), activation of the plasmalemmal Ca^{2+} pump and Ca^{2+} extrusion (Vrolix et al., 1988), and inhibition of $InsP_3$-induced Ca^{2+} release from the sarcoplasmic reticulum (Meisheri, Taylor & Saneii, 1986). The major mediator of cyclic GMP action is thought to be protein kinase G (PKG; Cornwell & Lincoln, 1989; Butt et al, 1993).

It is generally understood that cyclic AMP produces its effects via activation of protein kinase A (PKA), which mediates the cellular response by protein phosphorylation (for review, see Taylor, Buechler & Yonemoto, 1990). In most cell types however, cyclic AMP leads to an increase in $[Ca^{2+}]_i$ (Black et al., 1989; Lau & Bordeau, 1989; Staddon & Hansford, 1989). This is clearly not the case in vascular smooth muscle cells (Walter, 1981) and platelets (Waldman et al., 1986), and it is interesting that both of these cell types are rich in PKG (Lincoln & Cornwell, 1991). It is possible therefore that in vascular smooth muscle cyclic AMP activates PKG in addition to PKA, with the former action underlying the reduction in $[Ca^{2+}]_i$.

Cyclic AMP and cyclic GMP are important and powerful mediators of vascular smooth muscle relaxation and subsequent vasodilatation. Alterations in the regulation of these second messengers may therefore have functional consequences in hypertension, which has often been associated with a reduced ability of blood vessels to relax (Lockette, Otsuka & Carretero, 1986; Mayhan, Faraci & Heistad, 1987; Luscher et al., 1988).

Several studies in aortic smooth muscle from SHR have shown a reduced sensitivity to β-adrenoceptor agonists, when compared to muscle from normotensive, Wistar Kyoto (WKY) control rats (Triner et al., 1975; Cohen & Berkowitz, 1976; Godfraind & Dieu, 1978). Possible reasons include alterations in β-receptor density or coupling to adenylate cyclase, in adenylate cyclase activity, or in activity of the phosphodiesterases, a family of enzymes which metabolise cyclic nucleotides (Silver, Michalak & Kochmund, 1985).

A study on the role of PKA in the reduced sensitivity to β-agonists in SHR (Silver, Michalak & Kocmund, 1985) showed that there was no difference in the rate and extent of PKA activity between hypertensive and control rats, although arterial relaxation was markedly reduced in the SHR as compared to control. This suggests that a change in a component other than PKA is responsible for the reduced relaxation. Alternatively, this finding may support the hypothesis of Lincoln & Cornwell (1991), that PKG, rather than PKA, mediates cyclic AMP-induced vasodilatation in vascular smooth muscle.

The autonomic nervous system is one of the major physiological mechanisms controlling blood vessel diameter. The density of innervation of each blood vessel varies according to its location and function. The aorta is a conducting vessel and receives little innervation, whereas the main artery of the rat tail, a thermoregulatory organ, responds rapidly to electrical field stimulation as a consequence of its dense adrenergic innervation (Sittiracha, McLachlan & Bell, 1987).

The term "autonomic nervous system" was first proposed by Langley in 1898, and included both the sympathetic and parasympathetic divisions (Dale, 1954). The existence of the autonomic nervous system has in fact been recognised for several centuries. In 1732, the Danish anatomist Winslow applied the term "sympathetic" to the peripheral nerves (Bowman & Rand, 1981), which he believed regulated the interrelationships, or "sympathies" of organs in the body. Our present-day understanding of the anatomy and physiology of the autonomic nervous system is based on the work of Gaskell and Langley. Gaskell (1886) demonstrated the functional connection through the white rami communicantes between the central nervous system and the sympathetic chains, and the connections of the grey rami to spinal nerves. Gaskell labelled the cranial, sacral and thoracolumbar outflows from the prevertebral and other ganglia as the "involuntary nervous system".

The process by which signals are transmitted from the nerve to the effector tissue was the focus of much attention in the late 1800's. Du Bois Reymond (1877) proposed two alternative theories, the electrical theory and the humoral theory. The electrical theory, which held that transmission was brought about by the spread of nerve action currents to the tissue, was more generally believed. However, a key observation by Elliot (1904) was that degeneration of sympathetic nerve terminals did not abolish the sensitivity of smooth muscle

preparations to adrenaline (which the electrical theory predicted) but actually enhanced it. The humoral theory, which proposes that chemicals with specific actions are released from nerves, was then favoured, particularly since it explained the observation that both excitation and inhibition can be produced in one tissue by stimulation of both sympathetic and parasympathetic nerves (Loewi, 1921).

The chemical transmitter of the sympathetic nervous system was long believed to be adrenaline. This is true of the frog heart, where Loewi showed that sympathetic stimulation released a substance capable of accelerating a second heart. Later studies in other preparations, however, showed that the effects of injected adrenaline were not identical to the effects of sympathetic stimulation. The theory arose that adrenaline is released and combines with an excitatory or an inhibitory substance in the receptors to form two new complexes. These complexes, "Sympathin E" in cells excited by the transmitter, and "Sympathin I" in those inhibited by the transmitter, would then be the active substances (Cannon & Rosenblueth, 1933).

This early prediction of the intracellular production of second messengers was not recognised as such at the time, and the theory, which was only partially correct, was not generally accepted (Dale, 1954). The identity of the sympathetic transmitter was later revealed to be NA (von Euler, 1946).

The concept of chemical transmission was applied to parasympathetic fibres, following observations that the effects of the alkaloid muscarine and parasympathetic nerve stimulation were very similar, and could be blocked by atropine. The most likely candidate for the parasympathetic transmitter was shown to be ACh (Dale, 1914; Loewi, 1921; Dale & Dudley, 1929).

NON-ADRENERGIC, NON-CHOLINERGIC (NANC) TRANSMISSION

The main transmitters liberated from the post-ganglionic sympathetic and parasympathetic nerves of mammals are, respectively, NA and ACh. Over the years however, it has become increasingly evident that there are other substances which can function as neurotransmitters. Early studies hinted at such possibilities, indeed as far back as 1898, Langley noted that vagal stimulation of the stomach produced relaxation. This response was found to be "atropine-resistant" (McSwiney & Robson, 1929). Several other studies found that responses to parasympathetic nerve stimulation persisted in the presence of atropine (Langley & Anderson, 1895; Bayliss & Starling, 1899). The introduction of adrenergic neurone blocking drugs such as guanethidine revealed analogous responses from stimulation of some nerves which were anatomically sympathetic, but insensitive to block by these drugs.

Such anomalous effects could not be explained in terms of the "classical" transmitters NA and ACh. Experiments in the guinea pig taenia coli, for example, showed that following blockade of both adrenergic and cholinergic responses to nerve stimulation, large, transient hyperpolarisations were produced. These electrical responses, termed "inhibitory junction potentials" (i.j.p.s), were accompanied by relaxations (Burnstock et al, 1963, 1964). Thus the idea arose that "non-adrenergic, non-cholinergic" (NANC) nerves exist.

Since the 1960s, NANC nerves have been reported in a wide variety of organs and species, such as the gastrointestinal tract and rat anococcygeus muscle (Burnstock, Campbell & Rand, 1966; Gillespie, 1972), guinea pig trachea (Coburn & Tomita, 1973), rabbit portal vein (Hughes & Vane, 1967), and other parts of the cardiovascular system (Burnstock, 1969).

A systematic search for the identity of neurotransmitters other than NA and ACh then began. For a substance to be regarded as a neurotransmitter, it must fulfil certain criteria (Eccles, 1964), namely:-

(i)It must be synthesised and stored in nerve terminals,

(ii)It should be released during nerve stimulation,

(iii)There should be enzymes that inactivate the "transmitter" and/or an uptake system for the transmitter or its breakdown products,

(iv)Post-junctional responses should be produced by the exogenous "transmitter" that mimic responses to nerve stimulation,

and (v) drugs should produce parallel blocking or potentiating effects on the responses to both exogenous transmitter and nerve stimulation.

Several substances were investigated, including catecholamines, histamine, 5-HT , prostaglandins, various amino acids and some polypeptides. Most were rejected because, for example, specific blocking drugs for these compounds did not affect the nerve-mediated response, or their action was by stimulation of nerves and not by a direct action on smooth muscle (Burnstock, 1979). However, evidence accumulated that a likely contender for a non-adrenergic, non-cholinergic transmitter was a purine nucleotide, probably adenosine 5'-triphosphate (ATP; Burnstock et al., 1970; Burnstock, Dumsday & Smythe, 1972).

Nerves which use ATP as the main transmitter were termed "purinergic" (Burnstock, 1971), and the purinergic nerve hypothesis was formulated when Burnstock proposed a model for the storage, release and inactivation of ATP from purinergic nerves (Burnstock, 1972). Since then, there has been evidence both for and against the purinergic nerve hypothesis (Burnstock, 1979; Stone, 1981; Gillespie, 1982). In the stomach and oesophagus in particular, discrepancies arose in the ability of ATP to mimic the response to NANC nerve

stimulation (Gillespie, 1972; Ohga & Taneike, 1977; Daniel, Crankshaw & Sarna, 1979). This may be explained by the fact that ATP is rapidly broken down to adenosine monophosphate (AMP) and adenosine, thus accounting for its low potency (Ambache et al., 1977a; 1977b). In addition, ATP is a ubiquitous compound with a key role in cell metabolism and many workers were reluctant to accept an additional transmitter role for such a compound. However, Su, Bevan & Burnstock (1971) demonstrated that tritium-labelled adenosine was taken up by stomach and intestine preparations and converted to [^{3}H]-ATP. Analysis of the radioactivity in frozen sections showed that when the tissue was incubated in low concentrations of [^{3}H]-adenosine, most of the label was stored in ATP in nerves, thus supporting a role for ATP as a neurotransmitter.

Hints that substances other than ATP could function as the chemical messenger from NANC nerves came from morphological examination of neurones in the 1970s. Electron microscope studies revealed that some nerves contained a complex mixture of vesicles, suggesting that they may contain more than one transmitter (Gabella, 1972; Cook & Burnstock, 1976). Further histological studies, including fluorescence histochemistry, advanced the investigations, and in 1980, Hockfelt et al. described autonomic nerves containing various polypeptides.

Polypeptides and purines such as ATP may therefore function as NANC neurotransmitters in the autonomic nervous system. In addition, there is evidence that other compounds may fulfil such a role, including 5-HT (Wood & Mayer, 1979), γ-amino butyric acid (GABA; Jessen et al., 1979) and dopamine (Bell, 1982).

Recognition of NANC transmission marked a major advance in the study of autonomic control, and provided the basis for the next significant step

in our understanding of chemical transmission - the phenemenon of co-transmission.

CO-TRANSMISSION

The concept that a neurone may use more than one transmitter is now known as the co-transmitter hypothesis (Burnstock, 1976). It was possibly first proposed by Koelle in 1955, as an attempt to account for the effects of nerve stimulation which are often difficult to explain in terms of a single transmitter hypothesis.

For many years however, it was widely believed that a nerve cell makes and releases only one transmitter, and this became known as Dale's Principle. This is in fact a misconception of Dale's original proposal, which was that each neurone releases the same transmitter at all its synapses (Dale, 1935). Indeed, there is no example of a neurone known to release different transmitters at different terminals (Segal, 1983; Eccles, 1986). This however does not exclude the possibility that more than one transmitter can be liberated from each terminal (Eccles, 1986).

Following the early work of Burn and Rand (1965), there is now considerable evidence for the co-existence of purines or peptides together with classical neurotransmitters (Chan-Palay & Palay, 1984; O'Donohue et al., 1985). However, co-existence of more than one substance in a nerve terminal does not necessarily imply that each substance functions as a neurotransmitter. Nevertheless, it is now generally accepted than in many tissues the "classical" transmitters (NA and ACh) are stored together with the putative transmitters, and are co-released on nerve stimulation to act together post-junctionally.

Such a system provides sophisticated control of the target organ. The role of co-transmission in the control of target tissues such as vascular smooth

muscle is regulated by processes including ageing (Bao, Eriksson & Stjarne, 1989a) and hypertension (Vidal, Hicks & Langer, 1986). There is consequently a need to re-assess the existence and importance of co-transmission in many situations. One tissue on which there are conflicting reports about the existence and significance of co-transmission, is the rat tail artery.

THE RAT TAIL ARTERY

The rat tail is a major thermoregulatory organ (Dawson & Keber, 1974) with a complex vascular system (Gemmell & Hales, 1977). Blood flow through the tail is modified by changes in vascular resistance regulated mainly by variation in sympathetic discharge (Sittiracha, McLachlan & Bell, 1987). The main ventral artery, which lies superficially in a midline groove between the flexor muscle groups, is innervated by a dense varicose plexus of noradrenergic axons (Sittiracha, McLachlan & Bell, 1987). This vessel is often used as an example of a resistance vessel in studies of vasoactive drugs and mechanisms of sympathetic nervous control (Wade & Beilin, 1970; Surprenant, Neild & Holman, 1983; Medgett & Langer, 1984).

Both NA and ATP have been proposed to mediate contractions in response to sympathetic nerve stimulation in the rat tail artery (Sneddon & Burnstock, 1984a; Bao, Eriksson & Stjarne, 1989a, 1989b, 1989c, 1990; Bao, 1993), providing support for co-transmission in this vessel. Many studies however, do not address the possibility of ATP as a transmitter in this vessel (Cheung, 1984; Medgett, 1985; Papanicolaou & Medgett, 1986; Rajanayagam, Medgett & Rand, 1990). These studies focus on aspects of NA-mediated responses to nerve stimulation without considering possible effects of ATP, while the previous group of studies were enthusiastic in their support. An investigation by Kotecha and Neild (1987) used an interesting approach to address the question of co-transmission in the rat tail artery. It has been shown

that denervation causes an increase in receptors for the neurotransmitter (Axelsson & Thesleff, 1959; Kuffler, Dennis & Harris, 1971). Kotecha & Neild therefore reasoned that following denervation, an increase in receptors for α,β-Methylene ATP (α,β–MeATP), which binds to ATP receptors (P_{2x}-purinoceptors; Burnstock & Kennedy, 1985), would be consistent with these receptors being involved in neurotransmission. However, no evidence for an increase in receptors was found.

Clearly the issue for co-transmission in the rat tail artery has not been resolved. One of the main reasons for the controversy is that many blood vessels lack a distinct relationship between the electrical and mechanical responses to nerve stimulation (Burnstock, 1988).

In some preparations, NA/ATP co-transmission has been relatively well characterised, the rodent vas deferens being one of the best known examples. In the rodent vas deferens, following stimulation of the hypogastric nerve, a fast electrical response is produced on the post-junctional smooth muscle surface. It is now thought that this excitatory junction potential (e.j.p.) is mediated by ATP (Burnstock, 1988), and underlies the initial twitch contraction of a biphasic mechanical response (Meldrum & Burnstock, 1983; Burnstock, 1988). The second, slower phase of contraction is mediated by NA released from the same nerve, and probably the same vesicle, as ATP (Meldrum & Burnstock, 1983; Burnstock, 1988). The relevant phases of the response, both electrical and mechanical, are blocked by the appropriate antagonists. Both the twitch contraction and e.j.p. are inhibited by α,β-MeATP, a stable analogue of ATP which is thought to act by desensitising the receptor which mediates ATP-induced contraction, the P_{2x}-purinoceptor (Kasakov & Burnstock, 1982). The slow contraction is inhibited by adrenergic antagonists such as prazosin, an α_1-adrenoceptor blocker and phentolamine, an α-antagonist which is non-selective for the α_1- or α_2-adrenoceptor subtype (McGrath, Brown & Wilson, 1989).

In many blood vessels however, the nature of chemical transmission is much less well characterised. Excitatory junction potentials can be elicited in vascular smooth muscle such as the rat tail artery by stimulation of the perivascular nerves. Like the vas deferens, a distinctive feature of the e.j.p.s is their resistance to α-adrenergic blocking agents (Holman & Suprenant, 1980; Cheung, 1982). In contrast to the vas deferens however, in some blood vessels the e.j.p. is completely blocked by ATP antagonists, while the mechanical response is barely affected (Burnstock, 1988). Such dissociation between electrical and mechanical responses is not compatible with a causative relationship between the two, and leads to questions regarding the role or relevance of such an electrical change which apparently has little role in the ultimate response of the tissue, i.e. contraction.

An alternative hypothesis to co-transmission has been put forward to explain the origin of responses to nerve stimulation which are resistant to α-adrenoceptor blockade. A further subtype of adrenoceptor, the "gamma" (γ) receptor, was proposed to mediate such responses (Hirst & Jobling, 1989). In guinea pig arterioles, the contraction evoked by NA when applied to regions distant from the sympathetic nerves was mediated by α-adrenoceptors. In contrast, when NA was applied close to the sympathetic nerves, the response was resistant to α-adrenoceptor blockade (Hirst & Neild, 1980; 1981).This response was attributed to activation of a novel adrenoceptor, the γ-receptor. The theory received little support however, and was not pursued. Nevertheless, it did give rise to important questions regarding the receptor subtypes involved in the post-junctional response to neurally-released transmitter.

An early experiment by Bernard (1857) showed that nicotine applied to a certain part of a skeletal muscle surface produced twitching, and that this effect was prevented by curare. This prompted Langley (1905) to postulate the existence of a "receptor substance" located on the muscle rather than on the nerve endings. He also proposed that this "receptive substance" could exist in more than one form in autonomic effector cells.

Advances in receptor classification often came with the use of antagonists. Experiments by Dale (1914) showed that following blockade of muscarinic receptors by atropine, the nicotinic effects of ACh were revealed, thus demonstrating two different types of receptor for ACh. Similarly, a parallel with adrenergic receptors was shown (Dale, 1913). Dale's "adrenaline reversal" experiment demonstrated that adrenaline produces two different types of effect, vasoconstriction and vasodilatation, and that one could be converted into another depending on the experimental conditions. On injection of adrenaline into a cat, the arterial pressure rose. Dale showed that this vasoconstrictor component disappeared if the animal was first injected with an ergot derivative, and noticed that adrenaline then caused a fall, instead of a rise, in arterial pressure. It was later recognised that certain ergot alkaloids act as α-receptor antagonists, and that Dale's adrenaline reversal experiment reflected the unmasking of the β effects of adrenaline by α-receptor blockade. This experiment provided an early illustration of an important concept in physiology and pharmacology, namely, that the observed responses are not fixed and often depend on the experimental conditions.

Later work beginning with Ahlquist (1948) showed that several subclasses of adrenoceptor exist in the body. Ahlquist found that the rank order of potencies of various catecholamines fell into two distinct patterns and postulated the existence of two kinds of receptor, α and β, with the following

order of potency : α:- (NA) > adrenaline (adr) > isoprenaline (isop); β :-isop > adr > NA.

However, the classification of α- and β-adrenoceptors was not generally accepted until the introduction of specific β-adrenoceptor antagonist drugs, beginning with dichloroisoprenaline (Powell & Slater, 1958) followed by pronethalol (Black & Stephenson, 1962) and propranolol (Black et al., 1964). Lands et al. (1967) subsequently showed that β-adrenoceptors could be further divided into β_1- and β_2-subtypes.

A similar degree of selectivity among drugs acting on α–adrenoceptors has now been recognised, and these are generally subdivided into α_1– and α_2–adrenoceptors. Dubocovich & Langer (1974) found that α–adrenoceptors could be subdivided on the basis of their differential sensitivity to the alkylating agent phenoxybenzamine. This followed the discovery that pre-synaptic α–receptors differed from the post-junctional α–receptors (Starke, 1972; Langer, 1973). It was subsequently proposed that the post-junctional receptors should be termed α_1 and the pre-junctional, α_2 (Langer, 1974; Berthelsen & Pettinger, 1977).

For many years it was thought that receptors were confined to the post-synaptic membranes of the cells on which the neurotransmitters acted (Dale, 1952). Since then however, nerve terminals have been found to contain a wide variety of receptor types (Stjarne, 1975; Langer, 1977; Starke, 1977; 1981), which influence transmitter release (Starke, 1977; Westfall, 1977).

Experiments on the perfused cat spleen (Brown & Gillespie, 1956; 1957) demonstrated that drugs could increase sympathetic transmitter overflow. It was later shown that activation of pre-junctional α-adrenoceptors by transmitter noradrenaline reduces noradrenaline release, and that interruption of this "negative feedback loop" by α_2-adrenoceptor antagonists results in an increased transmitter outflow (Starke, 1977; 1981).

The original anatomical classification of adrenoceptor subtypes has now been rejected in favour of a more pharmacological approach. Studies using

specific α-adrenoceptor antagonists such as prazosin, which is highly selective for the α_1-subtype (Cambridge & Davey, 1980), revealed responses in the vascular smooth muscle of rat and cat which were resistant to blockade of α_1-adrenoceptors (Bentley, Drew & Whiting, 1977; Cambridge, Davey & Massingham, 1977). These contractile responses were thought to be mediated via a sub-population of α-adrenoceptors, and support the conclusion that α_2-adrenoceptors are located not only on nerve terminals but also post-junctionally on target tissues (Docherty, MacDonald & McGrath, 1979; Timmermans, Kwa & van Zwieten, 1979). The functional role of the post-junctional α_2-adrenoceptor is not as clear-cut as that of the α_1-adrenoceptor however, and many gaps in the literature still exist. These must be filled before the role of the α_2-adrenoceptor in post-synaptic events, such as regulation of the responsiveness of vascular smooth muscle, is fully accepted.

Since the original differentiation between α- and β- adrenoceptors by Ahlquist (1948) and the subsequent recognition of α_1- and α_2-adrenoceptor subdivision (Langer, 1974), various forms of technical progress have suggested further subdivisions. The advent of radioligand binding techniques enabled investigators to measure directly the binding of α-adrenoceptor-selective agents to their receptors. This led to the discovery that both α_1- and α_2-adrenoceptors could be subdivided further into at least two additional subtypes (Bylund, 1988; Minneman, 1988). Molecular and genetic techniques have since reported three clones of an α_2-adrenoceptor from human DNA. These clones are the α_2-C10 (Kobilka et al., 1987; Fraser et al., 1989), the α_2-C2 (Lomasney et al., 1990; Weinshank et al., 1990) and the α_2-C4 (Regan et al., 1988), with 10, 2 and 4 denoting chromosomal number. These genes encode for the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtypes respectively. An α_{2D}-adrenoceptor subtype has also been reported but it is thought that the gene encoding this subtype is a rat homologue of the human α_{2A}-adrenoceptor (MacKinnon, Spedding and Brown, 1994). Porcine and rat analogues of these clones have also been reported (Guyer et al., 1990; Zeng et al., 1990). Similarly, three different α_1-adrenoceptor clones have been discovered (Harrison, Pearson & Lynch, 1991).

This information should be interpreted cautiously however, and the relevance of such molecular data to functional receptors mediating physiological responses must not be overestimated.

For a long time, α_1-adrenoceptors were thought to be the only post-junctional adrenergic receptor subtype involved in the response of a blood vessel to circulating or neurally-released sympathomimetic agents. It is only in the last decade or so that the involvement of α_2-adrenoceptors in such responses has been recognised. Experiments demonstrating effects mediated via

post-junctional α_2-adrenoceptors have generally been conducted in situ in preparations such as the pithed rat (McGrath, 1982), conscious or pithed rabbits (Hamilton & Reid, 1980), and in the perfused hind limb of the rabbit (Madjar, Docherty & Starke, 1980) or dog (Langer, Massingham & Shepperson, 1981).

There is much less evidence available from in vitro experiments. The first report from an in vitro study for a vascular smooth muscle α_2-adrenoceptor was in the dog (De Mey & Vanhoutte, 1981). Venous smooth muscle was found to respond to α_1- or α_2-adrenoceptor agonists but arterial smooth muscle only to α_1. The effects of antagonists tended to support this but were complicated by the presence of non-competitive antagonism. At this stage therefore the classification of "post-junctional α_2-adrenoceptors" was still tentative.

Evidence from further in vitro work is steadily accumulating however that α_2-adrenoceptors do exist on vascular smooth muscle, as shown in the rat tail artery with selective labelling by tritiated clonidine (Weiss, Webb & Smith, 1983). Moreover, the authors suggested that these receptors do indeed have a functional role in mediating contraction of the vascular smooth muscle.

More recently, post-junctional α_2-adrenoceptors have been demonstrated in vitro in human blood vessels (Nielsen et al.,1992) where they mediate vasoconstriction.

It is now generally accepted that α_1-adrenoceptor activation leads to smooth muscle contraction both by promoting Ca^{2+} influx via Ca^{2+} channels on the plasma membrane, and by stimulating PI hydrolysis (Han, Abel & Minneman, 1987). It has been proposed that these effects are mediated via different subtypes of α_1-adrenoceptor, the α_{1A}- and α_{1B}-adrenoceptor respectively (Han, Abel & Minneman, 1987).

Despite the growing awareness that α_2- as well as α_1-adrenoceptor activation causes vascular smooth muscle to constrict, the mechanisms underlying α_2-adrenoceptor-mediated contraction are relatively unknown. Such

contractions have been difficult to demonstrate in vitro, and an interesting feature of some recent studies is that they are dependent upon a degree of vascular smooth muscle stimulation by some other receptor system (Dunn, McGrath & Wilson, 1991). In the isolated vascular bed of the rat tail for example, the α_2-agonist UK-14,304 was virtually inactive when added alone. In the presence of the hormone vasopressin, pressor responses to UK-14,304 were uncovered which were prazosin-resistant, but sensitive to inhibition by rauwolscine, an α_2-antagonist (Templeton et al., 1989). Similarly, contractions to UK-14,304 were revealed following precontraction of the rat tail vascular bed with the peptide endothelin -1 (Et-1; MacLean & McGrath, 1990). An analogous phenomenon has been described in the rabbit isolated distal saphenous artery, where contractions to UK-14,304 were dependent on prior exposure to the hormone AII or to the selective α_1-adrenoceptor agonist phenylephrine (PE; Dunn, McGrath & Wilson, 1989; 1991).

Clearly, whether or not a response to an α_2-adrenoceptor agonist is seen depends on the conditions under which the experiment is conducted. Several intriguing questions arise - why are other substances (e.g. AVP, AII, Et-1) apparently required before α_2-adrenoceptor-mediated contractions are seen, and is this linked to the observation that such contractions are more easily (although not exclusively) demonstrated in vivo rather than in vitro?

In the light of both the α_1- and the α_2-adrenoceptor being involved in vascular smooth muscle contraction, certain aspects of vascular control should be reconsidered. It is tempting to speculate on the possibility that prazosin-resistant contractions to sympathetic nerve stimulation, previously attributed to the action of co-transmitter ATP, may be mediated by the α_2-adrenoceptor. This however may be a simplistic explanation of the discrepancies in the purinergic hypothesis. Nevertheless, it is clear that the question of co-transmission in

blood vessels must be re-examined in order to understand more fully how the responsiveness of vascular smooth muscle is regulated by neural control.

SENSITIVITY CHANGES IN AUTONOMIC EFFECTORS

Smooth muscle sensitivity is not fixed, but is subject to change under certain circumstances, and can be influenced by both the autonomic nerves and the endocrine glands. One of the earliest observations of changes in effector sensitivity involved surgical denervation (Budge, 1855). This procedure resulted in a phenomenon which came to be known as supersensitivity.

(i) Supersensitivity.

Supersensitivity occurs when the amount of stimulant required to produce a response is less than normal (Cannon & Rosenblueth, 1949; Trendelenburg, 1972; Westfall, 1981), and generally results from removal of a tonic stimulus.

The type of supersensitivity most commonly seen in smooth muscle occurs when the responses to submaximal doses of an agonist are increased, but the maximum response is unchanged. This is reflected in a leftward displacement of the concentration-response curve for one or more agonists (Cannon & Rosenblueth, 1949). Another type of supersensitivity is reflected in an increased maximum response of the target tissue (Pollock et al., 1972; Gardiner, Gibson & Pollock, 1974; Gibson & Pollock, 1975). Mechanisms underlying an increased sensitivity include loss of inactivation of an agonist ("deviation" supersensitivity; Fleming, 1975), an increase in receptor number (Bito & Dawson, 1970), or changes in intracellular events distal to agonist-receptor interaction (Fleming, 1968).

(ii) *Subsensitivity.*

Subsensitivity of a tissue to a receptor agonist (desensitisation) results in a rightward displacement of the concentration-response curve for that agonist (Rang & Ritter, 1970). In addition, a reduction in the maximum response can be interpreted as subsensitivity (Waud, 1975). Two types of desensitisation are recognised, namely homologous and heterologous.

The term heterologous desensitisation describes the non-specific reduction in sensitivity that occurs when cells are incubated with one agonist and subsequently found to be subsensitive not only to that agonist, but to several others (Su, Cubeddu & Perkins, 1976). Heterologous desensitisation involves functional uncoupling of the receptors in the absence of sequestration or down-regulation (Harden, 1983).

Homologous desensitisation is the term used to described the specific desensitisation that occurs when cells are re-challenged with the same agonist used in the initial exposure. In this situation agonists other than that used in the initial exposure produced a normal response (Su et al., 1976). In the case of β-adrenoceptors, this type of desensitisation is thought to involve the sequestration of the receptors away from the plasma membrane. The sequestered receptors may then undergo degradation (Harden, 1983).

Alterations in receptor sensitivity therefore provide a means of regulating the responsiveness of vascular smooth muscle. One of the most important situations in which sensitivity can change is in hypertension, a major pathological disease affecting the vasculature.

Hypertension is characterised by an elevated systemic arterial blood pressure and is caused by an increased total peripheral resistance (Folkow et al, 1970; Lund-Johansen, 1980). This increased resistance is mainly due to changes that occur in small arteries and arterioles, particularly of the splanchnic region (Folkow, 1982). The mechanisms responsible for this phenomenon are still poorly understood but may include hypertrophy and hyperplasia of vascular smooth muscle and alterations in the release or response to neural or humoral factors that cause vasoconstriction or vasodilation (Korner, 1982).

The structural changes associated with hypertension were in fact noted from a very early date (Bright, 1836 and Johnson, 1868; cited by Sivertsson, 1988), but initially were regarded as having little functional importance. The haemodynamic impact was only estimated from morphological data, and it was concluded that the structural changes were too slight and too inconstant to cause the blood pressure elevation (Castleman & Smithwick, 1943). It was not until the haemodynamic effects of such structural alterations were studied (Folkow, Grimby & Thulesius, 1958; Conway, 1963; Sivertsson, 1970) that the significance of these vascular changes was realised. Indeed, Folkow (1982) and Sivertsson (1988) support the view that an increased responsiveness to vasoconstrictors in hypertension can be accounted for by structural changes alone. This is in contrast to evidence compiled by Winquist, Webb & Bohr (1982), who reviewed studies describing both functional and structural abnormalities in hypertension. It is now generally accepted that functional and structural factors are important in both the initiation and maintenance of hypertension.

However, there is great debate surrounding the precise nature of changes that occur in the sensitivity of vascular smooth muscle in hypertension. Much of the controversy has arisen from differences in experimental design,

including the model and time-course of hypertension studied, as well as the technique, agonist, and tissue utilised (Winquist, Webb & Bohr, 1982; Marin, 1993). It is not surprising therefore that a great variety of results have been reported in the literature.

Several studies found a decrease in endothelium-dependent relaxation in hypertension (Winquist et al., 1984; Lockette, Otsuka & Carretero, 1986), and a decrease in β-adrenoceptor-mediated vasorelaxation (Triner et al., 1975).

Other investigations however noted an increase in the response of vasculature from hypertensive animals (Werber & Fink, 1985; Webb, Vander & Henry, 1987) and humans (Hollenberg & Adams, 1976) to the vasodilator acetylcholine. These findings are somewhat surprising but have been suggested to be an adaption to the hypertension, as they clearly do not have a causative role. Still other studies report no difference in the vasodilator response to ACh between the mesenteric microvasculature of rats with steroid hypertension and their normotensive controls (King & Webb, 1988).

Similar conflicting results concerning vasoconstrictors in hypertension have also been reported. Various blood vessels and vascular beds from hypertensive animals and humans have been shown to exhibit increased, decreased, or indeed no change in sensitivity when compared to their normotensive controls (Clineschmidt et al., 1970; Hermsmeyer, 1976; Swamy & Triggle, 1980; Aalkjaer et al, 1987; Holck, 1988; King & Webb, 1988; 1991).

Hypertension is clearly a complex phenomenon. Further studies must be carried out to elucidate the nature of the sensitivity of vascular smooth muscle in hypertension. A systematic investigation is necessary before the discrepancies in the literature in this regard can be resolved.

AIMS OF THE STUDY

The responses of vascular smooth muscle are regulated by many factors, including receptors, neurotransmitters and disease processes such as hypertension. These factors are not independent of one another but are interconnected. Investigation of all these aspects of vascular control will seek to answer the following questions :-

1) Is the sensitivity of blood vessels from hypertensive rats altered with respect to vessels from control animals, and if so, how?

2)Do α_2-adrenoceptors exist on the smooth muscle of the rat tail artery? If so, what are the conditions required to demonstrate contractions mediated by these receptors, and what is their mechanism of action?

3)What is the nature of the neurotransmission in the rat tail artery? Does co-transmission have a role, and if so, is its involvement altered in hypertension?

Materials and Methods

1. EXPERIMENTAL ANIMALS

Preliminary experiments were carried out with two groups of male Wistar rats: (a) "older rats" : 300-350g, 12-17 weeks, and (b) "younger rats" : 145-225g, 6-9 weeks. For experiments where comparisons between older and younger rats were not being made, animals were usually 300-400g (10-12 weeks) or 180-240g (6-8 weeks). Experiments were also carried out with spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) control rats (200-300g, 10-12 weeks), supplied by Harlan Olac or Charles River.

Animal Pretreatments

(a) Reserpine

Rats were treated with reserpine, dissolved in vehicle (glacial acetic acid) and injected intraperitoneally (0.75 mg kg^{-1}, i.p.) daily for 7 days. Control rats received daily injections of the vehicle only. Experiments were carried out over the following two weeks.

(b) 6-Hydroxydopamine (6-OHDA)

Rats were treated with 6-OHDA as follows :-

2 x 25 mg kg^{-1} on day 1

2 x 100 mg kg^{-1} on days 3-5

Control rats received an equivalent volume of saline. Experiments were carried out over the following two weeks.

2. MEASUREMENT OF BLOOD PRESSURE

Systolic blood pressure was measured in conscious rats by the tail cuff method. The rats were maintained in a warming chamber at 37^{O}C for 15

minutes, prior to blood pressure measurement. Each rat was held in a restrainer and an inflatable cuff placed around the base of the tail. The cuff was inflated by means of a motor driven piston, and a pressure transducer, placed distal to the cuff, allowed the pulse of the tail artery to be detected accurately and displayed on a calibrated oscilloscope (Figure 12).

The point at which the tail artery pulse was blocked showed the systolic blood pressure and this was confirmed when the applied pressure fell and the arterial pulse reappeared.

3. MECHANICAL RESPONSES OF TISSUES

A. Aortic Rings

Rats were killed by stunning and exsanguination. The chest wall was cut along the midline and the thoracic cavity exposed. The descending thoracic aorta was removed, placed in a Krebs-filled petri dish and cleared of fat and connective tissue. Four adjacent transverse aortic rings (3-4mm) were obtained from each aorta, weighed and suspended between stainless steel hooks in 25ml organ baths containing Krebs' bicarbonate solution of the following composition (mM): NaCl (118.1), KCl (4.7), $MgSO_4$ (1.0), KH_2PO_4 (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25.0) and glucose (11.1). The Krebs solution was maintained at 37^{o}C by a thermostat and gassed continuously with 95% O_2 : 5% CO_2. The initial resting tension was adjusted to two grams and rings allowed to equilibrate for 90 minutes prior to the start of experiments. Isometric tension was measured using Statham force/displacement transducers and recorded on a Grass Polygraph (model 79D).

B. Isolated, perfused tail artery

Rats were killed as described, and the tails were cut off and skinned. Two 20mm segments of the proximal part of the main ventral artery were dissected from the ventral groove, and transferred to a Krebs-filled petri dish. The remaining length of artery was not used. Tail artery segments were perfused using modifications of the method of Medgett and Rajanayagam (1984), as follows:

(a) Single cannulation

In preliminary experiments, each artery segment was cannulated at one end with a fine polythene cannula. The artery was then passed through a pair of Ag/AgCl ring electrodes attached to a perspex holder, and the assembly inserted into a 25ml Krebs-filled organ bath, gassed and heated as before. The cannula was connected to a silicone rubber tube, through which Krebs buffer was pumped from a reservoir at a constant rate (0.5 or 1ml min^{-1}) by a Watson Marlow constant output pump, that had been previously calibrated. Preparations were allowed to equilibrate for 60 minutes prior to the start of experiments. Drugs were injected by bolus injection into the thick-walled rubber tubing, immediately preceding the cannula. The resistance of the artery to perfusion was measured by a Statham pressure transducer and the perfusion pressure was displayed on a Grass polygraph.

In later experiments, this arrangement was modified by addition of a heating coil between the reservoir and the cannula to maintain the temperature of the perfusing Krebs solution at 37^{o}C.

(b) Double cannulation

For all other perfusion experiments each artery segment was cannulated at both ends, with the second cannula allowing the perfusate to pass out of the organ bath without superfusion. Drugs were administered either in the

perfusing solution until each response reached equilibrium, or as a bolus injection.

C. Tail artery rings

(a) Proximal tail artery

Rings (3-4mm) of proximal tail artery were weighed, and mounted under 1g tension on a combined electrode and tissue holder. This was inserted into a 25ml organ bath containing Krebs solution, gassed and heated as described. One electrode was outside the artery lumen and the other inserted through the lumen to act as a fixed point for tension measurement. A stainless steel triangle inserted though the artery lumen was attached via a thread to a Statham force-displacement transducer, and isometric tension responses were displayed on a Grass polygraph. The artery rings were allowed to equilibrate for 60 minutes, then tension was removed and reapplied (1g as before) and the organ bath washed out and refilled. After a further 60 minutes equilibration, experiments were initiated.

(b) Distal tail artery

The tail artery was dissected out from the ventral groove along the entire length of the tail, then 3-4mm rings obtained from the distal part of the tail artery, 10-20mm from the tip. These rings were mounted on a small vessel, automated myograph. The method was adapted from that of Mulvany and Halpern (1976), using the myograph simply as a scaled-down organ bath in which to measure isometric tension responses from very small segments of blood vessel.

Using a dissecting microscope, the vessel segments were threaded onto two 40μm stainless steel wires attached, respectively, to a tension transducer and a micrometer translator under 1g resting tension. The myograph chamber was filled with Krebs solution maintained at 37^{O}C and gassed with 95% O_2 : 5% CO_2. As with the proximal tail artery rings in the organ bath, the distal tail

artery rings were allowed to equilibrate for 2 hours, with washout and re-setting of tension after the first hour, before any experimental procedures. Drugs were injected directly into the myograph chamber, and changes in isometric tension displayed on a Linseis chart recorder.

Addition of drugs

For experiments measuring isometric tension, drugs were injected directly into the organ bath. Concentration-response curves were carried out in two ways: a) Cumulatively, with each successive concentration of drug added when the response to the preceding concentration reached a plateau, or b) in the wash-out manner, with each concentration left in contact with the tissue until the response had peaked, then washed out. The next concentration was added when the preceding response returned to baseline.

For vasorelaxants, arteries were first precontracted with PE (EC_{75}), then cumulative concentration-response curves obtained.

When experiments were carried out in the presence of an antagonist, the antagonist was added to the organ bath at least 15 minutes before addition of the agonist, or as detailed in the Results.

For perfusion pressure experiments, concentration-pressure curves were obtained by either a) perfusion to equilibrium, where increasing concentrations of drug were perfused once the response to the previous concentration had reached a plateau, or b) by bolus injection, where increasing concentrations were injected into the Krebs-perfused artery once the preceding response had returned to baseline.

All other experimental protocols were as detailed in the Results.

Electrical field stimulation

Studies on the mechanical responses to electrical field stimulation in the rat tail artery were carried out using a Palmer square-wave stimulator. Non-cumulative frequency-response curves were constructed using a constant number of pulses (10), by varying the time of stimulation. The pulse width was 0.1 or 0.4ms, and the voltage supramaximal (usually 50V). The responses to trains of stimuli (10 Hz for 1s, 50V, 0.1ms, every 100s) were also studied. Other stimulation protocols were as detailed in the Results.

Expression of results

Results were expressed in a variety of ways, using the following units as appropriate: g tension; g mg^{-1} tissue; Δmm Hg; % maximum response; % of the maximum response to PE.

4. BIOCHEMICAL RESPONSES OF TISSUES

Phosphatidylinositol 4,5 -bisphosphate ($PtdInsP_2$) hydrolysis in the Rat Tail Artery.

The involvement of $PtdInsP_2$ hydrolysis in the mechanical response to noradrenaline in vitro was assessed by radiochemical measurement of total inositol phosphates released from segments of rat tail arteries incubated with myo-[2-^{3}H]-inositol.

A. Preparation of Ion Exchange Columns

Ion exchange resin (Dowex 1 x 8 - 400, chloride form) was prepared by washing with the following solutions: distilled water (1l), NaOH (2M, 2l), distilled water (2l), formic acid (2M, 0.2l), then distilled water until pH 4.5-5 was obtained. This produced the formate form of resin, which was added in 1ml aliquots to chromatography columns made from 5ml Pasteur pipettes, the

tips of which were plugged with glass wool. Columns were prepared by washing with ammonium formate (2.4M, 15ml), followed by distilled water until pH 4.5-5 was obtained. The products of $PtdInsP_2$ hydrolysis were examined using a modified version of the method of Akhtar & Abdel-Latif (1986).

B. Production and Measurement of Total Inositol Phosphates

Segments of rat tail artery (10mm) were weighed, and washed in ice-cold Krebs solution. Each segment was then placed in a test tube bubbled with 95% O_2 : 5% CO_2 and the following procedures carried out in a shaking water bath at 37°C. Tissues were pre-incubated in Krebs solution for 15-30 minutes, then labelled with 8μCi ml^{-1} inositol (in Krebs) for 2 hours. $PtdInsP_2$ hydrolysis was stimulated by incubation with NA (10^{-6}M, 10^{-5}M, 45-60 minutes), in Krebs solution containing 10mM LiCl to inhibit inositol phosphate phosphatase (InsP phosphatase) and hence prevent the breakdown of InsP to inositol and subsequent recycling of the PI cycle (Hallcher & Sherman, 1980). The reaction was terminated by addition of a chloroform/methanol mixture (1 : 2, v/v) and tissues homogenised in hand-held glass Potter-Elvehjem homogenisers. The test tubes containing the homogenised tissue were centrifuged at 2000g for 5 minutes at room temperature, then the lower organic phase discarded. The upper aqueous phase containing the inositol phosphates was taken for analysis.

Samples of the aqueous phase were applied to the previously-prepared Dowex resin columns, and washed with the following solutions: 20ml water, to elute [^{3}H]-inositol, 15ml of ammonium formate (50mM), to elute glycerophosphoinositides, and finally 8ml of ammonium formate (1M)/formic acid (0.1M) mixture, to elute the [^{3}H]-inositol phosphates.

A 1ml sample of the final eluate was added to scintillation fluid (Ecosint, 10ml) and total radioactivity determined by liquid scintillation counting

(Hewlett-Packard 2000CA Tri-carb). Results were expressed as disintegrations per minute (d.p.m.) per mg of tissue (wet weight).

5. HISTOLOGICAL EXAMINATION OF TISSUES

Blood vessel structure was studied using light microscopy, to compare the morphology of the aorta and tail artery from hypertensive animals with vessels from control animals.

A. Fixing

Blood vessels were dissected as described, then 1cm segments fixed in 4% buffered formalin (paraformaldehyde). This was prepared by mixing 4g formaldehyde with 90ml distilled water and 10ml 10x phosphate-buffered saline (PBS, containing 1.3M NaCl, 70mM Na_2HPO_4 and 30mM NaH_2PO_4 ; any precipitates or crystals formed were filtered off). The milky-white solution was stirred with a magnetic stirrer at 60^{O}C for 1 hour, until the solution became clear.

B. Sectioning and staining

After fixing, the vessels were sectioned and stained by David Russell and Dr. J. McGadey, of the Anatomy Department, University of Glasgow. Vessels were embedded in wax blocks, then from each segment, 5 x 50μm transverse sections were cut on a microtome. The sections were floated in a water bath and mounted onto slides, which were left overnight in a 37^{O}C oven to ensure sections were fixed securely.

Sections of rat tail artery were stained using Masson's trichrome technique, which stains nuclei blue/black, cytoplasm and muscle red, and connective tissue green. Sections of rat aorta were stained using Epple, which stains elastin purple, nuclei red, cytoplasm reddish orange, and connective tissue green. Coverslips were then mounted on the slides using the plastic mounting agent, DPX.

C. Examination

The slides were examined under a light microscope. Each section was projected onto paper and the image traced around to produce a drawing of the transverse section. Drawings were analysed using a Tandon computer and the Biquant System IV software package (R & M Biometrics). The parameters measured were media thickness, lumen diameter, media thickness to lumen ratio and media area (of a transverse section).

6. DRUGS USED

The following drugs were used: acetylcholine (Sigma), adenosine 5'-triphosphate (Sigma), 5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate (UK-14,304; Pfizer), carbamylcholine chloride (carbachol; Sigma), clonidine hydrochloride (Sigma), N^6, 2'-O-dibutyryladenosine 3':5'-cyclic monophosphate (Sigma), diltiazem hydrochloride (Sigma), forskolin (Sigma), 6-hydroxydopamine hydrobromide (Sigma), 5-hydroxytryptamine (creatinine sulphate complex; Sigma), [^{3}H]-inositol (Amersham International), 3-isobutyl-1-methylxanthine (Sigma), DL-isoprenaline sulphate (Sigma), ketanserin (Sigma), lithium hyrochloride (Sigma), [β-mercapto-β, β-cyclopentamethylenepropionyl^1, O-Me-Tyr^2, Arg^8]-vasopressin (Sigma), α,β-methylene ATP (Sigma), L-noradrenaline hydrochloride (Sigma), L-phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), reserpine base (Sigma), sodium nitroprusside (British Drug Houses), tetrodotoxin (Boehringer), yohimbine hydrochloride (Sigma).

All drugs were prepared in Krebs solution, with the following exceptions: Ketanserin was made up in abslolute alcohol and then diluted with Krebs, so that the final concentration of ethanol in the organ bath did not exceed 0.01%. Isoprenaline and noradrenaline were dissolved in Krebs solution containing 0.3mM L-ascorbic acid, to prevent oxidation and breakdown of

these catecholamines. Prazosin and yohimbine were dissolved in Krebs solution following ultrasonication. Reserpine was prepared in glacial acetic acid.

7. STATISTICAL ANALYSIS

All results were expressed as the mean ± standard error of the mean ($\chi \pm$ S.E.M.), for the number of observations, n. Statistical comparisons of the data were carried out using Student's t-test, the Mann-Whitney non-parametric t-test, or the paired t-test as appropriate, and a probability (P) of 0.05 or less was considered significant.

The following standard designation was applied to represent levels of significance:

* $P < 0.05$

* * $P < 0.01$

* * * $P < 0.001$

Results

PART I

PRELIMINARY EXPERIMENTS IN THE PERFUSED RAT TAIL ARTERY

In the first part of this study, the effects of drugs and field stimulation were studied in rats of two age groups, "older rats" (12-17 weeks, 300-350g) and "younger rats" (6-9 weeks, 145-225g).

A) RAT WEIGHT AND SYSTOLIC BLOOD PRESSURE

Male Wistar rats of two different age groups were weighed, and their systolic blood pressure (SBP) measured by the tail cuff method. Older rats were significantly heavier than rats in the younger age group (331±12g, c.f. 189±7g; $P<0.001$), and there was a close positive correlation between rat age and weight (r=0.956; Figure 4a). The SBP of the older rats was significantly higher than that of the younger rats (128±2mmHg cf. 114±3mmHg; $P<0.05$), but both sets of measurements were in the normotensive range. There was also a positive correlation between rat weight and SBP (r=0.375; Figure 4b), but this was not statistically significant.

B) PERFUSION PRESSURE MEASUREMENTS

1) *Single cannulation*

In preliminary experiments, segments of rat tail artery were cannulated at one end with a fine polythene cannula , passed through a pair of Ag/AgCl ring electrodes and inserted into an organ bath. Artery segments were perfused with Krebs solution at a constant flow rate (0.5 or 1ml min^{-1}) so that changes in vessel diameter were manifested as changes in perfusion pressure (PP).

a) Effect of noradrenaline :

Bolus injections of NA (10^{-3}-100μg) produced dose-related increases in perfusion pressure (Figure 5). Responses of vessels from the older, heavier rats

(300-350g) were greater than those from the younger, lighter rats (145-225g), with an increased maximum response (263.7±24.0 mmHg c.f. 151.9±14.9 mmHg , $P<0.01$). In addition, the EC_{50} in older rats was lower than that obtained in younger rats (0.07±0.01μg c.f. 0.63±0.05 μg , $P<0.01$).

b) Electrical field stimulation:

Electrical field stimulation (supramaximal voltage 80V, 50 pulses, 0.4ms duration, 0.5-80Hz) produced frequency-dependent increases in perfusion pressure in tail artery segments (Figure 6). The frequency-response curves obtained in arteries from both older and younger rats were biphasic, with the first peak at 5-10Hz and the second at 20-40Hz. At the lower frequencies (0.5-2Hz) there was clearly no difference in response between the two groups of rat . At higher frequencies (5-80Hz) responses from the older rats tended to be greater than those from younger rats (Maximum response 115.6±38.2 mmHg c.f. 60.5±6.6 mmHg). These values were not statistically significant however ($P>0.05$) due to the large variation in the responses from the older age group of rat.

2) *Double cannulation*

In other perfusion experiments, rat tail artery segments were cannulated at both ends, with the second cannula allowing the perfusate to pass out of the organ bath without superfusion. The effects of drugs were studied in rats of two age groups as above, using two different flow rates, 0.5ml min^{-1} and 1ml min^{-1}.

a) Effect of noradrenaline:

Perfusion of NA through the tail artery lumen in increasing concentrations (10^{-8} -10^{-5}M) produced corresponding increases in perfusion pressure (Figures 7,8,9). At each flow rate, there was no difference in the

EC_{50} or maximum response to NA between older and younger rats (Table 1a). In both age groups, responses at 0.5ml min^{-1} were significantly lower than at 1ml min^{-1} (Figures 8,9), with a greater EC_{50} at the lower flow rate ($P<0.05$), but there was no difference in the maximum response to NA when perfused at two different flow rates (Table 1b).

b) Effect of KCl:

KCl (1ml min^{-1}) produced concentration-dependent increases in perfusion pressure (Figure 10). Responses to KCl were much smaller than those to NA and occurred over a much higher and narrower concentration range (0.01-0.05M c.f. 10^{-8} -10^{-5}M). As with NA, there was no difference in the response to KCl between arteries from older and younger rats (EC_{50} 0.09 $\pm$ 0.01M and 0.09 $\pm$ 0.01M respectively; maximum response 90.2 $\pm$ 10.5mmHg and 114.7 $\pm$ 12.6 mmHg respectively).

3) *Single cannulation (modified set-up)*

When NA was administered as bolus injections in the Krebs-perfused tail artery (single cannulation), vessels from older rats were more responsive than were those from younger rats (Figure 5). This difference however was not seen when arteries were cannulated at both ends and NA perfused through the vessel lumen (Figure 7). It was therefore decided to repeat the single cannulation experiment, but this time the method was improved by the addition of a heating coil between the reservoir and the cannula to maintain the temperature of the perfused Krebs solution at 37°C. In addition, the Krebs solution in the reservoir, as well as that in the organ bath, was oxygenated.

