THE EFFECTS OF BLOOD PRESSURE VARIATION ON VASCULAR STRUCTURE AND FUNCTION IN GENETIC AND EXPERIMENTAL HYPERTENSION

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It is widely believed that vascular smooth muscle in spontaneously hypertensive rats (SHR) is prone to a greater genetically determined propensity to growth because antihypertensive drugs typically do not completely normalise the structural development of SHR resistance arteries. In order to investigate this further, the blood pressure rise in one hindlimb of SHR and normotensive Wistar Kyoto (WKY) control rats was attenuated by a partially constricting ligature around one external iliac artery at 5 weeks. Therefore blood pressure reduction was mediated by non-therapeutic means. At 12 and 24 weeks the femoral mean arterial pressure distal to the ligature was reduced in both strains, and the low pressure hindlimb in the SHR was subject to perfusion pressures similar to normally perfused WKY hindlimbs. Femoral resistance arteries were mounted in a myograph to permit measurements of morphology and reactivity. The smooth muscle content of arteries distal to the ligature was reduced in both strains and SHR arteries from the low pressure hindlimb were structurally indistinguishable from those of normally perfused WKY hindlimbs, suggesting that blood pressure was the major determinant of vascular structure and SHR femoral vascular smooth muscle is not subject to greater pressure independent influences. The reduced noradrenaline sensitivity in SHR arteries at 12 and 24 weeks was normalised distal to the ligature in 12 week rats. Noradrenaline-stimulated calcium sensitivity was increased only at 12 weeks but the ligature increased this sensitivity at both ages in SHR. The ligature did not influence noradrenaline or noradrenaline-stimulated calcium sensitivity in WKY rats.

Additional studies revealed that vasopressin sensitivity is normal in SHR mesenteric resistance arteries but vasopressin-stimulated calcium sensitivity is increased. Also, mesenteric resistance arteries from Wistar rats made hypertensive by 2-bromoethylamine-induced chemical renal medullectomy have reduced noradrenaline sensitivities - a possible mechanism for reduced pressor responses to noradrenaline in vivo.

ACKNOWLEDGEMENTS

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CONTENTS

FOREWORD

Hypertension has been described by Folkow (Folkow, 1975) as an 'irritatingly elusive disorder of regulation'. Whether it is a disease as such is debatable because high blood pressure merely describes a pressure situated towards the right of a population's blood pressure distribution curve. However, it is beyond doubt that an increased perfusion pressure in the vasculature will greatly increase the mortality and morbidity from cardiovascular disease, whatever the cause. In secondary forms of hypertension - those hypertensive states that can be ascribed to a known aetiology such as phaeochromocytoma or renal disease, the treatment can be tailored according to the cause. In essential hypertension (or 'primary hypertension' as described by Folkow) there is no known cause. Indeed in the past many researchers have tried to ascribe the problem to a single cause but our current state of knowledge suggests this is ambitious.

I shall begin by referring to the major historical landmarks in the field because as Folkow has reminded us 'to understand a science one must know its history' (Folkow, 1982). Then the introduction will more generally review the subject matter relevant to the research that I have performed which has been directed towards the examination of mechanisms which may influence blood pressure and how the structure of the vasculature is influenced by the blood pressure to which it is exposed.