Effect of noradrenaline:

NA (10^{-3}-100μg) produced dose-dependent increases in perfusion pressure but

using this modified set-up there was no difference in the response to NA between older (12-17 weeks) and younger (6-9 weeks) rats (Figure 11).

Preliminary experiments with rats of different ages initially showed that isolated blood vessels from older rats were more responsive to contractile agonists than vessels from younger rats. Later experiments revealed that there was no difference in the sensitivity of older and younger rats. It was therefore decided to investigate the responsiveness of isolated blood vessels from hypertensive rats. The model of hypertension studied was the SHR, and responses were compared to those obtained in the normotensive control strain, WKY.

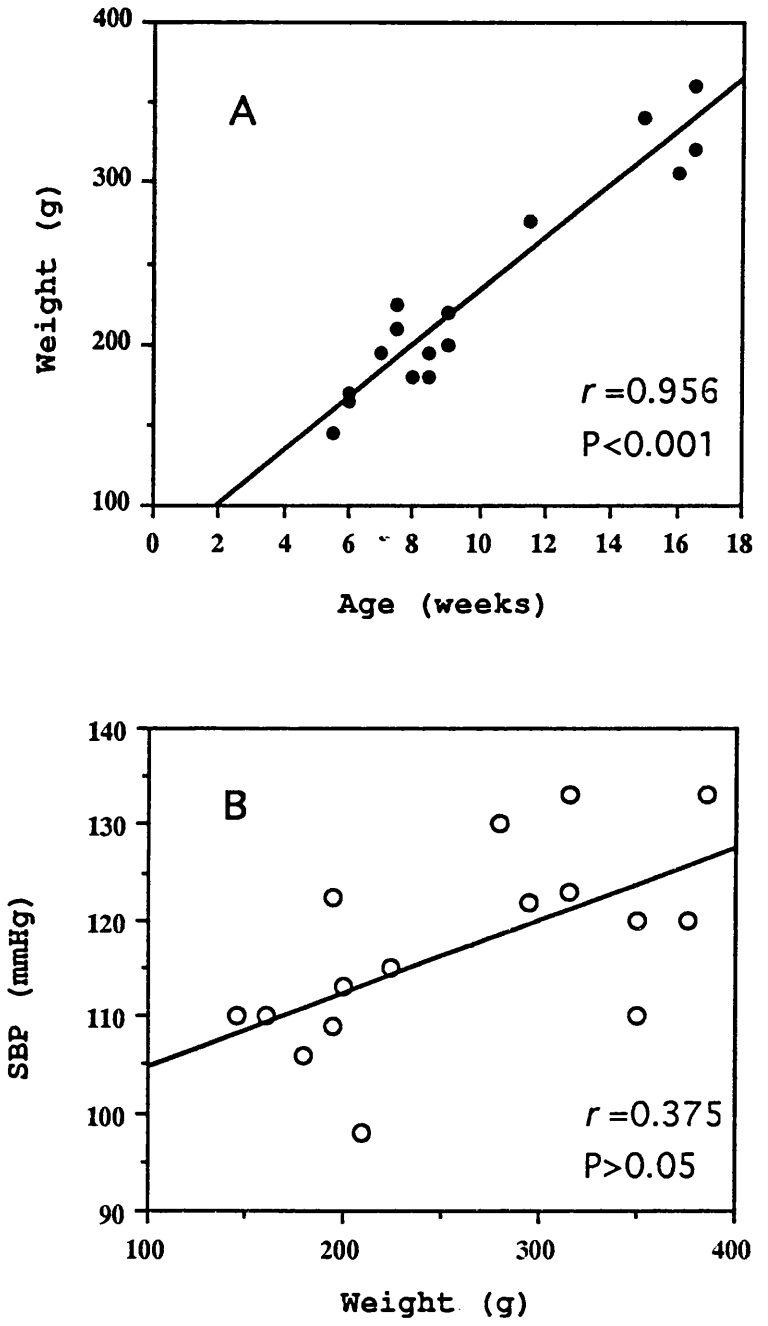


Figure 4. **Correlations between rat age and weight (A), and rat weight and systolic blood pressure (SBP) (B).** As rat age increased, there was a corresponding increase in rat weight (A). The SBP, measured by the tail cuff method, did not rise significantly with rat weight (B).

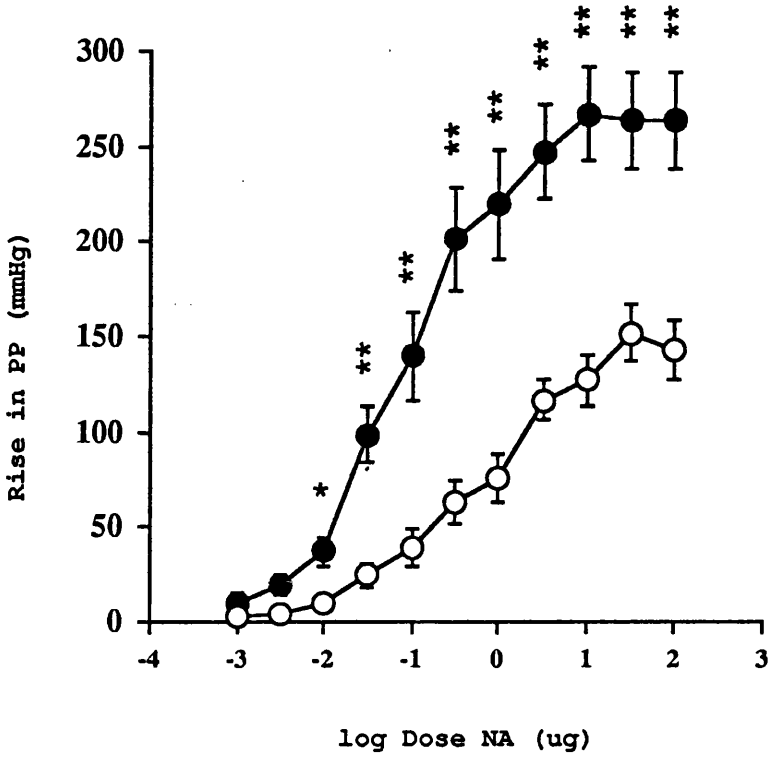


Figure 5. **Dose-response curve to noradrenaline (NA) in the perfused rat tail artery.** Segments of rat tail artery were cannulated at one end and perfused with Krebs solution at 1ml min^{-1}. NA was administered as bolus injections into the rubber tubing immediately preceding the cannula. Responses from the older rats (12-17 weeks; --●--) were significantly higher than those from the younger rats (6-9 weeks; --○--) as indicated by asterisks (*, $P<0.05$; **, $P<0.01$). Each point represents the mean ± s.e.m. of at least 8 observations. Where no error bars are shown, errors are contained within the symbols. (PP = perfusion pressure).

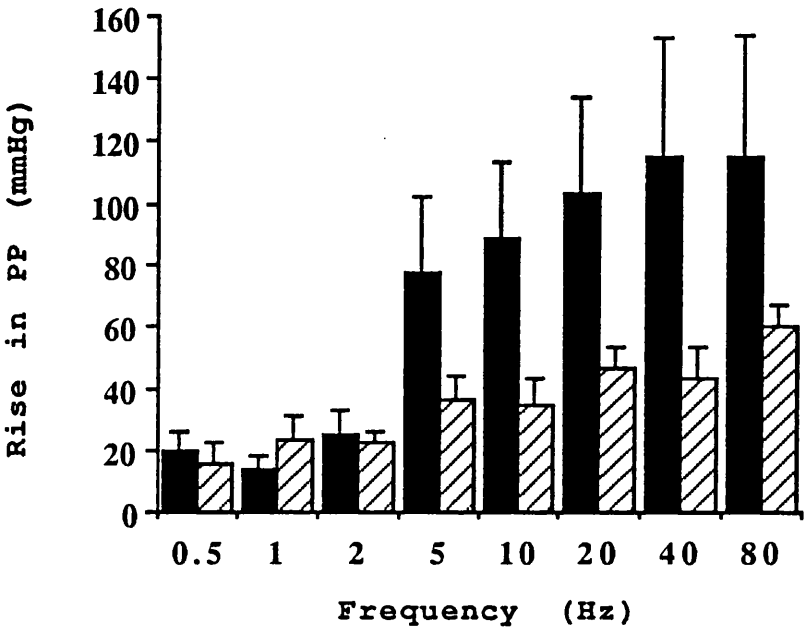


Figure 6. **Frequency-response curve in the perfused rat tail artery.** Segments of rat tail artery were cannulated at one end and perfused with Krebs solution at 1ml min^{-1}. Electrical field stimulation (supramaximal voltage 80V, 50 pulses, 0.4ms duration, 0.5-80Hz) was then applied. At the higher frequencies (5-80Hz) responses from the older rats (12-17 weeks, black bars) tended to be greater than those from younger rats (6-9 weeks, hatched bars) but the difference was not statistically significant ($P>0.05$). Each bar represents the mean $\pm$ s.e.m. of at least 6 observations.

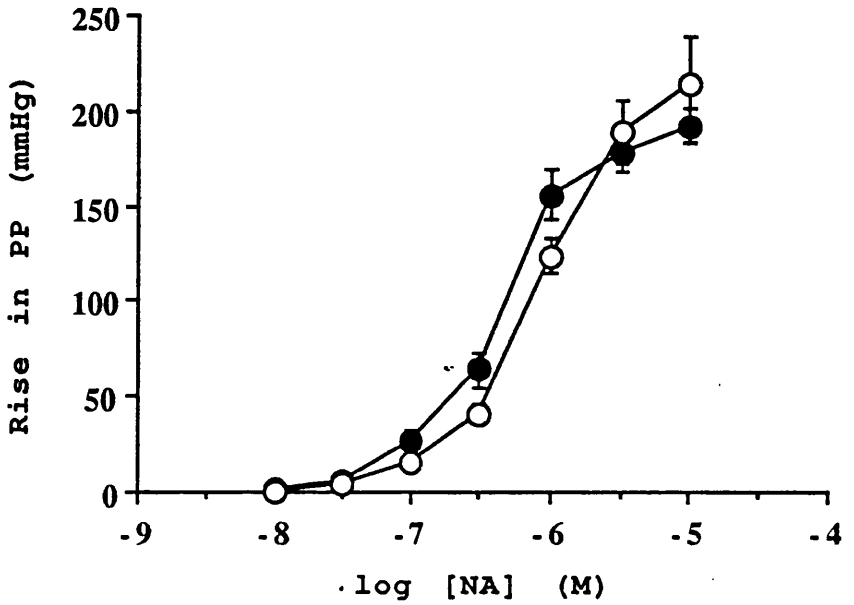


Figure 7. **Concentration-response curve to NA in the perfused rat tail artery.** Segments of rat tail artery were cannulated at both ends and perfused with noradrenaline (NA) at 1ml min^{-1} in increasing concentrations. There was no difference in response between arteries from older (12-17 weeks; --●--) and younger (6-9 weeks; --○--) rats. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, errors are contained within the symbols.

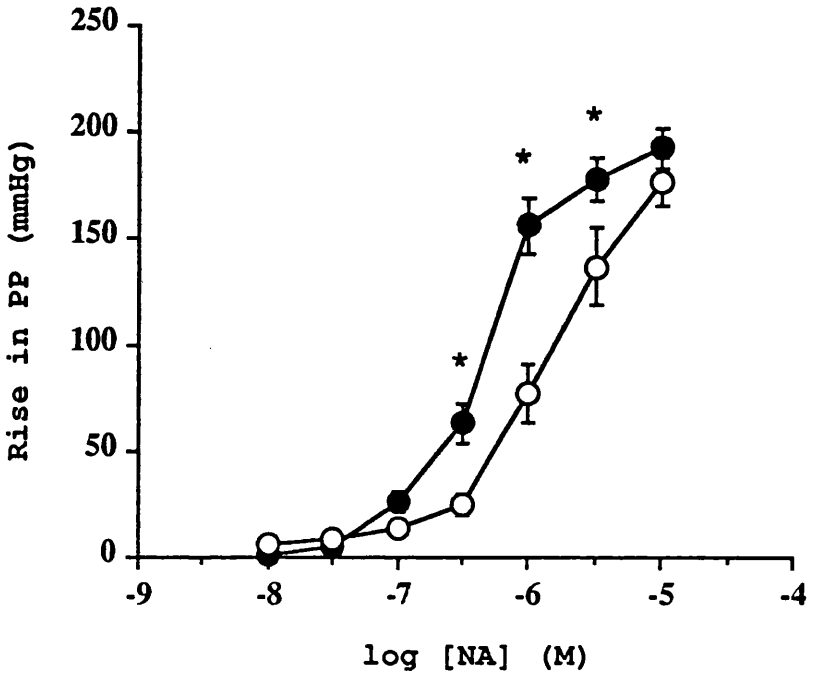


Figure 8. **The effect of flow rate on the concentration-response curve to NA in the perfused rat tail artery (i).** Segments of tail artery from 12-17-week old rats were cannulated at both ends and perfused with NA at either 1ml min^{-1} or 0.5ml min^{-1}. Responses to NA at 1ml min^{-1} (--●--) were greater than responses to NA at 0.5ml min^{-1} (--○--) (*; $P<0.05$). There was no difference in the maximum response. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, errors are contained within the symbols.

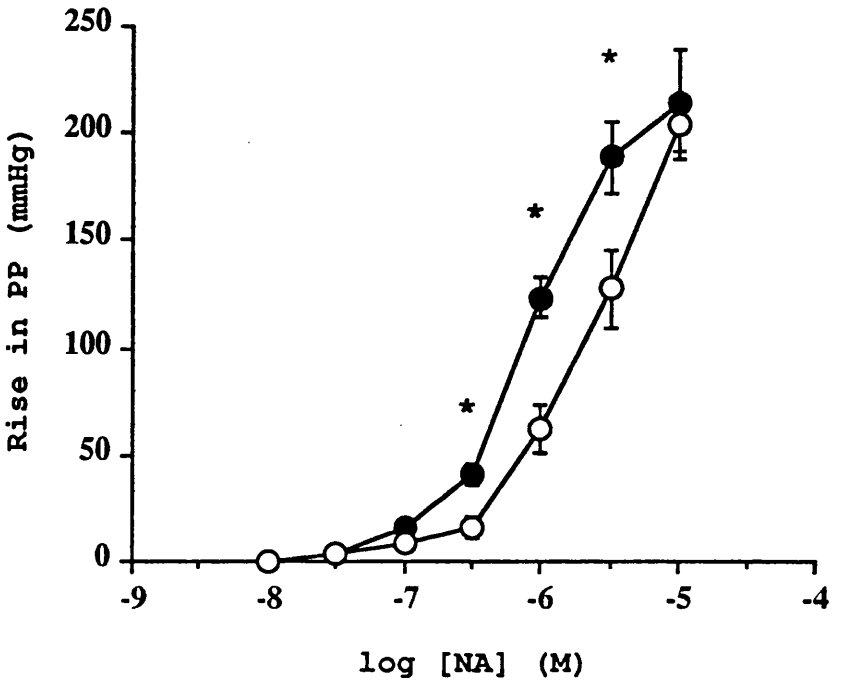


Figure 9. **The effect of flow rate on the concentration-response curve to NA in the perfused rat tail artery (ii).** Segments of tail artery from rats (6-9 weeks old) were cannulated at both ends and perfused with NA at either 1ml min^{-1} or 0.5ml min^{-1}. Responses to NA at 1ml min^{-1} (--●--) were greater than responses to NA at 0.5ml min^{-1} (--○--) (*; $P<0.05$). There was no difference in the maximum response. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, errors are contained within the symbols.

a)EC_{50}	**Flow Rate**		
Age of Rat	*1ml min^{-1}*	*0.5ml min^{-1}*	
6-9 weeks	0.76±0.07	1.8±0.20	*
12-17 weeks	0.63±0.06	1.15±0.12	*

b)**Maximum Response**	**Flow Rate**		
Age of Rat	*1ml min^{-1}*	*0.5ml min^{-1}*	
6-9 weeks	213.5±25.4	203.4±12.0	NS
12-17 weeks	192.0±9.7	176.2±11.6	NS

Table 1. **EC_{50} values in μM(a) and maximum responses in mmHg (b) in rat tail artery in response to NA.** Segments of rat tail artery were cannulated at both ends and perfused with NA at 0.5ml min^{-1} and 1ml min^{-1}. There was no difference between the two groups of rat in the EC_{50} or the maximum response, at either flow rate. However, the EC_{50} for NA was significantly lower at 1ml min^{-1} than at 0.5ml min^{-1} in both older and younger rats (*, $P<0.05$). There was no difference in the maximum response to NA when perfused at two different flow rates, in either older or younger rats (NS = not significant).

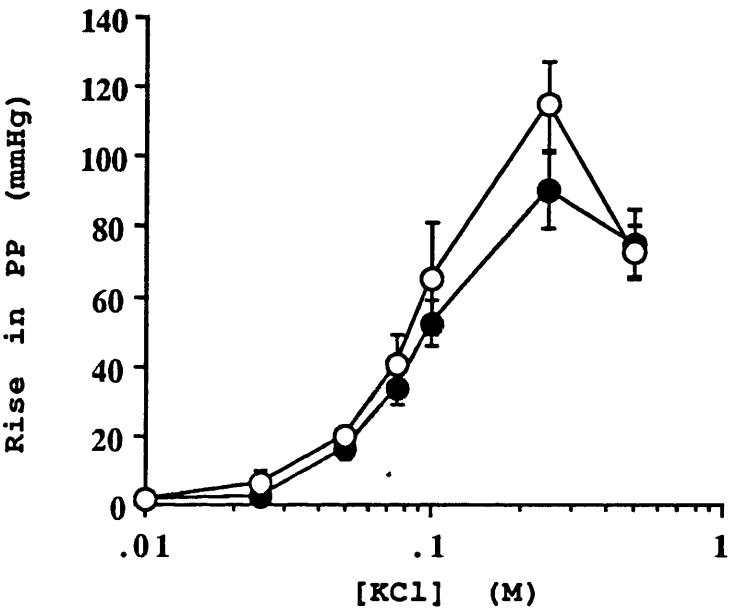


Figure 10. **Concentration-response curve to KCl in the perfused rat tail artery.** Segments of rat tail artery were cannulated at both ends and perfused with KCl in increasing concentrations. There was no difference in the response between arteries from older (12-17 weeks; --●--) and younger (6-9 weeks; --○--) rats. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, errors are contained within the symbols.

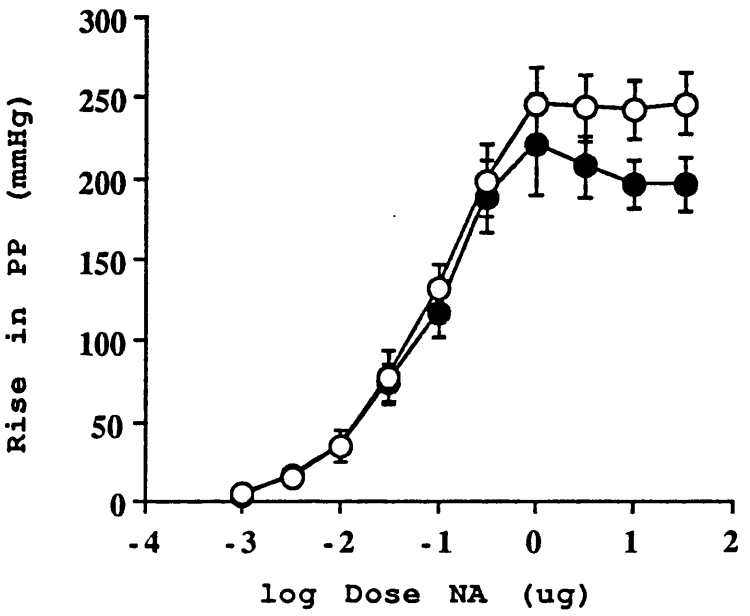


Figure 11. **Dose-response curve to NA in the perfused rat tail artery (modified set-up).** Segments of rat tail artery were cannulated at one end and perfused with Krebs solution from a reservoir at 1ml min^{-1}. The set-up was modified from previous experiments by inserting an additional heating coil between the reservoir and the cannula, and oxygenating the Krebs in the reservoir. NA was administered as bolus injections into the rubber tubing immediately preceding the canula. There was no difference in response between arteries from older rats (12-17weeks,--●--) or younger rats (6-9 weeks, --○--). Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, errors are contained within the symbols.

PART II

THE RESPONSIVENESS OF BLOOD VESSELS TO VASOACTIVE AGENTS IN HYPERTENSION

A) BLOOD PRESSURE MEASUREMENT

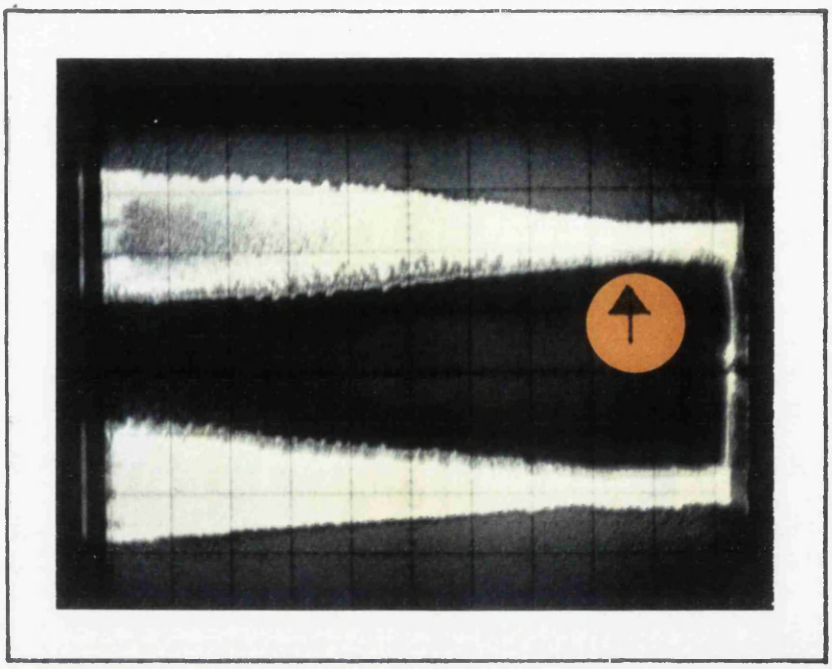
Systolic blood pressure (SBP) was measured in conscious SHR, WKY and Wistar rats by the tail cuff method. The SBP of the SHR rats (194±5.0 mmHg) was higher than that of the two control strains, WKY and Wistar (137±4mmHg and 121±3 mmHg respectively) ($P<0.001$; Figures 12, 13). The SBP of WKY rats was higher than that of Wistar rats ($P<0.01$), but both were in the normotensive range.

Both the rat isolated tail artery and the aorta were studied, as examples of a resistance vessel and a conducting vessel respectively.

B) STRUCTURE OF RAT BLOOD VESSELS

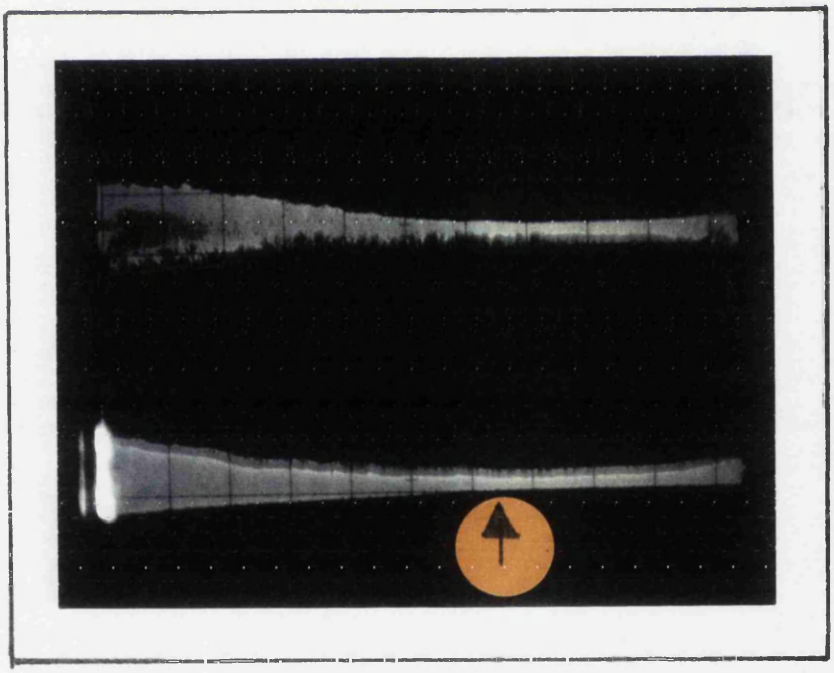
Aortic rings from hypertensive rats were heavier than those from control rats (6.9±0.4mg, c.f. 4.8±0.35mg, $P<0.001$; Figure 14a), despite being cut to the same length (3-4mm). Proximal tail artery rings from hypertensive rats were heavier than those from control rats (0.65±0.05g, c.f. 0.38±0.03g, $P<0.001$; Figure 14b), despite being cut to the same length (3-4mm).

These measurements suggested that there was a structural difference in the tail artery and aorta from SHR and WKY rats. This was studied using light microscopy.



A. SHR

215mmHg



B. WKY

130mmHg

Figure 12. **Typical blood pressure recordings from a spontaneously hypertensive rat (SHR; A) and a normotensive, Wistar Kyoto (WKY) rat (B), measured by the tail cuff method.** Arrows denote the point at which pulse oscillations stopped, indicating the systolic blood pressure. In (A), the screen was calibrated to read 40mmHg at the left hand edge. Pressure is shown to increase from left to right, and each horizontal division represents 20mmHg.

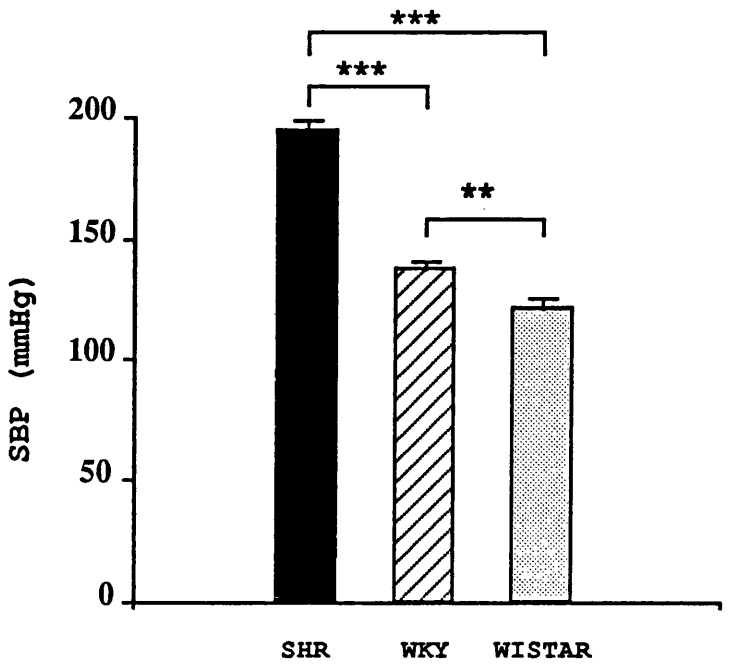


Figure 13. **Systolic blood pressure (SBP) measured in conscious rats.** SHR (n=8) had a higher systolic blood pressure than either WKY (n=8) or Wistar (n=10) rats. Asterisks represent significant differences between groups of rat joined by a horizontal bar (** , $P<0.01$; *** , $P<0.001$). Each bar represents the mean $\pm$ s.e.m. for the indicated number of rats.

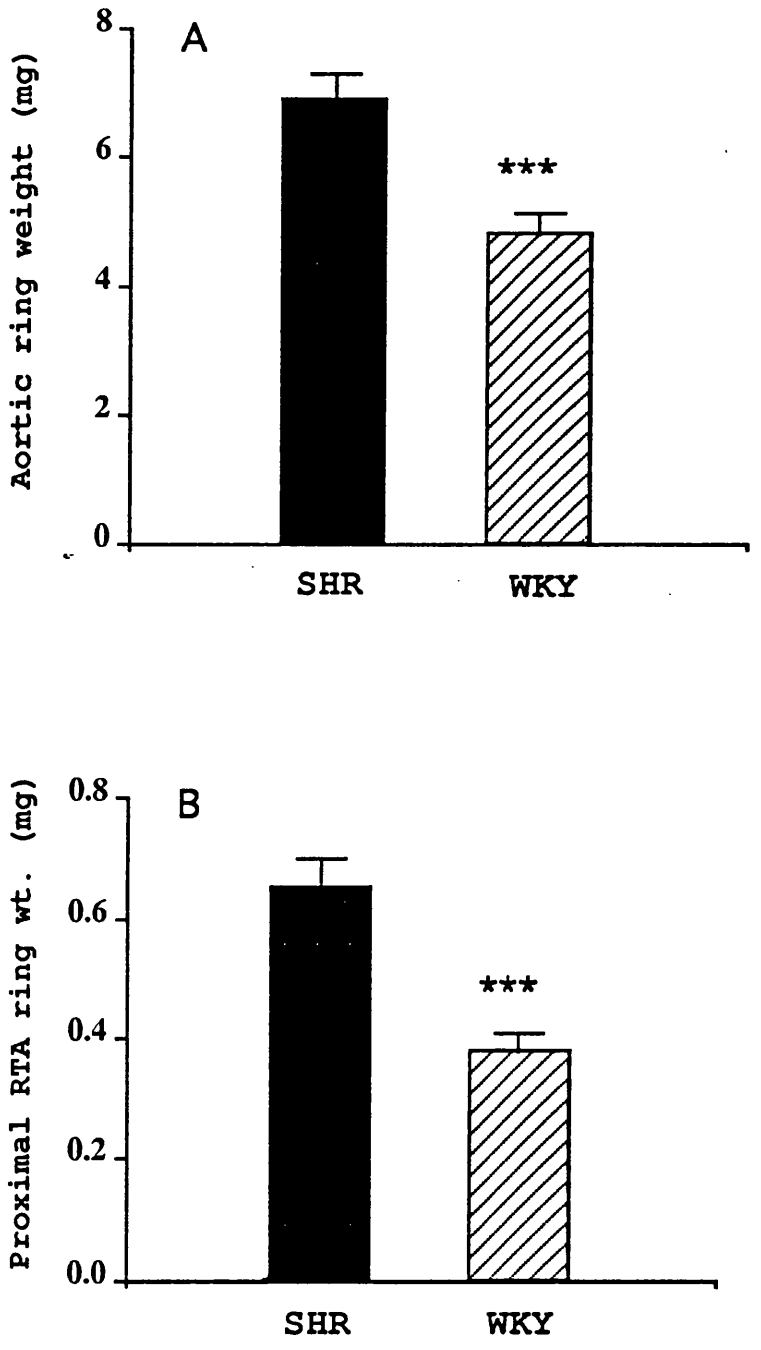


Figure 14. **Weights of artery rings from SHR and WKY rats.** Rings of aorta or rat tail artery (RTA; proximal region) were cut to a standard length (3-4mm). Rings of both aorta (A) and proximal tail artery (B) were heavier in SHR compared to WKY (***; $P<0.001$). Each bar represents the mean $\pm$ s.e.m. of 20-26 observations.

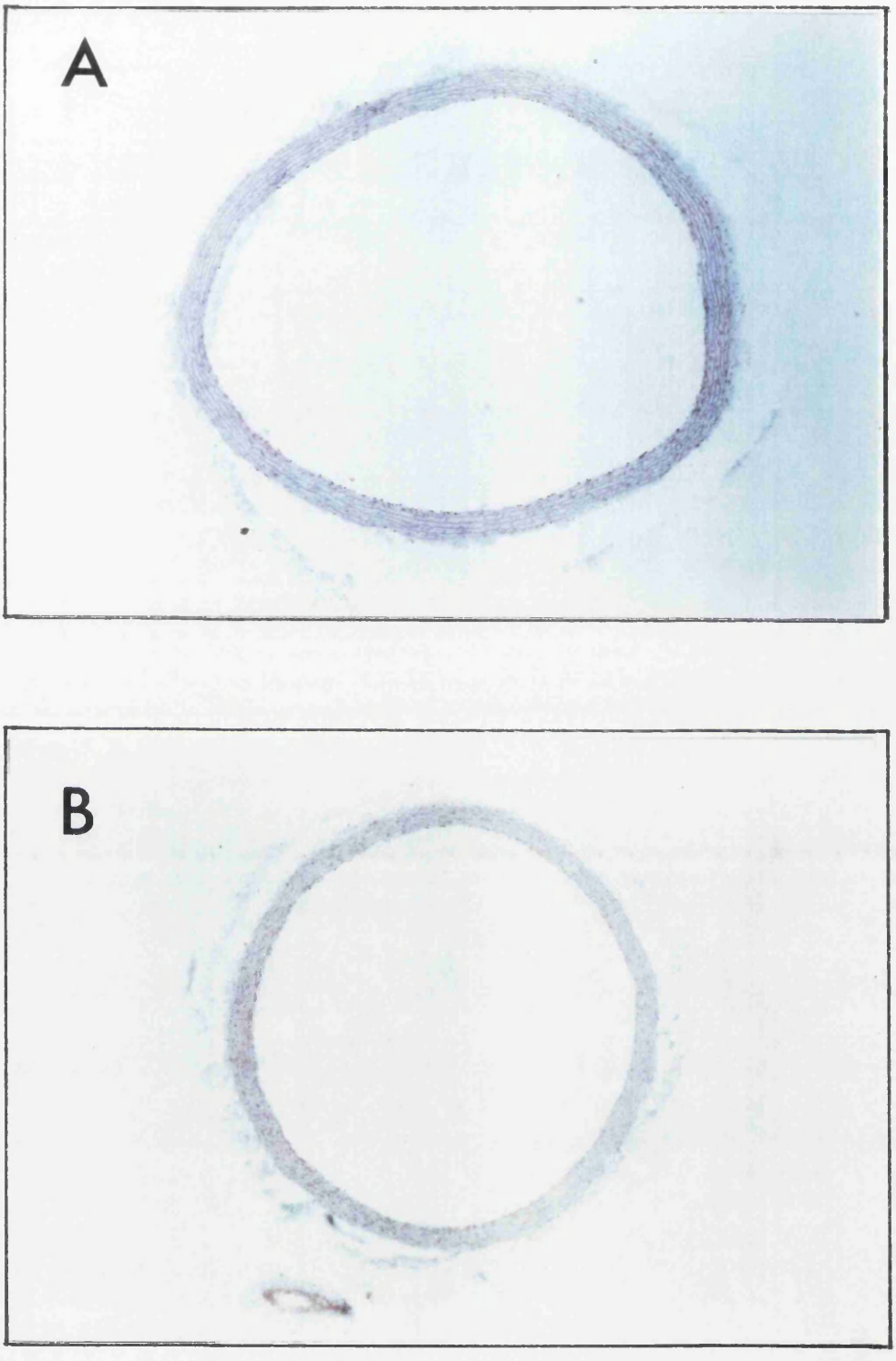


Figure 15. **Transverse sections of the aorta from SHR (A) and WKY rats (B).** Sections were stained with Epple, showing the elastic fibres of the aorta as purple rings. Both the wall thickness and lumen diameter were increased in aortae from SHR as compared to those from WKY.

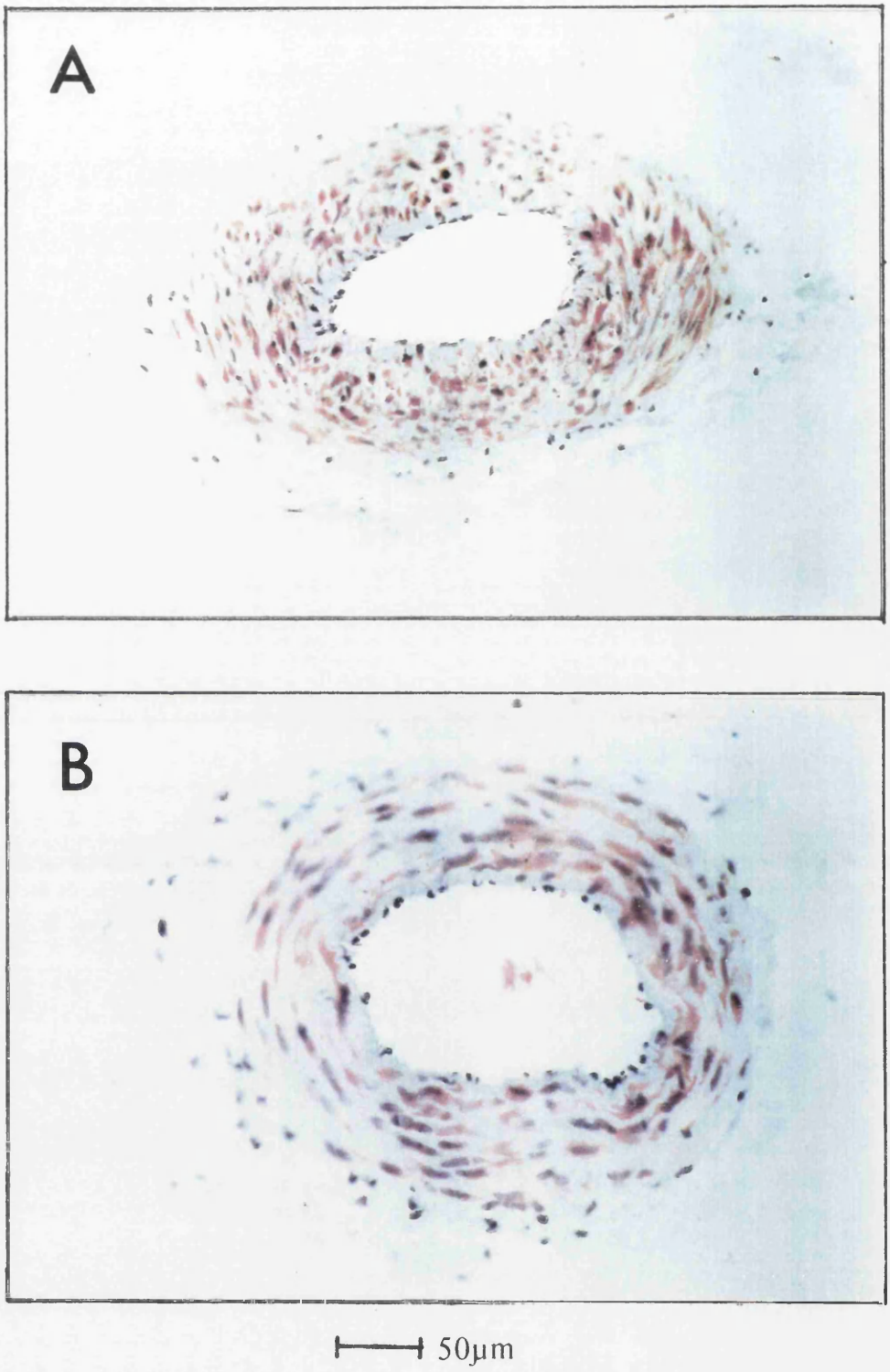


Figure 16. **Transverse sections of the proximal tail artery from SHR (A) and WKY rats (B)**. Sections were stained with Masson, showing the nuclei blue/black, cytoplasm and muscle purple/red, and connective tissue pale blue/green. There was no difference in the wall thickness between vessels from hypertensive and normotensive rats, but the artery wall from SHR appeared to have more layers of cells than that from WKY. The lumen diameter was decreased in SHR compared to WKY.

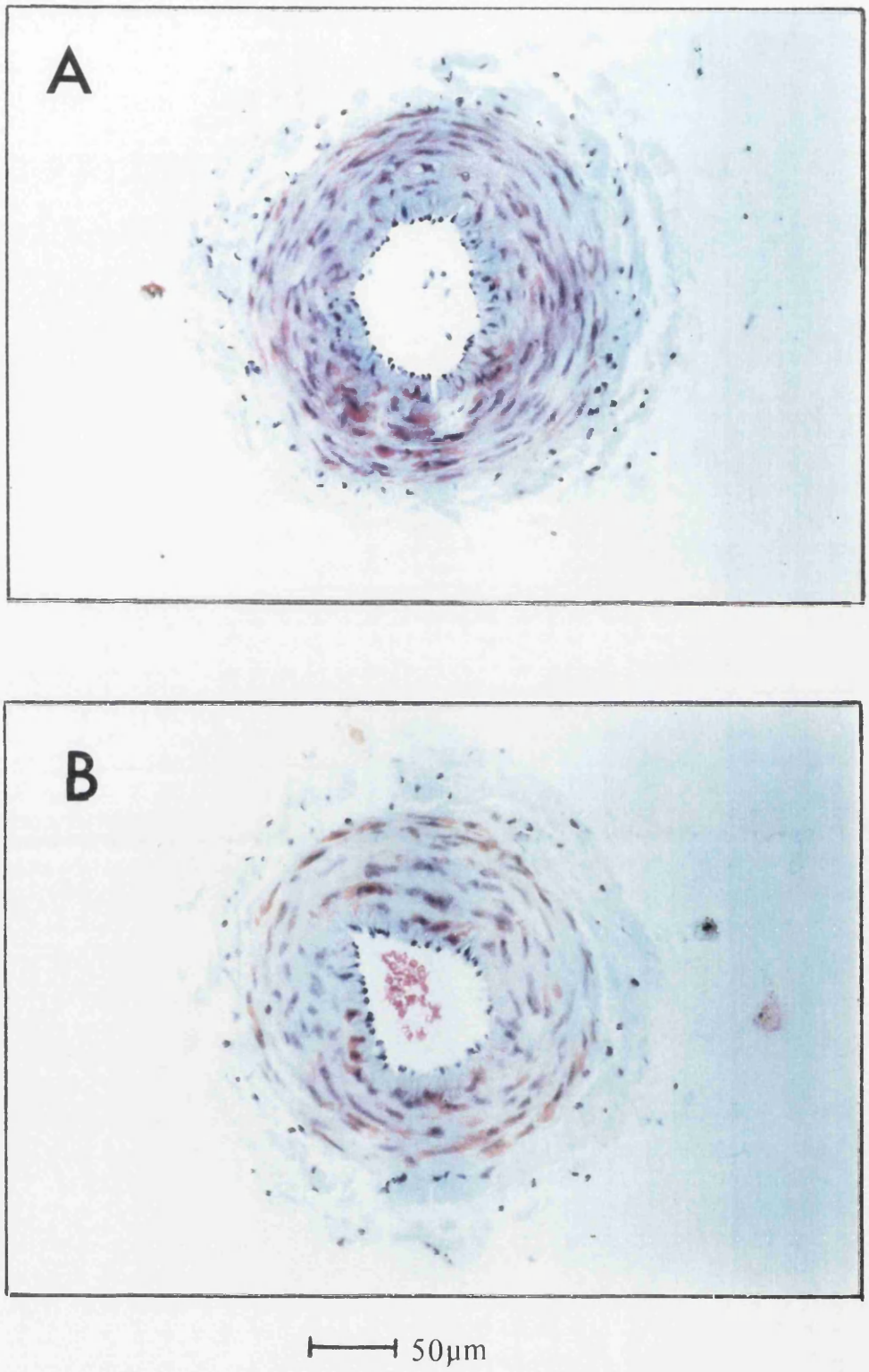


Figure 17. **Transverse sections of the distal tail artery from SHR (A) and WKY rats (B).** Sections were stained with Masson, showing the nuclei blue/black, cytoplasm and muscle purple/red, and connective tissue pale blue/green. The artery wall thickness was increased in hypertensive rats compared to control and there also appeared to be more layers of cells in SHR relative to control. The lumen diameter was decreased in SHR compared to WKY.

1) *Aorta*

The wall thickness of the aorta was greater in SHR when compared to WKY control rats (77.66±1.20 μm c.f. 64.63±1.04 μm; P<0.01), but the lumen diameter was greater in SHR compared to WKY (1252.05±16.69μm cf. 1176.69±9.60μm; P<0.01) (Figure 15). The media area was increased in SHR relative to control (331450.6±2445.83μm^2, c.f. 272845.5±4987.23 μm^2; P<0.001). There was no difference in the wall thickness to lumen ratio between hypertensive and control rats (0.06±0.006 and 0.05±0.005 respectively).

2) *Tail artery*

a) Proximal:

There was no difference in the wall thickness of the proximal part of the tail artery between SHR and WKY rats (84.64±0.95μm and 86.20±6.0μm respectively), but the lumen diameter was reduced in SHR relative to control (85.63±1.64μm cf. 121.25±4.94μm; P<0.001) (Figure 16). The media area was reduced in SHR relative to control (46921.78±943.56μm^2, c.f. 53287.8±2479.7μm^2; P<0.001). The wall thickness to lumen ratio was greater in SHR relative to control (0.99±0.08 c.f. 0.71±0.05; P<0.05).

b) Distal:

In the distal part of the tail artery, the wall thickness was greater in SHR compared to WKY control rats (69.61±0.95μm c.f. 52.8±0.67μm; P<0.001), and the lumen diameter was reduced in SHR relative to control (87.92±2.54μm cf. 127.64 ±2.39μm; P<0.001) (Figure 17). The media area was increased in SHR compared to control (35339.78±932.47μm^2, c.f. 30987.46±971.74 μm^2; P<0.05). The wall thickness to lumen ratio was greater in SHR compared to WKY control rats (0.79±0.06 cf. 0.41±0.03; P<0.001).

The above results of the histological measurements are summarised in Table 2.

a) SHR	*Wall Thickness* (μm) (n=12)	*Lumen Diameter* (μm) (n=8)	*Ratio* (n=8)	*Media Area* (μm^2) (n=5)
Proximal RTA	84.64 ±0.95 NS	85.63±1.64 ***	0.99±0.08 *	46921.78±943.56 ***
Distal RTA	69.61±0.95 ***	87.92±2.54 ***	0.79±0.06 ***	35339.78±932.47 *
Aorta	77.66±1.20 **	1252.05±16.69 **	0.06±0.006 NS	331450.6±2445.83 ***

b) WKY	*Wall Thickness* (μm)	*Lumen Diameter* (μm)	*Ratio*	*Media Area* (μm^2)
Proximal RTA	86.2±6.0	121.25±4.94	0.71±0.05	53287.8±2479.7
Distal RTA	52.8±0.67	127.64±2.39	0.41±0.03	30987.46±971.74
Aorta	64.63±1.04	1176.69±9.60	0.05±0.005	272845.5±4987.23

Table 2. **Table summarising structural measurements in the tail artery (RTA) and aorta from SHR (a) and WKY (b) rats.** Ratio refers to the ratio of wall thickness to lumen diameter. The media area is that of a transverse section through the vessel segment. Each value represents the mean ± s.e.m. for 5 vessel segments, with the indicated number of observations (n) from each segment. Asterisks indicate a significant difference between SHR and WKY (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS = not significant).

C) MECHANICAL RESPONSES 0F BLOOD VESSELS

1) *Aorta*

Aortic rings (3-4mm) were mounted in organ baths and changes in isometric tension were recorded in response to vasoactive agents.

a) Phenylephrine:

Increasing concentrations of PE (10^{-9}-$3x10^{-5}$M) produced corresponding increases in tension. Responses in aortic rings from hypertensive rats were lower than those from WKY control rats, with a lower maximum response (0.76±0.06g mg^{-1} c.f. 1.29±0.04g mg^{-1}: P<0.001) (Figure 18). There was no difference in the EC_{50} between hypertensive and control rats (0.04±0.006μM c.f. 0.02±0.004μM respectively).

For studies using vasorelaxants, aortic rings were first precontracted with the EC_{75} concentration of PE, which in SHR was 0.2μM and in WKY was 0.1μM, producing responses of 0.58g mg^{-1} and 0.97g mg^{-1} respectively.

b) Carbachol:

Increasing concentrations of carbachol (10^{-9}-$3x10^{-5}$M) produced corresponding relaxations of the PE-induced precontraction. Relaxations in aortic rings from hypertensive rats were greater than those from WKY control rats (Figure 19), with a greater maximum inhibition of the precontraction (106.4±2.1%, c.f. 76.4±4.5%; P<0.001), and a lower EC_{50} (0.10±0.01μM, c.f. 0.20± 0.02μM; P<0.01).

c) Sodium nitroprusside:

Increasing concentrations of SNP ($3x10^{-11}$ - $3x10^{-6}$M) produced corresponding relaxations of the PE-induced precontraction. At the lower end of the concentration-response curve ($3x10^{-11}$ - 10^{-8}M) there was no difference

between aortic rings from SHR and WKY rats (Figure 20). At the higher concentrations however ($3x10^{-8}$ - $3x10^{-6}$M), aortic rings from SHR were more responsive to SNP than were rings from WKY rats, with a greater maximum inhibition of the precontraction (127.8±3.1%, c.f. 107.0±1.60%; $P<0.001$). There was no difference in the EC_{50} for SNP between SHR and WKY (1.80±0.2nM c.f. 1.02±0.2nM; $P>0.05$).

d) Isoprenaline:

Increasing concentrations of isoprenaline ($3x10^{-10}$-10^{-5}M) produced corresponding relaxations of the PE-induced precontraction. There was no difference between hypertensive and control rats in the relaxation of aortic rings in response to isoprenaline (Figure 21 ; maximum responses 111.7±5.5% and 113.2±4.7% respectively; EC_{50} 0.42±0.04nM and 0.49±0.05nM respectively).