CHAPTER 1

INTRODUCTION

Historical Review

It is now over two hundred and fifty years since the Reverend Stephen Hales published his experiments demonstrating the measurement of blood pressure in the arteries of horses crudely cannulated with brass and glass piping (Hales, 1733). However, the systematic measurement of blood pressure and consequent clinical recognition of patients whose hypertension was not associated with renal disease, i.e. those with essential hypertension, did not occur until a little over one hundred years ago (Mahomed, 1881). Before this, Richard Bright had noted that pathological changes in the kidney were often associated with left ventricular hypertrophy (Bright, 1836). Although a relationship between pressure, renal disease and vascular structure was not proposed, he did suggest that changes in small vessels might require a greater cardiac force to counter the raised resistance in the diseased vessels. He also noted that the degree of left ventricular hypertrophy apparently matched the seriousness of the renal condition (Bright, 1836). Now we know that blood pressure would have been raised for longer or to a higher level to stimulate that cardiac enlargement. Johnson described both large and small vessel hypertrophy in patients with renal disease (Johnson, 1868) and attributed these structural changes to changes in the composition of the blood resulting from the disease although he did speculate that a pressure rise would occur from capillary obstruction. Gull and Sutton described vascular changes in the absence of renal disease (Gull and Sutton, 1872) before Mahomed described high blood pressure in the absence of renal disease (Mahomed, 1881).

Subsequently Frank described the term 'essential hypertension' (Frank, 1911). Therefore raised blood pressure and structural alterations of the cardiovascular system were being described with no renal contribution to either; thus the opportunity had arisen to consider a relationship between the architecture of the cardiovascular system and blood pressure. Changes in the composition of the blood could have been considered epiphenomena consequent upon the hypertrophy of the blood vessels and the pressure excess. However, it was Goldblatt's classic experiments that aroused so much interest by suggesting that a humoral factor was the prime cause for the increase in resistance (Goldblatt et al. 1934): an all embracing explanation for hypertension appeared to have been found. However, Pickering arrived at the conclusion that increased resistance to flow arose from the smallest precapillary vessels (Pickering, 1936) and that even after maximal vasodilation, resistance was still increased in hypertensives in comparison to controls: this finding was also noted by Prinzmetal and Wilson (Prinzmetal and Wilson, 1936). However ultimately these workers ascribed the increase in resistance to a stable circulating factor (Pickering, 1936; Prinzmetal and Wilson, 1936). However, using a rabbit model of renal secondary hypertension they later made observations that could not be explained by a single (or 'unitary') hypothesis (Wilson and Pickering, 1938; Pickering, 1945); they found that removal of the ischaemic kidney normalised blood pressure as expected but if the hypertension had been sustained for more than two or three weeks, then the hypertension could not be reversed. Similar findings were reported by Grollman who suggested that a deficiency in some renal vasodepressor agent was the cause of the renal hypertension

in the rabbit (Grollman, 1944) and in the dog (Grollman et al. 1949).

In 1945 Pickering did suspect that changes in vascular morphology were the cause of maintained hypertension (Pickering, 1945); and in their classic paper Folkow and co-workers (Folkow et al. 1958) described how structural alterations could account entirely for the raised resistance in essential hypertension, with no requirement at all for any alteration in vascular smooth muscle activity. For a given stimulus, vessels with an increased media thickness and either no luminal change or a reduction in lumen diameter would produce greater resistance to blood flow than control vessels, while if a lumen reduction occurred then this would also account for the raised resistance observed in the hypertensives at maximal vasodilatation (Folkow et al. 1958). Hence haemodynamic changes resulting from structural alteration could maintain a high blood pressure. But it is important to note that this does not imply that a structural change is the cause of the hypertension. Even today this remains very much a chicken-and-egg argument. In resistance arteries derived from gluteal skin biopsies of essential hypertensive patients there is an increased media thickness and an increased media:lumen ratio when compared with controls (Aalkjaer et al. 1987a), and when young offspring of hypertensives were studied in a similar fashion and the results compared with those from young subjects with no family history of hypertension it was found that they already had a tendency towards the vascular remodelling of the hypertensives (Aalkjaer et al. 1987b). However, in this study it was also noted that their blood pressures were already slightly raised and so it

appears that the structural alterations do not precede the hypertension. In fact blood pressure development and structural alteration of the resistance vasculature appear to proceed in parallel, possibly in a positive feedback mechanism as proposed by Folkow (Folkow et al. 1958).

Thus far a single factor which might lead to essential hypertension has not been found. It is increasingly clear that a multifactorial approach must be pursued in the elucidation of the abnormalities, a proposal advanced by Page in his 'mosaic theory' (Page, 1949). Indeed it may be that there is no identifiable abnormality: the raised pressure may be a result of a number of mechanisms acting in concert, the activity of each being not significantly altered in comparison with normal, but by a summation or synergy of their effects the disease ensues.

Obviously, human hypertension cannot be studied in a direct experimental approach; ethical considerations rightly restrict the procedures that may be performed on volunteers. Consequently a model is needed in which the nature of the hypertension apparently resembles that found in man.

The most commonly used model for this purpose is the spontaneously hypertensive rat developed from a Wistar rat population which contained rats which 'spontaneously' developed high blood pressure (Okamoto and Aoki, 1963). A selective breeding programme resulted in a strain of rat which regularly became hypertensive. Spontaneously hypertensive rats (SHR) share many similar features with patients with essential hypertension

(for review, see Frohlich, 1986), and relevant similarities will be described below. It is studies performed in these rats which comprise the major part of this thesis in conjunction with the chemical renal medullectomy model in normal Wistar rats. No attempt to identify initiating factors will be made, rather, I have investigated the inter-relationship between blood pressure and vascular structural and reactivity changes. Such studies are important because whilst they do not explain the causes of hypertension they help the understanding of the maintenance of the pressure excess. In the absence of maintaining factors the pressure excess might be reversed thus avoiding the excess mortality observed in hypertension.

The Vascular Structure - Blood Pressure Relationship

It is evident from the physique of certain athletes that the increased demand placed upon their muscles can result in marked skeletal muscular growth. Smooth muscle responds similarly to such increases in load; for example, if outflow from the urinary bladder is restricted in the rabbit (Uvelius, 1980) or rat (Mathiasson and Uvelius, 1982) then there is an hypertrophic response. Similarly, smooth muscle from guinea-pig small intestine responds with growth proximal to a constriction induced by wrapping of a section of the gut (Gabella, 1979). Cardiac and vascular smooth muscle are no exceptions: the vasculature of the giraffe is probably about the most obvious example of vascular smooth muscle adapting to the local load. As reviewed recently (Heagerty et al. 1988b) this animal requires a blood pressure of some 400-500 mmHg to perfuse its brain which may be up to 2-3 metres above heart level. The arteries in the legs have in consequence

extremely thick walls and pinpoint lumens. The left ventricular wall is 7.5 cm thick to provide the cardiac force required to produce the high pressure. Vascular restructuring also occurs in children. Following parturition and commencement of breathing the reduction of pulmonary pressure is associated with a regression of the wall thickness of the pulmonary vasculature (Folkow, 1982; Folkow,1984). In addition, as children adopt the standing posture more often there is an increase in the media thickness:lumen ratio of the vasculature in the legs - probably in response to the increase in hydrostatic pressure caused by standing from lying (Svejcar et al. 1962). Similarly, saphenous veins in adults have a greater smooth muscle content distally than proximally (Svejcar et al. 1962). In hypertension it is beyond doubt that the increased pressure is associated with a refashioning of the resistance vasculature. In 1958 Folkow and co-workers (Folkow et al. 1958) reported an increase in resistance in hypertension and suggested how the structural changes and blood pressure might be related in a positive feedback fashion. The experiment involved forearm plethysmography under conditions of reactive hyperaemia to induce maximal relaxation of the vasculature. It was shown that the resistance in hypertensive patients (both renal and essential) was greater than in normotensive controls, and importantly, the resistance was greater in hypertensives than in controls with artificially raised blood pressure produced by noradrenaline infusion. It was concluded the established increases in arterial pressure had produced a structural change and thus an increase in smooth muscle activity would not be required to maintain increased resistance. A thickened media, even if it encircles a similar sized lumen will result in greater