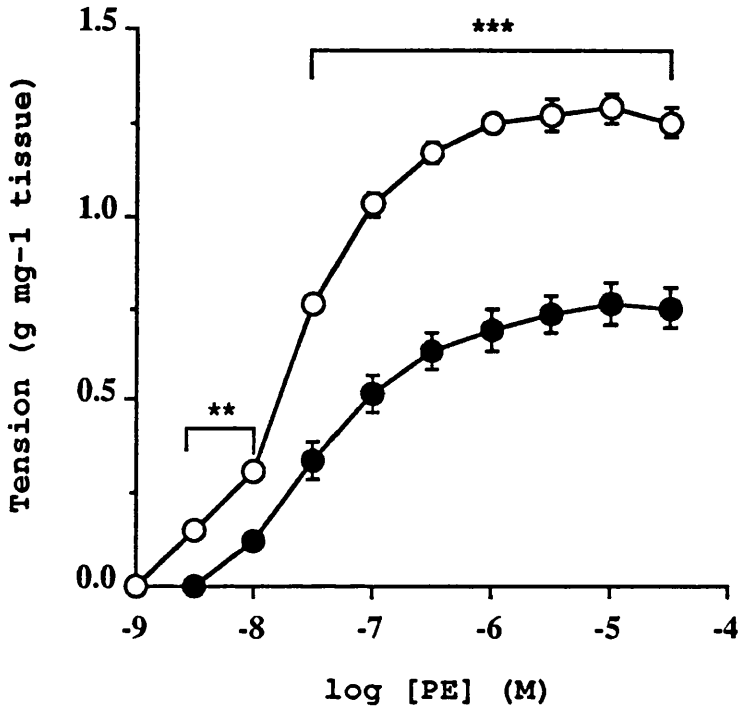


Figure 18. **Concentration-response curve to PE in rat aortic rings.** Responses were expressed as the tension developed per mg of tissue. Responses to PE in aortic rings from SHR (--●--) were lower than in those from WKY rats (--○--) (3-10nM: **, P<0.01; 30nM-30μM: ***, P<0.001). Each point represents the mean ± s.e.m of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

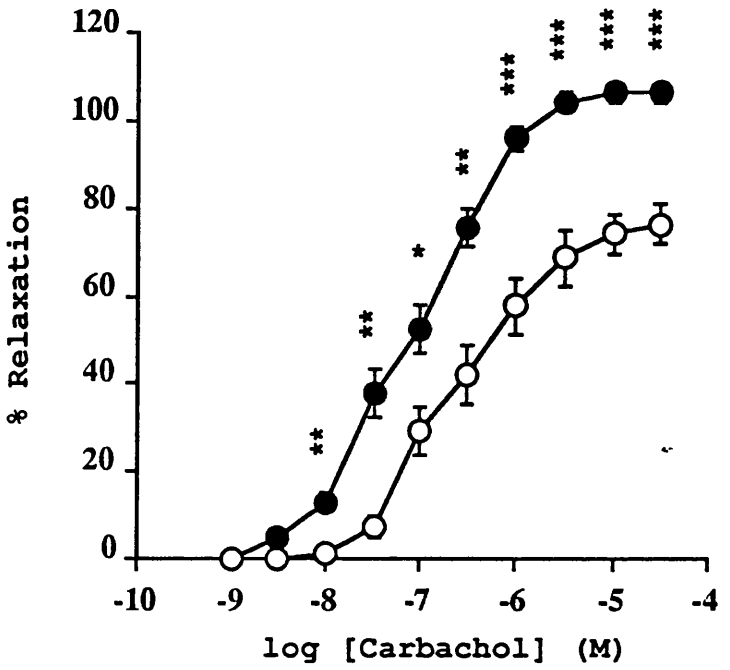


Figure 19. **Concentration-response curve to carbachol in rat aortic rings.** Responses were expressed as the percentage relaxation of the PE-induced (EC_{75}) precontraction. Responses to carbachol in rings from SHR (--●--) were greater than in those from WKY control rats (--○--) (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

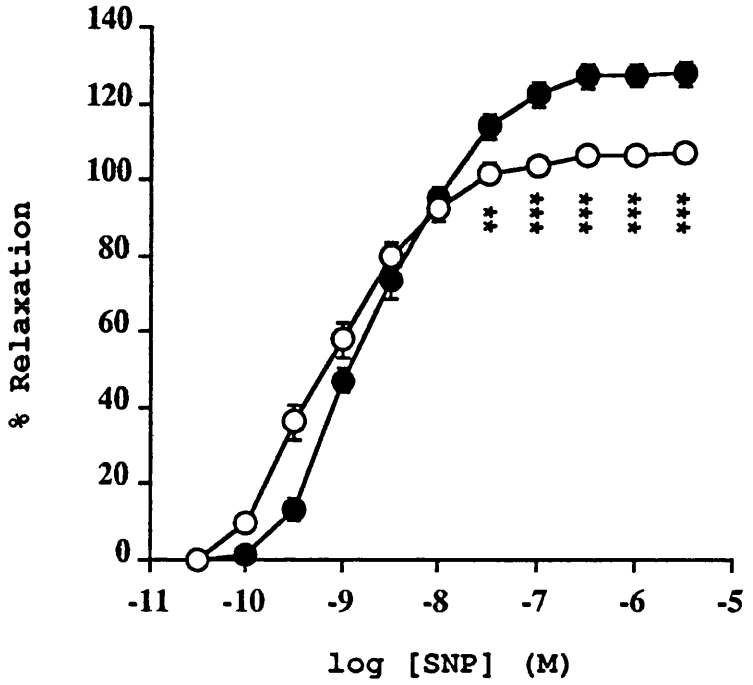


Figure 20. **Concentration-reponse curve to sodium nitroprusside (SNP) in rat aortic rings.** Responses were expressed as the percentage relaxation of the PE-induced (EC_{75}) precontraction. At the lower concentrations of SNP there was no difference in the relaxation between vessels from SHR (--●--) and WKY control rats (--○--). At higher concentrations of SNP, aortic rings from SHR were more responsive to SNP than were rings from WKY rats (**, $P<0.01$; ***, $P<0.001$). Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

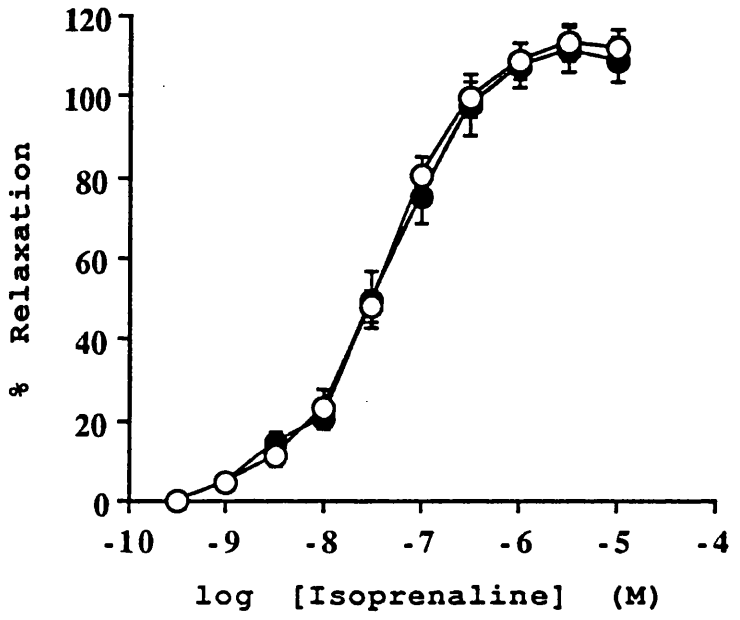


Figure 21. **Concentration-response curve to isoprenaline in rat aortic rings.** Responses were expressed as the percentage relaxation of the PE-induced (EC_{75}) precontraction. There was no difference in the response to isoprenaline in aortic rings between SHR (--●--) and WKY control rats (--○--). Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

2) *Tail artery*

The proximal part of the tail artery was used to investigate responses to vasoactive agents from SHR and WKY rats.

a) Phenylephrine:

Increasing concentrations of PE (10^{-8} - $3x10^{-5}$M) produced corresponding increases in tension. Responses in tail artery rings from hypertensive rats were greater than those from WKY control rats (Figure 22) with an increased maximum response (2.73 $\pm$ 0.32g mg^{-1} c.f. 1.72$\pm$0.13g mg^{-1}; $P<0.05$). The EC_{50} was unchanged between hypertensive and control rats (0.46$\pm$0.05μM and 0.54$\pm$0.05μM respectively).

b) UK-14,304:

Increasing concentrations of UK-14,304 ($3x10^{-10}$-$3x10^{-5}$M) produced corresponding increases in tension in rat tail artery rings. When responses were expressed as changes in tension, tail artery rings from SHR were more responsive to UK-14,304 than were rings from WKY, with an increase in the maximum response (0.72$\pm$0.09g, c.f. 0.42$\pm$0.09g; $P<0.05$) (Figure 23a; Table 3b). There was no difference in the EC_{50} for UK-14,304 between hypertensive and control rats (0.54$\pm$0.07μM and 0.57$\pm$0.06μM respectively; Table 3a). In contrast, when responses were expressed as the tension developed per mg of tissue (Figure 23b), there was no difference in the maximum response to UK-14,304 between SHR and WKY rats (1.20$\pm$0.06g mg^{-1} and 0.98$\pm$0.14g mg^{-1} respectively; $P>0.05$).

Yohimbine (50nM) produced a small but significant rightward shift in the concentration-response curve for UK-14,304 in tail artery rings from both SHR and WKY rats (Figure 24). In both groups of rat, the shift was non-parallel, with a greater effect at the lower end of the concentration-response curve. The EC_{50} for UK-14,304 was increased in both SHR and WKY rats in

the presence of yohimbine ($P<0.05$), but the maximum response to UK-14,304 was unchanged (Table 3).

c) Noradrenaline:

(i) Tension recording

NA (10^{-9}-10^{-5}M) produced concentration-dependent increases in tension in rat tail artery rings. When responses were expressed as changes in tension per mg of tissue (Figure 25a), tail artery rings from SHR were more responsive to NA than were rings from WKY, with an increase in the maximum response (0.50 ± 0.03g mg^{-1} cf. 0.36 ± 0.07g mg^{-1}; $P<0.05$. When these isometric tension responses were expressed as a percentage of the maximum reponse however, there was no difference between hypertensive and control rats (Figure 25b), with similar EC_{50} values ($0.59\pm0.06\mu$M and $0.46\pm0.06\mu$M respectively).

(ii) Perfusion pressure recording

The effect of NA was also studied in the perfused tail artery, where blood vessel segments were cannulated at both ends and perfused (1ml min^{-1}) with increasing concentrations of NA (10^{-9}-10^{-5}M). This produced corresponding increases in perfusion pressure. When expressed in absolute units, i.e. in mmHg, responses at the higher end of the concentration-response curve were greater in tail artery segments from SHR than in those from WKY ($P<0.01$; Figure 25c). A maximum response was not achieved in the SHR, so the EC_{50} could not be calculated. The EC_{50} for NA in the perfused tail artery of WKY was $0.56\pm0.07\mu$M, which was not significantly different from the value obtained in tail artery rings of WKY following tension recording. When responses were expressed as a percentage of the maximum (in the case of SHR, the highest response achieved), responses in vessels from SHR were lower than in those from WKY ($P<0.05$; Figure 25d).

For studies using vasorelaxants, tail artery rings were precontracted with the EC_{75} for PE, which in SHR was 2.05±0.28μM and in WKY was 1.66±0.30μM. These concentrations were not significantly different, and produced responses of 2.19±0.22g mg^{-1} and 1.29±0.10g mg^{-1} respectively. Responses to vasorelaxants were expressed as the percentage relaxation of the PE-induced precontraction.

d) Sodium nitroprusside:

Increasing concentrations of SNP ($3x10^{-10}$ - $3x10^{-5}$M) produced corresponding relaxations of the PE-induced precontraction (Figure 26a). Responses in tail artery rings from SHR were lower than in those from WKY rats, with a decreased maximum response (82.0±4.1%, c.f. 97.3±2.8%; $P<0.01$). There was no difference in the EC_{50} for SNP between hypertensive and control rats (40.77±5.20nM and 34.08 ±3.53nM respectively).

e) Carbachol:

The endothelium-dependent vasodilator carbachol (10^{-8} - $3x10^{-5}$M) produced little or no relaxation in tail artery rings from either hypertensive or normotensive rats (Figure 26b). The threshold was 10^{-5}M in each group. The highest concentration of carbachol added ($3x10^{-5}$M) produced relaxations of 7.9 ± 3.7% and 4.8±3.1% in SHR and WKY respectively. There was no evidence of contraction in response to carbachol in either group of rat.

f) Isoprenaline:

Isoprenaline (10^{-8} - 10^{-4}M) produced relaxation in tail artery rings from SHR and WKY, with a threshold of $3x10^{-7}$ - 10^{-6}M (Figure 26c). A maximum response was not achieved in either group of rat as the response was still increasing at 10^{-4}M, the highest concentration of isoprenaline which could be added to the organ bath, so the EC_{50} could not be calculated. Responses in

vessels from SHR were lower than those in vessels from WKY ($P<0.05$), the highest relaxations achieved being $41.0 \pm 4.1\%$ and $62.4 \pm 5.3\%$ respectively.

D) BIOCHEMICAL RESPONSES OF BLOOD VESSELS

Phosphoinositide (PI) hydrolysis

The biochemical response underlying NA-induced contraction was investigated by measuring accumulation of total inositol phosphates in response to NA (Figure 27). The basal level of PI hydrolysis in tail artery segments from SHR was significantly higher than in those from WKY rats (168.6 ± 7.99 dpm mg^{-1} tissue, c.f. 109.39 ± 8.78 dpm mg^{-1} tissue; $P<0.001$). NA (10^{-6}, 10^{-5}M) produced a concentration-dependent increase in PI hydrolysis in rings from both groups of rats. At 10^{-6}M NA, there was no difference in PI hydrolysis between the two groups of rat. At 10^{-5}M however, NA produced a significantly greater stimulation of PI hydrolysis in vessels from SHR than in vessels from WKY rats (2888.73 ± 187.33 dpm mg^{-1} tissue, c.f. 1734.4 ± 133.85 dpm mg^{-1} tissue; $P<0.001$).

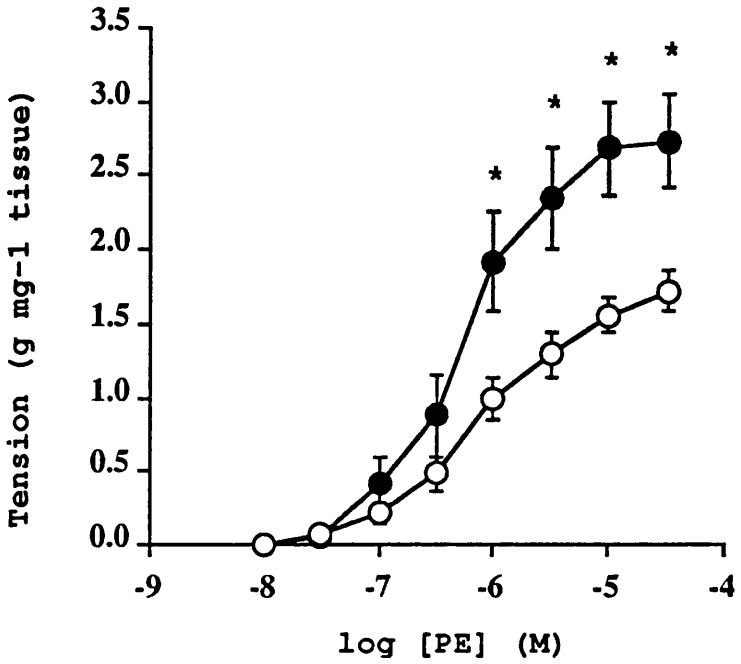


Figure 22. **Concentration-response curve to PE in rat tail artery rings.** Contractions were expressed as the tension developed per mg of tissue. Responses in tail artery rings from SHR (--●--) were greater than in those from WKY control rats (--○--) (*; $P<0.05$). Each point represents the mean ± s.e.m. of 6 observations. Where no error bar is shown, the errors are contained within the symbols.

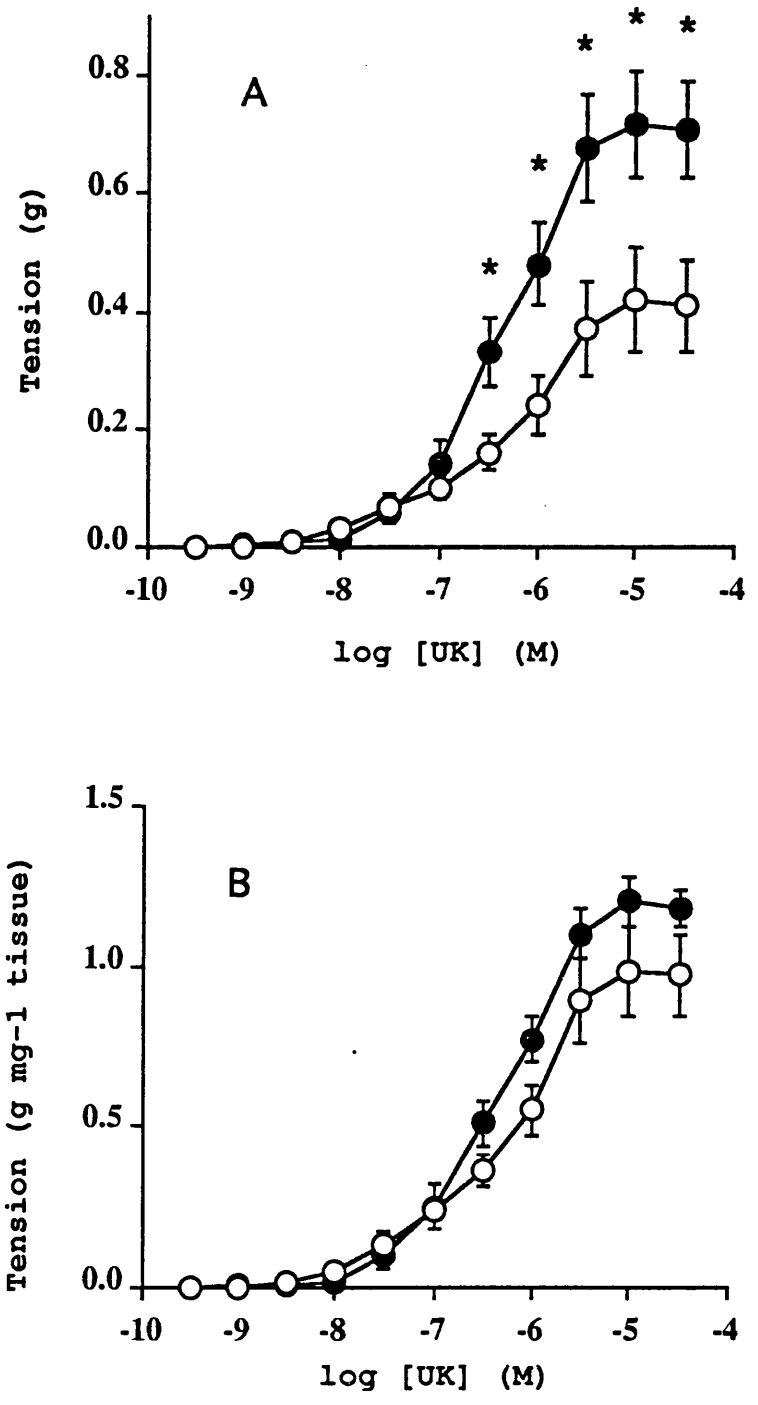


Figure 23. **Concentration-response curve to UK-14,304 (UK) in rat tail artery rings.** Responses were expressed both as the tension developed (A) and as the tension developed per mg of tissue (B). When expressed as tension, responses to UK in tail artery rings from SHR (--●--) were greater than in those from control WKY rats (--○--) (*, $P<0.05$). When expressed as the tension developed per mg of tissue, there was no difference between tail artery rings from SHR and WKY rats in the response to UK. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

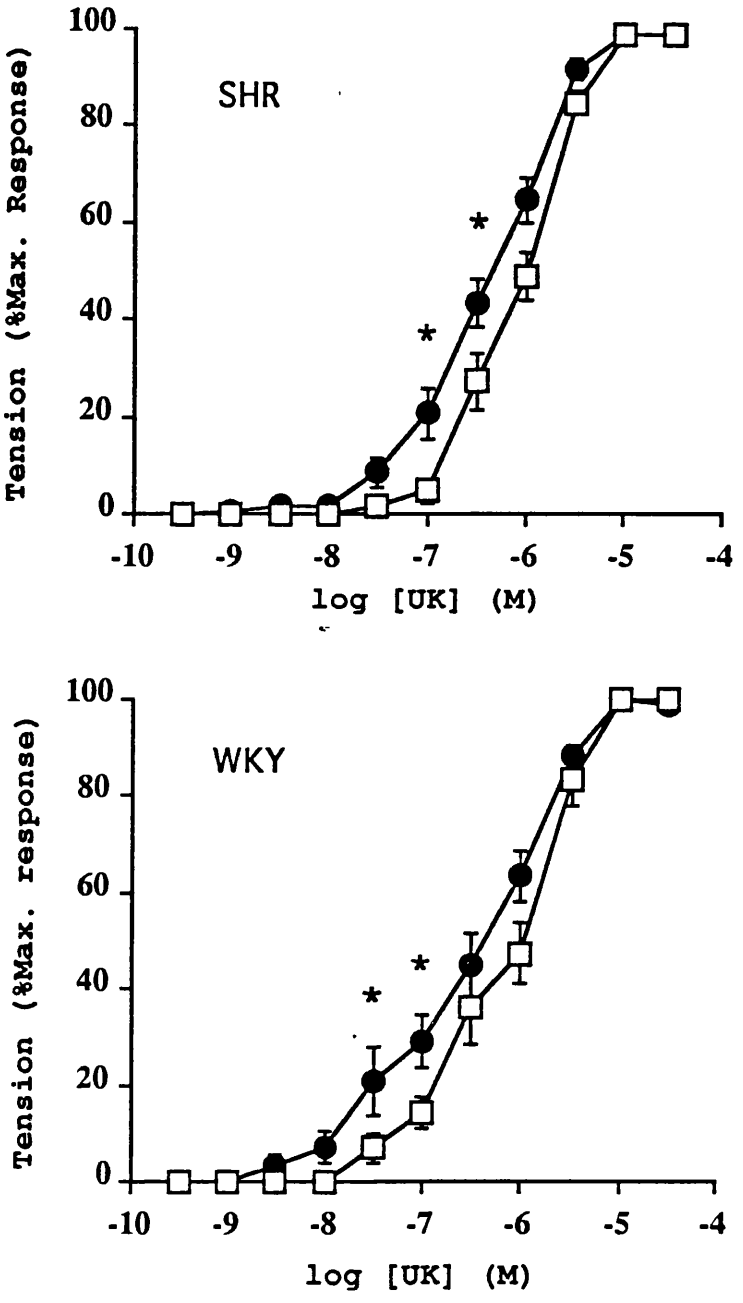


Figure 24. **The effect of yohimbine (50nM) on the response to UK-14,304 (UK) in rat tail artery rings.** Control concentration-response curves to UK were carried out (--●--) and repeated in the presence of yohimbine (--□--), in tail artery rings from SHR (upper panel) or control WKY rats (lower panel). Responses were expressed as a percentage of the maximum response. Yohimbine inhibited the response to UK in tail artery rings from both SHR and WKY (*, $P<0.05$). Yohimbine produced a non-parallel, rightward shift in the UK curve in both groups of rat, but did not affect the maximum response. Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

a)

EC_{50}	*Control*	*+ Yohimbine*	
SHR	0.54±0.07μM	1.01±0.01μM	*
WKY	0.57±0.06μM	1.02±0.01μM	*

b)

Maximum Response	*Control*	*+ Yohimbine*	
SHR	0.72±0.09g	0.75±0.10g	NS
WKY	0.42±0.09g	0.37±0.10g	NS

Table 3. **Table summarising the effect of yohimbine on the response to UK14,-304 in tail artery rings from SHR and WKY rats.** A concentration-response curve to UK-14, 304 was carried out and repeated in the presence of yohimbine. (a) The EC_{50} for UK-14,304 was increased in the presence of yohimbine in vessels from both SHR and WKY (*, $P<0.05$). (b) Yohimbine did not affect the maximum response to UK-14,304 in either group of rat (NS = not significant). Each value represents the mean ± s.e.m. of at least 6 observations.

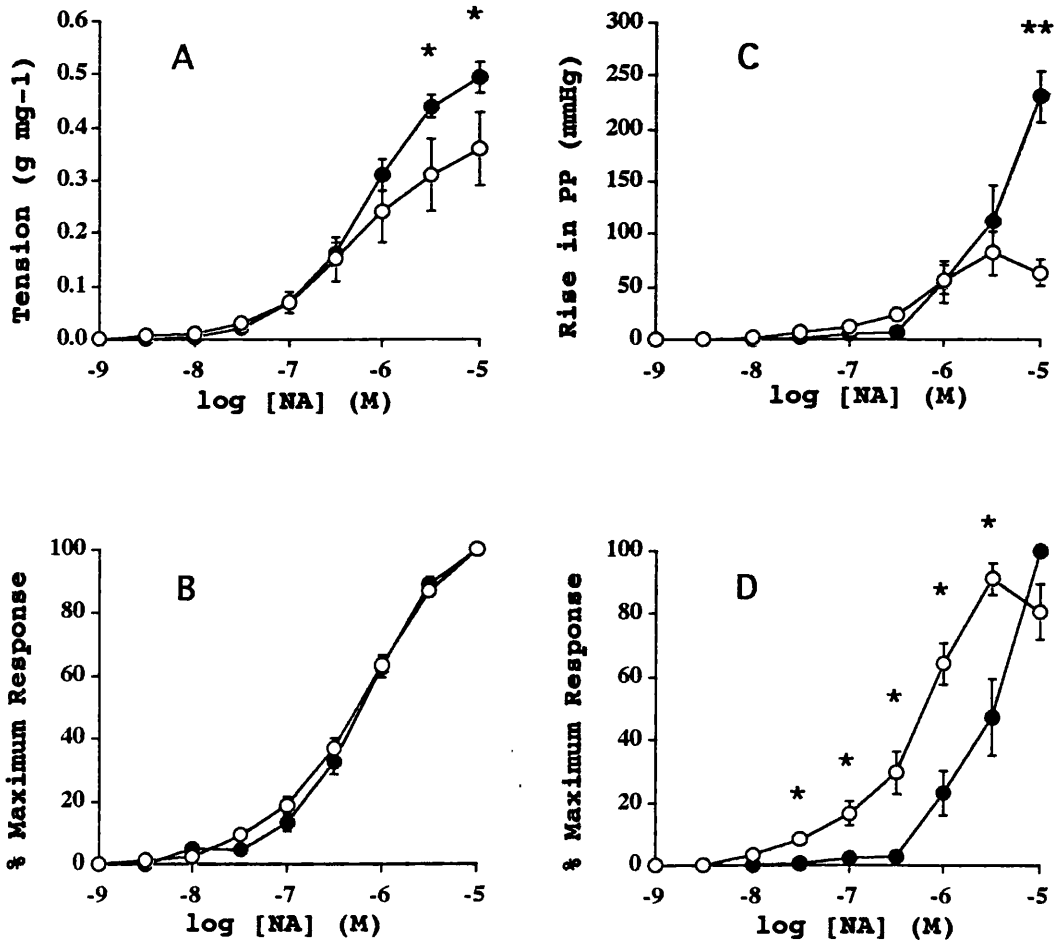


Figure 25. **Concentration-response curves to NA in the rat tail artery.** NA was administered either by cumulative addition into organ baths, where isometric tension was measured in artery rings (A, B), or by lumenal perfusion (1ml min^{-1}) in segments of tail artery, where rises in perfusion pressure (PP) were measured (C, D). In artery rings, tension responses to NA were greater in SHR (--●--) than in WKY rats (--○--) (*, $P<0.05$; A), but when expressed as a percentage of the maximum response there was no difference between SHR and WKY in the response to NA (B). In perfused artery segments (C, D) the rise in perfusion pressure in response to NA was greater in arteries from SHR (--●--) than in arteries from WKY rats (--○--) (**, $P<0.01$; C). When expressed as a percentage of the maximum response, responses from SHR were lower than those from WKY (*, $P<0.05$; D). Each point represents the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the errors are contained within the symbols.

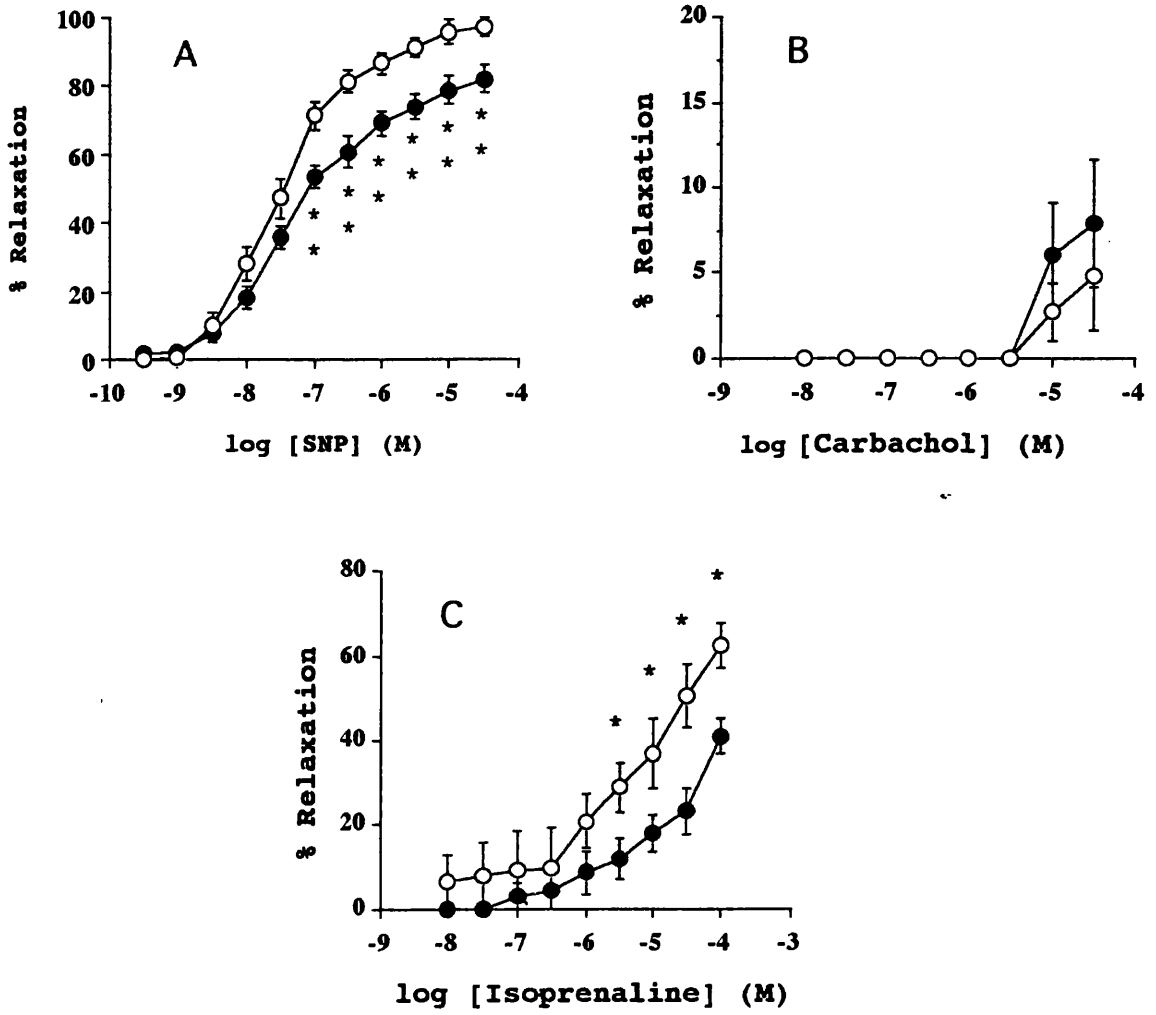


Figure 26. **Concentration-response curves to vasorelaxants in tail artery rings from SHR and WKY rats.** Responses were expressed as the percentage relaxation of the PE-induced (EC_{75}) precontraction. Responses to SNP (A) or to isoprenaline (C) in rings from SHR were lower than those in rings from WKY (A: **, $P<0.01$; C: *, $P<0.05$). There was little or no response to carbachol in vessels from either SHR or WKY (B). Each point represents the mean ± s.e.m. of at least 5 observations. Where no error bars are shown, the errors are contained within the symbols (--●-- = SHR; --○-- = WKY).

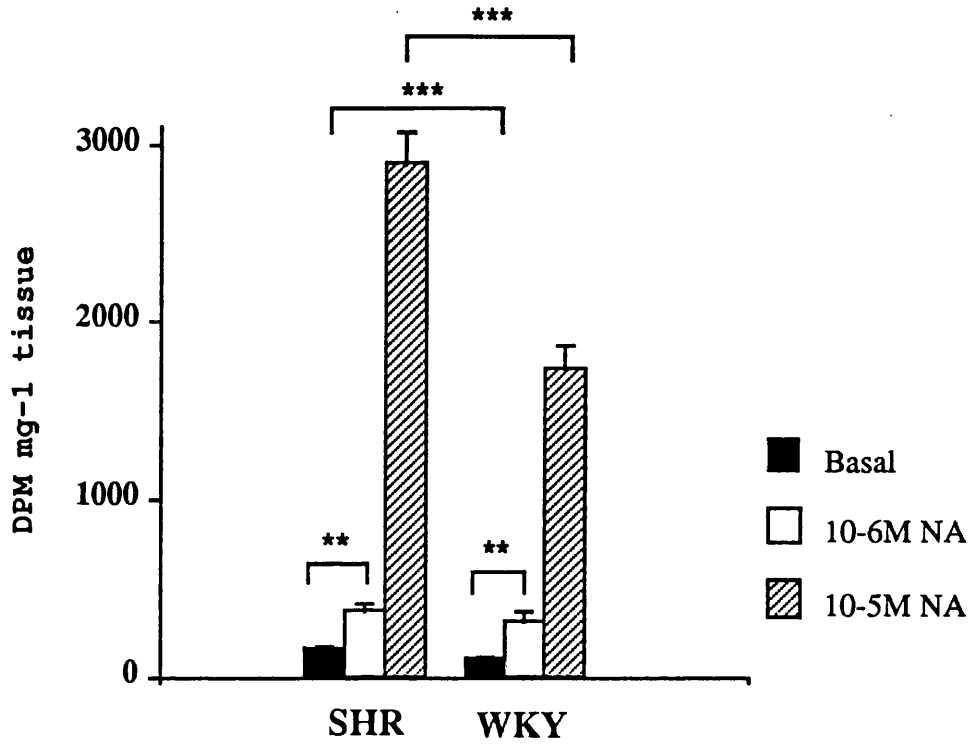


Figure 27. **NA-stimulated phosphoinositide (PI) hydrolysis in rat tail artery rings.** 1cm segments of artery from SHR or control WKY rats were labelled with (myo-^{3}H)-inositol and incubated with Krebs buffer containing lithium chloride (10mM) with or without NA for 1 hour. Vessels were then homogenised and the inositol phosphates extracted, separated on Dowex columns and measured in a liquid scintillation counter. The results were expressed as disintegrations per minute (DPM) per mg of tissue. The basal level of PI hydrolysis was higher in tail arteries from SHR than in those from WKY rats (***, $P<0.001$). 10^{-6}M NA increased the PI hydrolysis in tail arteries from both SHR and WKY rats (**, $P<0.01$), but there was no difference between the two groups of rat. 10^{-5}M NA produced a greater stimulation of PI hydrolysis in tail arteries from SHR than in those from WKY rats (***, $P<0.001$). Each bar represents the mean $\pm$ s.e.m. of 6 observations.

PART III

INVESTIGATION OF RESPONSES MEDIATED VIA POST-JUNCTIONAL ALPHA$_2$-ADRENOCEPTORS IN VASCULAR SMOOTH MUSCLE.

Contractions mediated via post-junctional α_2-adrenoceptors on vascular smooth muscle are often difficult to demonstrate. This part of the study investigated the conditions required to potentiate contractions of the rat tail artery in response to α_2-adrenoceptor agonists. Rings of tail artery were suspended in organ baths, and changes in isometric tension measured in response to α_2-adrenoceptor agonists, alone and in combination with other drugs.

A) CONCENTRATION-RESPONSE CURVES TO ALPHA$_2$-ADRENOCEPTOR AGONISTS

1) Proximal tail artery

a) Clonidine

Clonidine (10^{-8} -10^{-5}M) produced concentration-dependent increases in tension (Figure 28). The α_2-adrenoceptor antagonist yohimbine (30nM) produced a non-parallel rightward shift in the concentration-response curve to clonidine, with a greater effect at the lower end of the curve (Figure 28). The maximum response to clonidine (1.11$\pm$0.31g) was unaffected in the presence of yohimbine (1.24$\pm$0.25g), but the EC_{50} was increased in the presence of yohimbine as compared to control (0.18$\pm$0.03μM, c.f. 0.07$\pm$0.01μM; $P<0.05$).

b) UK-14,304

UK-14,304 (10^{-10} - 3x10^{-5}M) produced concentration-dependent

increases in tension in rat tail artery rings (Figure 29), with an EC_{50} of $0.41\pm0.05\mu M$.

2) Distal tail artery

Segments of rat tail artery taken from the distal part of the tail were mounted as ring preparations on a myograph and a concentration-response curve to UK-14,304 (10^{-10}-$3x10^{-5}M$) carried out. The curve was biphasic, with the first phase from 10^{-10} - $10^{-7}M$ (Figures 29, 30). The EC_{50} for this part of the curve was $2.18\pm0.32nM$. Following the plateau at 10^{-7} - $3x10^{-7}M$ UK-14,304, responses to increasing concentrations of UK increased and reached a second plateau at $3x10^{-5}M$. The EC_{50} for the second part of the curve was $30.0\pm6.2\mu M$.

Yohimbine (50nM) was added to the organ bath and the UK-14,304 concentration-response curve repeated 20 minutes later (Figure 30). The curve was monophasic in the presence of yohimbine, which produced a 174-fold rightward shift ($P<0.001$) in the first phase of the curve but did not affect the second phase. The EC_{50} for UK-14,304 in the presence of yohimbine was $0.38\pm0.45\mu M$.

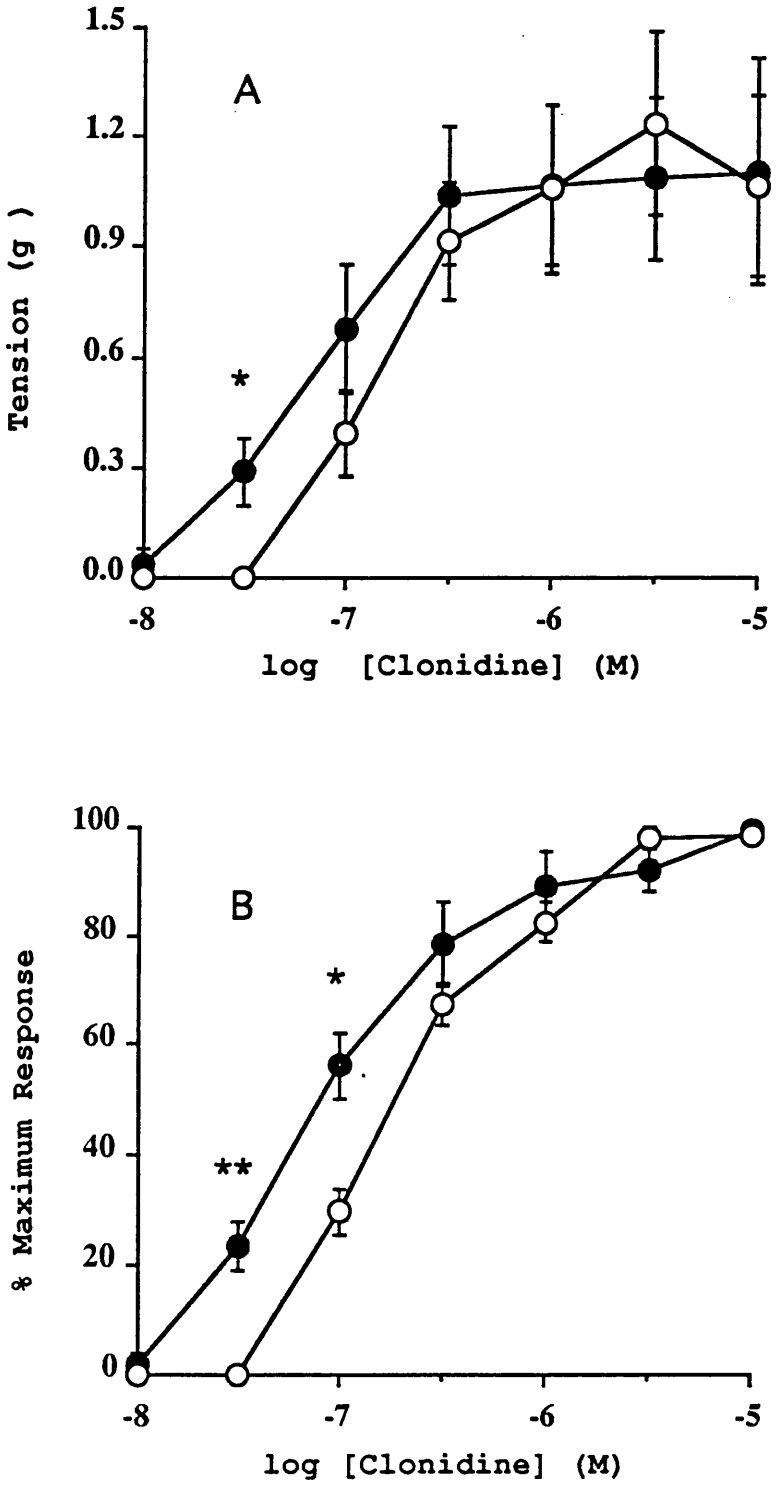


Figure 28. **The effect of yohimbine on the concentration-response curve to clonidine in rat tail artery rings.** A control curve to clonidine was carried out (--●--) and repeated in the presence of yohimbine (30nM; --○--). Responses were expressed as increases in isometric tension (A) and as a percentage of the maximum response (B). Yohimbine reduced the response to clonidine at the lower end of the curve (*, $P<0.05$; **, $P<0.01$) but did not affect either the maximum response or the concentration at which this was achieved. Each point represents the mean ± s.e.m. of 6 observations. Where no error bars are shown, the errors are contained within the symbols.

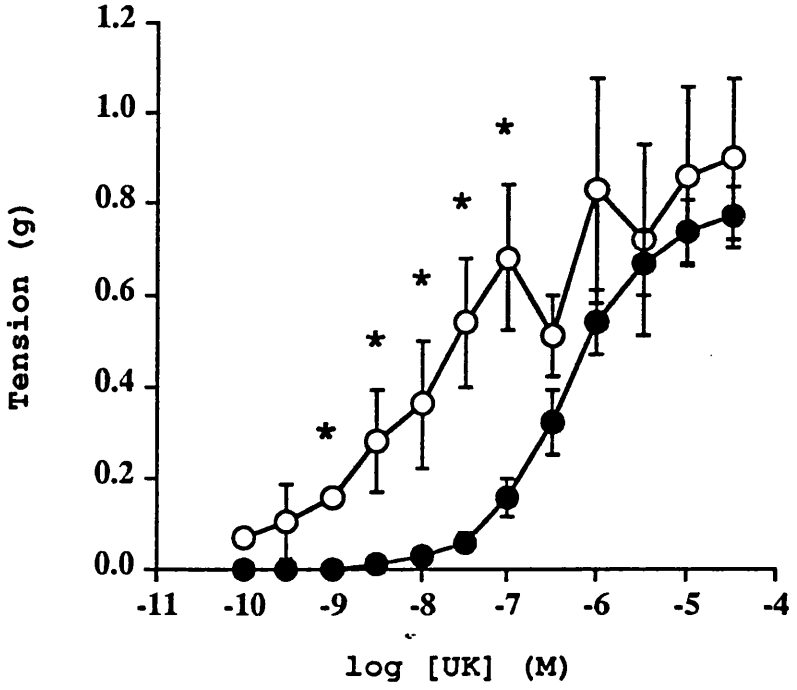


Figure 29. **Concentration-response curve to UK-14,304 in rat tail artery rings.** The lower concentrations of UK-14,304 (UK; 0.1-100nM) produced a smaller response in artery rings taken from the proximal part of the tail (--●--), than in rings taken from the distal part of the tail (--○--) (*, $P<0.05$). There was no difference in the response to the higher concentrations of UK (0.3-30μM) between rings from the proximal or distal regions of the vessel. Each point shows the mean ± s.e.m. of at least 5 observations. Where no error bars are shown, the errors are contained within the symbols.

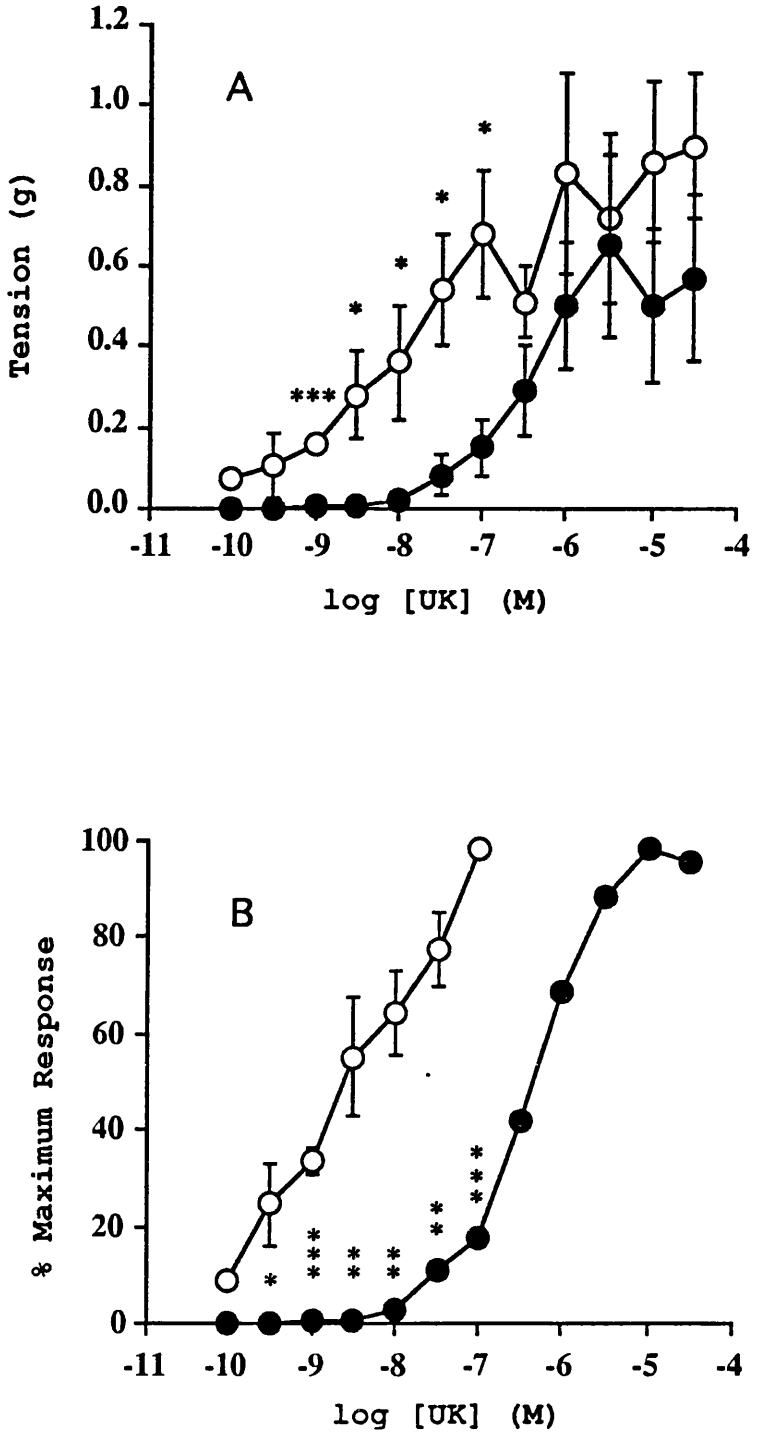


Figure 30. **The effect of yohimbine on the concentration-response curve to UK-14,304 in rat tail artery rings taken from the distal part of the tail.** When responses were expressed as changes in tension (A), the control curve (--○--) was biphasic, with the first maximum at 10^{-7}M UK-14,304 (UK) and the second at $3x10^{-5}$M UK. Responses to the lower concentrations of UK (0.3-100nM) were reduced in the presence of yohimbine (50nM) (--●--), but responses to the higher concentrations of UK (0.3-30μM) were unaffected. Responses were also expressed as a percentage of the maximum response achieved at the first plateau, i.e. at 10^{-7}M UK (B). Each point shows the mean ± s.e.m. of at least 5 observations. Where error bars are not shown, the errors are contained within the symbols. Asterisks indicate a significant reduction in the response to UK following yohimbine pretreatment (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

B)POTENTIATION OF RESPONSES TO ALPHA$_2$-ADRENOCEPTOR AGONISTS

This part of the study investigated some of the conditions under which enhanced responses to α_2-adrenoceptor agonists could be demonstrated, in order to understand the possible mechanisms of α_2-adrenoceptor-mediated contraction.

A protocol was established where a low concentration of α_2-adrenoceptor agonist (clonidine, 10nM, or UK-14,304, 50nM) was added to rings of rat tail artery, producing little or no response. This was then washed from the organ bath and the vessel contracted with a vasoconstrictor (the "precontracting agonist"). This contraction was relaxed with a vasodilator, and addition of the α_2-adrenoceptor agonist at this point, in the presence of the precontracting agonist and the vasodilator, now produced a large contraction.

1) Concentration-response curves for the precontracting agonists:

a) Vasopressin

AVP ($4x10^{-3}$-12mU ml^{-1}) produced concentration-dependent contractions, with an EC_{50} of 0.20 ± 0.03mU ml^{-1} (Figure 31a). Based on this, the concentration of AVP used to precontract the rat tail artery was 0.4mU ml^{-1}.

b) Phenylephrine

PE (10^{-9}-$3x10^{-5}$M) produced concentration-dependent contractions, with an EC_{50} of $0.42\pm0.05\mu$M (Figure 31b). Based on this, the concentration of PE chosen to precontract the rat tail artery was 0.5μM.

c) KCl

KCl (0.01-0.1M) produced concentration-dependent contractions which were biphasic, with a peak and plateau (Figure 31c). The EC_{50} for the peak phase of the response was 0.03±0.003M, and for the plateau phase was 0.04±0.005M. Based on this, the concentration of KCl chosen to precontract the rat tail artery was 0.03M.

d) 5-Hydroxytryptamine

5-HT (10^{-9}-$3x10^{-6}$M) produced concentration-dependent increases in tension, with an EC_{50} of 68.0±7.2nM (Figure 31d). Based on this, the concentration of 5-HT chosen to precontract the rat tail artery was 50nM.

2) The effect of different precontracting agonists:

The first series of experiments assessed the abilities of different precontracting agonists to enhance responses to the α_2-adrenoceptor agonists, clonidine and UK-14,304. The precontraction was inhibited by addition of the non-selective phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 10μM), then clonidine (10nM) or U-14,304K (50nM) was added. The response under these conditions was compared to the control response to clonidine or UK-14,304 alone.

Enhancement of the response to clonidine

a) Vasopressin as the precontracting agonist

Clonidine alone produced little or no response (0.07±0.01g). In the presence of AVP and IBMX, the response to clonidine was increased to 0.73±0.10g ($P<0.001$), an increase of 871.8±151.2%, and was inhibited by the α_2-adrenoceptor antagonist yohimbine (50nM: $P<0.01$). Further addition of

clonidine produced little response (Figures 32a, 33).

b) Phenylephrine as the precontracting agonist

Clonidine alone produced little or no response (0.03±0.01g). In the presence of PE and IBMX, the response to clonidine was increased to 0.50±0.06g ($P<0.001$), an increase of 810.4±93.9%, and was inhibited by yohimbine (50nM; $P<0.001$). Further addition of clonidine produced little response (Figures 32b, 34).

c) KCl as the precontracting agonist

Clonidine alone (10nM) produced little or no response (0.05±0.01g). In the presence of KCl and IBMX, the response to clonidine was increased to 0.28±0.04g, an increase of 231.5±65.3% ($P<0.01$), and was inhibited by yohimbine (50nM; $P<0.01$). Further addition of clonidine produced little response (Figures 32c, 35).

d) Effect of prazosin

The protocol using AVP as the precontracting agonist was repeated in the presence of the α_1-adrenoceptor agonist prazosin (50nM), which was added to the organ bath 20 minutes before the experiment. Prazosin had no effect on the size of the control response to clonidine (0.02±0.01g before prazosin; 0.02±0.01g after prazosin), or on the enhanced response to clonidine (0.48±0.05g before prazosin; 0.42±0.05g after prazosin). (Figure 36).

Enhancement of the response to UK-14,304

a) Vasopressin as the precontracting agonist

UK-14,304 alone produced little or no response (0.08±0.04g). In the presence of AVP (0.4mU ml^{-1}) and IBMX (10μM), the response to UK-

14,304 was increased (P<0.001) and often biphasic, with an initial peak which stabilised at a plateau. The enhanced response to UK-14,304 was, for peak then plateau, 1.12±0.12g then 1.01±0.12g, increases of 870±272% and 745±212.7% respectively. This enhanced response was blocked by yohimbine (50nM; P<0.001). (Figures 37a, 38).

b) Phenylephrine as the precontracting agonist

UK-14,304 alone produced little or no response (0.02±0.02g). In the presence of PE and IBMX, the response to UK-14,304 was increased to 0.69±0.11g (P<0.001), an increase of 2650%. This enhanced response was blocked by yohimbine (50nM; P<0.001). (Figures 37b, 39).