resistance to flow for any given level of smooth muscle activation purely as a result of the increased media:lumen ratio. Hence the positive feedback proposal; Folkow envisaged that an increased thickness of the smooth muscle bulk around the lumen of the resistance arteries might occur with either (i) no change, (ii) a reduction, or (iii) an increase in the internal diameter of the arteries. These three alternatives would produce three distinctive changes in the resistance curves as illustrated in Figure 1. The results obtained suggested that the structural change was described by condition (ii) above, and that the minimal resistance was increased in proportion to the mean arterial pressure; the final result is that hypertension is associated with an increased baseline resistance plus the amplifying effects of an increased media:lumen ratio.

As described in this study (Folkow et al. 1958) and later in his review (Folkow, 1982) the blood pressure - structure relationship is described simply by the scheme in Figure 2(a). It is possible that an increased arterial pressure has a greater effect on hypertrophy in some individuals,i.e. an exaggerated growth response to the pressure increase. Lever has reviewed mechanisms which may act directly on smooth muscle cells to induce growth (Lever, 1986). Non-pressure dependent growth may interact with pressure mediated hypertrophy such that schemes (b) and (c) in Figure 2 may represent a more complete picture of the state of affairs.

Sensitivity to Pressor Stimuli

It is also possible that alterations in agonist sensitivity may

FIGURE 1

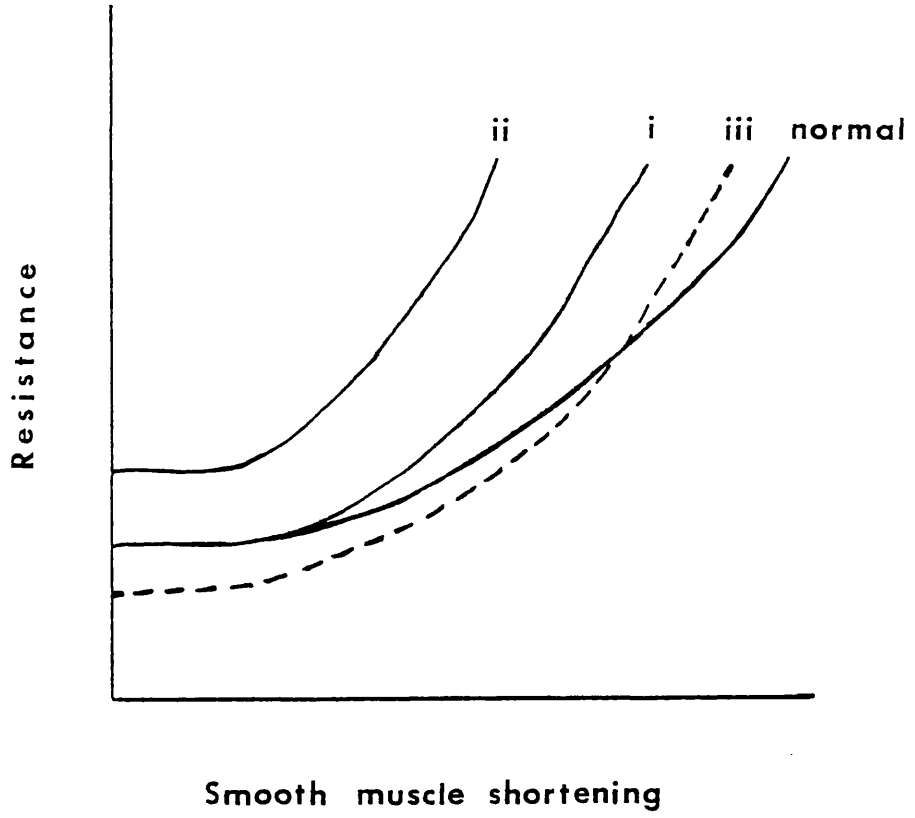
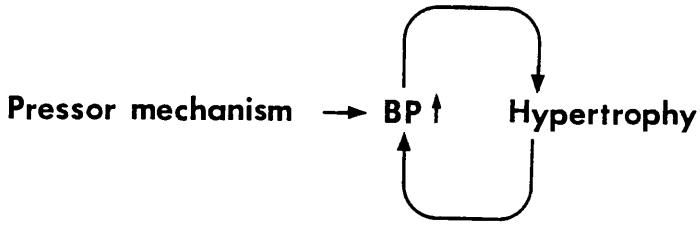