Lack of enhancement of the response to phenylephrine

The possibility that responses mediated via α_1-adrenoceptors could be potentiated in a similar way, was investigated using the α_1-adrenoceptor agonist PE. PE (0.2μM) alone produced little or no response (0.15±0.01g). In the presence of AVP and IBMX, the response to PE was no different from control (0.20±0.02g), while the response to clonidine was enhanced (0.82±0.08g, c.f. 0.12±0.01g control; P<0.001). This enhanced response to clonidine was blocked by yohimbine (50nM, P<0.001; Figures 40, 41).

3)*The effect of reversing the order of vasoconstrictor and vasodilator:*

The protocol was altered so that IBMX was added to the organ bath before AVP, to determine if the order of drug addition was important in producing an enhanced response to UK-14,304. Addition of IBMX (10μM) did not alter the tone of the tail artery rings. AVP (0.4mU ml^{-1}) now produced a small contraction (0.21±0.05g) in the presence of IBMX. UK-14,304 (50nM)

added at this point produced a response larger than the control response to UK-14,304 alone (0.85±0.11g c.f. 0.16±0.03g, P<0.01). This enhanced response was inhibited by yohimbine (50nM; P<0.01). (Figure 42).

4)*The effect of different vasodilators:*

The next series of experiments assessed the abilities of different vasodilators to enhance responses to clonidine and to UK-14,304. Tail artery rings were precontracted with AVP (0.4mU ml^{-1}) then relaxed with a variety of agents which act either via cyclic nucleotide-related mechanims, or by blocking calcium channels. The response to clonidine or to UK-14,304 under these conditions was compared to the control response to clonidine or UK-14,304 alone.

a) Diltiazem as the vasodilator

Clonidine alone (10nM) produced little or no response (0.004±0.003g). The calcium channel antagonist diltiazem (2μM) reduced the AVP-induced precontraction (0.54±0.05g) to 0.19±0.04g (P<0.01). In the presence of AVP and diltiazem the response to clonidine was increased to 0.34±0.07, an increase of 3650% (P<0.01), and was blocked by yohimbine (50nM; P<0.05) (Figures 43a, 45a).

b) Forskolin as the vasodilator

UK-14,304 alone (50nM) produced little or no response (0.09±0.03g). The adenylate cyclase activator forskolin (36nM) reduced the AVP-induced precontraction to 0.14±0.06g (P<0.01). In the presence of AVP and forskolin the response to UK-14,304 was increased to 0.91±0.09, an increase of 799±167% (P<0.001), and was blocked by yohimbine (50nM; P<0.001)

(Figures 43b, 45b).

c) Dibutyryl cyclic AMP as the vasodilator

UK-14,304 alone (50nM) produced little or no response (0.05±0.01g). The stable, permeant cyclic AMP analogue, dibutyryl cyclic AMP (0.2mM) reduced the AVP-induced precontraction to 0.02±0.01g ($P<0.001$). In the presence of VP and dibutyryl cyclic AMP, the response to UK-14,304 was increased to 0.36±0.04, an increase of 673±140% ($P<0.001$), and was blocked by yohimbine (50nM; $P<0.001$) (Figures 43c, 45c).

d) Sodium nitroprusside as the vasodilator

UK-14,304 alone produced little or no response (0.11±0.03g).The soluble guanylate cyclase activator SNP (0.1μM) reduced the AVP-induced precontraction to 0.02±0.01g ($P<0.001$). In the presence of AVP and SNP the response to UK-14,304 was increased to 0.75±0.05, an increase of 876±264% ($P<0.001$), and was blocked by yohimbine (50nM; $P<0.001$). Further addition of UK-14,304 produced little or no response (Figures 44a, 46a)

When tail artery rings were precontracted with PE (0.5μM), SNP reduced this contraction to 0.14±0.06g ($P<0.01$). Subsequent addition of UK-14,304 produced a response of 0.62±0.10g, an increase of 815±217% ($P<0.01$), which was blocked by yohimbine (50nM; $P<0.01$). Further addition of UK-14,304 produced little or no response (Figures 44b, 46b)

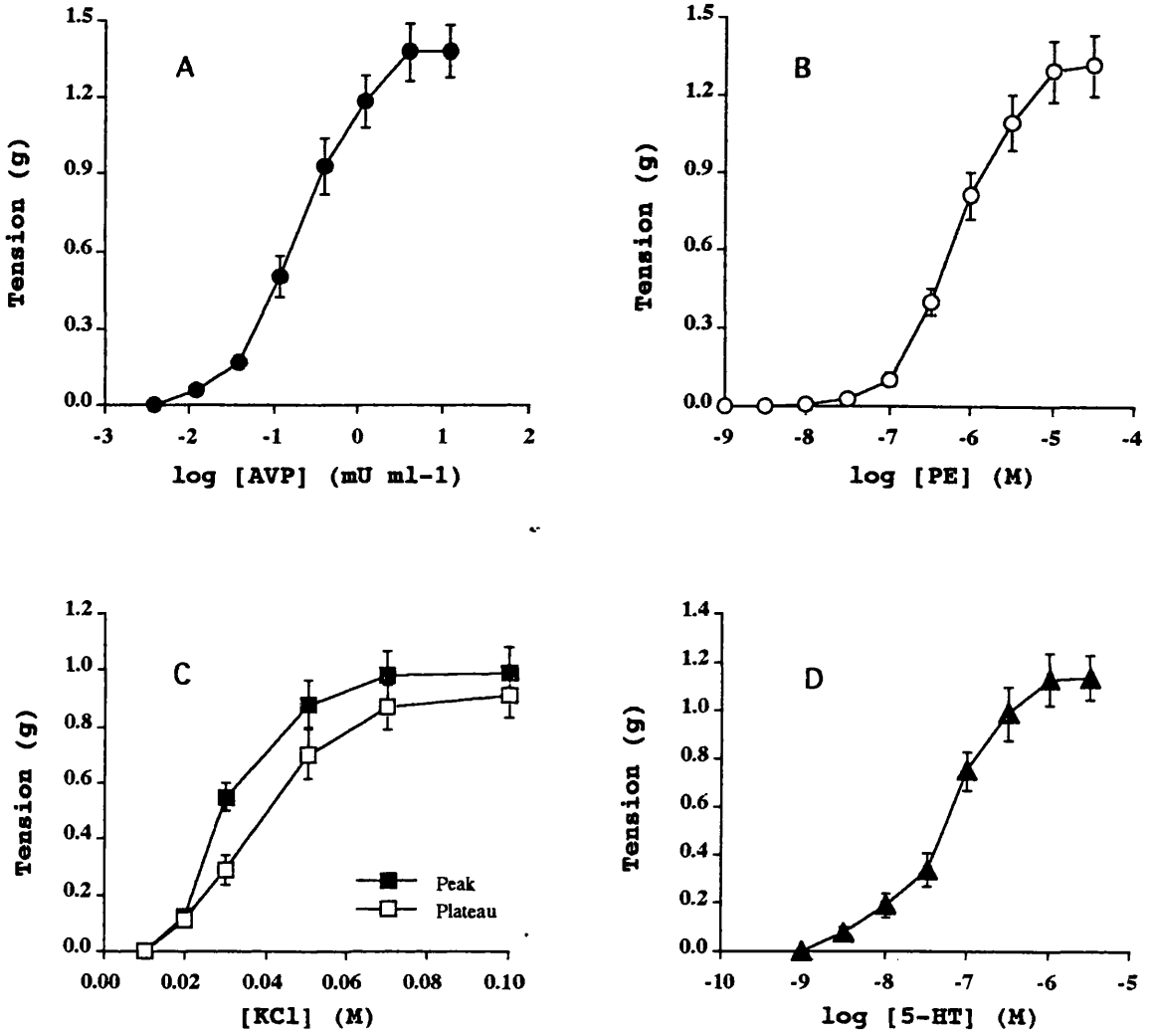


Figure 31. **Concentration-response curves for the agonists used to precontract rat tail artery rings.** Vasopressin (AVP; A; --●--, n=11), PE (B; --○--, n=11) and 5-hydroxytryptamine (5-HT; D; --▲--, n=8) were each added in a cumulative manner. KCl (C; n=12) produced biphasic responses consisting of a peak (--■--) then a plateau (--□--), so each concentration was washed out when the response had stabilised before the next concentration was added. Each point represents the mean ± s.e.m. of the indicated number of observations. Where no error bars are shown, the errors are contained within the symbols.

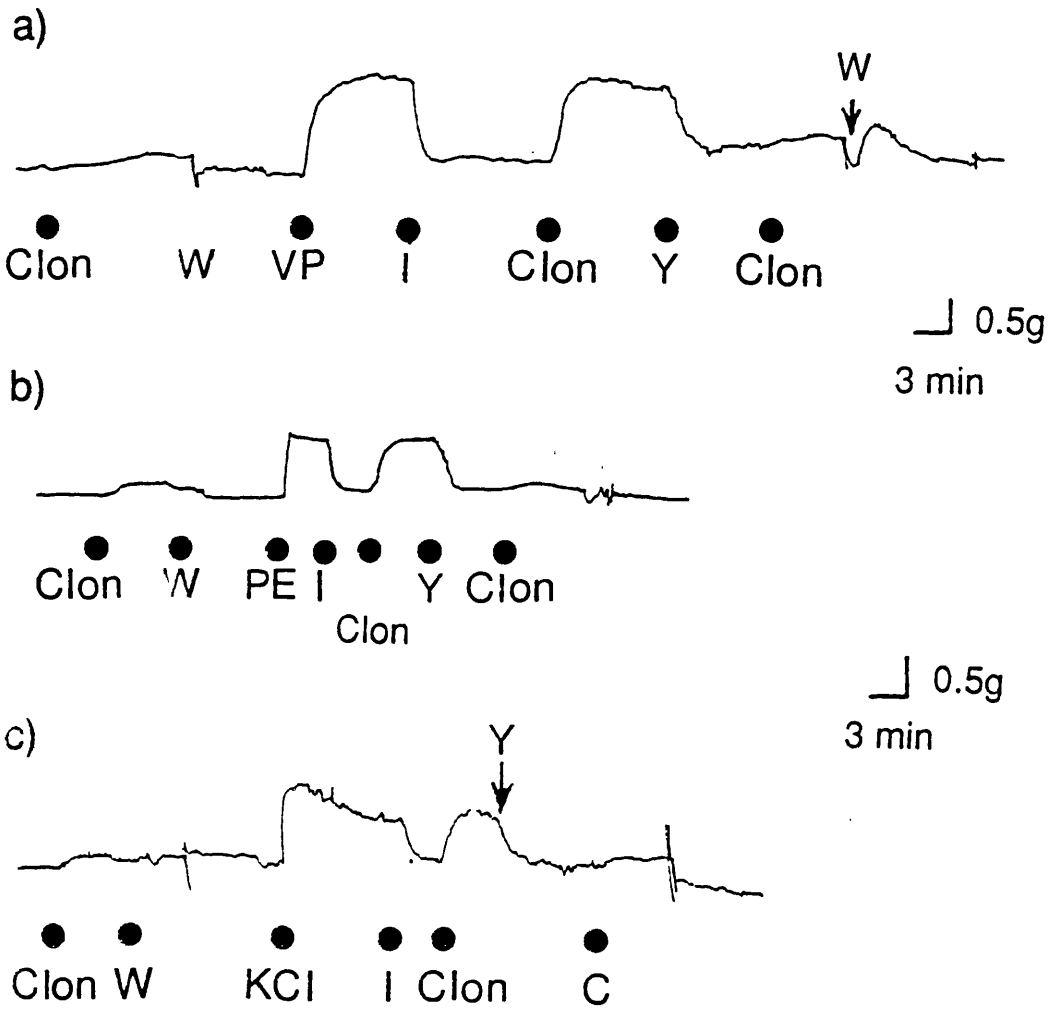


Figure 32. **Trace showing potentiation of the response to clonidine in rat tail artery rings.** A control response to clonidine (Clon, 10nM) alone was obtained, then clonidine was washed out (W; a,b,c). Artery rings were precontracted with AVP (0.4mU ml^{-1}; a), PE (0.5μM; b) or KCl (0.03M, c) then relaxed with isobutylmethylxanthine (I, 10μM; a,b,c). Addition of clonidine at this point produced a response significantly larger than the response to clonidine alone. This enhanced response was inhibited by yohimbine (Y, 50nM; a,b,c). Further addition of clonidine (+10nM; a, b, c) produced little or no response.

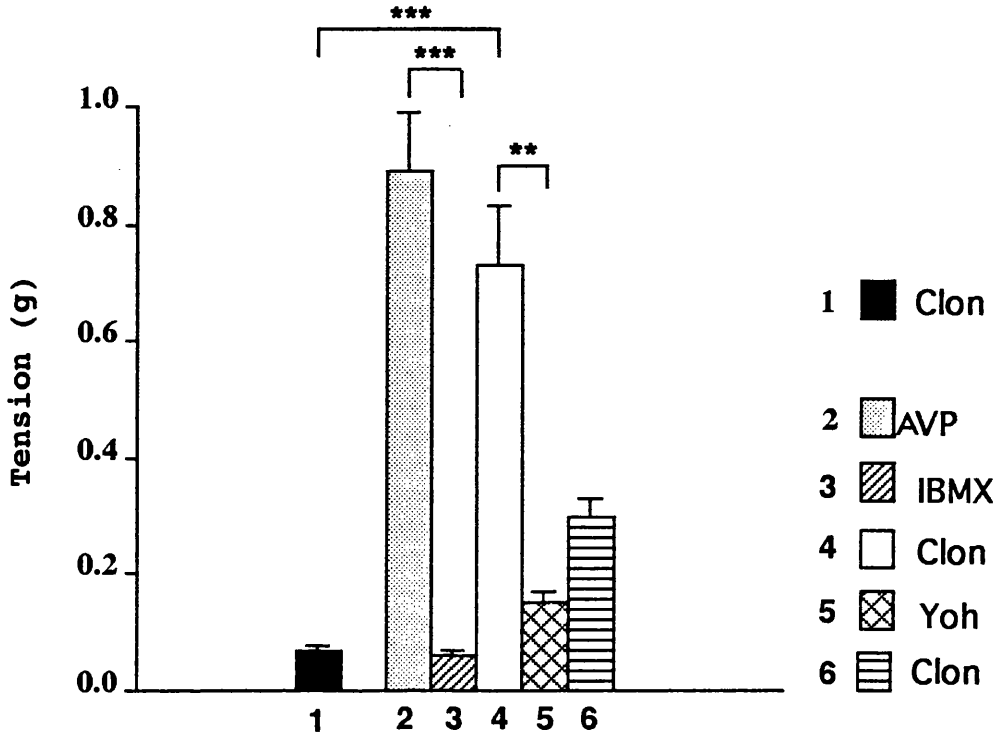


Figure 33. **Potentiation of the response to clonidine in rat tail artery rings, in the presence of AVP and IBMX.** A control response to clonidine alone (Clon, 10nM) was obtained (1), then clonidine was washed from the organ bath. Drugs were then added to the organ bath without being washed out, in the order shown in the key. AVP (0.4mU ml^{-1}; 2) produced a contraction which was inhibited by 3-isobutyl-1-methylxanthine (IBMX, 10μM; 3). Addition of clonidine at this point (4), in the presence of AVP and IBMX, produced a response significantly larger than the response to clonidine alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 5). Further addition of clonidine (+10nM) produced little or no response (6). Each bar shows the mean ± s.e.m. of 8 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (**, $P<0.01$; ***, $P<0.001$).

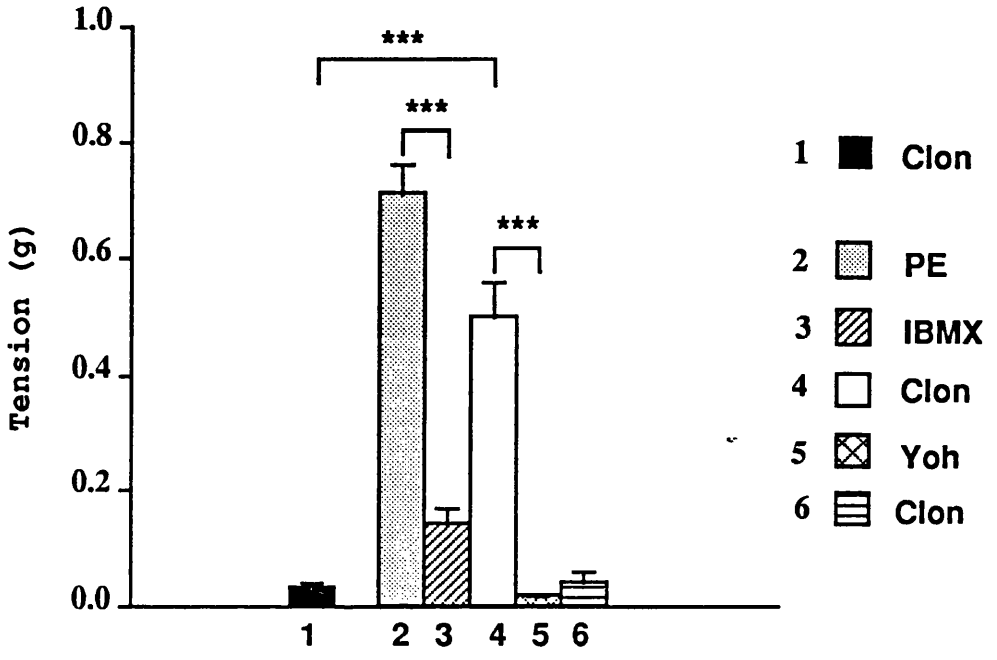


Figure 34. **Potentiation of the response to clonidine in rat tail artery rings, in the presence of PE and IBMX.** A control response to clonidine alone (Clon, 10nM) was obtained (1), then clonidine was washed from the organ bath. Drugs were then added to the organ bath without being washed out, in the order shown in the key. PE (0.5μM; 2), produced a contraction which was inhibited by IBMX (10μM; 3). Addition of clonidine at this point (4), in the presence of PE and IBMX, produced a response significantly larger than the response to clonidine alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 5). Further addition of clonidine (+10nM) produced little or no response (6). Each bar shows the mean ± s.e.m. of 8 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (***, $P<0.001$).

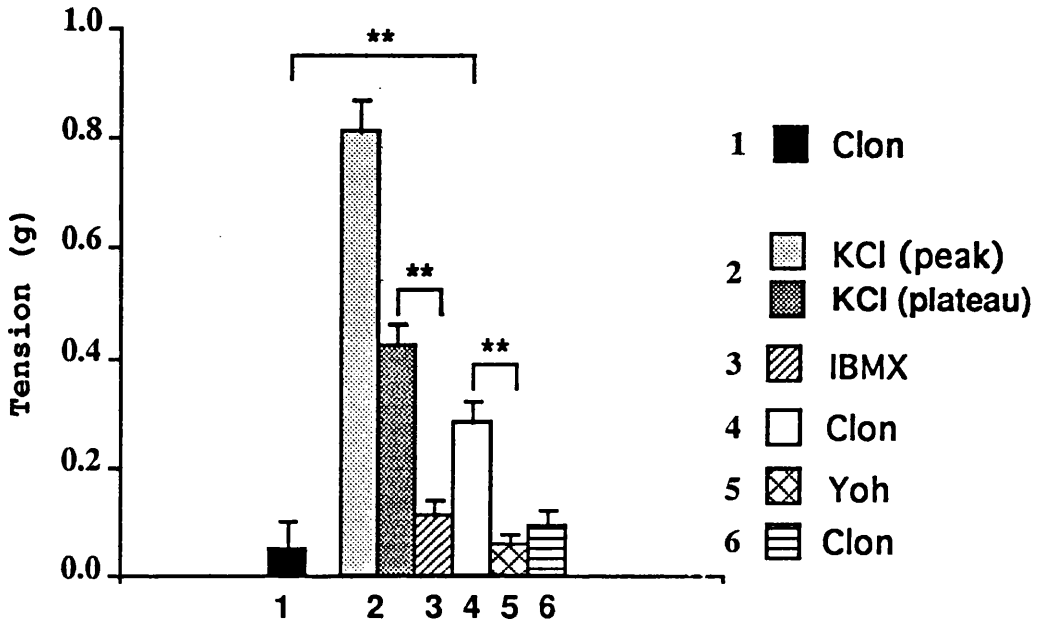


Figure 35. **Potentiation of the response to clonidine in rat tail artery rings in the presence of KCl and IBMX.** A control response to clonidine alone (Clon, 10nM) was obtained (1), then clonidine was washed from the organ bath. Drugs were then added to the organ bath without being washed out, in the order shown in the key. KCl (0.03M) produced a biphasic contraction consisting of a peak then a plateau (2), which was inhibited by IBMX (10μM; 3). Addition of clonidine at this point (4), in the presence of KCl and IBMX, produced a response significantly larger than the response to clonidine alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 5). Further addition of clonidine (+10nM) produced little or no response (6). Each bar shows the mean ± s.e.m. of 14 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (**, $P<0.01$).

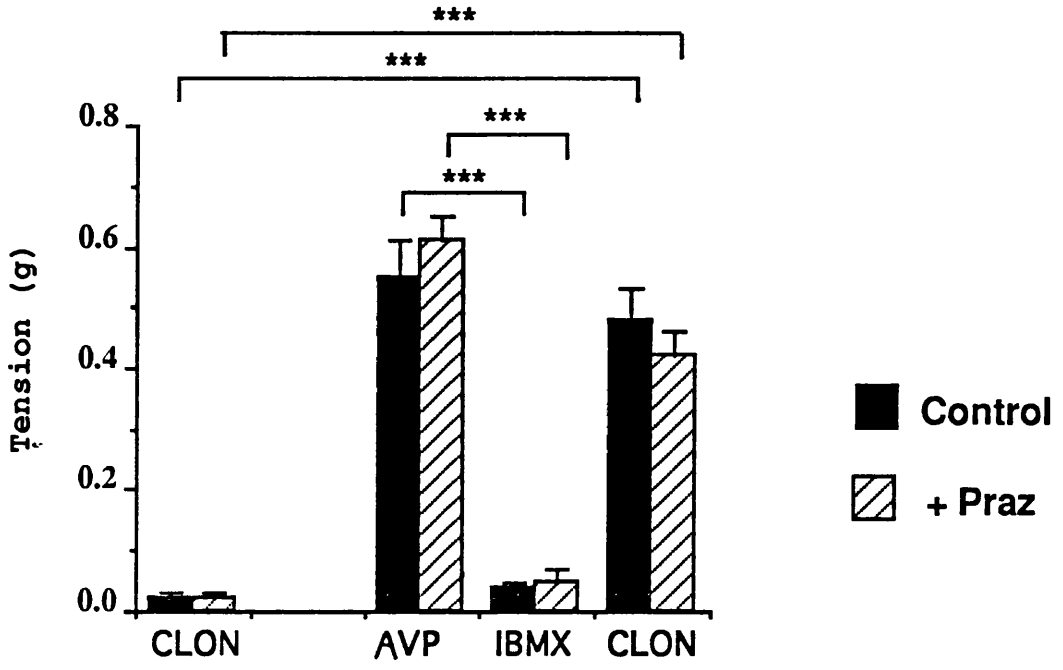


Figure 36. **Enhanced response to clonidine in rat tail artery rings, obtained in the presence of prazosin.** A control response to clonidine alone (Clon, 10nM) was obtained, then clonidine was washed from the organ bath. Drugs were then added cumulatively to the organ bath, in the order shown from left to right. AVP (0.4mU ml^{-1})-induced contraction was inhibited by IBMX (10μM). Addition of clonidine in the presence of AVP and IBMX produced a response larger than the response to clonidine alone. The drugs were washed from the organ bath, prazosin (Praz, 50nM) added and the experiment repeated 20 minutes later. Prazosin had no effect on either the control response to clonidine, or on the enhanced response produced in the presence of AVP and IBMX. Each bar shows the mean ± s.e.m. of 8 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (***, $P<0.001$).

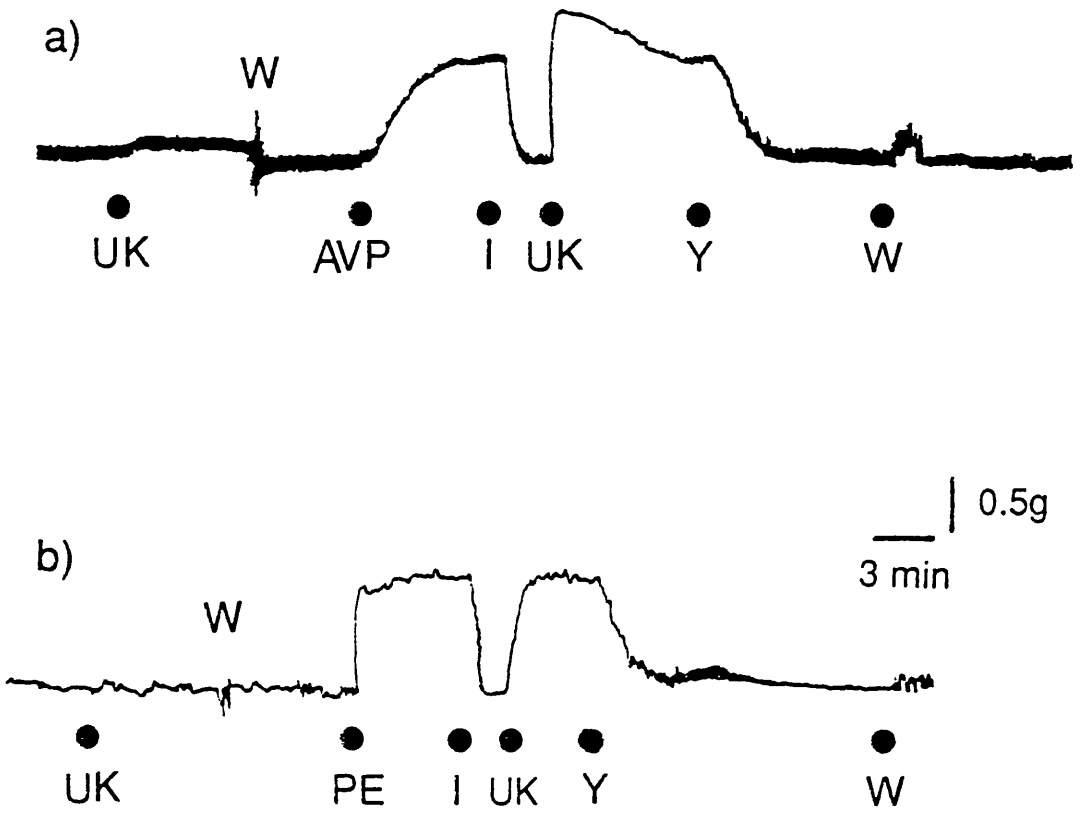


Figure 37. **Trace showing potentiation of the response to UK-14,304 in rat tail artery rings.** A control response to UK-14,304 (UK, 50nM) alone was obtained, then UK was washed out (W; a, b). Artery rings were precontracted with AVP (0.4mU ml^{-1}; a) or PE (0.5μM, b) then relaxed with IBMX (I, 10μM; a,b). Addition of UK at this point produced a response larger than that to UK alone (a, b). This enhanced response was inhibited by yohimbine (Y, 50nM; a,b).

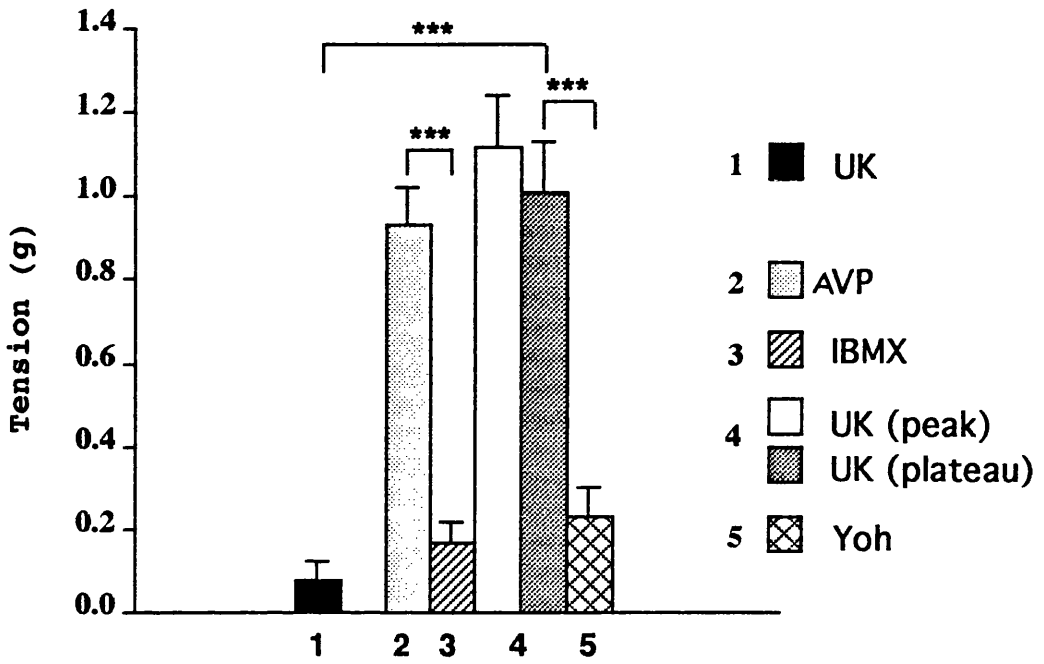


Figure 38. **Potentiation of the response to UK-14,304 in rat tail artery rings, in the presence of AVP and IBMX.** A control response to UK-14,304 (UK, 50nM) alone was obtained (1), then UK was washed from the organ bath. Drugs were then added cumulatively to the organ bath in the order shown in the key. AVP (0.4mU ml^{-1}; 2) produced a contraction which was inhibited by IBMX (10μM; 3). Addition of UK at this point (4), in the presence of AVP and IBMX, produced a response significantly larger than the response to UK alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 5). Each bar shows the mean ± s.e.m. of 6 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (***, $P<0.001$).

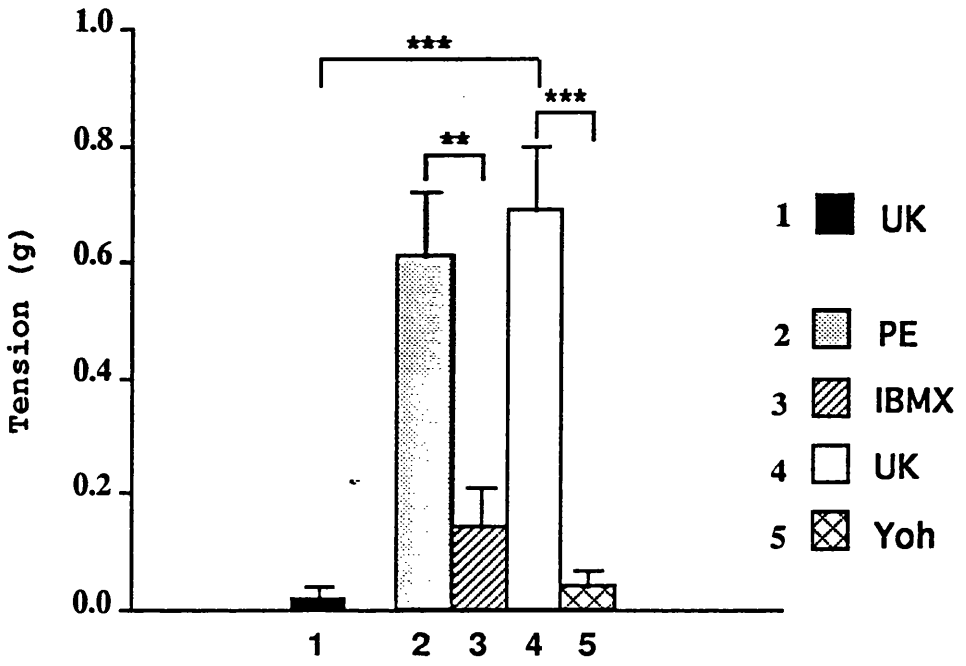


Figure 39. **Potentiation of the response to UK-14,304 in rat tail artery rings, in the presence of PE and IBMX.** A control response to UK-14,304 (UK, 50nM) alone was obtained (1), then UK was washed from the organ bath. Drugs were then added cumulatively to the organ bath in the order shown in the key. PE (0.5μM; 2) produced a contraction which was inhibited by IBMX (10μM; 3). Addition of UK at this point (4), in the presence of PE and IBMX, produced a response significantly larger than the response to UK alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 5). Each bar shows the mean ± s.e.m. of 6 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (**, $P<0.01$; ***, $P<0.001$).

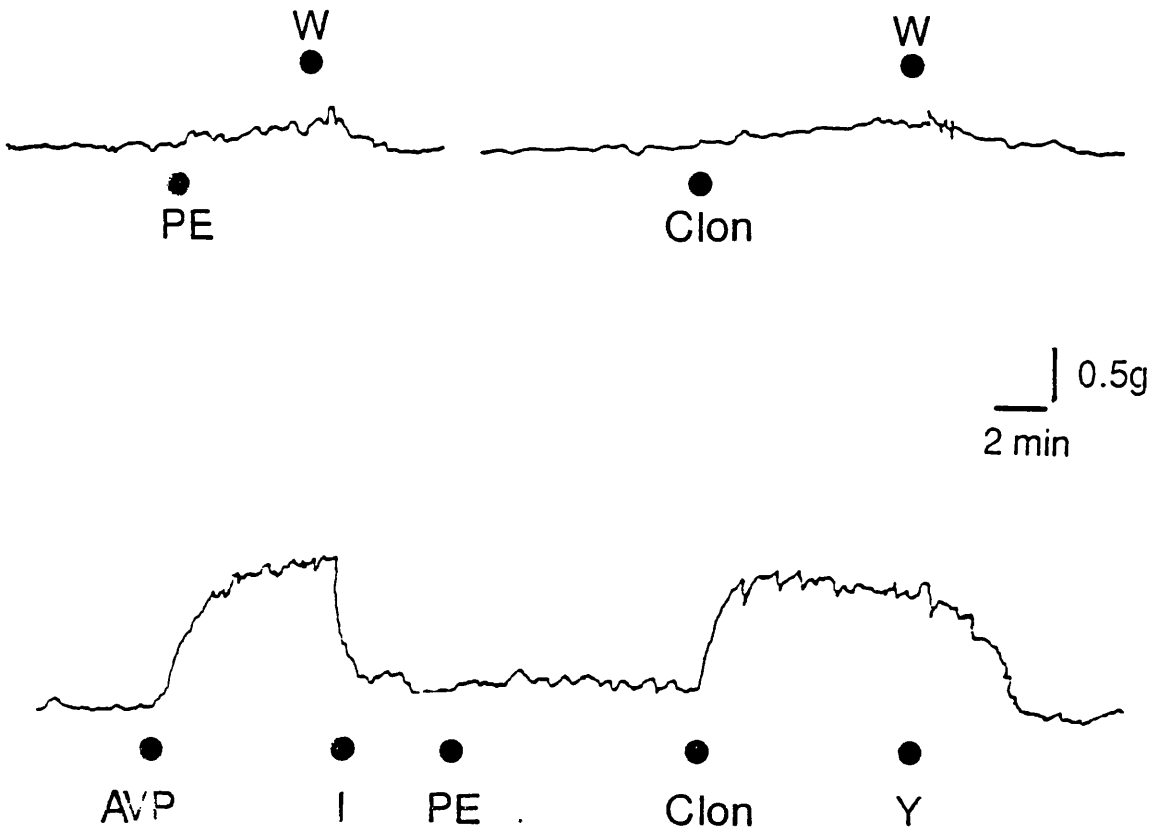


Figure 40. **Trace showing potentiation of the response to clonidine, but not to PE, in rat tail artery rings.** Control responses to PE (0.2μM) or to clonidine (Clon, 50nM) alone were obtained and washed out (W; upper trace). In the lower trace, a continuation of the same experiment, artery rings were precontracted with AVP (0.4mU ml^{-1}) then relaxed with IBMX (I, 10μM). Addition of PE at this point, in the presence of AVP and IBMX, produced a response which was no larger than the control response to PE. The response to clonidine however, was significantly larger than that to clonidine alone. This enhanced response was inhibited by yohimbine (Y, 50nM).

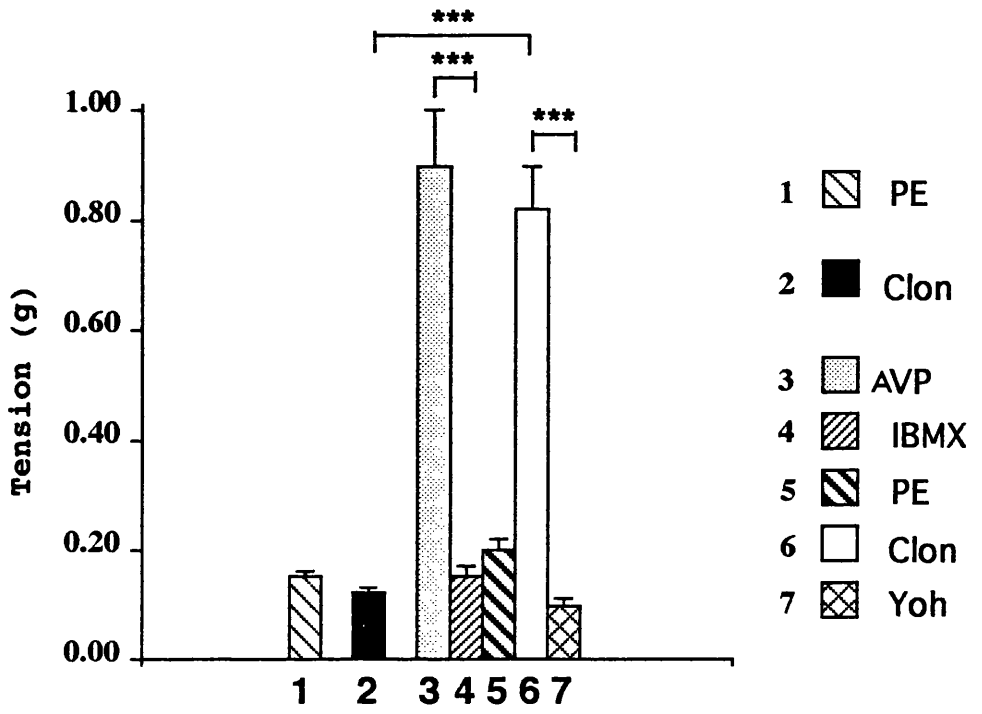


Figure 41. **Potentiation of the response to clonidine, but not to PE, in rat tail artery rings.** Control responses to PE (0.2μM) alone (1) and to clonidine (Clon, 10nM) alone (2) were obtained, then washed out. Drugs were then added cumulatively to the organ bath, in the order shown in the key. AVP (0.4mU ml^{-1}; 3) produced a contraction which was inhibited by IBMX (10μM, 4). Addition of PE at this point (5), in the presence of AVP and IBMX, produced a response no larger than control. The response to clonidine however (6), was significantly larger than the response to clonidine alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; 7). Each bar shows the mean ± s.e.m. of 6 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (***, $P<0.001$).

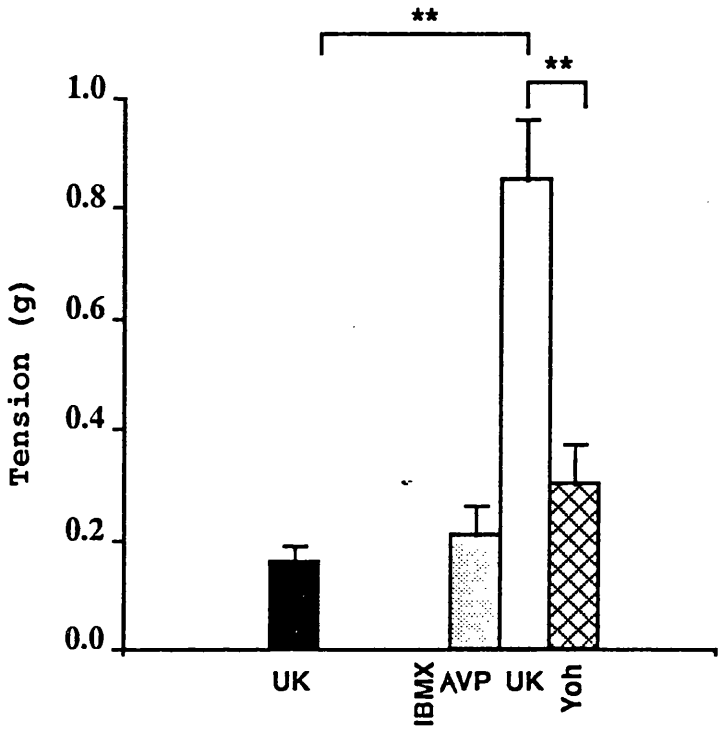


Figure 42. **Potentiation of the response to UK-14, 304 in rat tail artery rings following IBMX and AVP pretreatment.** A control response to UK-14,304 (UK, 50nM) was obtained, then UK was washed from the organ bath. Drugs were then added cumulatively to the organ bath, in the order shown from left to right. IBMX (10μM) produced no response, and AVP (0.4mU ml^{-1}) gave a small contraction in the presence of IBMX. Addition of UK in the presence of IBMX and AVP produced a response larger than that to UK alone. This enhanced response to UK was inhibited by yohimbine (Yoh, 50nM). Each bar shows the mean ± s.e.m. of 7 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (**, $P<0.01$).

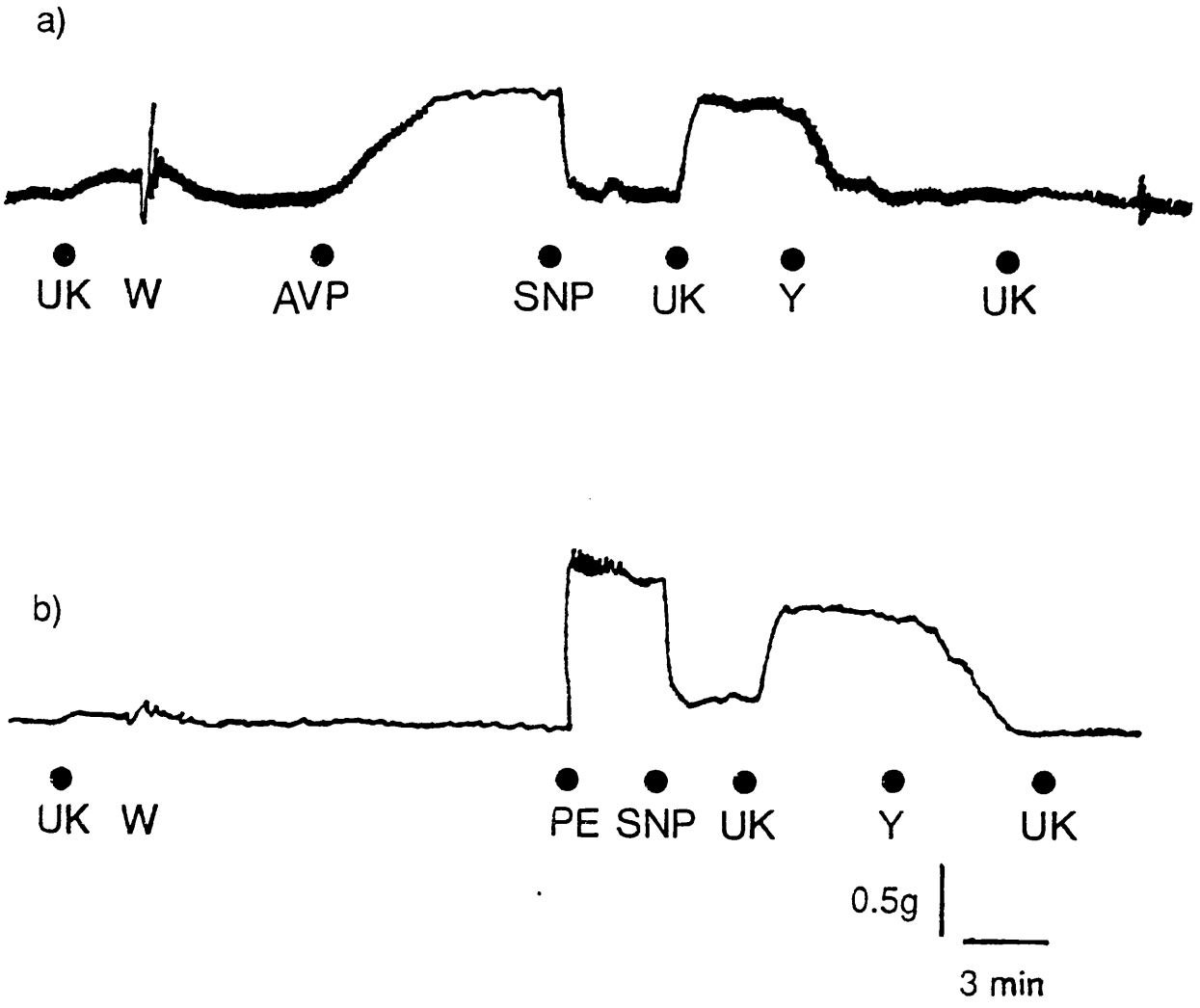


Figure 44. **Trace showing potentiation of the response to UK-14,304 in rat tail artery rings, in the presence of AVP (a) or PE (b) and SNP.** A control response to UK-14, 304 (UK, 50nM; a, b) alone was obtained and washed out (W). Artery rings were precontracted with AVP (0.4mU ml^{-1}; a) or PE (0.5μM; b) then relaxed with SNP (0.1μM; a, b). Addition of UK at this point, in the presence of AVP (a) or PE (b) and SNP (a, b), produced a response significantly larger than that to UK alone. This enhanced response was inhibited by yohimbine (Y, 50nM; a, b). Further addition of UK produced little or no response (a, b).

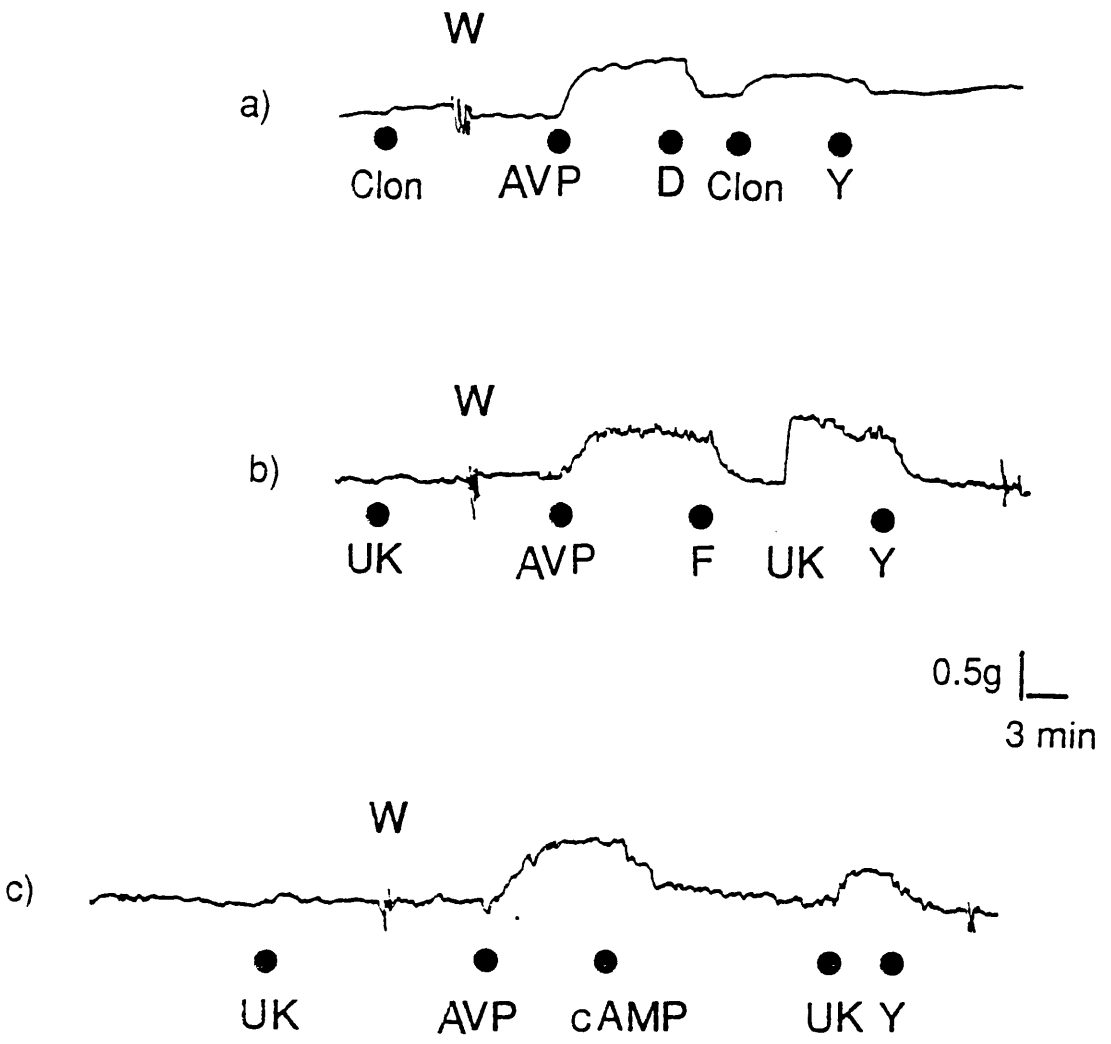


Figure 43. **Trace showing potentiation of the response to α_2-adrenoceptor agonists in rat tail artery rings, in the presence of AVP and different vasodilators.** A control response to clonidine (Clon 10nM; a) or to UK-14,304 (UK, 50nM; b, c) alone was obtained and washed out (W). Artery rings were precontracted with AVP (0.4mU ml^{-1}; a, b, c) then relaxed with diltiazem (D, 2μM; a), forskolin (F, 36nM; b) or dibutyryl cyclic AMP (cAMP, 0.2mM; c). Addition of clonidine (a) or UK (b, c) at this point, in the presence of AVP and the vasodilator, produced a response significantly larger than that to either clonidine (a) or to UK (b, c) alone. This enhanced response was inhibited by yohimbine (Y, 50nM; a, b, c).

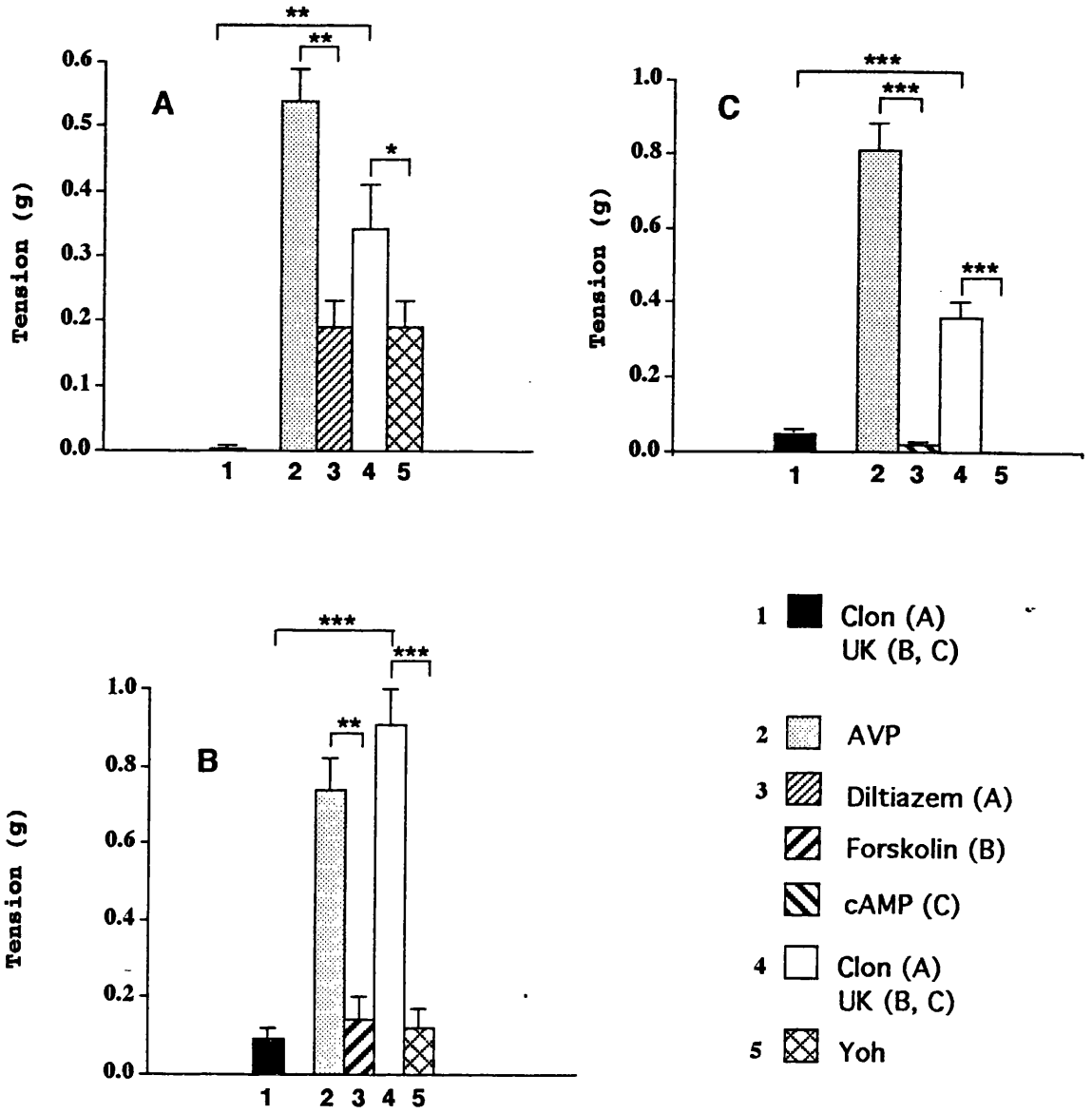


Figure 45. **Potentiation of the response to α_2-adrenoceptor agonists in rat tail artery rings, in the presence of AVP and different vasodilators.** A control response to clonidine (Clon, 10nM; A) or to UK-14,304 (UK, 50nM; B, C) alone was obtained (1) then washed from the organ bath. Drugs were then added cumulatively to the organ bath in the order shown in the key. AVP (0.4mU ml^{-1}, 2) produced a contraction which was inhibited by a vasodilator (3) as follows: diltiazem (2μM, A), forskolin (36nM, B) or dibutyryl cyclic AMP (cAMP, 0.2mM; C). Addition of clonidine (A) or UK (B, C) at this point (4), in the presence of AVP and the vasodilator, produced a response significantly larger than the response to UK alone (A, B, C). This enhanced response was inhibited by yohimbine (Yoh, 50nM; A, B, C; 5). Each bar shows the mean ± s.e.m. of at least 6 observations. Asterisks indicate significant differences between responses at either end of a horizontal bar (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).

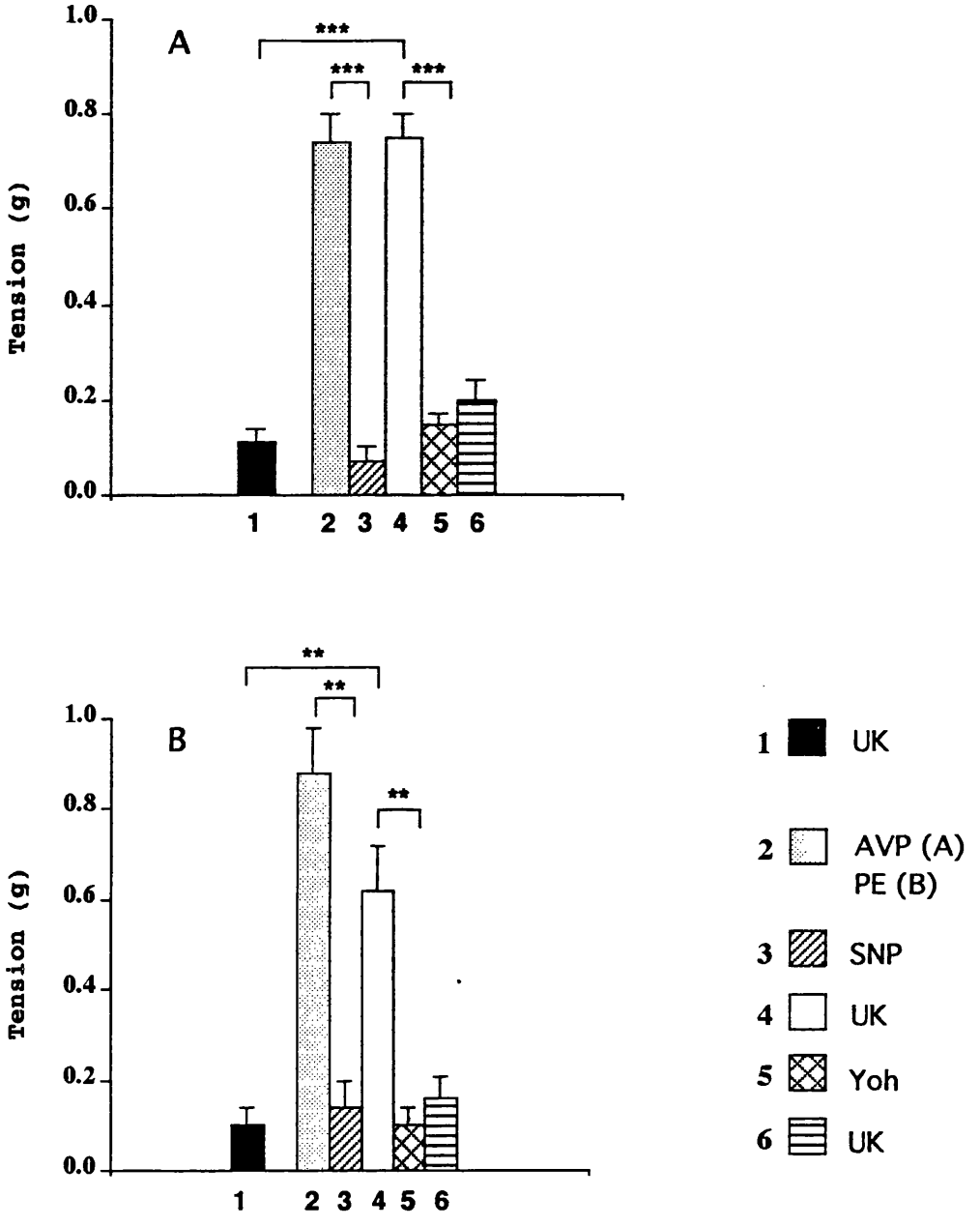


Figure 46. **Potentiation of the response to UK-14, 304 in rat tail artery rings, in the presence of AVP or PE and SNP.** A control response to UK-14,304 (UK, 50nM) alone was obtained (1), then UK was washed from the organ bath. Drugs were then added cumulatively to the organ bath in the order shown in the key. AVP (0.4mU ml^{-1}; A) or PE (0.5μM; B) produced a contraction (2) which was inhibited by SNP (0.1μM, 3). Addition of UK at this point (4), in the presence of AVP and SNP (A) or PE and SNP (B), produced a response significantly larger than the response to UK alone. This enhanced response was inhibited by yohimbine (Yoh, 50nM; A, B; 5). Further addition of UK (+50nM) produced little or no response (6). Each bar shows the mean ± s.e.m. of at least 6 observations. Asterisks indicate significant differences between the responses at either end of a horizontal bar (**, $P<0.01$; ***, $P<0.001$).

5)*Relationship between the precontraction and the enhanced response to α_2-adrenoceptor agonists:*

a) Correlations

From the experiments using IBMX as the vasodilator, the size of the enhanced response to clonidine (10nM) or to UK-14,304 (50nM) was plotted against the size of the response to the precontracting agonist (AVP, 0.4mU ml^{-1}; PE, 0.5μM; KCl; 0.03M). (Figures 47, 48).

Clonidine

There was a positive correlation between the size of the response to the precontracting agonist and the size of the enhanced response to clonidine, with the following correlation coefficients (r): VP/clonidine, r =0.946 ($P<0.001$, Figure 47a); PE/clonidine, r =0.840 ($P<0.01$, Figure 47b); KCl (peak response)/clonidine, r =0.697 ($P<0.01$, Figure 47c); KCl (plateau response)/clonidine, r =0.842 ($P<0.001$, Figure 47d).

UK-14,304

There was a positive correlation between the size of the response to the precontracting agonist and the size of the enhanced response to UK-14,304, with the following correlation coefficients: VP/UK, r =0.695 ($P<0.05$, Figure 48a); PE/UK, r =0.791 ($P<0.05$, Figure 48b).

b) Effect of the passage of time on the enhanced response to clonidine

The experimental protocol was carried out as normal, i.e. a control response to clonidine (10nM) was obtained, the drug washed from the organ bath and the tissue precontracted with AVP. The precontraction was relaxed with IBMX (10μM), then clonidine (10nM) added in the presence of AVP and

IBMX. The time between AVP/IBMX and clonidine addition was varied. 20 minutes after the addition of AVP, the enhanced response to clonidine was 74.0±6.3% of the initial response to AVP. As the time between AVP/IBMX and clonidine addition increased, the size of the enhanced response to clonidine declined, until 100 minutes after AVP addition it was only 32.8±7.5% of the initial response to AVP (Figure 49).

c) Effect of the passage of time on the response to vasopressin

During the above protocol, AVP was in the organ bath for prolonged periods of time, so it was important to establish whether the response to AVP itself varied with time. AVP (0.4ml min^{-1})-induced tone declined with time so that 120 minutes after addition the tone had fallen almost to baseline and was only 8.5±5% of the tone 10 minutes after addition (Figure 49).The reduction in the enhanced response to clonidine with time was closely matched by the reduction in the response to VP with time (Figure 49).

Effect of L-NAME:

The role of endothelium-derived relaxing factor (EDRF)/NO production in this phenomenon was investigated by repeating the time-course in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 0.1mM), an inhibitor of EDRF synthesis. L-NAME had no effect on the decline in the AVP response with time; 120 minutes after AVP addition, the AVP-induced tone in the presence of L-NAME was 9.9±4.0% of the initial response to AVP (Figure 50).

d) Effect of different antagonists on the enhanced response to UK-14,304

The above results indicated that the enhanced responses to α_2-adrenoceptor agonists were dependent on the precontraction. This was investigated further by assessing the abilities of antagonists of the precontracting agonist to inhibit the enhanced response. Control responses to UK-14,304 (50nM) were obtained, then rat tail artery rings were precontracted with AVP (0.4mU ml^{-1}), PE (0.5μM) or 5-HT (50nM) and relaxed with IBMX (10μM). Addition of UK-14,304 then produced an enhanced response, and the effects of antagonists added at this point were tested.

(i) Effect of prazosin:

AVP precontraction: UK-14,304 alone (50nM) produced little or no response (0.03±0.01g).The response increased to 0.75±0.06g in the presence of AVP and IBMX. Addition of the α_1-adrenoceptor antagonist prazosin (0.1μM) had no effect on the enhanced response to UK-14,304 (0.66±0.05g after prazosin) (Figures 51a, 52a). The enhanced response to UK-14,304 was completely blocked by yohimbine (50nM; $P<0.001$). Further addition of UK-14,304 produced little or no response.

PE precontraction: UK-14,304 alone (50nM) produced little or no response (0.03±0.02g).The response increased to 0.87±0.11g in the presence of PE and IBMX. Addition of prazosin (0.1μM) at this point completely abolished the enhanced response to UK-14,304 ($P<0.001$; Figures 51c, 52b). This is in contrast to the situation when AVP was used to precontract the tissue, where prazosin had no effect. Further addition of UK-14,304 produced no response.

5-HT precontraction: UK-14,304 alone (50nM) produced little or no response (0.05±0.01g).The response increased to 0.97±0.11g (peak) in the presence of 5-HT and IBMX, declining to 0.67 ± 0.09g (plateau) at the point of addition of prazosin. Following prazosin (0.1μM), the response further declined to 0.42±0.06g, but it is likely that this was due to the reduction in the enhanced response with time, rather than to the action of prazosin. The enhanced response to UK-14,304 was abolished by yohimbine (50nM; $P<0.001$) (Figure 52c).

(ii) Effect of the vasopressin antagonist, MCVP:

AVP precontraction: UK-14,304 alone (50nM) produced little or no response (0.09±0.02g).The response increased to 1.14±0.11g in the presence of AVP and IBMX. Addition of the AVP antagonist (β–mercapto-β,β–cyclopentamethylene-propionyl^1,O-Me-Tyr^2, Arg^8)-vasopressin (MCVP; 35nM) completely abolished the enhanced response to UK-14,304 ($P<0.001$; Figures 51b, 53a). Further UK-14,304 addition produced little or no response.

PE precontraction: UK-14,304 alone (50nM) produced little or no response (0.06±0.01g).The response increased to 0.80±0.12g in the presence of AVP and IBMX. Addition of MCVP had no effect on the enhanced response to UK-14,304 (0.65±0.11g after MCVP; Figures 51d, 53b). This is in contrast to the situation when AVP was used to precontract the tissue, where MCVP completely abolished the enhanced response to UK-14,304. The response was however blocked by the α_2-adrenoceptor antagonist yohimbine (50nM; $P<0.001$).

iii) Effect of ketanserin:

AVP precontraction: UK-14,304 alone (50nM) produced little or no response (0.05±0.01g).The response increased to 0.79±0.04g in the presence of AVP and IBMX. Addition of the 5-HT_2-receptor antagonist ketanserin (50nM) had no effect on the enhanced response to UK-14,304 (0.67±0.05g after ketanserin; Figure 54a). The enhanced response to UK-14,304 was however blocked by the α_2-adrenoceptor antagonist yohimbine (50nM; $P<0.001$).

PE precontraction: UK-14,304 alone (50nM) produced little or no response (0.06±0.01g; Figure 54b).The response increased to 0.70±0.06g in the presence of PE and IBMX. This enhanced response declined to 0.55±0.05g following ketanserin ($P<0.05$), but it is likely that this was due to a reduction in the enhanced response with time, rather than to the action of ketanserin. The enhanced response to UK-14,304 was however blocked by yohimbine (50nM; $P<0.001$). Further addition of UK-14,304 (+50nM) produced little or no response.

5-HT precontraction: UK-14,304 alone (50nM) produced little or no response (0.05±0.01g).The response increased to 0.62±0.04g in the presence of 5-HT and IBMX. Addition of ketanserin completely abolished the enhanced response to UK-14,304 ($P<0.001$; Figure 54c). Further UK-14,304 addition (+50nM) produced little or no response.

The effects of antagonists on the enhanced response to UK-14,304 are summarised in Table 4.

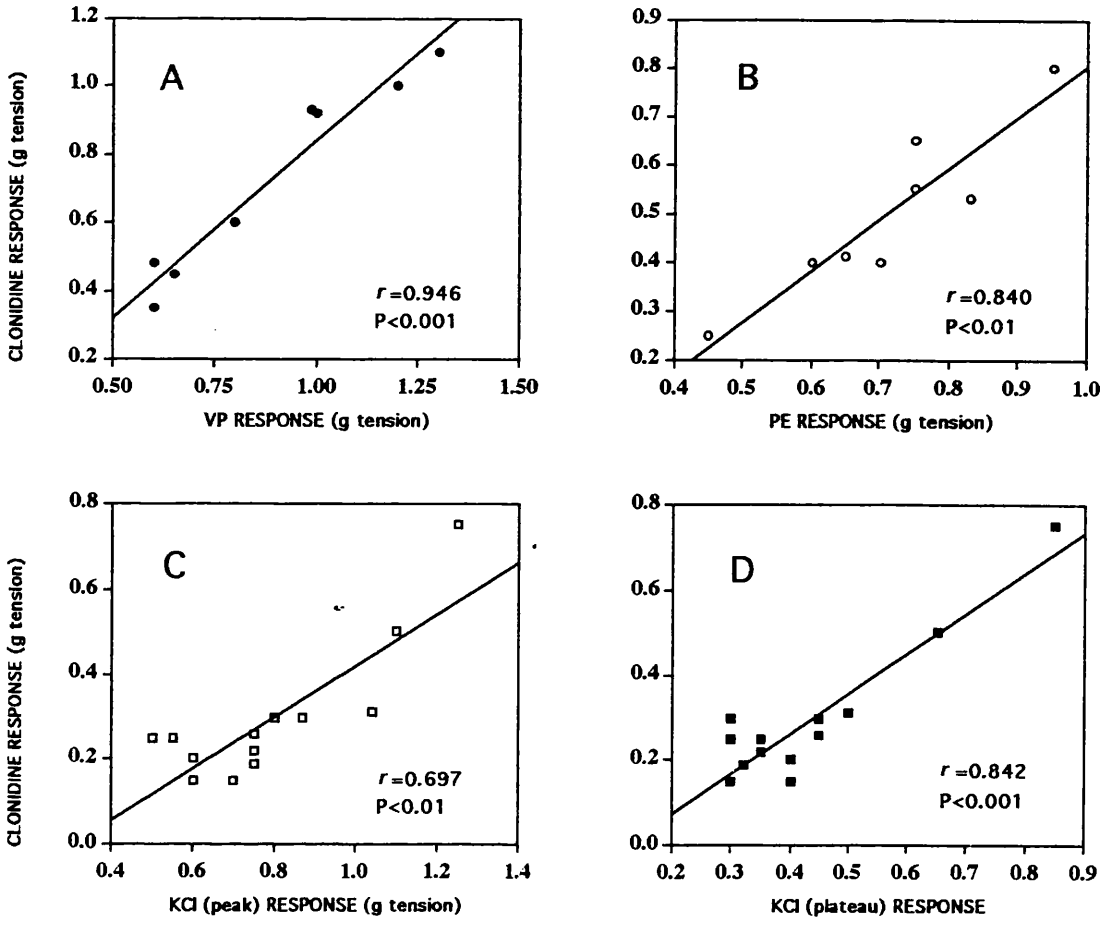


Figure 47. **Correlation between the size of the response to the precontracting agonist and the size of the enhanced response to clonidine in rat tail artery rings.** Artery rings were precontracted with AVP (0.4mU ml^{-1}; A), PE (0.5μM; B) or KCl (0.03M; C, D) and then relaxed with IBMX (10μM). Subsequent addition of clonidine produced a large contraction which restored the IBMX-induced relaxation to the level of tone produced by the precontracting agonist. Each point represents the response to the precontracting agonist (x-axis) plotted against the response to clonidine obtained in the presence of the precontracting agonist and IBMX (y-axis). r indicates the correlation coefficient for each pair of variables.

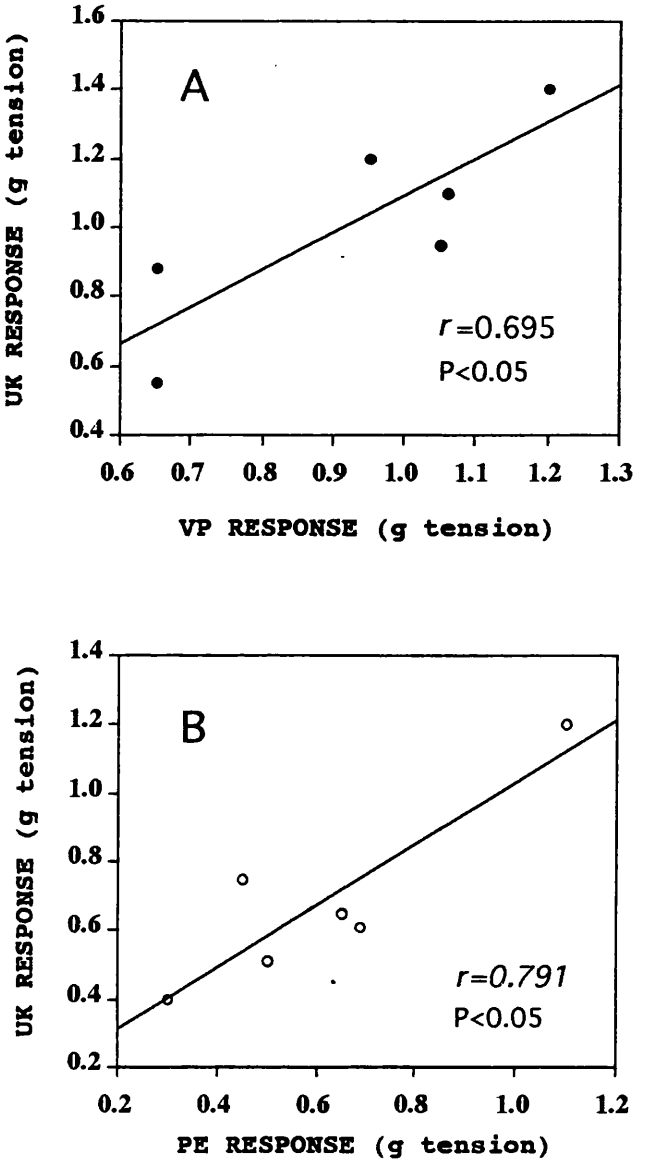


Figure 48. **Correlation between the size of the reponse to the precontracting agonist and the size of the enhanced response to UK-14,304 in rat tail artery rings.** Artery rings were precontracted with AVP (0.4mU ml^{-1}; A) or PE (0.5μM; B) and then relaxed with IBMX (10μM). Subsequent addition of UK-14,304 (UK, 50nM) produced a large contraction which restored the IBMX-induced relaxation to the level of tone produced by the precontracting agonist. Each point represents the response to the precontracting agonist (x-axis) plotted against the response to UK obtained in the presence of the precontracting agonist and IBMX (y-axis). r indicates the correlation coefficient for each pair of variables.

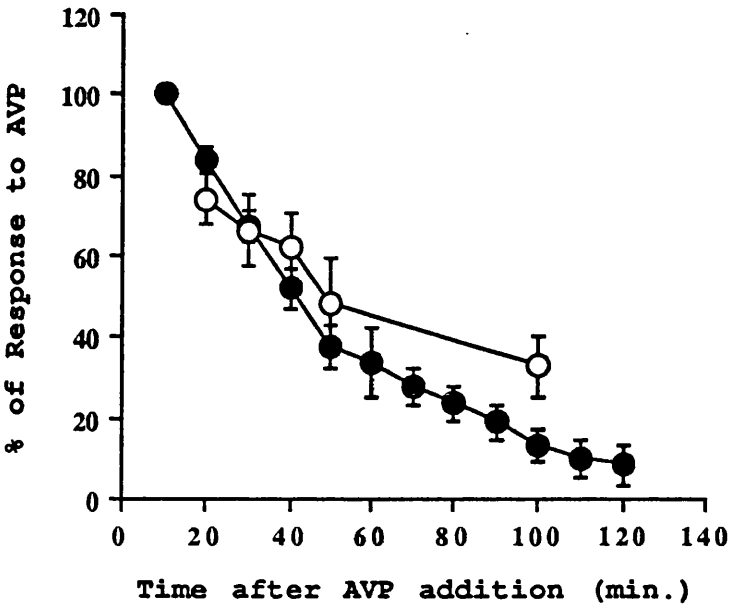


Figure 49. **The effect of the passage of time on the response to AVP and on the enhanced response to clonidine in rat tail artery rings.** Responses were expressed as a percentage of the AVP-induced contraction 10 minutes after AVP addition. AVP (0.4mU ml^{-1}) alone produced a large contraction which declined over time (--●--). In separate experiments, tail artery rings were precontracted with AVP and then relaxed with IBMX (10μM). Addition of clonidine (10nM) at this point, in the presence of AVP and IBMX, produced a large contraction (--○-). The size of this contraction decreased as the interval between VP/IBMX and clonidine addition was increased. Each point represents the mean ± s.e.m. of at least 6 observations.

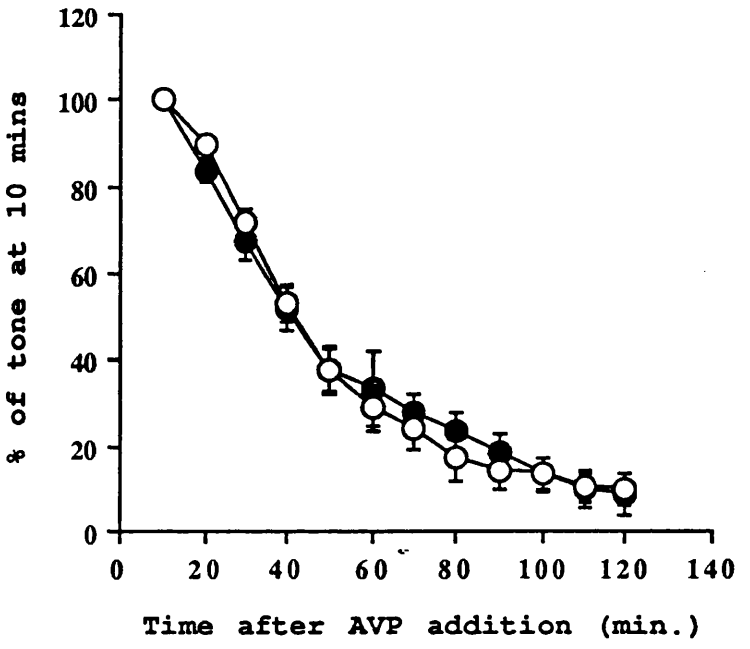
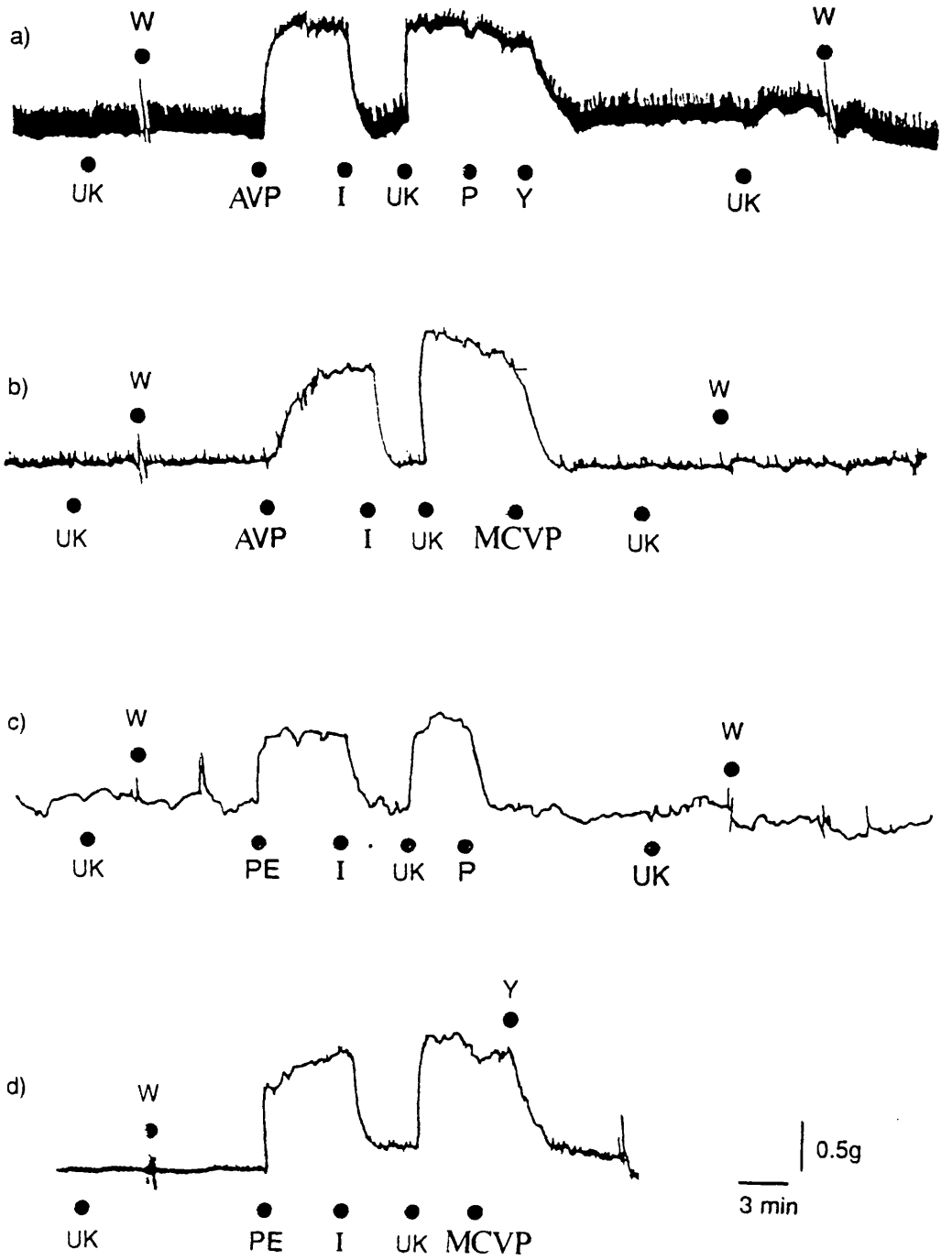


Figure 50. **The effect of L-NAME on the time course of the response to AVP in rat tail artery rings.** Responses were expressed as the percentage of the AVP-induced contraction 10 minutes after AVP addition. AVP (0.4mU ml^{-1}) produced a large contraction which declined over time (--●--). The time course was repeated in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 0.1mM; --○--), added to the organ bath 20 minutes before AVP. L-NAME had no effect on the decline of the AVP-induced contraction with time. Each point represents the mean ± s.e.m. of at least 6 observations.

Figure 51. **Trace showing the effect of prazosin or a vasopressin antagonist on the enhanced response to UK-14,304 in rat tail artery rings.** A control response to UK-14,304 alone (UK, 50nM) was obtained and washed out (W; a, b, c, d). Artery rings were precontracted with either AVP (0.4mU ml^{-1}; a, b) or PE (0.5μM; c, d) then relaxed with IBMX (I, 10μM; a, b, c, d). UK in the presence of AVP and IBMX (a, b) or PE and IBMX (c, d) produced a response larger than that to UK alone. When the vessels were precontracted with AVP (a, b) the enhanced response to UK was not inhibited by prazosin (P, 0.1μM; a) but was inhibited by yohimbine (Y, 50nM; a) or by the AVP antagonist, [β-mercapto-β,β–cyclopentamethylenepropionyl[1], O-Me-Tyr[2], Arg[8]]-vasopressin (MCVP, 35nM; b). When the vessels were precontracted with PE (c, d), the enhanced response to UK was not inhibited by MCVP (d) but was completely abolished by prazosin (c) or by yohimbine (d). Further addition of UK (+10nM) produced little or no response (a, b, c).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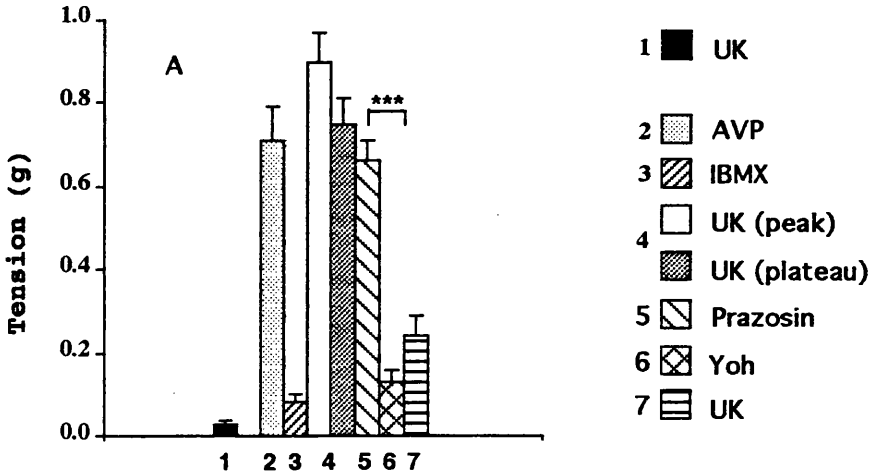
b)
W
W
UK
AVP
I
UK
MCVP
UK
c)
W
W
UK
PE
I
UK
P
UK
d)
Y
W
UK
PE
I
UK
MCVP
0.5g
3 min

Figure 52. **The effect of prazosin on the enhanced response to UK-14,304 in rat tail artery rings.** A control response to UK-14,304 alone (UK, 50nM) was obtained (1) and washed out. Drugs were then added cumulatively in the order shown in the key. AVP (0.4mU ml^{-1}; A), PE (0.5μM; B) or 5-HT (50nM, C) produced a contraction (2) which was inhibited by IBMX (10μM; 3). Addition of UK at this point (4), in the presence of AVP and IBMX (A), PE and IBMX (B) or 5-HT and IBMX (C) produced a response larger than the response to UK alone. When the vessels were precontracted with AVP (A) or 5-HT (C), the enhanced response to UK was not inhibited by prazosin (0.1μM; 5) but was inhibited by yohimbine (50nM; 6). When the vessels were precontracted with PE (B), the enhanced response to UK was completely abolished by prazosin (5), and further addition of UK (+50nM) produced no response (6). Each bar shows the mean ± s.e.m. of at least 6 observations. Asterisks indicate significant differences between responses at either end of a horizontal bar (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$).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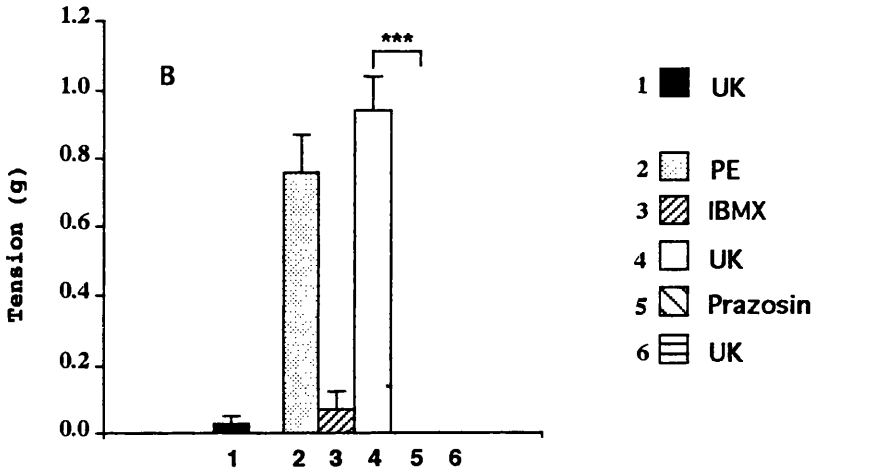
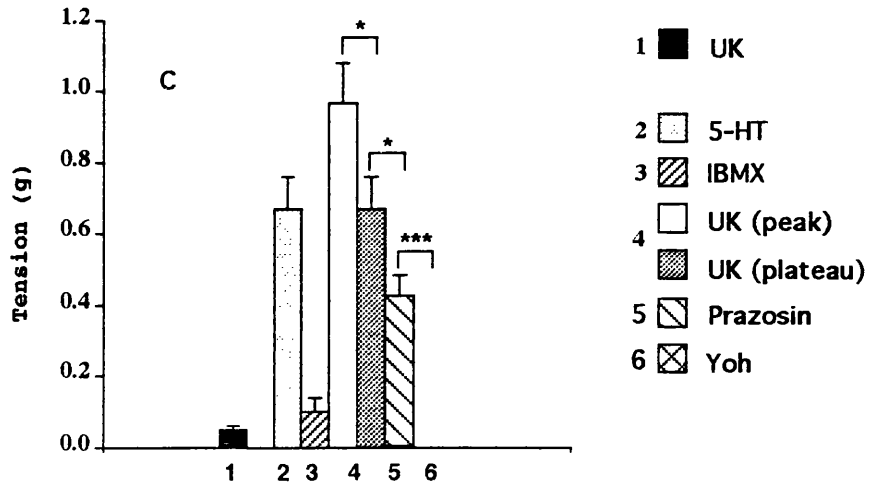
1.0
0.8
0.6
0.4
0.2
0.0
***
1 2 3 4 5 6 7
1 UK
2 AVP
3 IBMX
4 UK (peak)
UK (plateau)
5 Prazosin
6 Yoh
7 UK


B
Tension (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
1 2 3 4 5 6
1 UK
2 PE
3 IBMX
4 UK
5 Prazosin
6 UK


C
Tension (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
***
1 2 3 4 5 6
1 UK
2 5-HT
3 IBMX
4 UK (peak)
UK (plateau)
5 Prazosin
6 Yoh

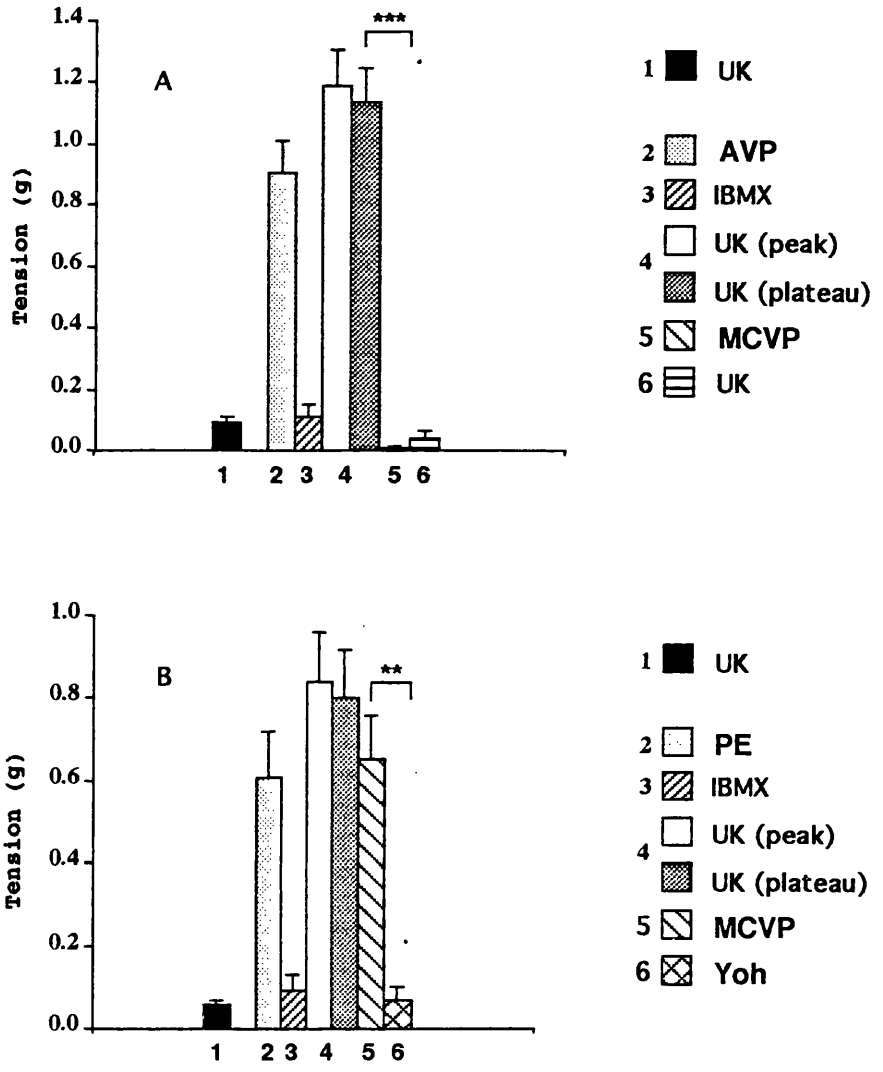
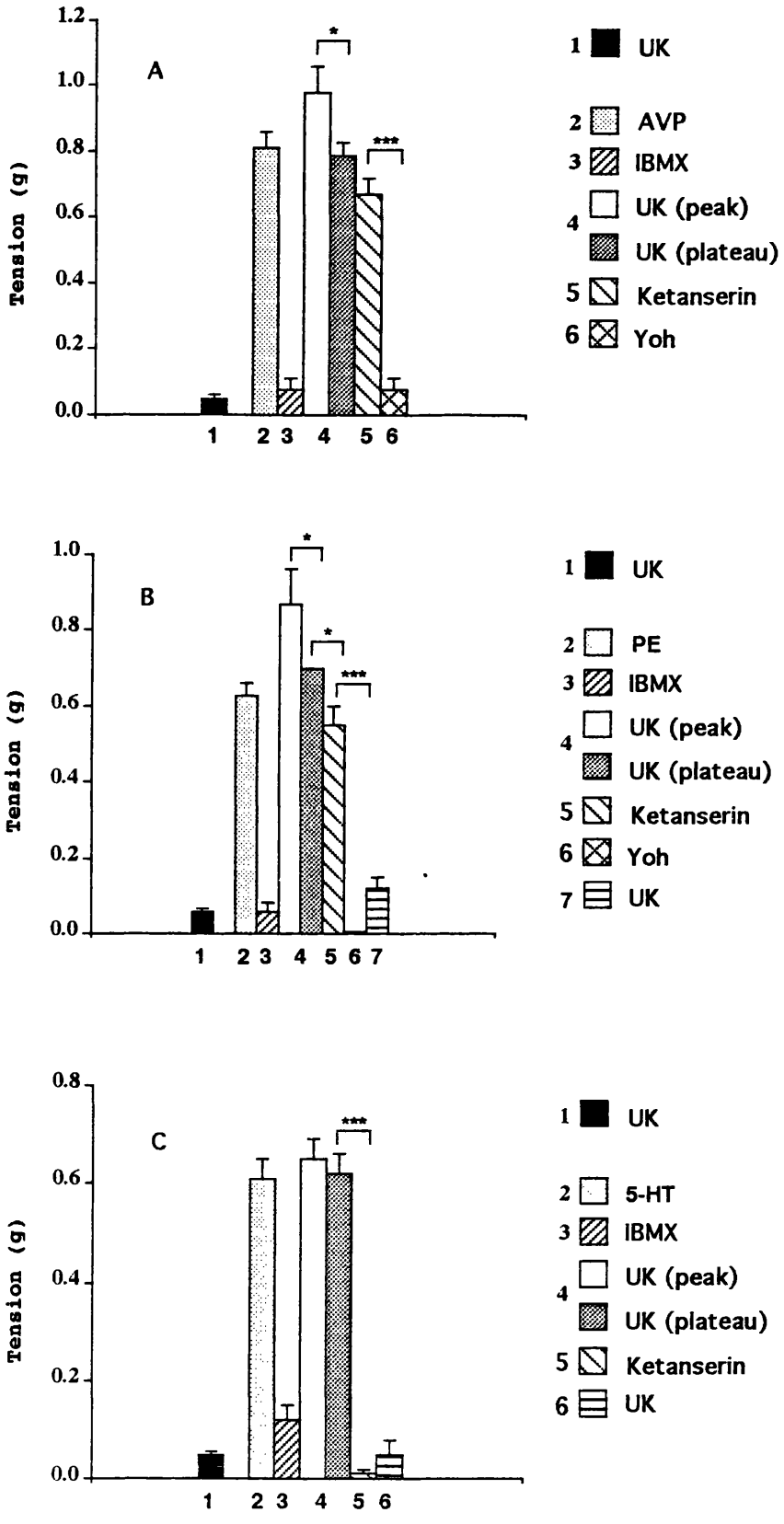


Figure 53. **The effect of a vasopressin antagonist on the enhanced response to UK-14,304 in rat tail artery rings.** A control response to UK-14,304 (UK, 50nM) alone was obtained (1) and washed from the organ bath. Drugs were then added cumulatively in the order shown in the key. AVP (0.4mU ml^{-1}; A) or PE (0.5μM; B) produced a contraction (2) which was inhibited by IBMX (10μM, 3). Addition of UK at this point (4), in the presence of AVP and IBMX (A) or PE and IBMX (B), produced a response significantly larger than the response to UK alone. When the vessels were precontracted with AVP (A), the enhanced response to UK was completely abolished by the AVP antagonist (β-mercapto-β, β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8) vasopressin (MCVP, 35nM; 5). Further UK addition (+10nM) produced little or no response (6). When the vessels were precontracted with PE (B), the enhanced response to UK was unaffected by MCVP (5) but was inhibited by yohimbine (Yoh, 50nM; 6). Each bar shows the mean ± s.e.m. of at least 6 observations. Asterisks indicate significant differences between responses at either end of a horizontal bar (**, $P<0.01$; ***, $P<0.001$).

Figure 54. **The effect of ketanserin on the enhanced response to UK-14,304 in rat tail artery rings.** A control response to UK-14,304 alone (UK, 50nM) was obtained (1) and washed out. Drugs were then added cumulatively in the order shown in the key. AVP (0.4mU ml^{-1}; A), PE (0.5μM; B) or 5-HT (50nM; C) produced a contraction (2) which was inhibited by IBMX (10μM; 3). Addition of UK at this point (4), in the presence of AVP and IBMX (A), PE and IBMX (B) or 5-HT and IBMX (C) produced a response significantly larger than the response to UK alone. The enhanced response to UK was unaffected by ketanserin (50nM; 5) when AVP or PE was used to precontract the tissue (A, B) but was abolished by yohimbine (Yoh, 50nM, A, B; 6). Further addition of UK (+50nM; 7) produced little or no response (B). When 5-HT was used to precontract the tissue (C), the enhanced response to UK was abolished by ketanserin (5), and further UK addition produced little or no response (6). Each bar shows the mean ± s.e.m. of at least 6 observations. Asterisks indicate significant differences between responses at either end of a horizontal bar (*, $P<0.05$, ***, $P<0.001$).


A
Tension (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6
*
***
1 UK
2 AVP
3 IBMX
4 UK (peak)
UK (plateau)
5 Ketanserin
6 Yoh
B
Tension (g)
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7
*
*
***
1 UK
2 PE
3 IBMX
4 UK (peak)
UK (plateau)
5 Ketanserin
6 Yoh
7 UK
C
Tension (g)
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6
***
1 UK
2 5-HT
3 IBMX
4 UK (peak)
UK (plateau)
5 Ketanserin
6 UK

	Antagonist			
Precontracting Agonist	*Prazosin*	*MCVP*	*Ketanserin*	*Yoh*
AVP	✗	✔	✗	✔
PE	✔	✗	✗	✔
5-HT	✗	✗	✔	✔
KCl	---	---	---	✔

Table 4. **Table summarising the effects of different antagonists in inhibiting the enhanced response to UK-14,304 in rat tail artery rings.** Vessels were precontracted with the agonists AVP (0.4mU ml^{-1}), PE (0.5μM), 5-HT (50nM) or KCl (0.03M) then relaxed with IBMX (10μM). In the presence of a precontracting agonist and IBMX, UK-14,304 (50nM) produced an enhanced response which was (✔) or was not (✗) inhibited by prazosin (0.1μM), the AVP antagonist (β-mercapto-β,β-cyclopentamethylenepropionyl[1], O-Me-Tyr[2], Arg[8])-vasopressin (MCVP; 35nM), ketanserin (35nM) or yohimbine (Yoh, 50nM). (---) indicates an agonist-antagonist combination which was not tested.

PART IV

INVESTIGATION OF THE NATURE OF THE NEUROTRANSMISSION IN THE RAT TAIL ARTERY

The rat tail artery receives a dense sympathetic innervation, but the precise nature of the transmitter(s) involved is unclear. This part of the study examined the possibility that ATP may act along with the main transmitter NA as a co-transmitter. Responses to electrical field stimulation were examined in rat tail artery rings, under a range of conditions designed to determine the roles of NA and ATP in the contractile response to field stimulation.

A) THE EFFECT OF EXOGENOUS AGONISTS

1) *Noradrenaline*

Cumulative addition of NA (10^{-9}- $3x10^{-4}$M) produced concentration-dependent increases in tension (Figure 55a) with an EC_{50} of $0.76\pm0.09\mu M$. The α_1-adrenoceptor antagonist prazosin ($0.1\mu M$) produced a 30-fold, rightward shift in the NA curve and an increase in the EC_{50} for NA ($22.00\pm0.30\mu M$; $P<0.01$) (Figure 55a).

2) *ATP*

ATP ($3x10^{-7}$-$3x10^{-3}$M) produced concentration-dependent increases in tension (Figure 55b). The response to ATP was rapid but was not maintained, declining sharply, so the concentration-response curve was carried out in the washout manner. A maximum response was not achieved as the highest concentration which could be added to the organ bath was 3mM and the response was still indreasing, so the EC_{50} for ATP could not be calculated.

B) ELECTRICAL FIELD STIMULATION

1) *Effect of tetrodotoxin*

Trains of electrical stimuli (supramaximal voltage 50V, 10Hz for 1s, 0.1ms duration, every 100s) were applied and then tetrodotoxin (TTX, 0.1μM) added to the organ bath. TTX completely abolished the response to field stimulation, which was restored when TTX was washed from the organ bath (Figure 56).

2) *Frequency-response curve*

Electrical field stimulation (supramaximal voltage 50V, 10 pulses, 0.1ms, 0.1-40Hz) produced frequency-dependent increases in tension. The frequency-response curve (FRC) was biphasic, with the first peak at 5-10Hz and the second at 30-40Hz (Figures 57, 58).

a) Effect of prazosin

A control FRC was obtained, then prazosin (0.1μM) was added to the organ bath and the FRC repeated 20 minutes later. In the presence of prazosin, the contractile response to field stimulation was abolished at all frequencies, with no evidence of a residual response (Figures 57, 58).

b) Effect of α,β–MeATP

A control FRC was obtained, then addition of α,β–MeATP (0.5μM) produced a transient contraction (0.45±0.10g) which returned to baseline within 5-10 minutes (Figure 59). The FRC was then repeated in the presence of α,β–MeATP but was no different from control (Figures 59, 60). In some experiments a further addition of α,β–MeATP (+0.5μM) was given following the second FRC (Figure 59). This did not produce a contraction and had no effect on the FRC subsequently carried out . Addition of prazosin (0.1μM) at this point however, abolished the contraction at all frequencies of stimulation .