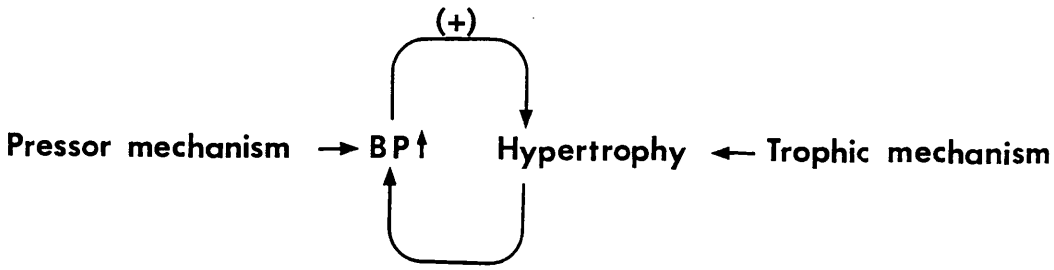
Diagram to illustrate how the basal resistance may be influenced by three forms of lumen changes associated with an increased media thickness, i.e. (i) no change, (ii) reduced lumen diameter, or (iii) an increased lumen diameter.

FIGURE 2

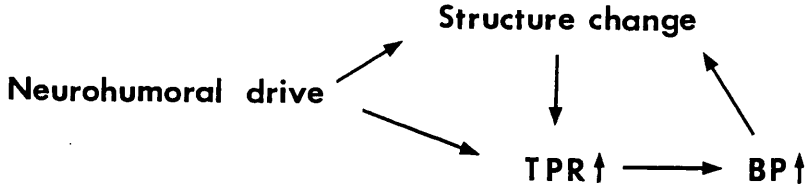
(a)



(b)



(c)



(a) Simple relationship between raised blood pressure and vascular structure. (Adapted from Folkow et al. 1958 and Folkow, 1982).

(b) Non-pressure dependent growth may influence vascular structure directly and some individuals may have an exaggerated growth response for a given pressure increase, as indicated by (+). Adapted from Folkow et al. 1958 and Lever, 1986).

(c) Scheme adapted from Mulvany, 1987. Note that structure change may be a media:lumen ratio increased bysmooth muscle rearrangement rather than a media growth.

Abbreviations: BP, blood pressure; TPR, total peripheral resistance.

play a role in the initiation and/or maintenance of the hypertensive state. In human essential hypertension there is a paucity of data concerning sensitivity differences between hypertensives and normotensive controls. Firstly considering noradrenaline, there appears to be no difference in vascular sensitivity to this agonist. In temporal arteries (Horwitz, 1974), digital arteries (Moulds, 1980), cystic arteries (Wyse, 1984) and abdominal wall arteries (Thulesius, 1983) no difference has been found. In resistance arteries ($<$300 um diameter) from skin biopsies taken from the gluteal region of essential hypertensive patients and normotensive control subjects noradrenaline sensitivities are similar in the absence and presence of cocaine (used to eliminate the effect of noradrenaline uptake by the nerve terminals), although the increase in sensitivity induced by cocaine is greater in the arteries from patients (Aalkjaer et al. 1987a). In vivo measurements of vascular bed noradrenaline sensitivities also reveals little differences when assessed by forearm, (Hulthen, 1984) and hand plethysmography (Sivertsson and Olander, 1968).