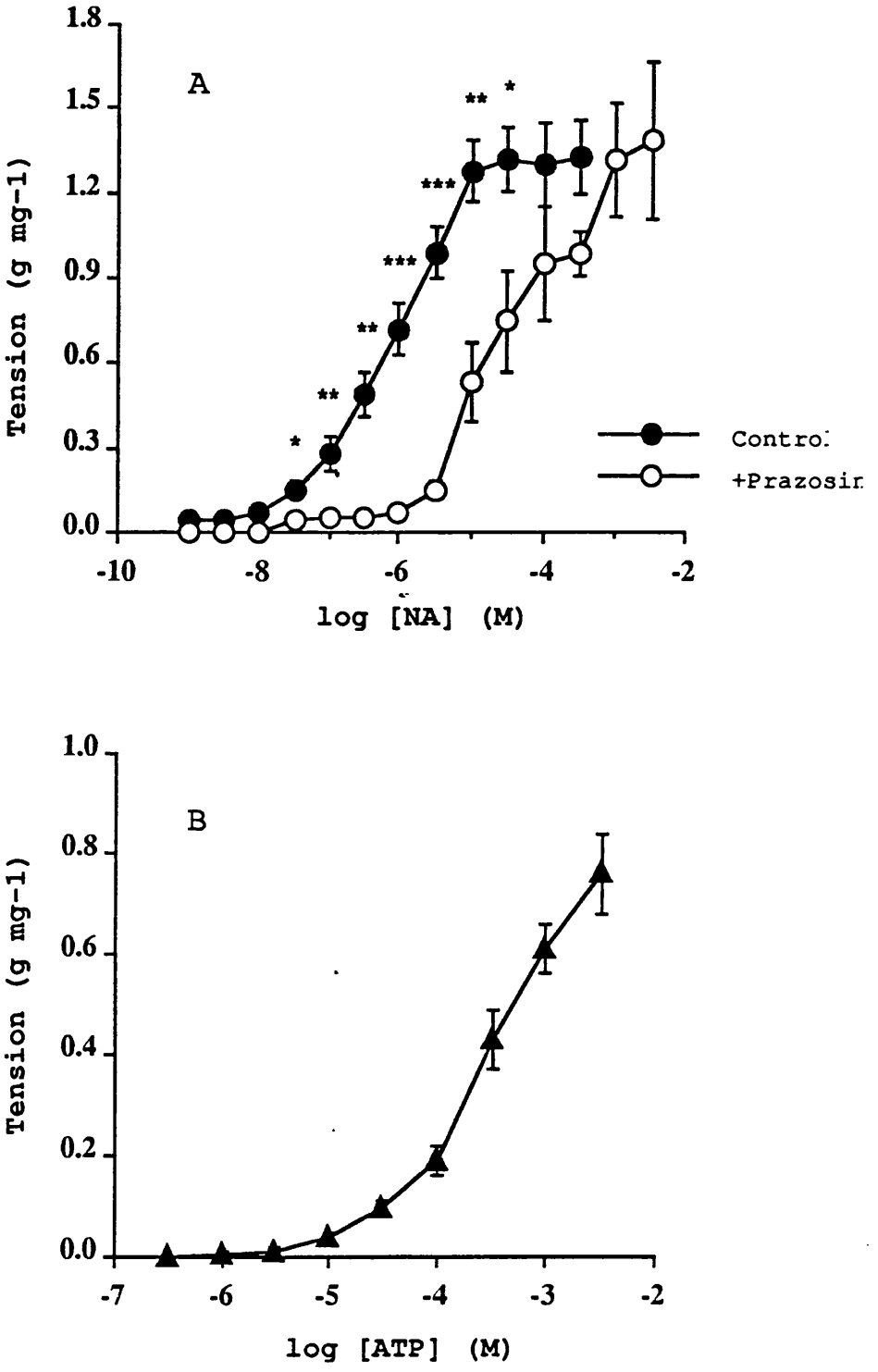


Figure 55. **Concentration-response curves to NA (A) and to adenosine triphosphate (ATP; B) in rat tail artery rings.** Prazosin (0.1μM) produced a rightward shift in the NA concentration-response curve (A). Asterisks indicate a significant reduction in the response to NA by prazosin (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). Each point is the mean ± s.e.m. of at least 6 observations. Where no error bars are shown, the error bars are contained within the symbols.

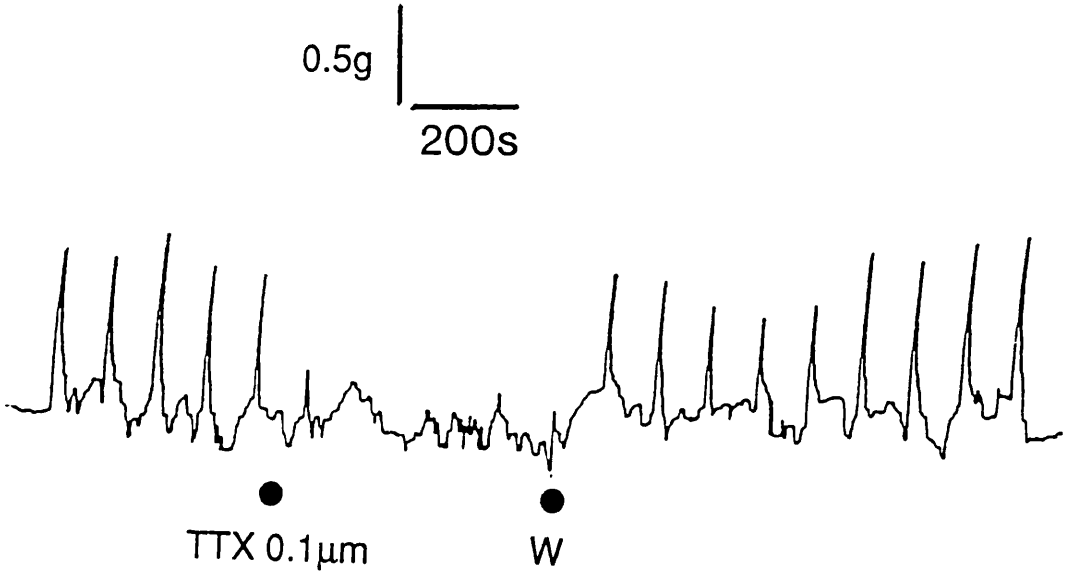


Figure 56. **Trace showing the effect of tetrodotoxin on the contractile response to electrical field stimulation in rat tail artery rings.** Contractions to field stimulation (supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms, every 100s) were abolished by tetrodotoxin (TTX), suggesting a neural origin. The responses were restored when TTX was washed from the organ bath (W).

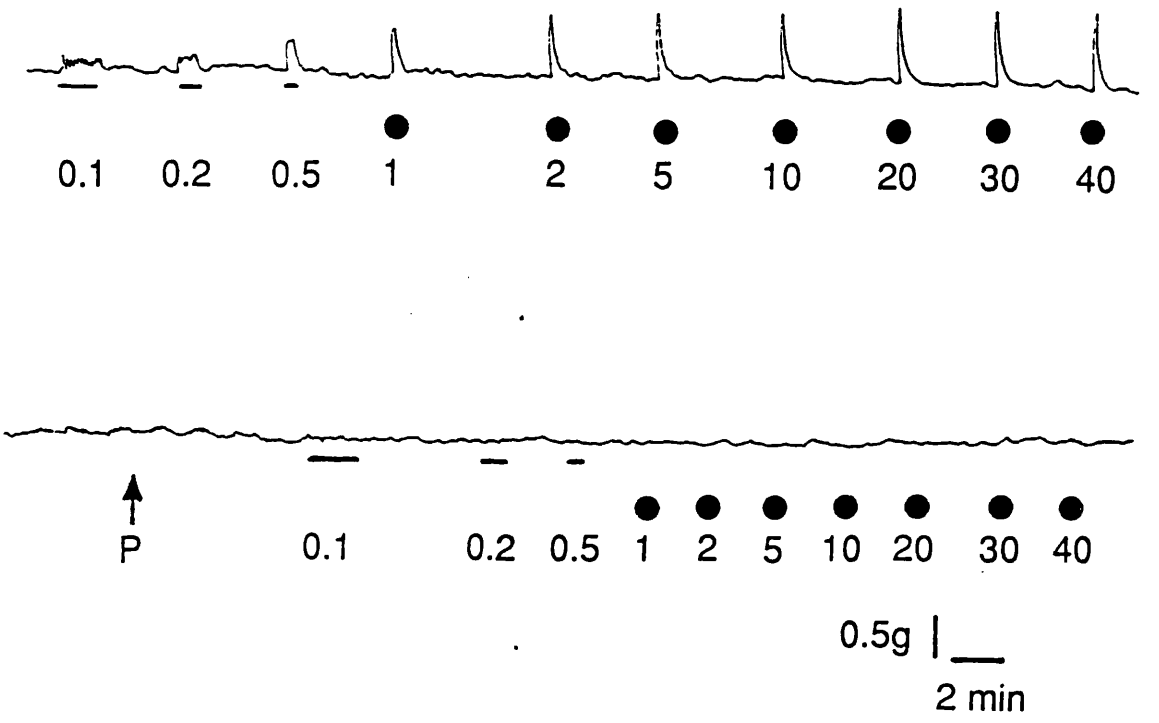


Figure 57. **Trace showing the effect of prazosin on the frequency-response curve in rat tail artery rings.** The upper panel shows a control frequency-response curve (FRC; supramaximal voltage 50V, 0.1-40Hz, 10 pulses, pulse width 0.1ms). The lower panel is a continuation of the same experiment, where the FRC was repeated in the presence of prazosin (P, 0.1μM). Responses at all frequencies were abolished by prazosin.

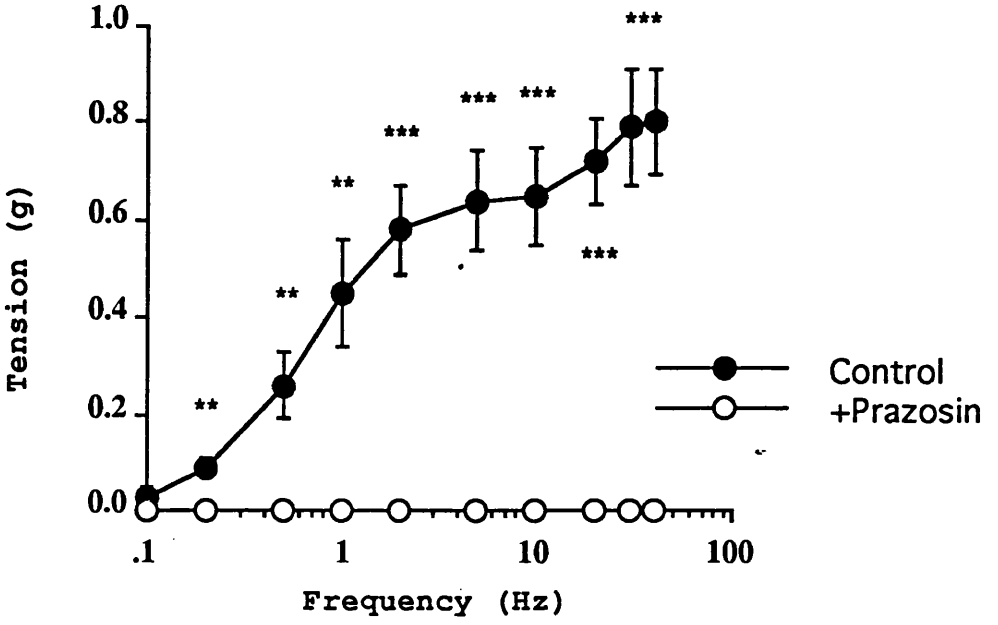


Figure 58. **The effect of prazosin on the frequency-response curve (FRC; Supramaximal voltage 50V, 10 pulses, 0.1ms) in rat tail artery rings.** The control FRC (--●--) was biphasic. Prazosin (0.1μM) abolished the response at all frequencies (--○--) (**, P<0.01; ***, P<0.001). Each point is the mean ± s.e.m. of 6 observations. Where no error bars are shown, the errors are contained within the symbols.

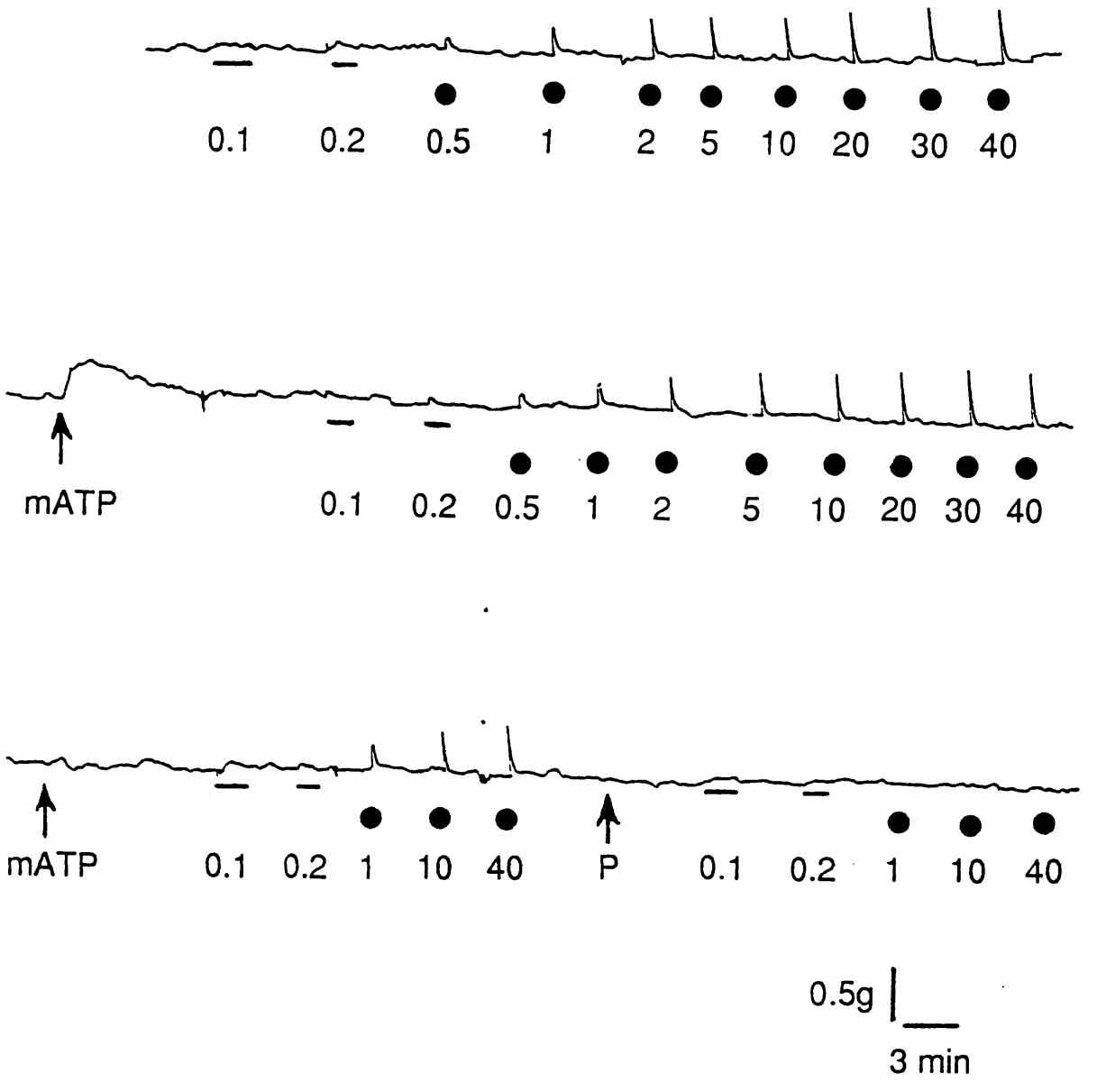


Figure 59. **Trace showing the effect of α, β-methylene ATP on the frequency-response curve in rat tail artery rings.** The upper panel shows a control frequency-response curve (FRC; supramaximal voltage 50V, 0.1-40Hz, 10 pulses, pulse width 0.1ms). The middle panel shows a continuation of the same experiment, where the FRC was repeated in the presence of α, β-methylene ATP (mATP, 0.5μM) which produced a transient contraction. The lower panel shows the FRC in the presence of a second addition of mATP (+0.5μM), which did not produce a contraction. Responses at all frequencies were unaffected by mATP. Subsequent addition of prazosin (P; 0.1μM) abolished the response at all frequencies of stimulation.

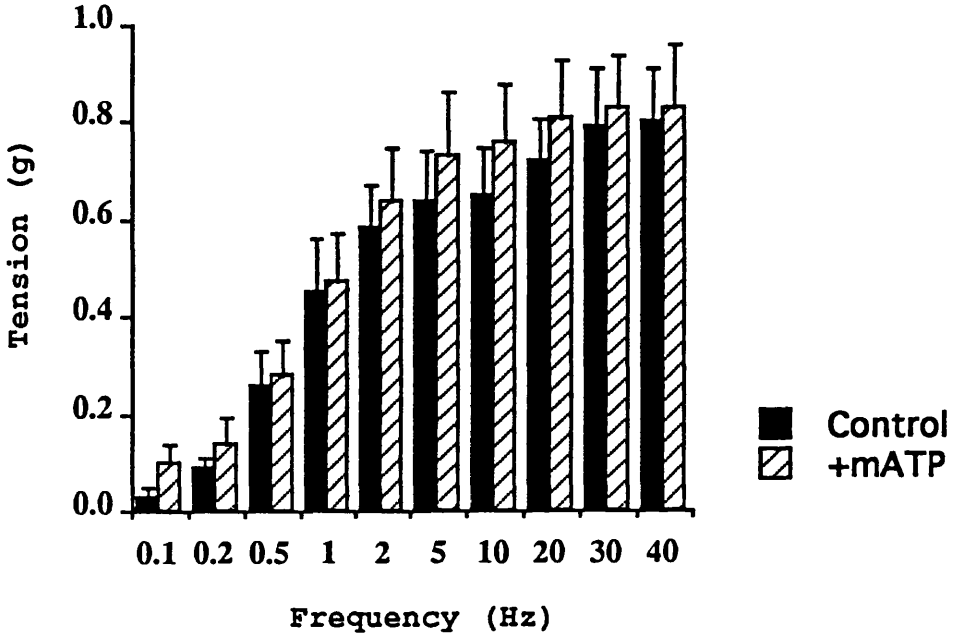


Figure 60. **The effect of mATP on the frequency-response curve (FRC; Supramaximal voltage, 10 pulses, 0.1ms) in rat tail artery rings.** mATP (0.5μM) had no effect on the FRC. Each bar shows the mean ± s.e.m. of 6 observations.

d) Effect of prazosin plus yohimbine

Prazosin (0.1μM) abolished the contractile response to field stimulation (Figure 65). A lower concentration of prazosin (5nM) however, produced a 45-70% reduction in the field stimulation-induced contraction (0.50± 0.01g control; 0.28±0.03g following prazosin 5nM; $P<0.01$). The remaining response was completely abolished by yohimbine (50nM; $P<0.001$) (Figures 65, 66).

e) Effect of α,β-MeATP on the response to field stimulation and to ATP

Two control responses to field stimulation (50V, 10Hz for 1s, 0.1ms duration) then a control response to ATP ($3x10^{-4}M$) were obtained, then α,β–MeATP (0.5μM) added (Figure 67). α,β–MeATP produced a transient contraction (0.45±0.10g) and the sequence was repeated when the α,β–MeATP-induced contraction returned to baseline. The response to field stimulation was unaffected in the presence of α,β–MeATP (0.88±0.10g; c.f. 0.86±0.10g control). The response to exogenous ATP was significantly reduced in the presence of α,β–MeATP (0.17±0.06g, c.f. 0.62±0.03g control; $P<0.01$) (Figures 67, 68).

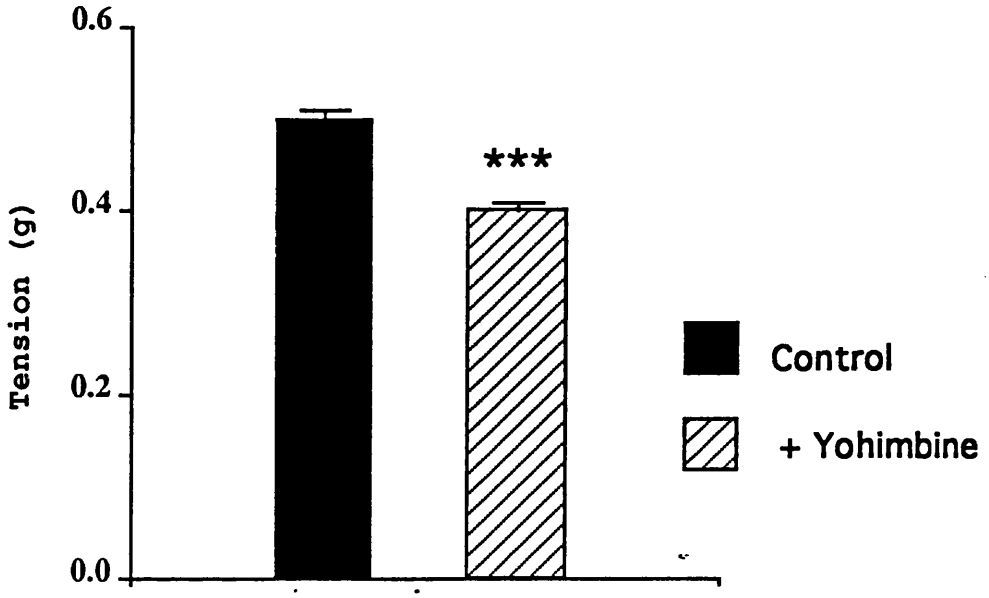


Figure 61. **The effect of yohimbine on the contractile response to electrical field stimulation in rat tail artery rings.** Trains of stimuli (Supramaximal voltage 50V, 10Hz for 1s, 0.1ms duration, every 100s) gave reproducible responses, which were inhibited in the presence of yohimbine (50nM) (***, $P<0.001$). Each bar represents the mean ± s.e.m. of at least 12 observations.

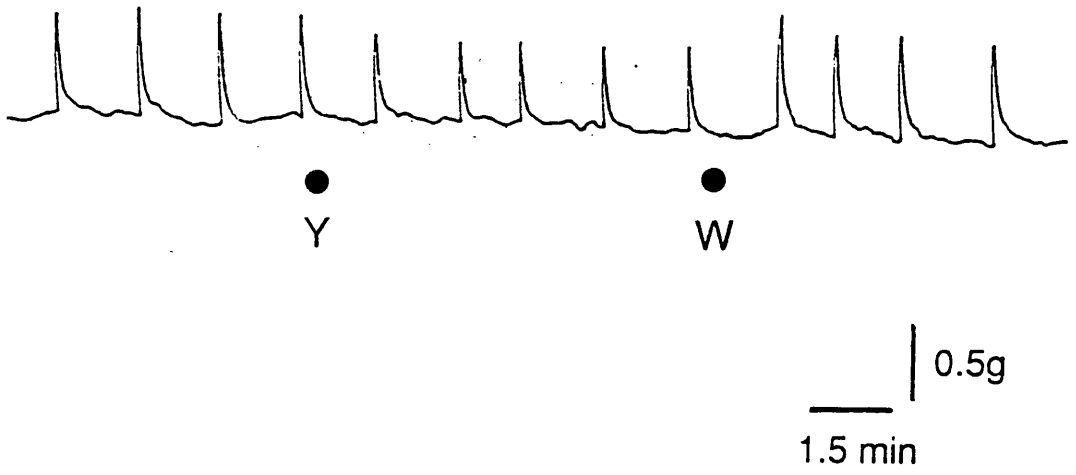


Figure 62. **Trace showing the effect of yohimbine on the contractile response to electrical field stimulation in rat tail artery rings.** Yohimbine (Y, 50nM) reduced the height and curtailed the duration of the response to field stimulation (supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms, every 100s). Responses returned to the control height and duration when yohimbine was washed from the organ bath (W).

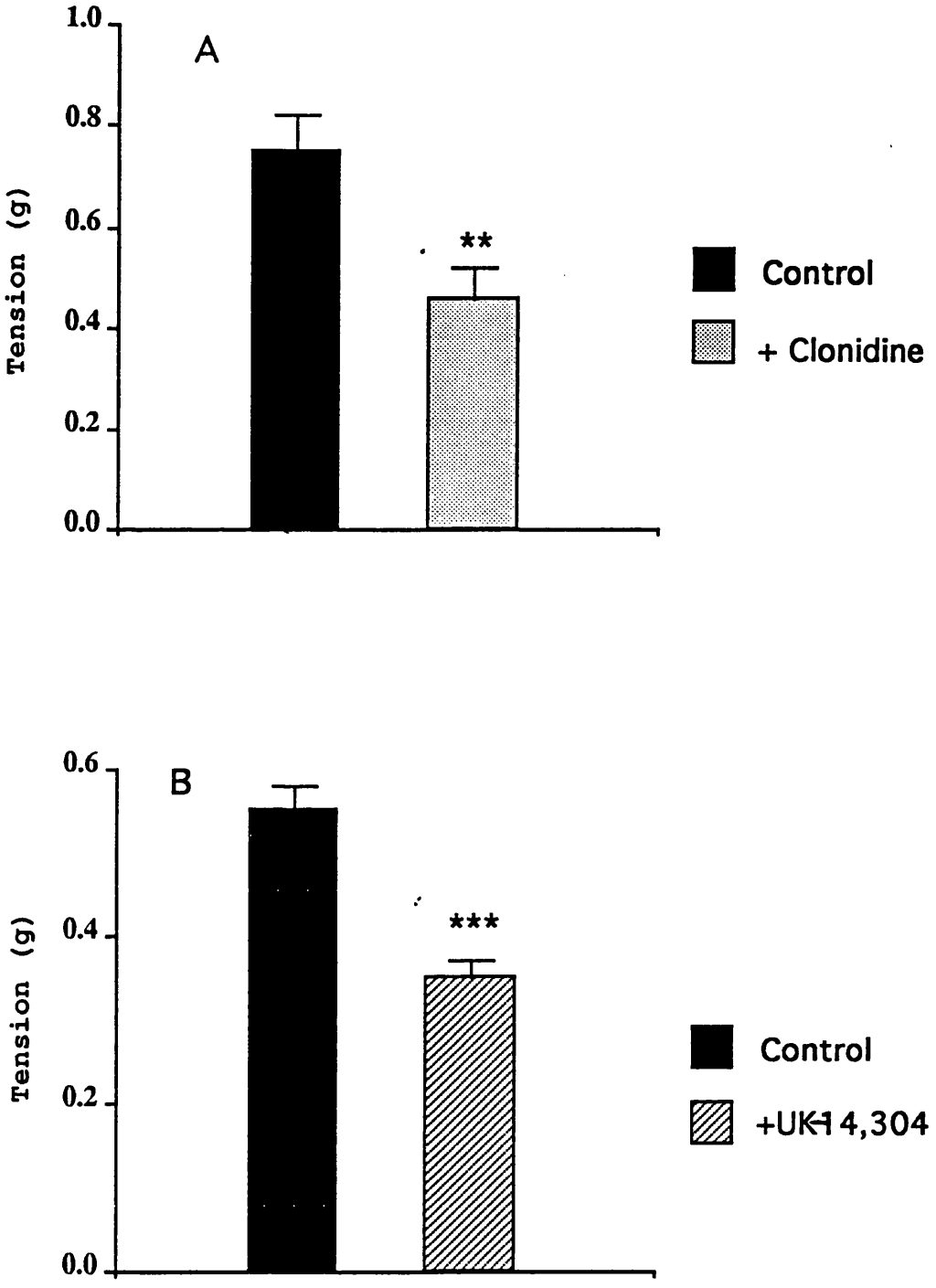


Figure 63. **The effects of clonidine and of UK-14,304 on the contractile response to electrical field stimulation in rat tail artery rings.** Field stimulation (Supramaximal voltage 50V, 10Hz for 1s, 0.1ms duration) gave reproducible responses (Control; A, B). Clonidine (50nM; A) and UK-14,304 (50nM; B) inhibited the field stimulation-induced contraction (**, $P<0.01$; ***, $P<0.001$). Each bar represents the mean ± s.e.m. of at least 12 observations.

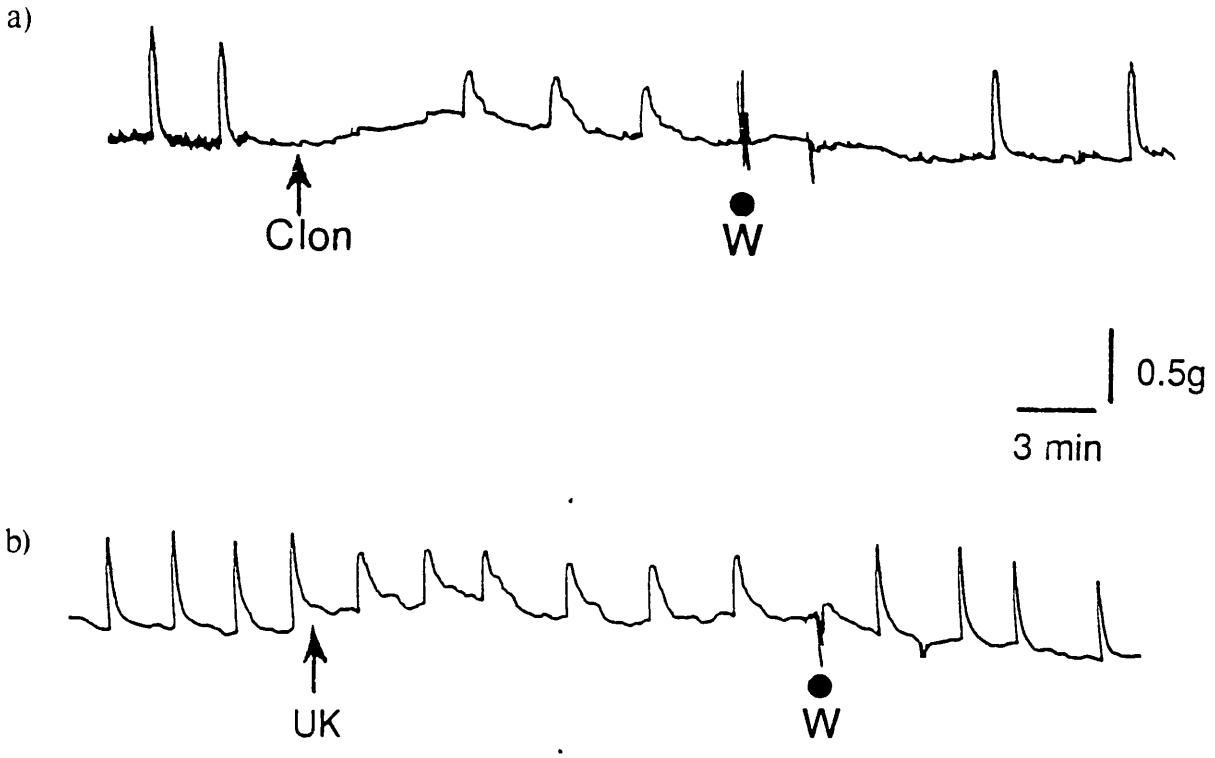


Figure 64. **Trace showing the effects of clonidine and UK-14,304 on the contractile response to electrical field stimulation in rat tail artery rings.** Vessels were stimulated with supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms. Both clonidine (Clon, 50nM; a) and UK-14,304 (UK, 50nM; b) produced small contractions, and reduced the height and prolonged the duration of the field stimulation-induced contraction. The responses returned to control height and duration when the drugs were washed from the organ bath (W).

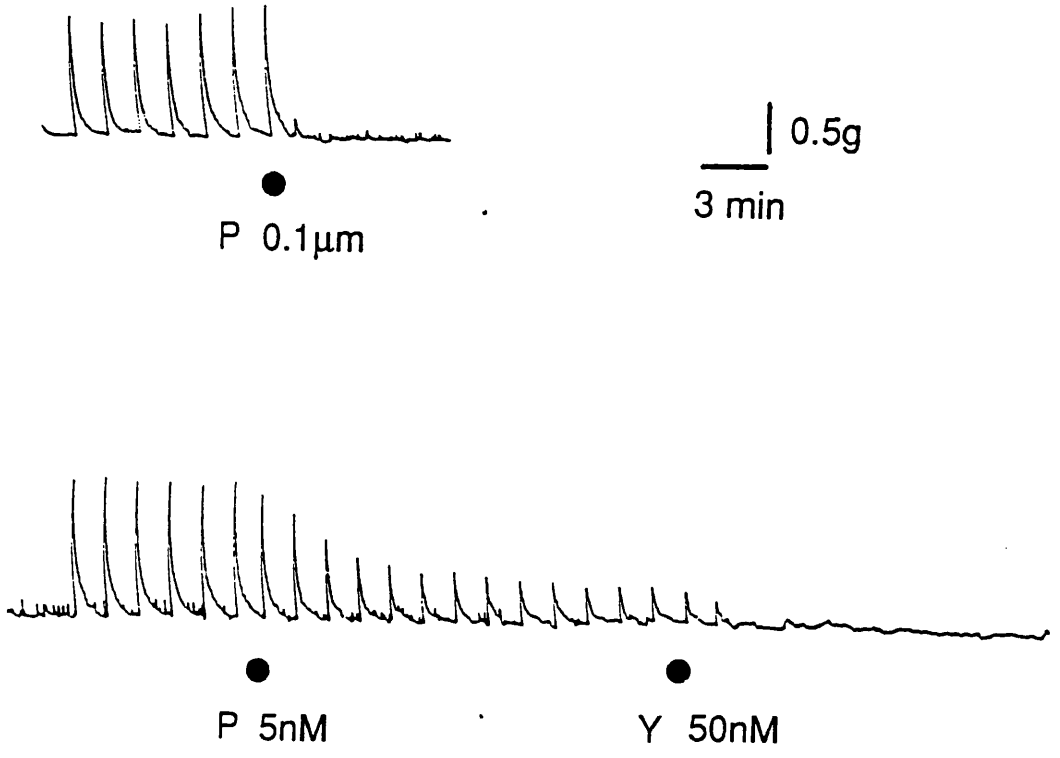


Figure 65. **Trace showing the effects of prazosin and yohimbine on the contractile response to electrical field stimulation in rat tail artery rings.** Vessels were stimulated with supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms, every 100s. In the upper panel, the response was abolished by prazosin (P; 0.1μM). The lower panel shows the effect of a lower concentration of prazosin (P, 5nM), which inhibited the contraction to field stimulation. The residual response was abolished by yohimbine (Y, 50nM).

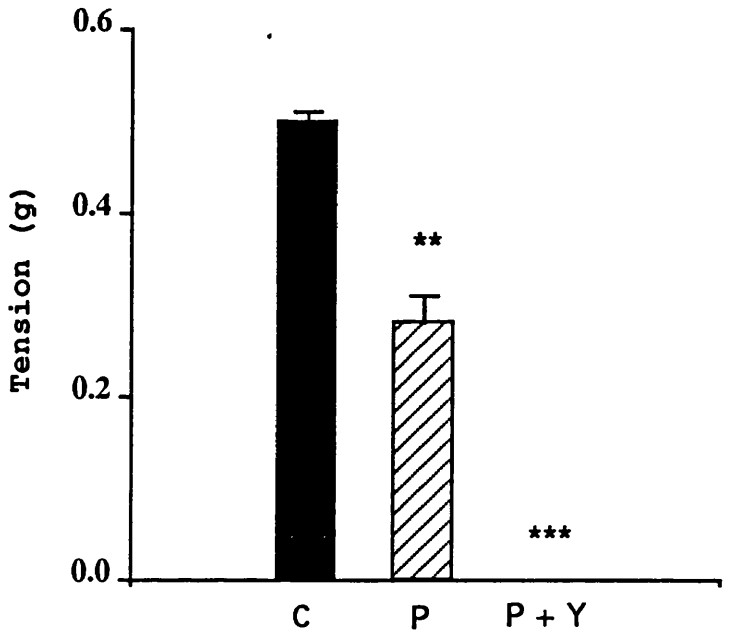


Figure 66. **The effect of prazosin and yohimbine on the contractile response to electrical field stimulation in rat tail artery rings.** Field stimulation (Supramaximal voltage 50V, 10Hz for 1s, 0.1ms duration) gave reproducible control responses (C). Prazosin (5nM, P) inhibited the field stimulation-induced contraction (**, $P<0.01$). The residual response was abolished by yohimbine (50nM, P+Y; ***, $P<0.001$). Each bar represents the mean $\pm$ s.e.m. of at least 12 observations.

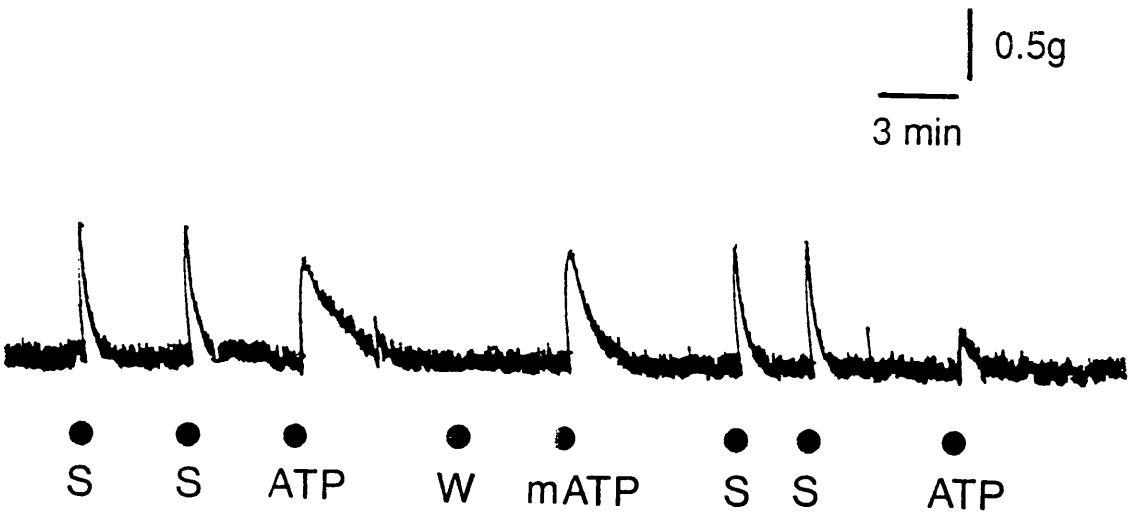


Figure 67. **Trace showing the effect of mATP on the contractile responses to electrical field stimulation and to ATP in rat tail artery rings.** Two control responses to field stimulation (S; supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms) were obtained, then ATP (0.3mM) added. ATP produced a transient contraction, then was washed out (W). Addition of mATP (0.5μM) produced a transient contraction, then the sequence was repeated in the presence of mATP. mATP did not affect the response to field stimulation but inhibited the response to exogenous ATP.

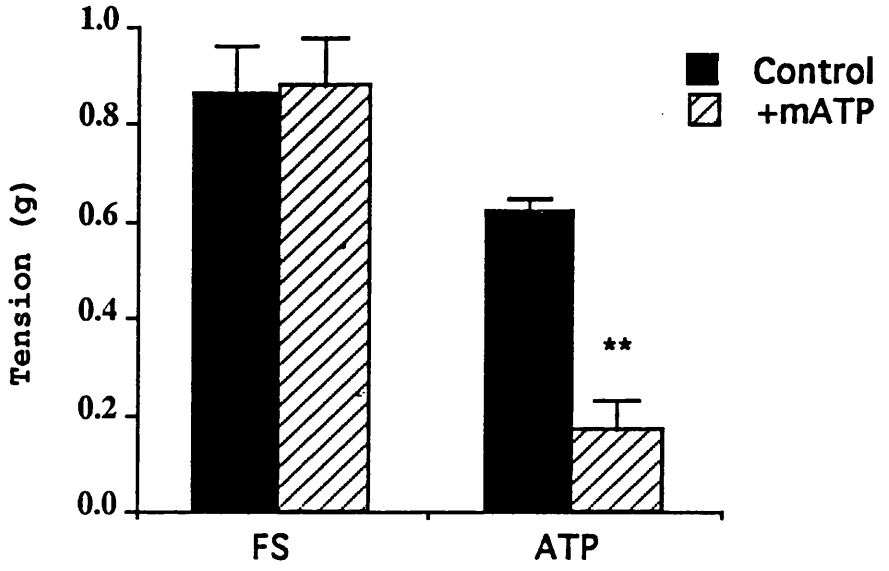


Figure 68. **The effect of mATP on the contractile responses to electrical field stimulation and to ATP in rat tail artery rings.** Control responses to field stimulation (FS; Supramaximal voltage 50V, 10Hz for 1s, 0.1ms duration) and to ATP (0.3mM) are shown as black bars. Responses in the presence of mATP (0.5μM) are shown as hatched bars. mATP did not affect the field stimulation-induced contraction, but markedly reduced the response to exogenous ATP (**, $P<0.01$). Each bar represents the mean ± s.e.m. of at least 6 observations.

4) *Responses to field stimulation following precontraction and subsequent relaxation*

Previous experiments (Part III) established conditions in the rat tail artery for potentiating contractile responses mediated via post-junctional α_2-adrenoceptors. The effects of clonidine, UK-14,304 and yohimbine suggested that these receptors may have a role in the field stimulation-induced contraction. Field stimulation was therefore carried out in vessels which had been precontracted by AVP (0.4mU ml^{-1}) and then relaxed by IBMX (10μM).

a) Control

Two control responses to field stimulation (Supramaximal voltage 50V, 10Hz for 1s, 0.1ms pulse width) and a control response to clonidine (50nM) were obtained (Figure 69a). Artery rings were then precontracted with AVP and relaxed with IBMX. In the presence of AVP and IBMX there was no difference in the height of the response to field stimulation as compared to control (0.33±0.08g and 0.42±0.09g respectively). The response was, however, prolonged as compared to control. Subsequent addition of clonidine (50nM) produced a response greater than control (0.83±0.22g, c.f. 0.12±0.01g; $P<0.01$), which was abolished by yohimbine (50nM; $P<0.01$). Field stimulation then produced no response.

b) Effect of prazosin

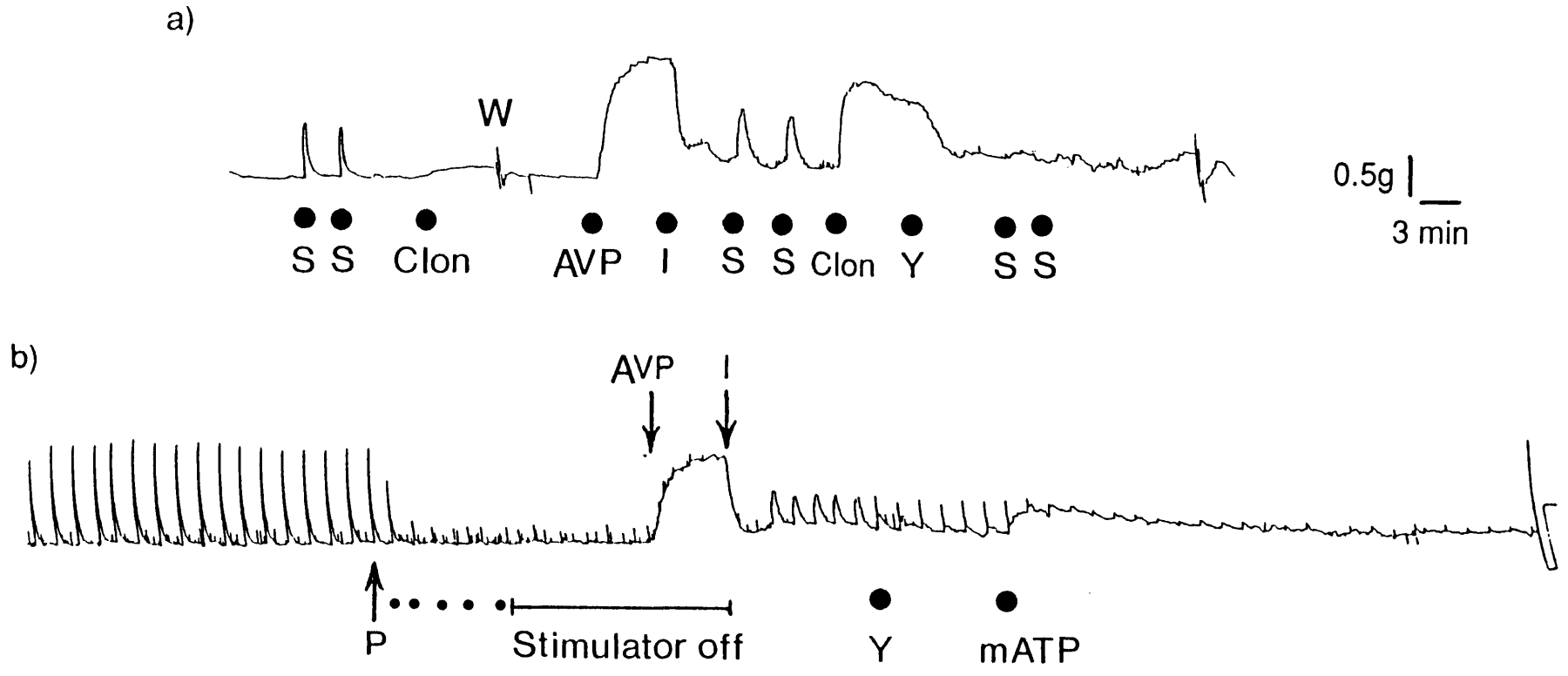
Trains of electrical stimulation (10Hz) gave reproducible responses, which were completely abolished by prazosin (0.1μM; Figure 69b). The remainder of the experiment was carried out in the presence of prazosin. Tail artery rings were precontracted with AVP and relaxed with IBMX, and field stimulation now produced a response in the presence of prazosin. This response was biphasic, with an initial spike followed by a more prolonged second

component, and the amplitude was reduced (0.39±0.04g, c.f. 1.15±0.01g control; $P<0.001$). Addition of yohimbine (50nM) at this point had no effect on the amplitude of this revealed response, but abolished the second component of the response, leaving a spike. This spike was almost completely abolished by addition of α,β–MeATP (0.5μM).

Figure 69. **Trace showing the effect of AVP and IBMX on the response to field stimulation in rat tail artery rings, in the absence (a) and presence (b) of prazosin.**

(a) Two control responses to field stimulation (S; supramaximal voltage 50V, 10Hz for 1s, pulse width 0.1ms) were obtained, then clonidine (Clon; 50nM) added. This gave a small contraction and was washed out (W). The vessel was then precontracted with AVP (0.4mU ml^{-1}) and relaxed with IBMX (I, 10μM). Field stimulation in the presence of AVP and IBMX then produced a response which was prolonged as compared to control. Clonidine gave an enhanced response, which was inhibited by yohimbine (Y; 50nM). Field stimulation then produced no response.

(b) Prazosin (P; 0.1μM) abolished the response to field stimulation. The stimulator was switched off for the period indicated, then the vessel precontracted with AVP and relaxed with IBMX. Field stimulation (every 100s until the end of the experiment) now produced a biphasic response. Yohimbine (Y; 50nM) abolished the second phase, leaving a spike. Addition of mATP (0.5μM) gave a transient contraction and almost abolished the spike.


a)
W
0.5g
3 min
S S Clon AVP I S S Clon Y S S
b)
AVP I
P
Stimulator off
Y
mATP

C) FIELD STIMULATION IN HYPERTENSIVE RATS

It has been suggested that the importance of ATP as a co-transmitter is increased in hypertension (Vidal, Hicks & Langer, 1986). Contractile responses to electrical field stimulation were therefore investigated in tail artery rings from SHR and compared with responses from normotensive control rats (WKY).

1) *Frequency-response curve*

Electrical field stimulation (supramaximal voltage 50V, 10 pulses, 0.1ms, 0.1-40Hz) produced frequency-dependent increases in tension. In both groups of rat the frequency-response curve was biphasic with the first maximum at 2-10Hz, after which the response again increased from 10-40Hz (Figure 70). When expressed as increases in tension (g), responses in tail artery rings from SHR were greater than in those from WKY at all frequencies ($P<0.05$; Figure 70a). When expressed as a percentage of the response to PE (10μM) however, only responses at the lower frequencies (0.1Hz, 0.2Hz) were increased in hypertensive rats relative to control (0.1Hz: $18.6\pm3.4\%$ c.f. $5.0\pm1.9\%$, $P<0.05$; 0.2Hz: $22.3\pm3.5\%$ c.f. $10.9\pm3.0\%$, $P<0.05$) (Figure 70b).

2) *Time controls*

Three consecutive frequency-response curves were carried out in tail artery rings from hypertensive and control rats. In SHR the responses at each frequency diminished with each repetition (Figure 71). From 0.1-2Hz the decrease in response between the first and third curves was statistically significant ($P<0.05$). In WKY, there was no difference in response comparing the first, second and third curves (Figure 71).

3) *Effect of α,β-MeATP*

A control FRC was obtained, then α,β–MeATP (1μM) added to the organ bath. This produced a transient contraction (SHR: 0.75±0.11g; WKY: 0.62±0.10g), and when this returned to baseline, a second FRC was carried out in the presence of α,β–MeATP. Addition of a further 1μM α,β–MeATP produced little or no response (SHR: 0.10±0.04g; WKY: 0.07±0.03g) and a third FRC was then obtained. Responses were corrected for any changes occurring during the time control and expressed as a percentage of the response to PE (10μM).

In SHR, α,β–MeATP had no effect on the response to field stimulation at 0.5-40Hz. At 0.1-0.2Hz the response increased following the first addition of α,β–MeATP ($P<0.05$), but returned to control following the second α,β–MeATP addition (Figure 72). In WKY, α,β–MeATP had no effect on the field stimulation-induced contraction at any of the frequencies examined (Figure 72).

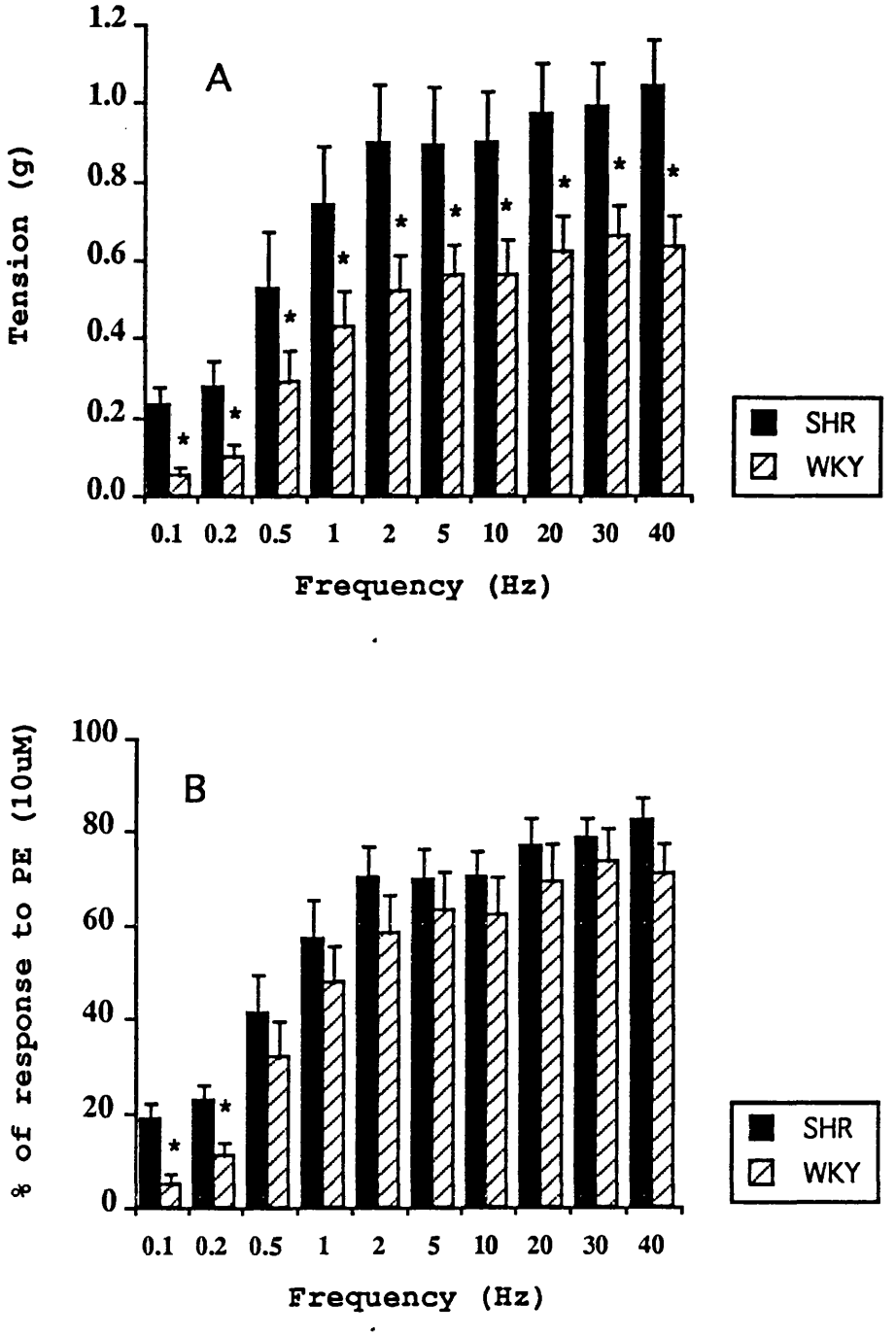


Figure 70. **Frequency-response curves in tail artery rings from SHR (--■--) and WKY (--▨--) rats.** Vessels were stimulated with supramaximal voltage (50V), 10 pulses, 0.1ms. When expressed as increases in tension (A), responses from SHR were greater than those from WKY at all frequencies (*, $P<0.05$). When expressed as a percentage of the response to PE (10μM; B), only responses at 0.1Hz and 0.2Hz were greater in SHR compared to WKY (*, $P<0.05$). Each bar represents the mean ± s.e.m. of at least 6 observations.