Angiotensin II sensitivity may be increased in the renal vasculature when estimated from measurements of renal vascular resistance (Ljungman, 1983); but isolated vessels from other vascular beds do not have an altered sensitivity to this hormone (Moulds, 1980; Aalkjaer et al. 1987a). Indeed when a variety of agonists has been investigated sensitivity is normal in human essential hypetension (Moulds, 1980; Aalkjaer et al. 1987a). Consequently it seems unlikely that an increase in vascular sensitivity to any agonist contributes to any

substantial degree to the maintenance of the disease. However, small changes acting on a genetically altered vasculature may still of course act in concert with other blood pressure controlling mechanisms.

In the case of the SHR, on the other hand, the literature contains many examples of increased vascular agonist sensitivity, although there is no consistent pattern between different vessel beds. The normotensive control rat strain used in comparison with the SHR is the Wistar Kyoto rat (WKY) which was also developed from the Wistar rat strain. Therefore the inbred SHR strain has an inbred normotensive control in contrast to the outbred Wistar control. However the WKY was not released at the same time as the SHR and consequently many of the earlier studies utilised SHR-Wistar comparisons.

In comparison to WKY rats, isolated perfused kidneys from SHR have normal sensitivity to noradrenaline as well as normal angiotensin II and Ba^{2+} sensitivities (Collis and Vanhoutte, 1978; Collis et al. 1980). More recently, while supporting the conclusions on noradrenaline, angiotensin II and Ba^{2+} in older rats, it has been reported that younger rats of 4-5 weeks have a reduced noradrenaline sensitivity in the presence of cocaine in comparison to WKY controls when isolated kidneys were perfused (Smeda et al. 1988a). Isolated perfused kidneys from stroke prone SHR (SPSHR) have increased sensitivity to noradrenaline, vasopressin and serotonin but not angiotensin II (Berecek et al. 1980a). Berecek also demonstrated increased agonist sensitivity in isolated perfused kidneys from deoxycorticosterone acetate

(DOCA) salt hypertensive rats (noradrenaline, vasopressin and angiotensin II) (Berecek et al. 1980b). Thus, it is clear that the type of model of hypertension studied may give rise to divergent conclusions regarding vascular sensitivity and its relation to blood pressure.

Isolated perfused hindquarter preparations may exhibit either unchanged or increased agonist sensitivities when compared with similar preparations from normotensive controls. Moreover Folkow and co-workers reported no differences in noradrenaline sensitivity when Wistar rats were used as controls (Folkow et al. 1970; 1971; 1972). In addition Lais and Brody found similar noradrenaline and angiotensin II sensitivities in SHR and Wistar isolated perfused hindquarters, although interestingly if autologous blood was used as the perfusate the SHR preparation became more responsive (Lais and Brody, 1975), suggesting that in vivo the SHR vasculature may be more sensitive but when perfused with artificial media this difference in sensitivity is lost. Therefore it may be possible that some circulating factor(s) may influence agonist sensitivity, at least in the hindquarter vasculature. When WKY rats are used as controls, hindquarter noradrenaline sensitivity is normal in SHR in the absence of cocaine (Finch and Hauesler, 1974; Folkow and Karlstrom, 1984; Gothberg et al. 1980; Jandhyala et al. 1980) or increased (Lais and Brody, 1978). An increased α_1 adrenoceptor sensitivity is observed until 14 weeks of age in SHR preparations during methoxamine perfusions (Adams et al. 1989). Also, in comparison to WKY, SPSHR noradrenaline sensitivity is increased in this preparation (Schomig et al. 1978). In addition, sensitivity to

Ba^{2+} is unaltered in SHR hindquarter preparations (Gothberg et al. 1980; Jandhyala et al. 1980; Lais and Brody, 1978); the same is true for vasopressin (Jandhyala et al. 1980) but serotonin sensitivity may be decreased in comparison with Wistar controls (Ahlund et al. 1977).

The perfused mesenteric circuit of the SHR has increased noradrenaline sensitivity if surgically denervated preparations are used when compared with Wistar controls, that is when the influence of noradrenaline reuptake is eliminated (Haeusler and Haefely, 1970). This result is also found commonly in isolated resistance arteries of the mesenteric circuit. For example, in the absence of cocaine the SHR preparation noradrenaline sensitivity did not differ to that observed in WKY but when cocaine was present in the bathing medium then an increased sensitivity was observed (Jespersen et al. 1985; Mulvany et al. 1980; Mulvany et al. 1981a; Nyborg and Mulvany, 1985). If presynaptic uptake is eliminated by in vitro denervation with 6-hydroxydopamine then the noradrenaline hypersensitivity of the SHR smooth muscle cells is revealed (Whall et al. 1980). While no data were presented concerning pre cocaine treatment, Gray and DeMey provided further support for there being an enhanced noradrenaline sensitivity in SHR mesenteric resistance arteries when compared with WKY (Gray and DeMey, 1985). It is of interest that it has been shown that the noradrenaline sensitivity of mesenteric resistance arteries in Wistar rats increases with increased wall tension (Nilsson and Sjoblom, 1985). Consequently there may be a role in vivo for the increased pressure in the SHR vasculature in vivo to induce a greater noradrenaline sensitivity

which might be lost when arteries from SHR and WKY are compared under standardised conditions in vitro. However, since the medial layer of SHR resistance arteries is increased, the tension load per unit volume of smooth muscle will not necessarily be raised. Thus a role for pressure-induced hypersensitivity remains conjecture.

Isolated femoral arteries from SHR are more sensitive to noradrenaline in comparison with WKY (Asano et al. 1982; Field and Soltis, 1985) and a similar finding is observed in the SPSHR (Soltis and Bohr, 1987). This observation may be the consequence of a reduced β receptor-mediated relaxation which is suggested by the finding of decreased noradrenaline induced relaxations in the absence of α receptor-mediated contraction (Asano et al. 1982), or decreased isoprenaline induced relaxation (Asano et al. 1982; Field and Soltis, 1985; Soltis and Bohr, 1987). More recently it has been demonstrated that a reduced β -mediated relaxation occurs in a variety of other arteries from the SHR and it has been suggested that this may reflect a reduced Gs protein function since adenylate cyclase activity was not different between SHR and WKY as deduced from equal sensitivities to forskolin-mediated relaxations (Masuzawa et al. 1989). However, if one considers the resistance arteries of the femoral bed there does not appear to be a sensitivity abnormality in the SHR and cocaine could only induce a minimal enhancement in noradrenaline responsiveness in both WKY and SHR (Mulvany et al. 1982); the small cocaine shift in noradrenaline sensitivity was a result of sparse innervation which was revealed by fluorescence histochemistry (Mulvany et al. 1982). Therefore these isolated

resistance arteries appear to have behaved in a similar manner to those in the hindquarter perfusion experiments reported by Folkow's group as quoted above.

While it appears that noradrenaline sensitivity in the SHR vasculature is in general either increased or unchanged, that of the aorta is unchanged or decreased. Using Wistar rats as controls there is apparently no difference (Clineschmidt et al. 1970; Hallback et al. 1971) or in comparison to WKY sensitivity is decreased (Noon et al. 1978). Large tail arteries also yield variable results, possibly depending on the preparation used. For example, perfused arterial segments in cocaine and propranolol are not different between SHR and WKY (Fouda et al. 1987). Helical tail artery strips from SHR possess a normal noradrenaline sensitivity but after in vitro denervation with 6-hydroxydopamine they displayed a greater leftward shift in the noradrenaline dose response curve to reveal a greater vascular smooth muscle sensitivity (Webb and Vanhoutte 1979; Webb et al. 1981) in a similar fashion to that observed in mesenteric resistance arteries (Whall et al. 1980). However, SHR tail artery rings mounted in a myograph were less sensitive than those from WKY in the absence or presence of cocaine (Mulvany et al. 1982). Tail artery strips may also reveal an increased noradrenaline sensitivity, possibly since the agonist could depolarise myocyte membrane potentials to a greater degree in SHR preparations (Hermsmeyer, 1976). Noradrenaline topically applied to the cremasteric bed in situ reveals no difference in sensitivity in this hindquarter bed as judged from changes in lumen diameter although the presence of anaesthetic may have confounded vascular