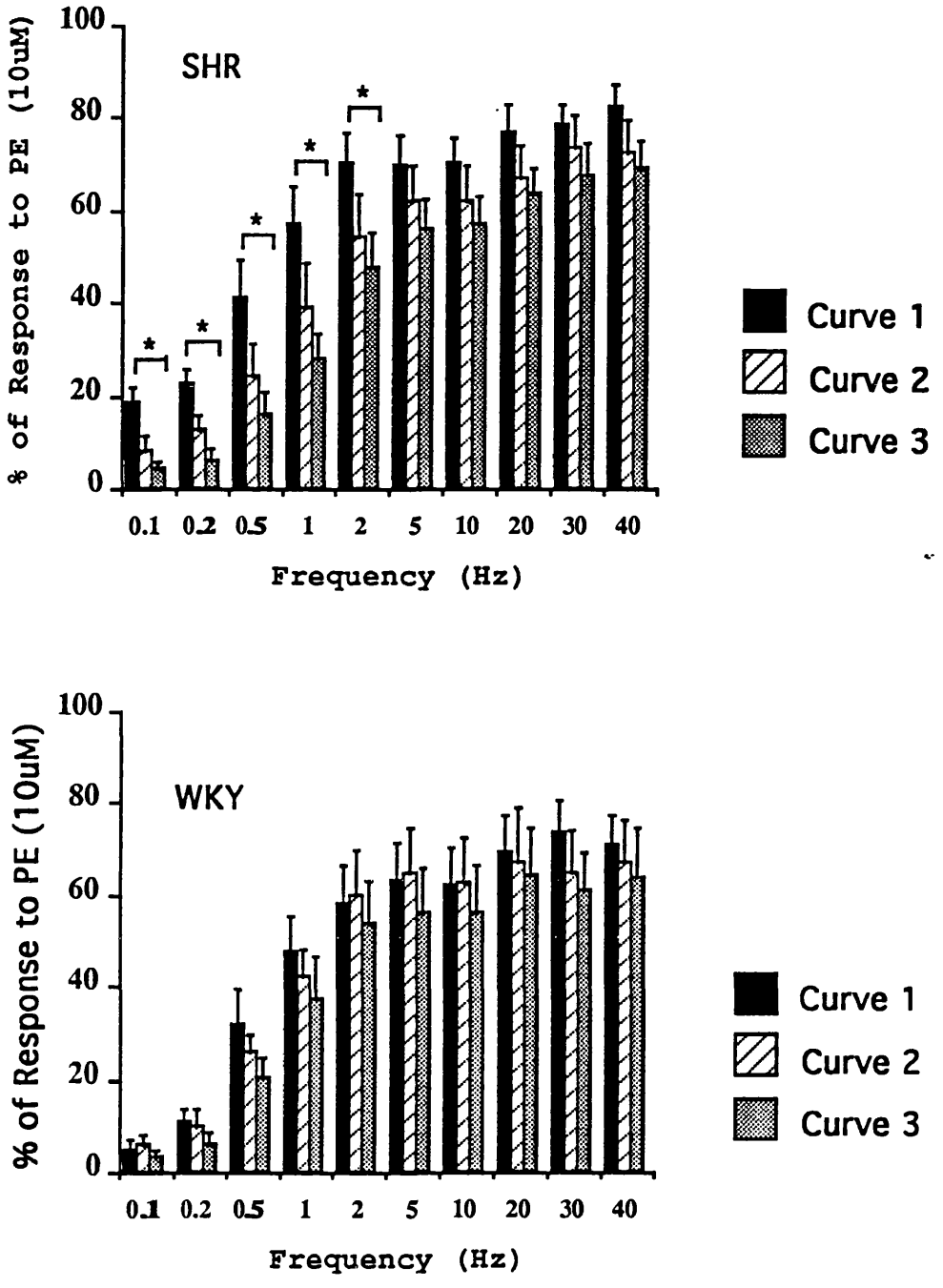


Figure 71. **Time control for the frequency-response curves in tail artery rings from SHR (upper panel) and WKY (lower panel) rats.** Three consecutive frequency-response curves were obtained (Supramaximal voltage 50V, 10 pulses, 0.1ms; Curves 1-3). In SHR, the response decreased with each repetition and the decrease was significant between the first and third curves from 0.1-2Hz (*, $P<0.05$). In WKY, the responses were maintained with each repetition. Each bar represents the mean $\pm$ s.e.m. of at least 6 observations.

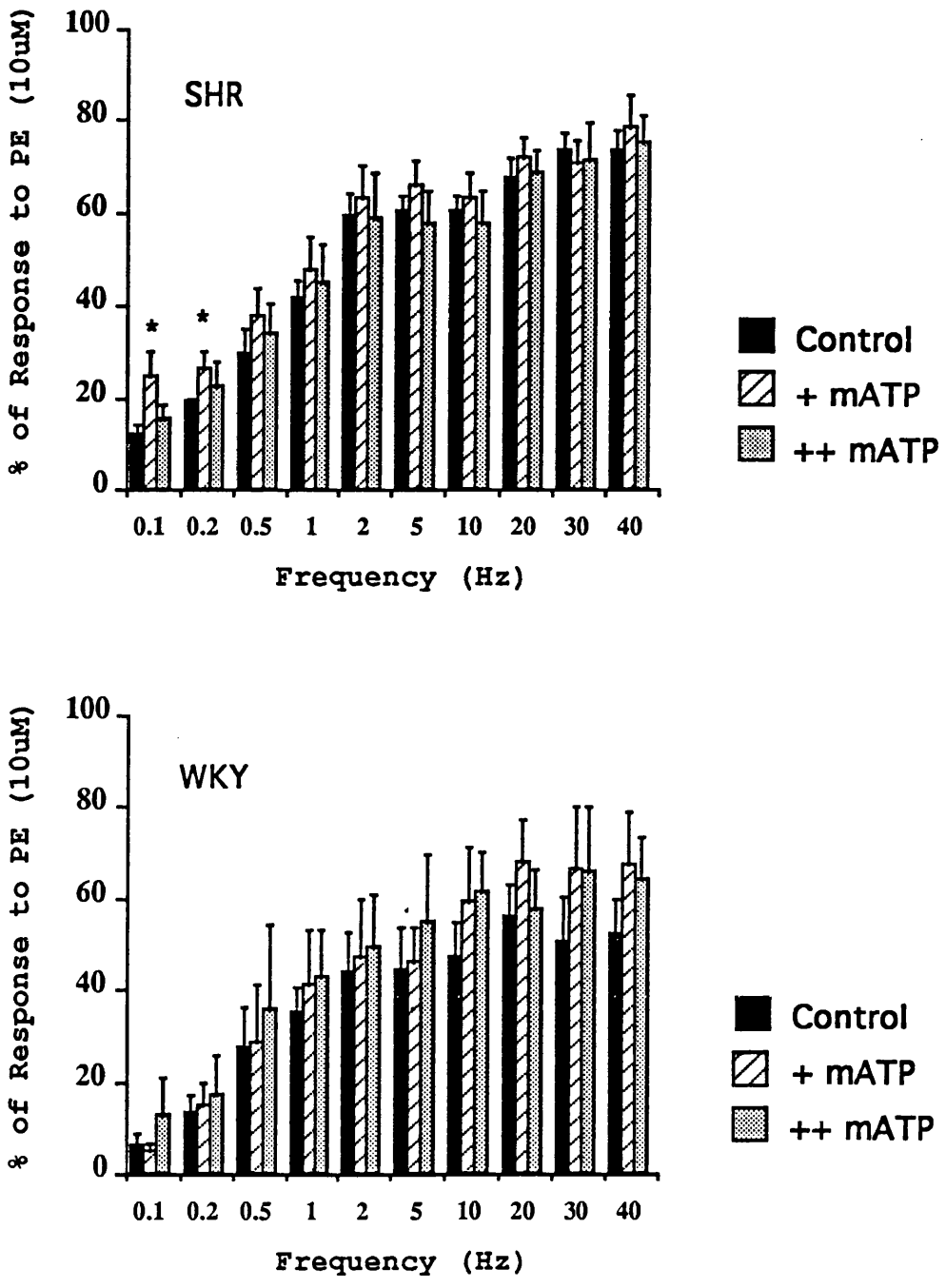


Figure 72. **The effect of mATP on the frequency-response curve in tail artery rings from SHR and WKY rats.** Vessels were stimulated with supramaximal voltage (50V), 10 pulses, 0.1ms, and responses were controlled for time. mATP (1μM) was added after the first (Control) curve, and a second curve (+mATP) was carried out. A further addition of mATP (+1μM) was given and the third curve (++mATP) carried out. In SHR (upper panel), the responses at 0.1Hz and 0.2Hz increased following the first addition of mATP (*, $P<0.05$), but returned to control after the second addition. In WKY (lower panel), mATP had no effect on the response to field stimulation at any frequency. Each bar represents the mean ± s.e.m. of at least 6 observations.

D) FIELD STIMULATION IN RATS PRETREATED WITH RESERPINE OR 6-HYDROXYDOPAMINE

Reserpine and 6-OHDA are two drugs known to inhibit sympathetic transmission, reserpine by depleting nerve terminals of NA, and 6-OHDA by destroying sympathetic nerve terminals. Reserpine and 6-OHDA may distinguish between nerves which use only NA and nerves where the actions of an additional transmitter may be unaffected by NA depletion. Responses to field stimulation were therefore investigated in tail artery rings from rats pretreated with reserpine or 6-OHDA.

1) *Rat weight and systolic blood pressure*

All rats were age- and weight-matched, then treated with drug or vehicle control. Reserpine-treated rats were significantly lighter than rats which had received vehicle control (235±17g, c.f. 338±11g, $P<0.01$). There was no difference in weight between 6-OHDA-treated rats as compared to control (305±9g, c.f. 322±15g). Systolic blood pressure was not affected by either reserpine (120±10mmHg, c.f. 132±12mmHg control) or 6-OHDA treatment (114±13mmHg, c.f. 125±14mmHg control).

2) *Electrical field stimulation*

Field stimulation (supramaximal voltage 70V, 0.5Hz, 10Hz or 20Hz, pulse width 0.4ms, 10 pulses) produced a contractile response in tail artery rings from both reserpine- and 6-OHDA-treated rats. The shape of the response was wider than control responses, and although rising sharply to a peak, did not return to baseline until 5-8 minutes after stimulation (Figures 73, 75, 76, 78). The stimulation-induced contraction was not frequency-dependent, with responses at 0.5Hz similar to those at 20Hz (Figures 75, 76, 78). The effects of drugs or drug combinations were tested on this contractile response.

a) Effect of TTX

Tail artery rings from all groups of rat were stimulated at 10Hz then TTX (0.1μM) was added to the organ bath and field stimulation was repeated. TTX did not affect the field stimulation-induced contraction in vessels from either reserpine- or 6-OHDA-treated rats (Figures 73a, 73c). Responses in vessels from the control groups of rat were abolished by TTX (Figures 73b, 73d).

b) Time control

Two responses each to 0.5Hz then 20Hz were obtained, then this stimulation sequence was repeated twice. For rats from both treatment groups there was no difference in response with each repetition (Figures 74a, 74b). Responses from the respective control groups diminished with each repetition at 0.5Hz ($P<0.05$) but not at 20Hz (Figures 74a, 74b).

c) Effect of prazosin and yohimbine

Two responses each to 0.5Hz then 20Hz were obtained. Prazosin (0.1μM) was added to the organ bath and ten minutes later the field stimulation sequence was repeated. Responses in tail artery rings from both treatment groups were unaffected by prazosin (Figures 75, 76, 77) but responses in vessels from the respective control groups were abolished (Figures 75, 76). Yohimbine (50nM) was then added to the organ bath and stimulation repeated twenty minutes later. Responses in tail artery rings from both treatment groups were unaffected by yohimbine (Figures 75, 76, 77).

d) Effect of α,β–MeATP

Two responses each to 0.5Hz, then 20Hz were obtained. α,β–MeATP (1μM) produced a transient contraction in vessels from treated and control groups, then the stimulation sequence was repeated. Responses in tail artery rings from all groups of rat were unaffected by α,β–MeATP (Figures 78, 79).

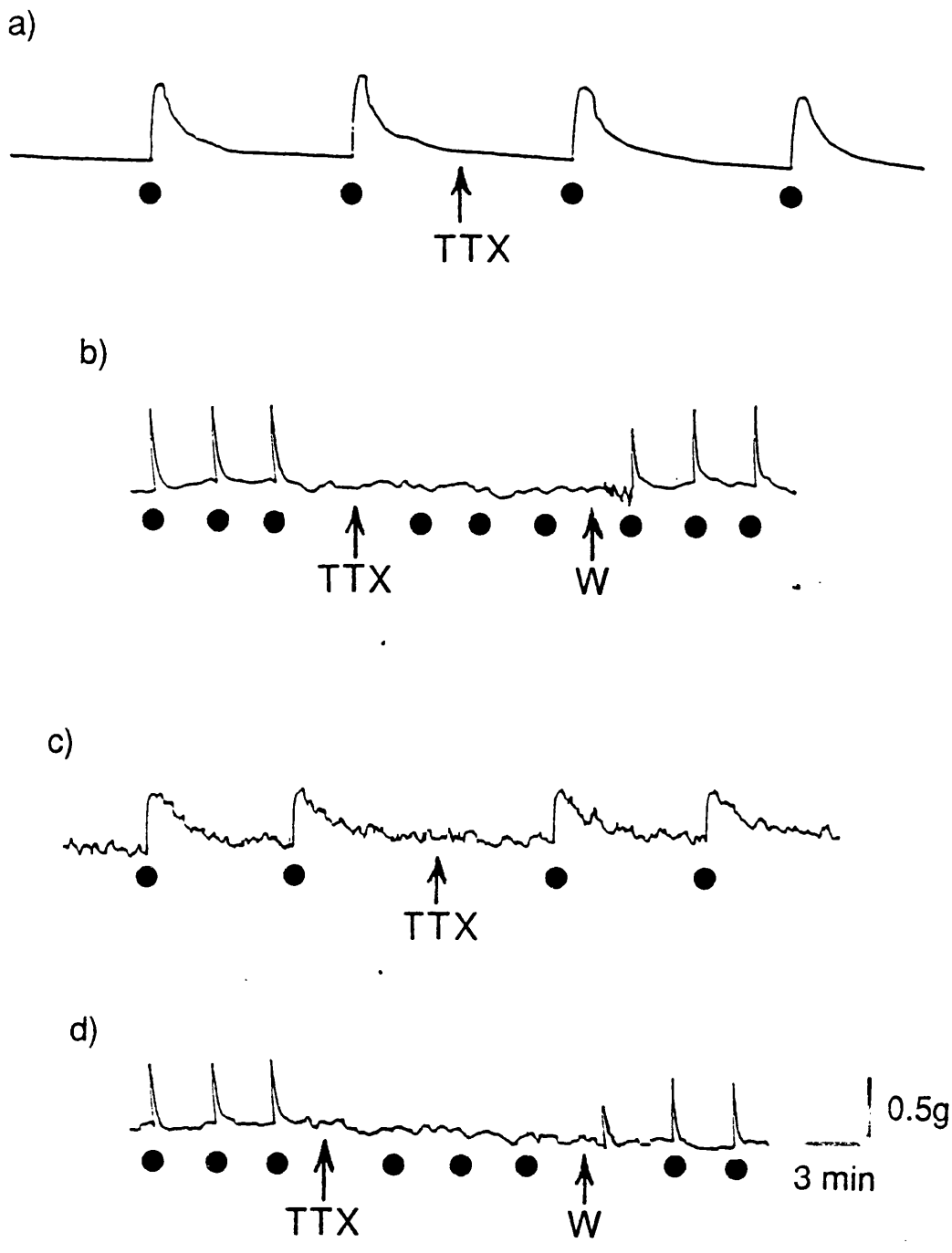


Figure 73. **Trace showing the effect of TTX on the contractile response to electrical field stimulation in tail artery rings from rats pretreated with reserpine (a) or 6-hydroxydopamine (6-OHDA; c).** Each train of stimulation (supramaximal voltage 70V, 10Hz for 1s, pulse width 0.4ms) is represented by a dot. TTX (0.1μM)had no effect on responses from rats pretreated with reserpine (a) or 6-OHDA (c). Responses from rats pretreated with vehicle controls for reserpine (b) or for 6-OHDA (d) were abolished by TTX, and restored after the TTX was washed out (W).

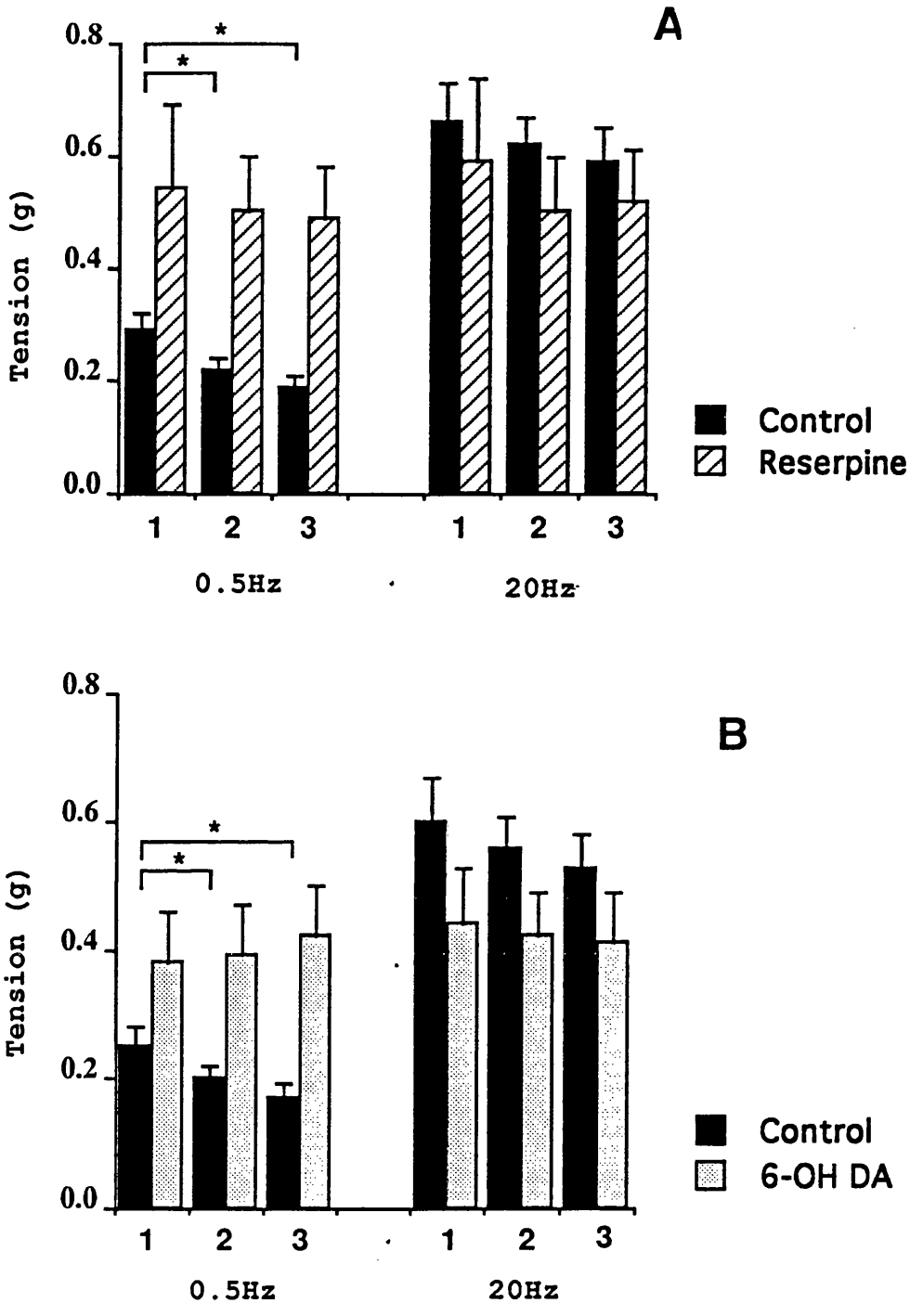


Figure 74. **Time control for the contractile response to electrical field stimulation in tail artery rings from rats treated with reserpine (A), 6-OHDA (B) or the appropriate vehicle control (Control; A, B).** Vessels were stimulated using 70V, 0.5Hz or 20Hz, pulse width 0.4ms, 10 pulses. Two responses were obtained at each frequency (Response 1) and the sequence repeated twice (Responses 2 and 3 respectively). In the control groups, responses 2 and 3 were smaller than the initial response at 0.5Hz but not at 20Hz (*, $P<0.05$). In the treatment groups, there was no difference in response with each repetition, at either frequency. Each bar represents the mean $\pm$ s.e.m. of at least 6 observations.

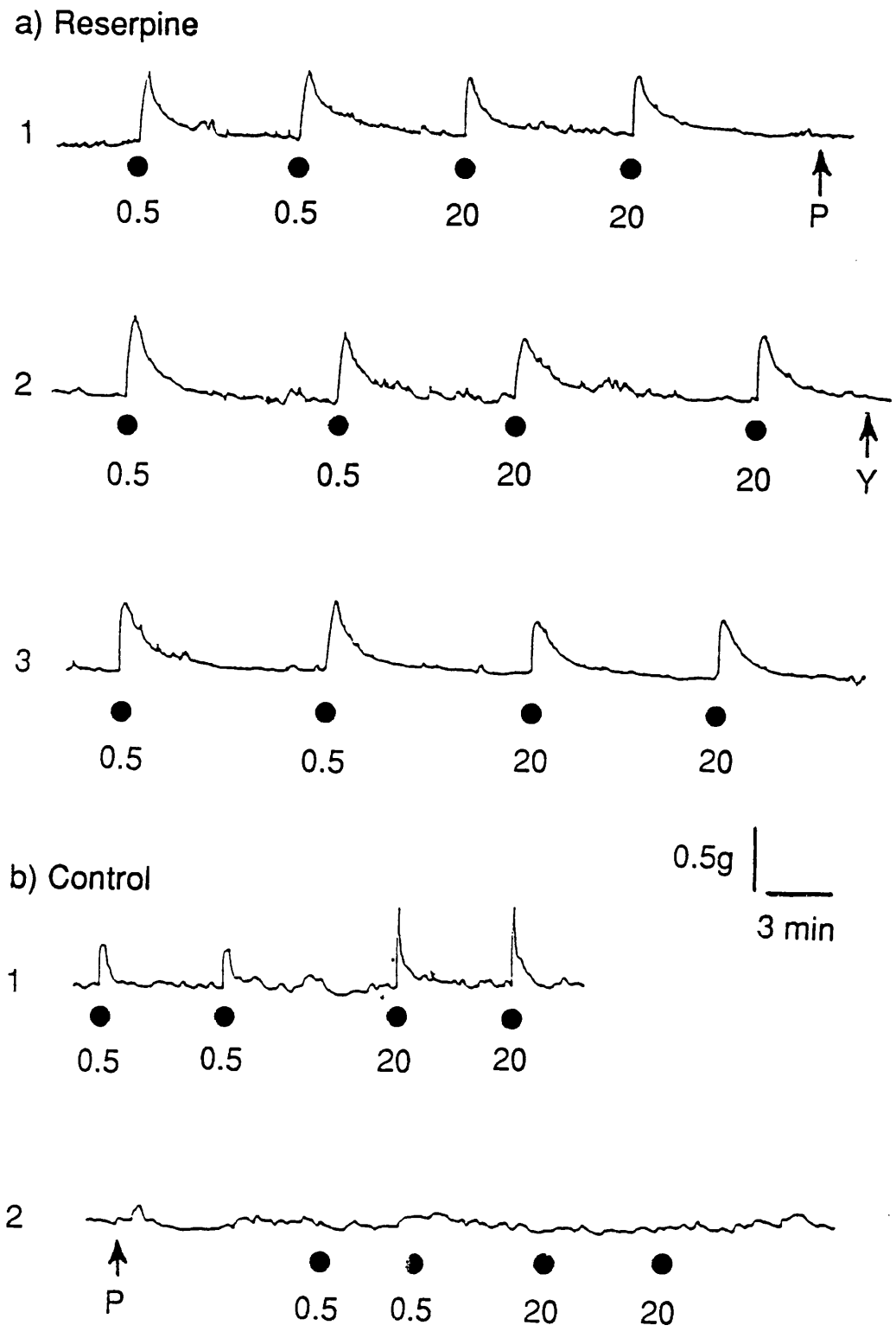


Figure 75. **Traces showing the effect of prazosin and yohimbine on the contractile response to electrical field stimulation in tail artery rings from rats pretreated with reserpine (a) or vehicle control (b).** Each train of stimulation (supramaximal voltage 70V, 0.5Hz or 20Hz, 10 pulses, pulse width 0.4ms) is represented by a dot. In (a), trace 1 shows responses from reserpinised rats before drug addition. Traces 2 and 3 are continuations of the experiment. Prazosin (P; 0.1μM) and yohimbine (Y; 50nM) had no effect on the response. In (b), trace 1 shows responses from control rats before drug addition. Trace 2 is a continuation of the experiment. Prazosin abolished the response to field stimulation.

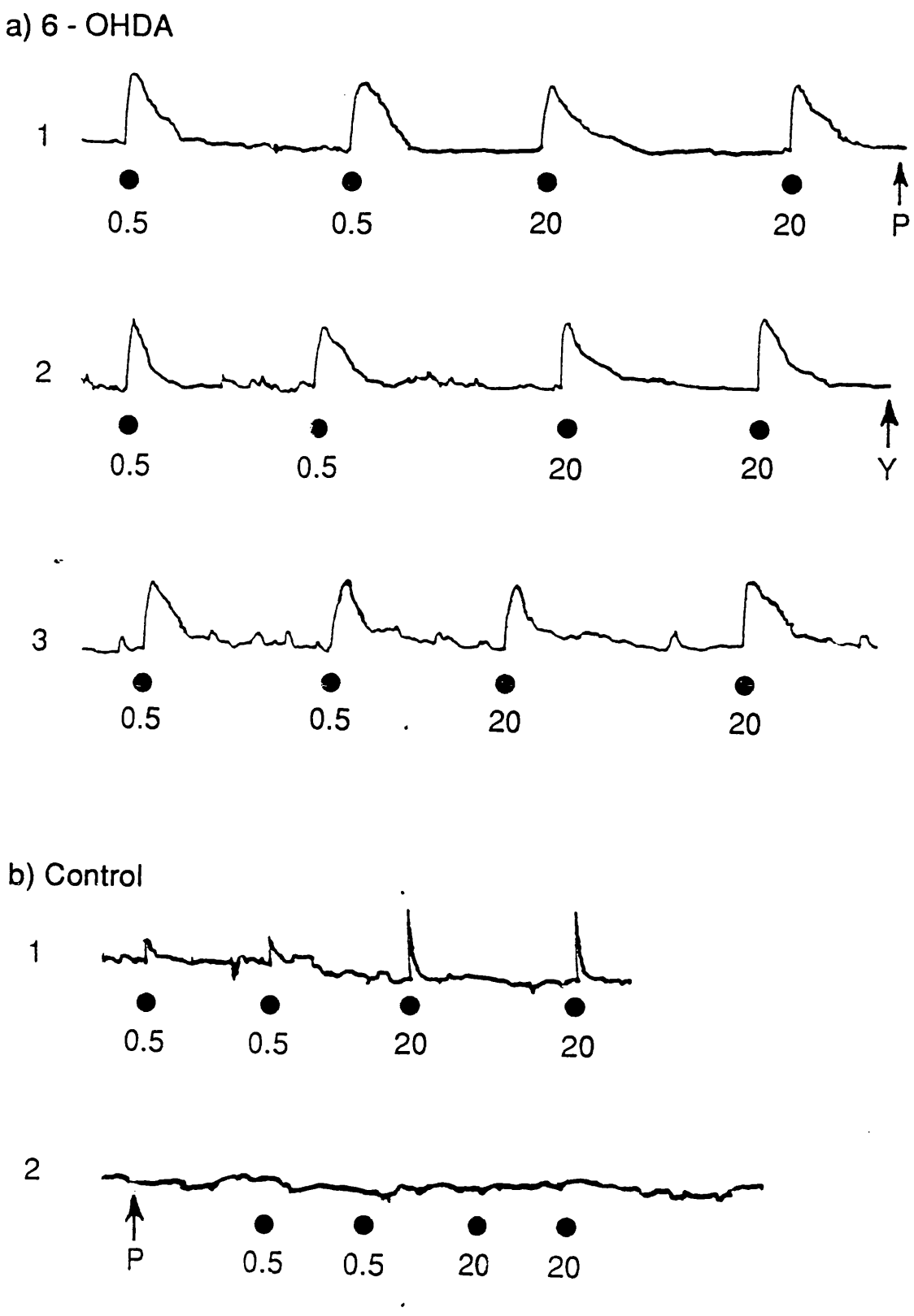


Figure 76. **Traces showing the effects of prazosin and yohimbine on the contractile response to field stimulation in rats pretreated with 6-OHDA (a) or vehicle control (b).** Each train of stimulation (Supramaximal voltage 70V, 0.5Hz or 20Hz, 10 pulses, pulse width 0.4ms) is represented by a dot. In (a), trace 1 shows responses from 6-OHDA-treated rats before drug addition. Traces 2 and 3 are continuations of the experiment. Prazosin (P; 0.1μM) and yohimbine (Y; 50nM) had no effect on the response. In (b), trace 1 shows responses from control rats before drug addition. Trace 2 is a continuation of the experiment. Prazosin abolished the response to field stimulation.

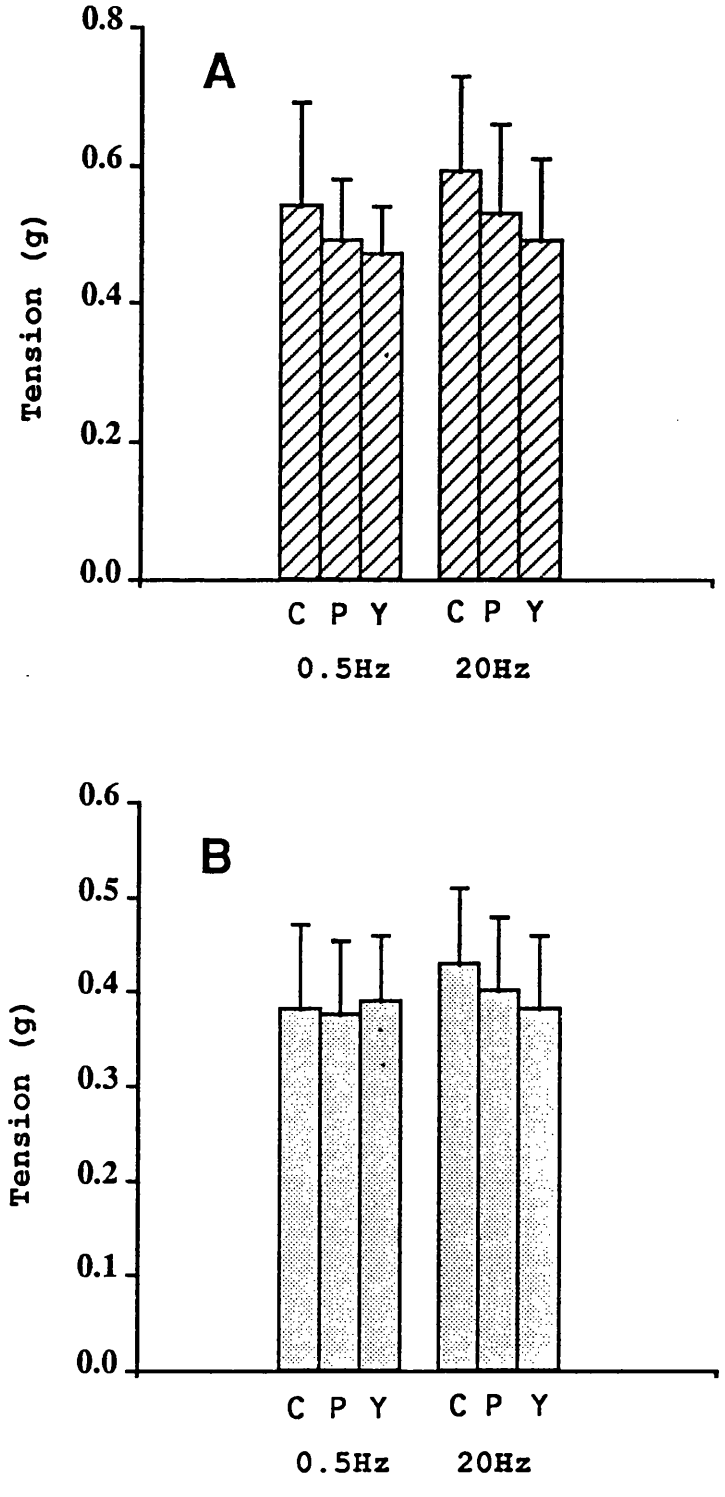


Figure 77. **The effect of prazosin and yohimbine on the contractile response to electrical field stimulation in tail artery rings from rats treated with reserpine (▨; A) or 6-OHDA (▩; B).** Vessels were stimulated using 70V, 0.5Hz or 20Hz, pulse width 0.4ms, 10 pulses. Two control responses were obtained at each frequency (C) and the sequence repeated in the presence of prazosin (P; 0.1μM). Yohimbine (Y; 50nM) was then added to the organ bath and the sequence repeated. Neither prazosin nor yohimbine affected the field stimulation-induced contraction at either frequency, in either treatment group. Each bar is the mean ± s.e.m. of at least 6 observations.

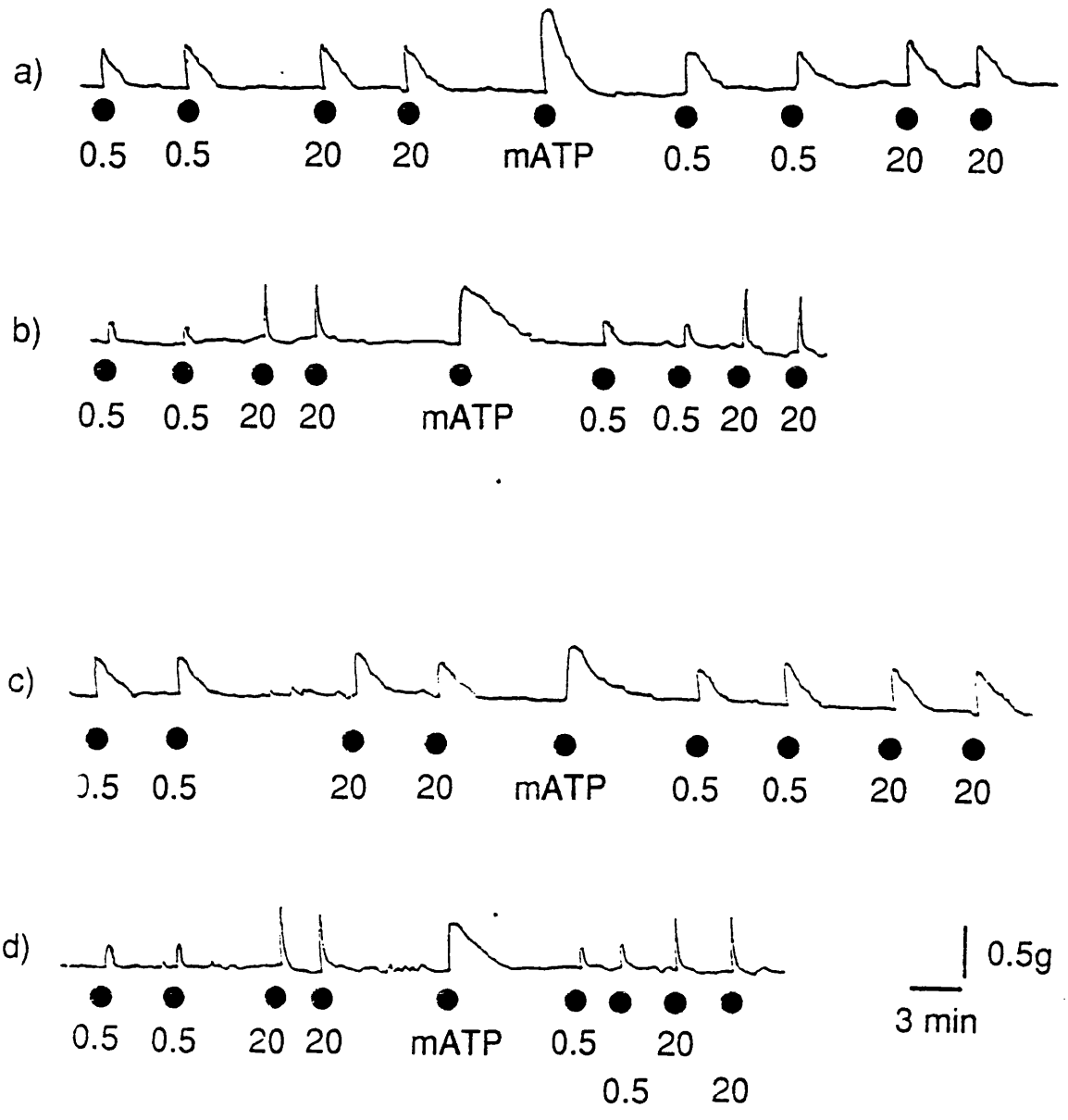


Figure 78. **Traces showing the effect of mATP on the contractile response to electrical field stimulation in tail artery rings from rats pretreated with reserpine (a) or 6-OHDA (c).** Each train of stimulation (supramaximal voltage 70V, 0.5Hz and 20Hz, 10 pulses, pulse width 0.4ms) is represented by a dot. Traces (b) and (d) show responses from rats pretreated with vehicle controls for reserpine and 6-OHDA respectively. Responses from each group of rat were unaffected by mATP (1μM).

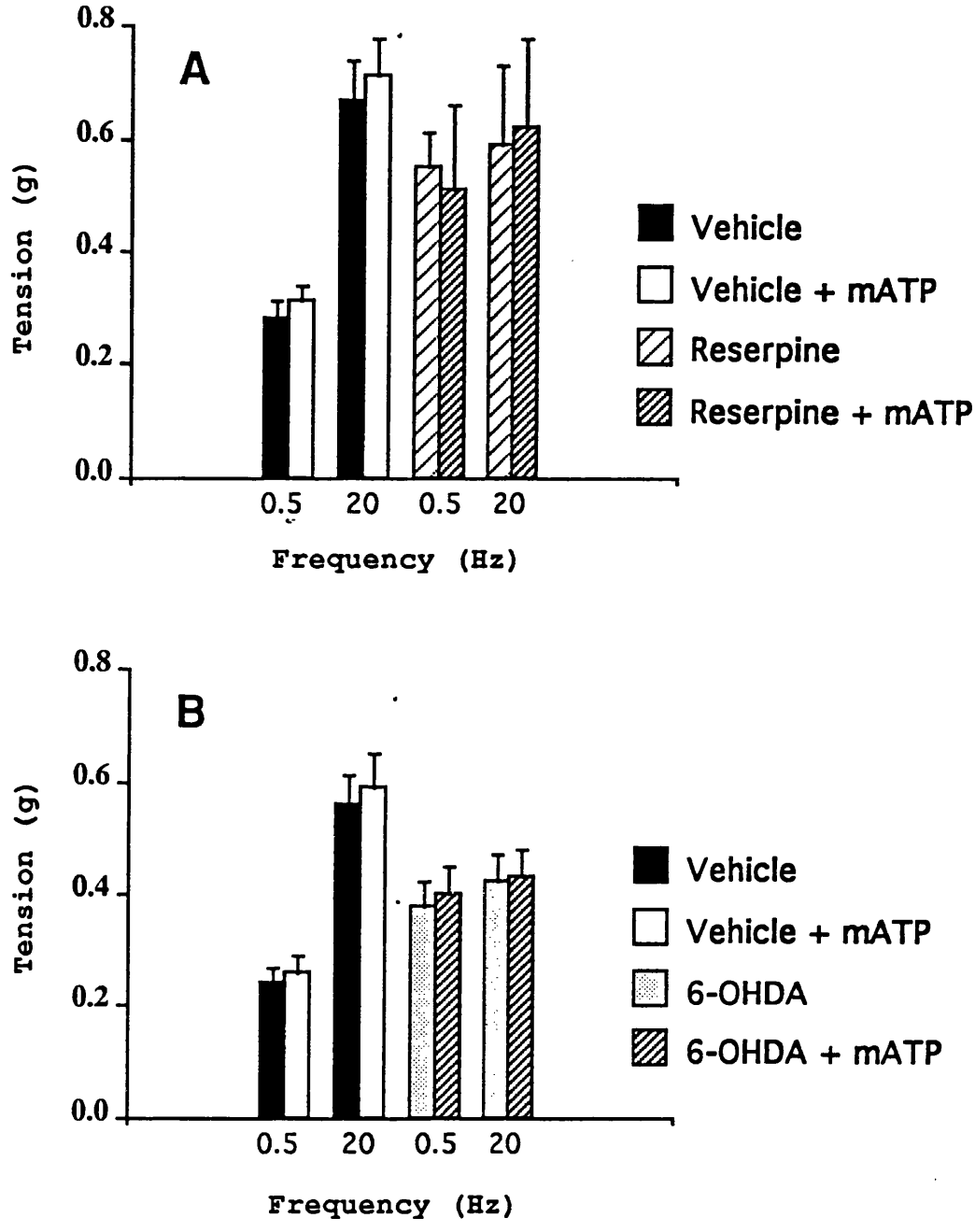


Figure 79. **The effect of mATP on the contractile response to electrical field stimulation in tail artery rings from rats treated with reserpine (A), 6-OHDA (B) or the appropriate vehicle control (A, B).** Vessels were stimulated using 70V, 0.5Hz or 20Hz, pulse width 0.4ms, 10 pulses Two responses were obtained at each frequency, then the stimulation sequence was repeated in the presence of mATP (1μM). The field stimulation-induced contraction from all groups of rat was unaffected by mATP. Each bar represents the mean ± s.e.m. of at least 6 observations.

Discussion

Since vascular responsiveness depends on complex interactions between various physiological mechanisms and sometimes various pathological processes, it is not surprising that gaps in our knowledge exist concerning its regulation. This study has provided answers for some key questions regarding blood vessel control.

The presence of post-junctional α_2-adrenoceptors in the rat tail artery was confirmed. An original experimental protocol allowed elucidation of the conditions under which contractions mediated via α_2-adrenoceptors could be demonstrated, thus extending current knowledge of the functional significance of these receptors.

This study also established that α_2-adrenoceptors located on vascular smooth muscle contribute to the field stimulation-induced contraction in the rat tail artery, providing evidence that these receptors have a role in the neural regulation of blood vessels. This finding partially explains why some neurogenic contractions are prazosin-resistant, a phenomenon which has previously been attributed to the action of a transmitter other than NA, for example, adenosine triphosphate (ATP). The present study did not find any direct evidence for NA/ATP sympathetic co-transmission in the rat tail artery. Under certain circumstances however, neurogenic contractions could be inhibited by desensitisation of purinoceptors. These experiments, while not resolving the controversial issue of co-transmission in this vessel, provide valuable information on the neural control of blood vessels.

Part of this study investigated the changes which occur in blood vessels during hypertension. Structural, mechanical and biochemical features were examined in a conducting and a resistance vessel, the rat aorta and tail artery respectively. Using this integrated approach, interesting results were

obtained which confirm and extend previous work in this laboratory and elsewhere.

2. HYPERTENSION

Arterial blood pressure is the product of cardiac output and total peripheral resistance (Lund-Johansen, 1980), the latter being largely determined by the small arteries and arterioles, which may therefore be termed "resistance arteries". Blood pressure is maintained within defined limits by homeostatic mechanisms such as the baroreceptor reflex, or longer-term systems such as the renin-angiotensin-aldosterone system (Docherty, 1990). These mechanisms, involving neural and humoral actions at the central and peripheral levels, operate in the whole animal or human. Clearly, isolated blood vessels are not exposed to the same regulatory influences as entire vascular beds in the intact animal. It was therefore of interest to determine, under conditions where blood pressure control is deranged, i.e. hypertension, whether changes associated with this phenomenon are manifested at the level of the blood vessel itself.

The model of hypertension chosen for this study was the spontaneously hypertensive rat (SHR), a genetically hypertensive strain first developed by Okamoto and Aoki in 1963. The SHR is a convenient model, requiring no physiological, pharmacological or surgical intervention, and as with human primary hypertension, exhibits a slowly progressing and naturally developing hypertension.

There are criticisms of this model however, which are generally levelled at the fact that the SHR is not an exact replica of human primary hypertension (Malik & McGiff, 1975; McGiff & Quilley, 1981). However, given that both diseases are polygenetic in origin and that the control of

normal arterial pressure in both man and rat is multifactorial (Trippodo & Frohlich, 1981), it is inevitable that differences exist.

Bearing these limitations in mind, the study of any model of naturally-occurring hypertension should provide useful and important information regarding primary hypertension in humans. Since both structural and functional factors are important in the hypertension of humans and SHR, the SHR was considered to be an appropriate model for the study of vascular reactivity in hypertension, a major focus of this investigation.

This study demonstrated that the blood pressure of the spontaneously hypertensive rats was indeed elevated in relation to the normotensive control strain, Wistar Kyoto (WKY), as measured by the tail cuff method. This method, which is non-invasive, produces a reliable measurement of systolic blood pressure highly comparable to direct readings from the rat carotid artery (Bunag, 1973). This change in the resting blood pressure was associated with a number of interesting changes in the blood vessels.

Structural changes in blood vessels during hypertension

Aortic and tail artery rings from SHR were heavier than those from WKY, despite being cut to the same standard length, indicating a structural alteration. Transverse sections of tail artery (proximal and distal) and aorta were then examined further using light microscopy.

There was no difference in the wall thickness of the proximal tail artery from SHR and WKY rats, which was unexpected considering the increase in weight of the SHR vessels. Moreover, the area of the medial layer was actually reduced in proximal tail artery sections from hypertensive rats as compared to control. These observations are apparently conflicting, but may be explained if there is an increase in the number of cells within the same volume or area, i.e., an increase in tissue density. This suggestion is supported

by evidence from the photographs of the histological sections. The number of smooth muscle layers is increased in the proximal and indeed distal tail artery of SHR compared to those of WKY rats, and it appears that these cell layers are much more densely packed. It is therefore proposed that smooth muscle hyperplasia occurs in the tail artery of spontaneously hypertensive rats, along the entire length of the vessel. These results are consistent with previous observations that in a typical resistance vessel, increases in both the size and number of myocytes contributes to the hypertrophy associated with hypertension (Mulvany, Baandrup & Gundersen, 1985; Lever, 1986; Lyall, Lever & Morton, 1988).

In this study, vessel thickening occurred in the aorta of SHR as compared to WKY, confirming the findings of previous workers (Limas, Westrum & Limas, 1980). It was not possible to determine in this study if the wall thickening in SHR aorta was due to hypertrophy or to hyperplasia. However, evidence in the literature indicates that aortic medial hypertrophy in SHR is due primarily to cellular hypertrophy rather than to hyperplasia (Owens, Rabinovitch & Schwartz, 1981; Owens, 1985; Owens, 1987).

An important structural feature of blood vessels in hypertension is the ratio of the wall thickness to lumen diameter (W/r) (Folkow, 1978; 1982; 1993). This study demonstrated that in the rat tail artery, the wall thickness to lumen ratio was increased in hypertensive animals compared to controls, along the entire length of the vessel. In proximal regions this was a consequence of a reduced lumen diameter, while in distal regions, both vessel thickening and lumen reduction contributed to the increased W/r. This is in contrast to the aorta, where there was no difference in W/r between vessels from SHR and those from WKY, since wall thickness and lumen diameter both increased.

The haemodynamic consequences of alterations in the wall thickness to lumen diameter ratio become apparent when the law of Laplace is considered (Folkow, 1993). According to Laplace's Law, the perfusion pressure in a blood vessel (P) is proportional to the wall tension (T) and wall thickness (W), and inversely proportional to the radius of the lumen (r), ie.

$$P = T \times W/r.$$

Clearly then, when W/r is increased, there is an exaggerated increase in perfusion pressure for any given degree of muscle contraction. The response of a blood vessel to a given vasoactive stimulus can therefore be augmented by this structural amplification factor.

It is likely that such a change in the geometry of a blood vessel will have a more profound effect on the response to a vasoactive agent if measured as the change in perfusion pressure in a perfused vessel, rather than as the isometric tension change in an artery ring. In this study therefore, responses to NA were measured using these two different methods, to determine if the technique used to record responses could affect the results obtained. This was indeed found to be the case, as will be demonstrated. Ring preparations were used for the most part however, as tension recording provides a more direct measure of muscle contraction, uncomplicated by geometric factors.

Responses to vasoconstrictors and vasodilators in blood vessels during hypertension

This study demonstrated that blood vessels from rats which were genetically predisposed to have hypertension do indeed respond differently to both vasoconstrictors and vasodilators than do vessels from normotensive control animals. In addition, the type of changes seen depend on which vessel is being studied. In the rat tail artery, there was an increase in response to the vasoconstrictors phenylephrine (PE), NA and UK-14,304 in SHR compared to

WKY, while the responses to the vasodilators SNP and isoprenaline were decreased. In contrast, the opposite pattern was found in the aorta, where aortic rings from SHR were markedly less responsive to PE than were aortic rings from WKY rats. Responses to carbachol and to sodium nitroprusside were enhanced in aortic rings from SHR compared to control, while interestingly, there was no difference in isoprenaline-induced relaxation between the two groups of rat.

What are the possible explanations for the differences in the responsiveness both between the aorta and tail artery, and between vessels from hypertensive and normotensive animals?

The present experiments demonstrated that PE produced weaker contractions in aortic rings from SHR than in those from WKY. This is consistent with the results of several other studies, which have found impaired contractile responses to NA in aortae from SHR as compared to their WKY controls (Konishi & Su, 1983; Sim & Singh, 1987) and agrees with previous studies carried out in this laboratory (Morrison, 1988; Aidulis et al., 1990). Interestingly, the latter two studies also showed a similar impairment of KCl-induced contraction in aortic rings from SHR. KCl contracts arteries by a non-receptor mechanism, depolarising the smooth muscle cell membrane and opening voltage-dependent calcium channels. The subsequent rise in intracellular free calcuim levels ($[Ca^{2+}]_i$) initiates the calcium-calmodulin cascade leading to activation of the contractile proteins, and to blood vessel contraction.

It is therefore possible that the mechanism responsible, at least in part, for a reduced contraction in SHR aortae lies at a level distal to receptor stimulation.

Evidence in the literature indicates that there is indeed a defect in the force generating ability of vascular smooth muscle from hypertensive animals, and that this is associated with vascular hypertrophy. Berner and co-workers (1981) described an increase in intermediate filaments in the rabbit portal-anterior mesenteric vein resulting from partial ligation of the vein. This was accompanied by a decrease in the number and distribution of myosin filaments. It is conceivable therefore that the hypertrophic cells express different isoforms of contractile proteins, thus producing less maximal force. Alternatively, Papageorgiou & Morgan (1991) showed that in the aorta, hypertrophy was accompanied by the presence of polyploidy and that maximal force generation was less than normal. Polyploid cells may possess unique physiological properties (Schwartz, Campbell & Campbell, 1986) which could account for the reduced force production of hypertrophic smooth muscle.

In the present study on hypertension, tissue responses were corrected on a tissue weight basis. Hypertrophy however is not only associated with changes in contractile material but also with increased connective tissue or tissue water (Heagerty, Ollerenhaw & Swales, 1986). It is possible therefore that if this correction were inappropriate, it may have contributed to the apparent reduction in the contractile response of aortic rings from SHR. This is unlikely however, as even before responses from SHR were divided by the increased tissue weight, the absolute tension developed was still less than that developed in WKY aortic rings.

The present investigation also showed that in the aorta, while the response to the vasoconstrictor PE was reduced in hypertension, the responses to vasodilators, both endothelium-dependent and directly-acting, were enhanced in the SHR. This increase in responsiveness applied only to

vasodilators whose actions are mediated via cyclic GMP, i.e. carbachol and SNP, but not to isoprenaline, whose actions are mediated via cyclic AMP.

Carbachol is a cholinergic agonist less susceptible to breakdown by acetylcholinesterase than is the endogenous compound, ACh (Rang & Dale, 1991). Carbachol relaxes blood vessels by the same mechanism as acetylcholine, which activates muscarinic receptors on the endothelial cells to liberate a compound identified as nitric oxide (NO; Palmer, Ferridge & Moncada 1987).

This labile substance diffuses to the vascular smooth muscle cells where it activates the soluble enzyme guanylate cyclase (Dinerman, Lowenstein & Snyder, 1993). This increases synthesis of cyclic GMP, which relaxes the smooth muscle by a number of mechanisms (Dinerman, Lowenstein & Snyder, 1993).

The results of the present study, where responses to the endothelium-dependent vasodilator carbachol were enhanced in the aorta of SHR, do not agree with previous studies which showed that endothelium-dependent relaxations were impaired during chronic hypertension (Lockette, Otsuka & Carretero, 1986; Luscher & Vanhoutte, 1986). Indeed, the latter study described a vasoconstrictor substance, possibly prostanoid in origin, released from aortic endothelial cells in the SHR in response to ACh (Luscher & Vanhoutte, 1986). There was no evidence of such an endothelial-derived constricting agent released in response to carbachol in this study. It is not clear why the results of the present study differ from the findings of previous work (Lockette, Otsuka & Carretero, 1986; Luscher & Vanhoutte, 1986), as all these investigations involved the same blood vessel, the aorta, and one model of hypertension, the SHR. However, it is possible that other differences in the experimental design of the studies may account for the different results. For example, Luscher and Vanhoutte (1986) reported a reduced maximum

contraction in response to NA in aortic rings from SHR compared to those from WKY, agreeing with the findings of the present study. However, for relaxation studies these workers then precontracted aortic rings from SHR and WKY to the same absolute level (1.4g), then carried out concentration-response (relaxation) curves to ACh. Consequently, aortic rings from SHR were precontracted to 50% of their maximal response to NA, whereas those from WKY were precontracted to only 25% of their maximal response. It may therefore have been more difficult for ACh to overcome a relatively greater contraction in the SHR, leading to a reduced ACh response for this reason. In the present study such a situation did not arise because tissues were always precontracted to 75% of the maximal contractile response to PE, obtained in each individual tissue.

This study demonstrated that cyclic GMP-mediated relaxations are enhanced in aortic rings from SHR. In addition, endothelial disruption has been reported to produce greater potentiation of NA-induced vasoconstriction in vessels from deoxycorticosterone acetate (DOCA)-hypertensive rats than in normotensive controls (King & Webb, 1988), implying that the ability of the endothelium to limit vasoconstriction is enhanced in the DOCA form of hypertension. Clearly then, the generalisation that dilator responsiveness is impaired in hypertension (Webb, 1984) is not valid. An earlier study in this laboratory (Morrison, 1988) showed that relaxations to SNP at the higher end of the concentration-response curve were enhanced in aortic rings from SHR compared to those from WKY rats, agreeing with the results of the present investigation. Furthermore, Morrison (1988) also showed that this enhanced relaxation was associated with increased cyclic GMP levels in response to SNP in the SHR. It is possible therefore that SHR aortae have an enhanced ability to accumulate cyclic GMP, and that increased levels of this

biochemical second messenger may underlie the enhanced mechanical response to nitrovasodilators in aortic rings from SHR.

An interesting study by Moncada et al.. (1991) demonstrated increased nitrovasodilator-stimulated relaxations and cyclic GMP levels in rat vasculature, both in vivo and in vitro, following endothelial denudation or treatment with inhibitors of NO synthase. Furthermore, response to isoprenaline or to 8-bromo-cyclic GMP were unchanged following these procedures. The authors concluded that a specific supersensitivity to nitrovasodilators developed after the removal of endogenous NO. It is tempting therefore to speculate on the role of this phenomenon in hypertension. If diminished NO occurs in hypertension (Dinerman, Lowenstein & Snyder, 1993) then soluble guanylate cyclase, the intracellular "receptor" for NO (Moncada, Radomski & Palmer, 1988), may become up-regulated. Such a situation would be analogous to the post-junctional supersensitivity of denervated tissues (Cannon & Rosenblueth, 1949), observed after removal of a basal mediator tone. This would be consistent with the findings of the present study, where only relaxations to SNP and to carbachol, but not to isoprenaline, were enhanced in aortic rings from SHR compared to those from WKY.

The rat tail artery was used in this investigation as an example of a resistance vessel, and it was found that the pattern of response to vasoactive agents in hypertension in this vessel was the opposite of that obtained in the aorta. In the rat tail artery, mechanical responses to the vasoconstrictors NA and PE, and to a lesser extent UK-14,304, were increased in the SHR compared to control. However, relaxations to the vasodilators SNP and isoprenaline were reduced in vessels from hypertensive rats compared to control.

An important observation of this study, which must be borne in mind when interpreting the results, was that all rat tail artery rings were endothelium-denuded, as indicated by the lack of response to the endothelium-dependent vasodilator carbachol (Figure 26b). This was found in tail artery rings from both hypertensive and normotensive rats, and presumably was a consequence of the small size of the vessel, where the insertion of two wires into the lumen resulted in substantial endothelial damage despite careful dissection. Alternatively, or in addition, the presence of wires in the lumen may have reduced the access of drugs to the endothelial layer while leaving access to the muscle layers unaffected, but this is unlikely. It may therefore have been better to carry out this part of the study on a myograph, rather than in a 25ml organ bath, as the insertion of much finer wires (40μm diameter) into the vessel lumen would have permitted investigation of endothelium-dependent responses in a vessel of this size.

There have been studies on the effects of vasoconstrictors, mainly NA, in tail arteries from spontaneously hypertensive rats. Unfortunately, many different experimental protocols have been used (helical strips, perfused segments and ring preparations), in the absence and presence of drugs which inhibit various adrenergic and non-adrenergic processes (cocaine, 6-hydroxydopamine (6-OHDA), propranolol, indomethacin). Consequently, the results in the literature encompass both an increased (Medgett, Hicks & Langer, 1984) or decreased (Webb, Vanhoutte & Bohr, 1981) maximum response to NA in the tail artery of the SHR. Similarly, the EC_{50} for NA has been reported to be increased (Mulvany et al.., 1982), reduced (Hermsmeyer, 1976) or unchanged (Fouda et al.., 1987) in the isolated tail artery of SHR compared to WKY.

Part of the present study was therefore designed to illustrate how the way in which an experiment is carried out can influence the results obtained. It was shown that in the rat isolated tail artery the increase in isometric tension in response to NA was greater in artery rings from SHR compared to those from WKY. However, when tension responses were expressed as a percentage of the maximum, this difference disappeared, giving identical concentration-response curves for SHR and WKY . This observation indicates that the adrenoceptor affinity for NA in the rat tail artery is unchanged in the SHR. When responses to NA were measured as increases in perfusion pressure following lumenal perfusion of NA, the response to the highest concentration of NA was much greater in SHR compared to WKY. Consequently, when these values were expressed as a percentage of the maximum response achieved, the concentration-response curve for SHR lay to the right of that for WKY. Careful interpretation of this result is therefore required, as this would seem to indicate subsensitivity to NA in the SHR, but it merely reflects that the response in the SHR is a much smaller fraction of an increased maximum response in the perfused vessel, which is perhaps amplified by structural alterations leading to an increased wall-to-lumen ratio.

In this study, the increased contractile response to PE and NA in rings of rat tail artery from SHR was associated with an increase in the maximum response compared to control. This is therefore suggestive of a change beyond agonist-receptor interaction. Such a distal event may be PI hydrolysis, since this second messenger pathway is known to exist in the rat tail artery where both NA and adrenaline caused an increase in [^{3}-H]-inositol phosphate accumulation (Fox, Abel & Minneman, 1985; Nally, 1990).

This study demonstrated that NA produced a concentration-dependent increase in PI hydrolysis in tail arteries from both hypertensive and control rats. In addition, NA was shown to produce a greater stimulation of PI

hydrolysis in SHR compared to WKY, at a concentration (10^{-5}M) which produced an increase in the contractile response in tail artery rings from hypertensive rats relative to control. Furthermore, unstimulated, basal levels of inositol phosphates were enhanced in SHR relative to control. Indeed, it has been shown that the activity of phospholipase C, the enzyme which cleaves $PtdInsP_2$ and initiates the cycle of inositol phosphate hydrolysis, is enhanced in the vascular wall of SHR (Uehara et al., 1988).

It seems likely therefore, that increased (basal and stimulated) PI hydrolysis has a role in the vascular abnormalities associated with hypertension. In addition to the increased $InsP_3$ produced, which liberates Ca^{2+} from intracellular stores (Berridge, 1987) and may therefore mediate enhanced contraction, concomitant raised DAG levels may have a role in the vascular hypertrophy associated with hypertension (Marin, 1993). Aalkjaer (1990) showed that protein kinase C stimulation by DAG activates Na^+ - H^+ exchange on the vascular smooth muscle membrane, increasing cell alkalinity. This rise in intracellular pH is thought to be a stimulus for cell growth (Owen, 1984), and it has recently been shown that activity of the Na^+ - H^+ antiporter is increased in cultured vascular smooth muscle cell from stroke-prone SHR (Kobayashi et al., 1990). Therefore, increased membrane phosphoinositide hydrolysis may be involved in both the increased contractile responses and vascular hypertrophy in hypertension observed in this study.

It is important to note however, the temporal characteristics of the increased PI response. Heagerty, Ollerenshaw & Swales (1986) showed that in rat aorta from 5-week old SHR, both basal and NA (10^{-4} M)-stimulated [^{3}H]-inositol phosphate accumulation were enhanced relative to WKY, but by 19 weeks basal levels were similar, whereas NA-stimulated accumulation was reduced in SHR aortae relative to WKY. It was proposed that increased PI

hydrolysis is an important signal in the early stages of hypertension (Heagerty, Ollerenshaw & Swales, 1986; MacIver et al., 1993). In addition, different patterns of PI hydrolysis in hypertension may occur, depending on the type of blood vessel (Vila, MacRae & Reid, 1991, MacIver et al., 1993) or model of hypertension studied (MacIver et al., 1993).

Compared to the α_1-adrenoceptor agonists NA and PE, there is little evidence available concerning the action of α_2-adrenoceptor agonists on PI hydrolysis. In the present study, the increased mechanical response to the alpha$_2$-agonist UK-14,304 in tail artery rings from SHR compared to those from WKY was less marked than the increase observed with NA or PE. Indeed, the apparent enhanced response to UK-14,304 in SHR tail artery rings disappeared when the increase in tissue weight in hypertension was accounted for. Furthermore, previous work in this laboratory (Dowell, 1990) showed that there was no difference in the contractile response to KCl in tail arteries from SHR and WKY rats, following correction for the increased tissue weight in SHR.

In the present investigation therefore, combining evidence from histological, biochemical and mechanical studies, the following hypothesis is proposed; that the increased contractile response to adrenergic agonists in tail artery rings from SHR can be explained, at least in part, by a combination of changes in vascular structure and an enhancement of the second messenger system underlying α_1-adrenoceptor-mediated contraction, i.e. PI hydrolysis.

The present study showed that, in contrast to the rat aorta, two vasodilators with different mechanisms of action (SNP and isoprenaline) had a reduced ability to relax tail artery rings from SHR compared to those from

WKY. SNP is metabolically degraded within the vascular smooth muscle cell to liberate NO, which raises cyclic GMP levels by direct activation of soluble guanylate cyclase. Isoprenaline increases cellular cyclic AMP via activation of $ß_2$-adrenoceptors on the smooth muscle cell membrane leading to adenylate cyclase stimulation.

Defects at any level in the pathways of the generation or effects of these cyclic nucleotides are possible, and have not been described specifically for the rat tail artery in the literature as yet. A study by Silver, Michalak & Kocmund (1985) of several rat blood vessels found that in the rat tail artery, basal protein kinase A (PKA) activity was found to be similar between SHR and WKY rats. Unfortunately however, the study did not measure either relaxation or PKA activity stimulated by isoprenaline or forskolin in the rat tail artery. The present study therefore is important as it represents one of the first reports concerning relaxation of the rat tail artery, and in particular, the impaired relaxation of a resistance vessel in hypertension.

An interesting observation in the present investigation was that isoprenaline was unable to completely relax tail artery rings from both hypertensive and normotensive rats. The endothelium-dependent vasodilator, carbachol, was unable to relax vessels from either group of rat, suggesting that these preparations were endothelium-denuded. The results with isoprenaline may be connected to this observation.

Recent studies in conduit arteries support a role for the endothelium and NO in ß-adrenoceptor-mediated relaxation (Kamata, Miyata & Kasuya, 1989; Gray & Marshall, 1991), but there is some disagreement (Moncada et al, 1991). A detailed study in rat mesenteric resistance arteries however, revealed that in this vessel, $ß_1$-adrenoceptor activation causes relaxation via NO release from the endothelium (Graves & Poston, 1993). It is possible therefore, that the failure of isoprenaline to fully relax PE-constricted tail

arteries from SHR and WKY resulted from endothelial damage during tissue manipulation, and consequent removal of a putative site of action for isoprenaline.

SNP-induced relaxation of SHR tail artery rings was reduced compared with rings from WKY. In the aorta the opposite pattern was shown and it was suggested that the increased responsiveness to SNP in SHR may have been due to increased sensitivity of guanylate cyclase. Clearly then, if such a phenomenon exists, it may be vessel-specific, with not all vascular beds becoming supersensitive to NO in hypertension.

In any case, it appears that relaxation in the rat aorta and tail artery are differently affected in hypertension, as is the vasoconstriction. This is perhaps not surprising when the different natures of these vessels are considered. The aorta is a large elastic artery specialised for conducting blood flow. The tail artery of the rat is a much smaller vessel, specialised for contracting, and is highly innervated. Although some workers consider the tail artery as a conduit vessel, it is clearly regulated differently from the aorta in hypertension, and is certainly more representative of a resistance vessel than the aorta.

In summary, this study showed that in SHR compared to WKY, aortic rings were less responsive to vasoconstrictors, but more responsive to vasodilators. The rat tail artery however, was more responsive to vasoconstrictors, primarily those which activate PI hydrolysis, and showed a reduced ability to relax. In addition, although vessel thickening was observed in SHR aortae, an increase in the wall to lumen ratio was only observed in the tail artery from SHR. Thus, it appears that the changes which occur in the tail artery in hypertension are consistent with mechanisms which are associated with an increase in peripheral resistance and hence blood pressure.

3. RESPONSES TO FIELD STIMULATION AND VASOCONSTRICTORS IN TAIL ARTERIES FROM RATS OF DIFFERENT AGES

Preliminary experiments with normotensive Wistar rats of different ages demonstrated that there was a positive correlation between rat age and weight, and that systolic blood pressure tended to increase with age. This latter observation was not statistically significant, but it is likely that a greater separation of age groups or a larger population would have yielded a significant correlation. Indeed, in most large scale studies of the human population, systolic blood pressure tends to increase with age throughout life (Robertson, 1989).

Initial experiments suggested that isolated, perfused tail arteries from older rats were more responsive to exogenous NA than were vessels from younger rats (Figure 5). It is possible that this may reflect a thickening of the vascular walls, which is known to occur with ageing in rats (Docherty, 1990). However, this is unlikely since there was no difference in responsiveness when tail arteries were cannulated at both ends and perfused with NA (Figure 7).

The possibility that the route of administration of exogenous NA may have led to a difference in response between vessels from older and younger rats was considered. In the first set of experiments (when responses from older rats were greater than those from younger rats), tail arteries were cannulated at one end and the NA solution, which was perfused luminally, was allowed to superfuse over the muscle surface. In such a situation, the NA would perhaps be more susceptible to neuronal uptake than when a second cannula carried the perfusing NA out of the organ bath without allowing superfusion. If uptake 1 decreases with age, a reduction in this inactivation mechanism for

NA may therefore partly explain the increase in response in older animals. Although the evidence for a decline in neuronal uptake with age is equivocal (Docherty, 1990), there is evidence that in the rat tail artery, the uptake blocker cocaine had a reduced ability to potentiate responses to adrenergic nerve stimulation in ageing (Duckles, 1987), indicating reduced uptake. This might also explain the observation in this study that a greater response to field stimulation was obtained in tail arteries from older rats compared to responses in those from younger rats.

When the single cannulation experiment was repeated to investigate this phenomenon further, surprisingly there was now no difference in the response to exogenous NA with age (Figure 11). There was however a small difference in the set-up, as an additional heating coil was introduced between the NA reservoir and the cannula. It is known that temperature can influence a number of processes involved in vascular control, for example in the dog saphenous vein, increases in α_2-adrenoceptor- and ATP-mediated constriction were shown when the temperature was reduced from 37°C to 24°C. (Flavahan and Vanhoutte, 1986). In addition, some of these processes are affected by age, for example both pre- and post-junctional α_2-adrenoceptor-mediated effects decrease with age in the rat tail artery (Buchholz et al., 1992).

However, it is unlikely that the addition of a heating coil to the perfusing NA solution created a temperature difference sufficient to account for the experimental result, especially since each preparation was in a Krebs-filled organ bath maintained at 37°C.

Caution must therefore be exercised in interpreting these results, and although a number of interesting observations have been made, no firm conclusions can be drawn from these experiments on the effects of age on vascular control.

4. ALPHA$_2$-ADRENOCEPTOR-MEDIATED CONTRACTIONS IN VASCULAR SMOOTH MUSCLE

The involvement of post-synaptic α_2-adrenoceptors in the regulation of the responsiveness of vascular smooth muscle is an area of research which has generated increasing interest over the last few years. As both α_1- and α_2-adrenoceptors can mediate vasoconstriction in most vascular beds (Timmermans, Chiu & Thoolen, 1987), much of the interest has focused on possible alterations in receptor number and affinity in hypertension. However, the evidence to date has been unconvincing (Michel, Brodde & Insel, 1990; Marin, 1993). Using EC_{50} concentrations for NA, PE and UK-14,304 as an index of adrenoceptor affinity, the present study showed no evidence of altered adrenoceptor number or affinity in either the aorta or tail artery from hypertensive rats as compared to control. In addition, the α_2-adrenoceptor antagonist, yohimbine, produced rightward shifts of the lower end of the UK-14,304 concentration-response curves in both SHR and WKY rat tail arteries, indicating that contractions to the lower concentrations of UK-14,304 were indeed mediated by α_2-adrenoceptors. However, the rightward shifts were of equal magnitude between the two groups of rat, suggesting no altered α_2-adrenoceptor density or affinity in hypertension.

In this study, experiments in normotensive Wistar rats with the α_2-adrenoceptor agonist clonidine, and the more selective compound UK-14,304, revealed that the rat tail artery contracted in response to these agents in a concentration-dependent manner. However, in the proximal tail artery, only the responses to low ($\leq 10^{-7}$M) concentrations of clonidine were reduced by the α_2-adrenoceptor antagonist yohimbine. In distal segments of the vessel, UK-14,304 produced a biphasic curve. This became monophasic in the

presence of yohimbine, which markedly reduced the lower part of the curve, and resembled the control UK-14,304 concentration response curve of the proximal tail artery. These results are similar to findings in the dog mesenteric vein, where UK-14,304 produced a biphasic concentration-response curve, the lower end of which was blocked by the α_2-adrenoceptor antagonist rauwolscine (Shimamoto et al., 1992). Hence, it seems likely that only contractions to low concentrations of UK-14,304 are mediated via α_2-adrenoceptors in the rat tail artery. This may be explained by binding studies in the dog mesenteric vein, which revealed that UK-14,304 exhibited a 10-fold difference in affinity for displacing [^{3}H]- rauwolscine or [^{3}H]-prazosin from membranes (Daniel et al., 1991). Thus, UK-14,304 in higher concentrations can bind to α_1-adrenoceptors. However, the α_2- vs. α_1- adrenoceptor selectivity of UK-14,304, as demonstrated in a variety of functional in vitro assays, is much higher than the 10-fold difference detected by the radioligand binding experiment (Shimamoto et al., 1992). UK-14,304 can therefore be regarded as a potent and selective α_2-adrenoceptor agonist (Cambridge, 1981).

It is noteworthy that in the present study, UK-14,304 was more potent in distal regions of the rat tail artery than in more proximal sections, with a threshold of 0.1nM in distal compared to a 3-10nM in proximal regions. This is in agreement with a previous report in the rat tail artery which showed that adrenaline was more potent in distal than in proximal segments. In addition, the α_2-adrenoceptor antagonist idazoxan was more effective in distal than in proximal regions against adrenaline-induced constriction (Rajanayagam & Medgett, 1987). Therefore, it is likely that smooth muscle α_2-adrenoceptors are more prevalent in the distal than in the proximal end of the rat tail artery. This may have functional significance in the thermoregulatory properties of the rat tail, where cooling has been shown to enhance α_2-adrenoceptor-mediated constriction (Harker et al., 1991). Such a receptor distribution would

favour constriction of the more distal regions of the vascular bed in response to a drop in temperature, thus conserving heat nearer to the body.

Potentiation of alpha$_2$-adrenoceptor-mediated contractions in the rat tail artery

It is only relatively recently that α_2-adrenoceptors have been accepted as mediators of vascular smooth muscle contraction. This has partly arisen because these receptors have often proved to be "elusive" and evidence for their existence is derived from functional studies, in which various techniques have been used to "uncover" them. For example, in the rabbit saphenous artery, α_2-adrenoceptor-mediated responses were only revealed when the vessel was stimulated with an agonist which acted via a different receptor system (Dunn, McGrath & Wilson, 1991). These observations raise several questions, especially about what the term "uncover" means. For example, are α_2-adrenoceptor recognition sites literally uncovered by these treatments, or are they present in the membrane but normally not coupled to the intracellular mechanism, through which they operate, until $[Ca^{2+}]_i$ is raised? A third possibility is that these receptors are normally present in the membrane and are coupled, but special conditions are required to demonstrate their existence and functional capacity. This study examined the third possibility and considered the conditions required to "uncover" the α_2-adrenoceptor-mediated contractile mechanism in the rat tail artery.

An experimental protocol was established, based on previous investigations which had revealed α_2-adrenoceptor-mediated responses following vascular preconstriction (Templeton, 1988; Templeton et al. 1989; MacLean & McGrath, 1990). The present study investigated whether the ability of a contractile agonist to enhance α_2-adrenoceptor-mediated

contractions was determined by its mechanism of action. It was shown that responses to low concentrations of clonidine (10nM) or to UK-14,304 (50nM) were enhanced in the presence of AVP, PE or 5-HT, agonists known to increase $[Ca^{2+}]_i$ by causing PI hydrolysis (Cobbold et al., 1990). However, responses to clonidine (10nM) were also enhanced in the presence of KCl, which depolarises vascular smooth muscle cells causing Ca^{2+} entry. Therefore, stimulation of the PI cycle by a precontracting agonist is not essential in producing enhanced α_2-adrenoceptor-mediated responses. This finding is consistent with a previous report on the rat tail artery, where contractions to the α_2-agonist B-HT 920 were augmented in the presence of either KCl or prostaglandin $F_{2\alpha}$ (Harker et al., 1991).

It must be noted however, that an important difference exists between the present study and previous reports concerning α_2-adrenoceptor "uncovering" in blood vessels. That is, in the present study, preconstriction was relaxed by a vasodilator before addition of the α_2-agonist to the tissue. The working hypothesis underlying this protocol was based on the finding that in a wide variety of tissues (platelets, neuroblastoma cells, renal cortex cells, pancreatic islet ß-cells), inhibition of adenylate cyclase has been proposed as the primary α_2-adrenoceptor coupling mechanism (Ruffolo et al., 1991). Clearly then, the capacity of clonidine or UK-14,304 to activate α_2-adrenoceptors would be more apparent if adenylate cyclase was stimulated. Studies have shown that adenylate cyclase can be activated by Ca^{2+} (Harrison, Hewlett & Gnegy, 1989). It is possible then that during the precontraction, which elevates intracellular Ca^{2+} levels, adenylate cyclase is activated. This therefore would provide an opportunity for an α_2-adrenoceptor agonist to exert its effect in inhibiting the enzyme and may partially explain why α_2-adrenoceptor-mediated contractions are better observed following preconstriction. In this investigation, IBMX was used to amplify the adenylate

cyclase/cyclic AMP-mediated inhibitory mechanism, by inhibition of cyclic nucleotide phosphodiesterase.

IBMX however is a relatively non-specific compound, as it inhibits the phosphodiesterases which metabolise cyclic GMP as well as cyclic AMP, and possesses a number of other actions unrelated to its phosphodiesterase activity, such as inhibition of agonist-induced prostaglandin synthesis (Spry et al, 1985). Subsequent experiments revealed that responses to UK-14,304 were also enhanced following AVP-induced preconstriction which was relaxed by more specific agents. These were forskolin, which activates adenylate cyclase (Seamon & Daly, 1983), SNP, which activates guanylate cyclase following metabolic activation to NO in vascular smooth muscle (Kowaluk, Seth & Fung, 1992), and the stable, cell-permeant cyclic AMP analogue, dibutyryl cyclic AMP.

The results of the present study suggest that, in the described protocol, the effect of α_2-receptor stimulation is to reverse the effect of the vasorelaxant used to inhibit the precontraction. Knowing the mechanism of action of the vasodilator, whose effects were shown to be reversed by clonidine or UK-14,304, may therefore provide information on the mechanisms stimulated by α_2-receptor activation.

It is clear from these results therefore that the enhanced responses to clonidine and to UK-14,304, obtained in the presence of AVP and any one of these vasorelaxants, does not simply result from inhibition of adenylate cyclase via α_2-receptors. This is because clonidine and UK-14,304 reversed the effects of vasodilators which do not rely on adenylate cyclase activation to relax blood vessels. The reversal of forskolin-induced relaxation by UK-14,304 is consistent with inhibition of adenylate cyclase by α_2-adrenoceptor stimulation. It does not however rule out the possibility that UK-14,304 opposed the effects of the cyclic AMP synthesised during forskolin-induced

adenylate cyclase activation. The results with dibutyryl cyclic AMP indicate that α_2-adrenoceptor stimulation can indeed oppose the effects of cyclic AMP, rather than (or in addition to) its synthesis. In addition, results with SNP, the guanylate cyclase activator, indicate that α_2-adrenoceptor activation can reverse either the synthesis, or more likely the effects, of cyclic GMP.

To understand how α_2-adrenoceptor activation may reverse cyclic nucleotide-mediated relaxation in the rat tail artery, it is important to understand how the cyclic nucleotides can cause vascular smooth muscle relaxation. Currently, cyclic GMP is thought to relax muscle by reducing $[Ca^{2+}]_i$, via activation of PKG, but the mechanism by which PKG acts is still not understood (Lincoln & Cornwell, 1993). Mechanisms which have been reported include :- (1) Inhibition of $InsP_3$ generation (Rapoport, 1986); (2) stimulation of intracellular Ca^{2+} sequestration (Lincoln, 1983); (3) Reduced phosphorylation of myosin light chain (Rapoport, Draznin & Murad, 1983); (4) Inhibition of voltage- and receptor- operated Ca^{2+} channels (Godfraind, 1986; Lincoln, 1989); (5) stimulation of membrane Ca^{2+}-ATPase (Fiscus, 1988) and (6) increase of K^+ permeability through K^+ channels causing membrane hyperpolarisation (Komori & Suzuki, 1987; Lincoln & Cornwell, 1993).

Many of these actions are similar to, or shared by, the actions of cyclic AMP in relaxing vascular smooth muscle. This is perhaps not surprising when it is considered that, in addition to activating PKA, cyclic AMP can also activate PKG under physiological conditions (Jiang et al., 1992; Lincoln & Cornwell, 1991). It is likely that cyclic AMP regulates smooth muscle relaxation, at least in part, by activating PKG.

How then can α_2-adrenoceptor activation reverse the powerful relaxations induced by cyclic nucleotides in the smooth muscle of the rat tail artery? Little is known about α_2-adrenoceptor coupling in the vasculature to date (Aburto, LaJoie & Morgan, 1993). Although inhibition of adenylate cyclase has been demonstrated in many cell types following α_2-receptor activation (Ruffolo et al., 1991), this mechanism does not explain many of the physiological effects of α_2-receptor stimulation (Aburto, LaJoie & Morgan, 1993). Other signal transduction pathways for α_2-adrenoceptors include mobilisation of intracellular Ca^{2+} (Nielsen et al., 1992; Michel et al., 1989), modulation of Ca^{2+} channels and Ca^{2+} influx (Timmermans , Chiu & Thoolen, 1987; Daly et al., 1990) and activation of the Na^{+}-H^{+} antiporter (Limbird, 1988). This latter effect has been shown to increase vascular tone by a Ca^{2+}-dependent mechanism (Marin, 1993). Such mechanisms activated by α_2-adrenoceptors could conceivably cause contraction, and have the potential to oppose the inhibitory mechanisms of the cyclic nucleotides. It must be remembered however that in the present study, rat tail artery rings were first precontracted; both processes, contraction and relaxation, must be considered together in producing the conditions required to reveal α_2-adrenoceptor-mediated responses.

Several observations in this study showed that the potentiated responses to the α_2-agonist were dependent on the initial precontraction. Firstly, positive correlations were demonstrated between the size of the response to the precontracting agonist (AVP, PE or KCl), and the size of the enhanced response to clonidine or UK-14,304 obtained in the presence of the precontracting agonist and IBMX. Secondly, the size of the enhanced α_2-mediated response decreased as the time between vessel pretreatment (AVP and IBMX) and UK-14,304 addition increased; this was strongly paralleled by the reduction in the response to AVP alone with time. Finally, the enhanced

responses to UK-14.304, obtained in the presence of a precontracting agonist (AVP, PE or 5-HT) and IBMX, were abolished by an antagonist selective for the precontracting agent (MC-AVP, prazosin, or ketanserin, respectively). This is similar to an observation by Zimmerman & Kraft (1979), who found that the facilitatory effect of AII on α_2-adrenoceptor responses in the rabbit isolated saphenous vein was blocked by the AII receptor antagonist, saralasin.

An important finding of this study is that raised tone per se is not necessary for the "uncovering" of post-junctional α_2-adrenoceptors, since α_2-stimulation produced a large response when vasodilators (IBMX, forskolin, SNP or dibutyryl cyclic AMP) reduced the AVP-induced tone to basal levels.

Several studies have demonstrated that α_2-receptor mediated responses were unmasked following elevation of vascular tone (Furuta, 1988; Templeton et al., 1989; MacLean & McGrath, 1990). However, post-junctional α_2-receptor-mediated contractions have also been revealed in the presence of concentrations of agonists which did not cause contraction by themselves (Sulpizio & Hieble, 1987; Dunn, McGrath & Wilson, 1989). Therefore, it seems that the conditions currently operating within the vascular smooth muscle cell are more important than the absolute level of tone produced. Vascular tone is a net result of the sum of positive and negative influences. It would appear that, following the protocol of the present study, the vascular smooth muscle cell is "primed", with excitatory and inhibitory mechanisms in balance, and that this balance is upset by the stimulation of α_2-adrenoceptors. It may be that the "potentiators" in this and other studies produce a particular pattern of effects on the signalling process which selectively synergises with the coupling involved in α_2-adrenoceptor-activated processes.

Several pieces of evidence from this study demonstrated that the enhanced contractions to clonidine or to UK-14,304 were mediated by

adrenoceptors of the α_2-subtype. Low concentrations of clonidine (10nM) and UK-14,304 (50nM) were used which were sub-threshold or just threshold for contraction, and which lay on the yohimbine-sensitive part of the concentration-response curve. Therefore, the large contractions observed following pretreatment were unlikely to have been due to a non-selective α_1-adrenoceptor stimulation. In addition, all enhanced responses were inhibited by a low concentration of yohimbine (50nM) likely to be selective for α_2-adrenoceptors. Moreover, even if clonidine or UK-14,304 did act on α_1-adrenoceptors, the enhanced response was unaffected by prazosin, showing the potentiation to be mediated by α_2-adrenoceptors. The only case where the enhanced response to UK-14,304 was prazosin-sensitive, was when the precontracting agonist was PE. Furthermore, responses to the α_1-adrenoceptor agonist phenylephrine were not potentiated in the present investigation. This finding agrees with the results of Templeton et al. (1989), who demonstrated that responses to UK-14,304, but not those to PE, were enhanced following AVP-induced preconstriction in the rat tail artery. Indeed, several other studies support a preferential interaction between AII and responses mediated via post-junctional α_2-adrenoceptors rather than α_1-adrenoceptors (Schumann & Lues, 1983; Dunn, McGrath & Wilson, 1991; Dunn et al., 1991a).

An interesting study by Xiao and Rand (1989) revealed that vasoconstrictor responses in the rat tail artery to AVP, KCl, NA and PE were enhanced by α_2-adrenoceptor agonists. In addition, Shepperson (1984) demonstrated potentiation of a PE-induced contraction by UK-14,304 in a non-vascular smooth muscle preparation, the nictitating membrane of the cat. Evidently then, the ability of an agonist response to be potentiated does not depend on its ability to stimulate α_2-receptors.

Indeed, these findings prompt a new or alternative interpretation of the results in the present study. In this study, responses to AVP, 5-HT, PE and

KCl were inhibited by vasorelaxants, i.e. responses to the agonists at this point were minimal. Subsequent stimulation of α_2-adrenoceptors (by clonidine or UK-14,304) then produced a large contraction which was sensitive to an antagonist of the precontracting agent. It could be argued therefore that stimulation of α_2-adrenoceptors potentiated the response to the precontracting agonist, which was minimal in the presence of the vasorelaxant. It is possible therefore that the enhanced response, which was certainly triggered by α_2-receptor activation, was a continuation of the precontraction, which had been "interrupted" by the vasorelaxant.

Because of the apparently reciprocal nature of the potentiation, it is probable that the two receptor types involved reside on the same cells, and interact at the level of a common post-receptor site in the events leading to contraction. A mechanism which is becoming increasingly recognised as important in α_2-receptor-mediated signal transduction is the influx of extracellular Ca^{2+} (Aburto, LaJoie & Morgan, 1993; Daly et al., 1990) via both dihydropyridine-sensitive and dihydropyridine-resistant Ca^{2+} channels (Dunn et al., 1991b). It is perhaps not surprising therefore that such a wide variety of agents, in the present study and in the literature (AVP, PE, 5-HT, KCl, NA, AII, Bay K 8644) can potentiate α_2-adrenoceptor-mediated contractions. All of these agonists elevate intracellular Ca^{2+} levels by intracellular release and/or membrane translocation. The Ca^{2+} which enters the cell following α_2-receptor stimulation may then synergise with the Ca^{2+} raised by the agonists, to produce an enhanced activation of the contractile proteins.

This proposal however is too general to explain why, for example, responses to PE cannot be potentiated by AVP (Templeton et al, 1989; present study). A further property of α_2-agonists is the ability to increase the Ca^{2+}-

sensitivity of the contractile apparatus in vascular smooth muscle (Aburto, LaJoie & Morgan, 1993). These authors showed that significantly elevated force could be maintained in the presence of resting $[Ca^{2+}]_i$ levels. It is possible therefore that in the present experiments, when resting tone (and presumably resting $[Ca^{2+}]_i$ levels) was basal following preconstriction and relaxation, α_2-receptor activation increased the contractile apparatus sensitivity such that tone was restored. This would indeed imply that the α_2-agonist enhanced the response to the precontracting agent, rather than the reverse. Sensitisation of the contractile apparatus by α_2-receptor stimulation however does not explain why UK-14,304 or clonidine produced little or no contraction when added alone.

It seems then that the present explanations are insufficient to account for potentiation of α_2-adrenoceptor-mediated responses both in this study and in the literature. It is clear that synergy occurs between responses mediated by α_2-receptors and other receptor types. What is not evident as yet is exactly where this synergy occurs, in the pathways leading to vascular smooth muscle contraction.

This study has thus given an insight into the complex nature of the regulation of vascular smooth muscle by post-junctional α_2-adrenoceptors. The results emphasise the dynamic nature of the events within the vascular smooth muscle cell, illustrating interactions involving a wide variety of agents to create conditions in the cell such that the final response is effected.

5. NEUROTRANSMISSION IN THE RAT TAIL ARTERY

One of the major vascular control systems addressed in this study was the nature of the neurotransmission in the rat tail artery. This vessel receives a dense adrenergic innervation, but the question of whether ATP functions as a co-transmitter together with the main transmitter NA in this vessel remains unresolved.

In this study, exogenous addition of both NA and ATP produced contraction in the rat tail artery, fulfilling one of the criteria for a neurotransmitter (Eccles, 1964) and confirming the findings of other studies (Bao, Erikson & Stjarne, 1989c; Bao, 1993; Dalziel et al., 1990). Responses to exogenous NA and ATP were inhibited by prazosin and α,β-MeATP respectively, indicating that they were mediated via α_1-adrenoceptors and P_{2x}-purinoceptors respectively. α,β–MeATP produced an initial contraction as it is in fact a potent agonist. Its blocking action is thought to arise from desensitisation of the P_{2x}-purinoceptor (Meldrum & Burnstock, 1983; Sneddon & Burnstock, 1984b; Kennedy, Saville & Burnstock, 1986).

Electrical field stimulation produced a frequency-response curve (0.1-40Hz) which appeared biphasic, reaching a plateau at 5-10 Hz, with responses increasing again at the higher frequencies, 20-40Hz. It is possible that this increased response at higher frequencies is due to an action by neuropeptide Y (NPY). NPY is often co-stored with NA in the large dense cored vesicles (LDVs) of sympathetic nerve endings (Stjarne, 1989), and can be co-released with NA by nerve stimulation (Lundberg et al., 1984). NPY by itself produces little or no contractile response (MacLean & McGrath, 1990; Bao, 1993), but has been shown to potentiate the response to field stimulation at 20 Hz in the rat tail artery (Bao, 1993). In addition, there is some evidence that high-

frequency stimulation favours the secretion of neuropeptide-containing vesicles (Stjarne, 1989). Unfortunately however, the lack of antagonists specific for NPY to date meant that this possibility was not investigated further in the present study, and must therefore remain speculative.

Contractile responses to field stimulation at supramaximal voltage (50V) and 0.1ms pulse duration in tail artery rings from Wistar rats were shown to be abolished by TTX, confirming that responses were neurogenic in origin. Thus these stimulation parameters (50V, 0.1ms) did not result in direct activation of the smooth muscle, and were used throughout the study.

Field stimulation-induced contractions were abolished at all frequencies (0.1-40Hz) by prazosin (0.1μM), with no evidence of a residual response. In contrast, α,β-MeATP had no effect on any part of the frequency-response curve. The possibility that P_{2x}-receptors were not desensitised was ruled out by the observation that repeated addition of α,β–MeATP did not produce a response apart from the initial transient contraction, but subsequent frequency-response curves remained unaffected by α,β–MeATP. These results therefore suggest that the field stimulation-induced contraction in rat tail artery rings is mediated solely by NA acting on post-synaptic α_1-adrenoceptors. However, experiments with a lower concentration of prazosin (5nM), revealed a prazosin-resistant component, which was completely abolished by yohimbine (50nM), indicating the involvement of post-junctional α_2-adrenoceptors in the response to sympathetic stimulation in the rat tail artery. This conclusion was supported by the observation that yohimbine alone caused a small but significant reduction in the amplitude of the response. Indeed, it is likely that this inhibitory effect of yohimbine on post-junctional α_2-adrenoceptors was underestimated, due to the well-documented pre-synaptic action of yohimbine to increase transmitter release by blockade of

auto-inhibition (Starke, 1981). These results support the growing awareness that the α_2-adrenoceptors are both (1)located on vascular smooth muscle and (2)involved in the contractile response to sympathetic nerve stimulation (Rajanayagam, Medgett and Rand, 1990; Medgett, 1985). Previously, it was generally accepted that α_2-adrenoceptors were mainly located on nerve terminals, and any post-junctional α_2-adrenoceptors were extrasynaptic, mediating responses to circulating catecholamines, whereas neurally-released NA acted on post-junctional α_1-receptors (Timmermans & Van Zwieten, 1982; Jie et al., 1987). However, recent data indicate that both α_1- and α_2-adrenoceptors mediate contraction in response to sympathetic nerve stimulation in human and rat resistance vessels (Ohyanagi, Faber & Nishigaki, 1991; Parkinson et al., 1992), confirming the results of the present study.

In addition to the α_2-adrenoceptor antagonist yohimbine, the α_2-receptor agonists clonidine and UK-14,304 also reduced the amplitude of the field stimulation-induced contraction in the present study. This is probably as a consequence of the well-documented role of presynaptic α_2-receptor activation to reduce transmitter output (Starke, 1981; Starke, 1987). However, increasing evidence indicates that clonidine and UK-14,304 may also decrease transmitter release by activating presynaptic imidazoline binding sites (Kalsner, 1985; Gothert and Molderings, 1992).

The α_2-receptor agonists clonidine and UK-14,304, and the antagonist yohimbine, although all reducing the amplitude of the response, had different effects on the duration of the field stimulation-induced response, which was prolonged by clonidine and UK-14,304, but curtailed slightly by yohimbine.

It is possible that this reflects the proposed effects of α_2-receptor stimulation on Ca^{2+} influx on vascular smooth muscle, which may somehow act to facilitate or prolong the post-synaptic response to released transmitter.

However, this must remain purely speculative in the absence of data on presynaptic transmitter output.

Conditions previously described in this study which potentiated post-synaptic α_2-receptor-mediated contractions, i.e. precontraction with AVP and subsequent relaxation by IBMX, were shown to prolong the response to field stimulation at 10 Hz. In the presence of prazosin, at a concentration which abolished control stimulation-induced contractions, a field stimulation-induced contraction was revealed following AVP and IBMX pretreatment. This prazosin-resistant contraction was biphasic, with an initial spike followed by a more prolonged contraction. Yohimbine abolished the second phase, indicating that an α_2-adrenoceptor-mediated contraction had been uncovered in response to field stimulation, in a manner analogous to the response to exogenous α_2-agonists as previously described. Interestingly however, the prazosin- and yohimbine-resistant spike which remained was abolished by α,β–MeATP. This therefore suggests that ATP is indeed released from sympathetic nerves in the rat tail artery, and under certain circumstances, can mediate contraction.

Since the effects of neurally-released ATP were only revealed following blockade of α_1-adrenoceptors and conditions conducive to observing post-synaptic α_2-mediated effects, it is possible that α_2-receptor activation facilitated the contractile effects of ATP. This proposal is consistent with the findings of Xiao & Rand (1989), who demonstrated that contractions to ATP in the rat tail artery were enhanced by UK-14,304. However, in the present study, the contraction to ATP (i.e. that inhibited by α,β–MeATP) was not dependent on α_2-receptor activation since it persisted in the presence of yohimbine. Presumably, AVP and IBMX create conditions in the cell such that both α_2- and ATP-mediated contractions are potentiated in a similar manner.

In this study, responses to field stimulation were investigated in tail artery rings from SHR and WKY rats. Frequency-response curves from both groups of rat were biphasic, as was described for Wistar rats. Responses at all frequencies were greater in vessels from hypertensive rats compared to control, when expressed as the absolute tension developed. However, when responses were expressed as a percentage of the maximum response to PE, only the responses at the lower frequencies (0.1 & 0.2 Hz) were increased in SHR relative to control. Therefore, the increase in response at frequencies above 0.2 Hz can be explained by the increase in both tissue weight and responsiveness to α_1-adrenergic stimulation in the SHR previously demonstrated in this study.

These observations suggest that there is some component other than α_1-adrenoceptor-mediated contraction which contributes to the field stimulation-induced motor response in the SHR. Previous reports have suggested that the role of post-junctional α_2-receptors in neurogenic contraction is increased in hypertension (Medgett, Hicks & Langer, 1984). In addition, studies have shown that the receptors involved in the field stimulation-induced contractions in the rat tail artery vary with the stimulation parameters, such that the α_2- component is greater at lower frequencies (Bao, Eriksson & Stjarne, 1990). The results from this study with Wistar rats show that α_2-adrenoceptors mediate part of the field stimulation-induced contraction at higher frequencies, since yohimbine inhibited the response to trains of stimuli at 10 Hz. It is possible therefore, that an α_2- component exists at all frequencies of stimulation in tail artery rings from normotensive (Wistar, WKY) and hypertensive (SHR) rats, but is increased in SHR relative to WKY only at lower frequencies. However, this conclusion must remain speculative, as the effects of adrenergic antagonists were not assessed in SHR or WKY rats.

The purinoceptor desensitising agent α,β–MeATP had no effect on the frequency-response curve in WKY tail artery rings, consistent with the results obtained in Wistar rats. This agrees with findings in other tissues where α,β–MeATP had no effect on the end-organ response to field stimulation (Duval, Hicks & Langer, 1985; Allcorn et al., 1985). In contrast, the response to the lowest frequency of stimulation (0.1 Hz) was enhanced by α,β–MeATP in tail artery rings from hypertensive rats. This implies that ATP released from nerves upon electrical stimulation is inhibitory to contraction, and agrees with findings by Bao, Eriksson & Stjarne (1990) and Bao (1993) in tail artery rings from normotensive Sprague-Dawley rats. This is surprising in view of the recognised electrical effects of ATP (exogenous and endogenous); in the guinea pig vas deferens, ATP produces a fast excitatory junctional potential (e.j.p.) which is abolished by α,β–MeATP (Sneddon & Burnstock, 1984b).

Bao et al. (1990) concluded from the effects of α,β–MeATP that despite a reduction in the amplitude and duration of the contraction, ATP accelerated the onset of the contraction. This may be because neurally-released NA produces a small, slow and long-lasting depolarisation (Cheung, 1984), and the cumulative effects of ATP and NA may cause the membrane potential to reach the threshold for firing a propagated muscle action potential, which is one mechanism for triggering a fast contraction (Holman and Surprenant, 1980).

The explanation for the increase in amplitude of field stimulation-induced contractions by α,β-MeATP in the present study remain unclear. Previous studies in the rat tail artery have shown that α,β-MeATP had no effect on the overflow of [^{3}H]-NA caused by nerve stimulation (Vidal, Hicks & Langer, 1986; Bao, Eriksson & Stjarne, 1990). In addition, neurogenic contraction was unaffected by the adenosine antagonist, 8-PSøT (Bao, Eriksson & Stjarne, 1990). It is unlikely therefore that neurally-released ATP

autoinhibits transmitter release, either via pre-junctional P_{2x}-receptors or following metabolism to adenosine to act on pre-junctional adenosine receptors.

The use of α,β-MeATP can be criticised as a suitable tool to assess the role of transmitter ATP, since some effects of α,β-MeATP may be unrelated to P_{2x}-receptors (Wiklund & Gustafsson, 1988), and can inhibit responses to substances other than ATP. However, α,β-MeATP was used in this study as it allows comparison with other work in the rat tail artery, and is generally accepted to be the most suitable compound available as yet.

In an attempt to avoid reliance on antagonist specificity to delineate the neurotransmitter profile, this study therefore employed an additional approach. If co-transmission of NA and ATP does occur in the rat tail artery, then depletion of the NA stores should reveal a residual response, which should be resistant to α-adrenoceptor blockade. Experiments in normotensive rats pretreated with reserpine to deplete sympathetic nerve terminals of NA did indeed reveal a residual response to field stimulation, which was resistant to both prazosin and yohimbine. However, this contraction was also resistant to TTX and therefore was not due to the action of neurally released transmitter. This non-neurogenic contraction was probably produced by direct stimulation of the smooth muscle, since smooth muscle sensitivity to a variety of agents has been reported to increase following reserpine pretreatment (Nasseri et al., 1985). Similar results were obtained in rats pretreated with 6-OHDA, which destroys sympathetic nerve terminals. These findings are consistent with the work of Vidal, Hicks & Langer (1986), where reserpine pretreatment in SHR and WKY rats revealed a purinergic component in the hypertensive rats only.

The results of the present investigation therefore support the conclusion that NA is the main sympathetic transmitter in the rat tail artery. The role, if any, of ATP as a sympathetic cotransmitter in this vessel is minor. ATP may become more important as a transmitter in certain circumstances, for example in the presence of agents which potentiate its effects, or in the pathological condition of hypertension.

6. CONCLUSIONS

This study has investigated many of the aspects of vascular control, and has explored how the responsiveness of vascular smooth muscle can be regulated. A variety of techniques (mechanical, biochemical and structural) were used, and interesting and useful information was gained from this integrated approach. It was demonstrated that α_2-adrenoceptors exist on the smooth muscle of the rat tail artery, under conditions which may occur in vivo, for example in the presence of vasopressin. In addition, post-junctional α_2-adrenoceptors were shown to contribute to the contraction of this vessel following sympathetic stimulation. The role of ATP as a cotransmitter with NA in the rat tail artery was only apparent in hypertensive rats, and to a very minor extent.

Hypertension was associated with other interesting changes in the vasculature, such that in a resistance vessel (the rat tail artery), responses to vasoconstrictors were increased while those to vasodilators were reduced. In contrast, the opposite pattern was found in the aorta, a conducting vessel, where vasoconstrictor responses were reduced while vasodilator responses were enhanced.

Perhaps one of the most important conclusions of this investigation is that the circumstances in which an experiment is carried out has such a profound effect on the observed results, which must be interpreted accordingly.

Thus, this investigation has answered many of the questions surrounding blood vessel control. However, several other intriguing problems were raised during the course of the study. For example, how exactly do agents such as AVP and PE or NA synergise with α_2-receptor agonists; how important is NA/ATP co-transmission in disease states such as hypertension, and does this contribute to the pathology of the disease? Such questions may therefore provide the basis of further research on the mechanisms of vascular responsiveness, and their relevance in both human and animal physiology and pathology.

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