reactivity studies in this preparation (Wiegman et al. 1979).

Depolarisation-induced contractions in the hindquarters of SHR are not different compared with controls. Although the controls used were poor (male Sprague Dawley rats compared with female SHR) the hypertensive rat femoral artery helical strips high $[K^+]$-induced contractions were similar to those of the normotensive rat (Hansen and Bohr, 1975). Similar results are described for SPSHR in comparison with WKY (Soltis and Bohr, 1987). Tail artery potassium-induced contractions also revealed no strain differences between SHR and WKY (Webb and Vanhoutte, 1979). Consequently when $[Ca^{2+}]$ is not limiting, spontaneous hypertension is not associated with a defect in depolarisation induced contraction of vascular smooth muscle. Nor is there a generalised defect in serotonin sensitivity since it is increased in resistance sized basilar arteries from SHR in comparison with WKY (Winquist and Bohr, 1983) and in portal veins and aorta when Wistar rats are used as controls (Ahlund et al. 1977); however serotonin sensitivity is reduced in perfused hindquarter preparations of SHR compared with Wistar preparations (Ahlund et al. 1977).

Calcium Sensitivity

The rate limiting factor in any contraction is free cytoplasmic $[Ca^{2+}]$ and consequently it is often desirable to determine the calcium sensitivity of a vascular preparation. The principle is to apply a maximum dose of agonist but limit the concentration of external calcium. In this way calcium concentration response curves can be determined. In human essential hypertension the

subcutaneous resistance vasculature has a depressed noradrenaline-stimulated calcium sensitivity (Aalkjaer et al. 1987a) but this may be an adaptive process to limit the increased reactivity resulting from the increased media:lumen ratios in these arteries, since the offspring of essential hypertensive patients did not show altered calcium sensitivity in these arteries when compared with arteries obtained from normotensive controls with no familial predisposition to hypertension (Aalkjaer et al. 1987b). In comparison with Wistar control rats, the SHR hindquarters appear to change in the opposite direction with regard to noradrenaline stimulated calcium sensitivity and age, since at 6 weeks the calcium sensitivity is normal but at 6 months the sensitivity was enhanced in isolated hindquarter preparations (Folkow et al. 1977). This raises the possibility that in the SHR at least the calcium sensitivity increases with the blood pressure, however there is no evidence to suggest that one leads to the other.

As previously described the noradrenaline sensitivity in the SHR vasculature is generally increased or normal in comparison with controls, but that for aortic preparations is generally unchanged or reduced. A similar state of affairs exists for calcium sensitivity. WKY helical aortic strips can maintain noradrenaline induced contractions more effectively in low [Ca^{2+}] media than those from SHR (Noon et al. 1978) i.e. the SHR strips were less sensitive. In addition, aortic rings from SHR had reduced noradrenaline - and K^{+}-stimulated calcium sensitivities in comparison with Wistar rat controls (Pedersen et al. 1978). In tail arteries (Aqel et al. 1986) and mesenteric resistance

arteries (Mulvany and Nyborg, 1980) K^+-stimulated calcium sensitivities are unchanged in SHR compared with WKY, as is observed for femoral helical strips from SPSHR (Soltis and Bohr, 1987) and SHR femoral helical strips in comparison with Sprague-Dawley control rats (Hansen and Bohr, 1975), while the K^+-stimulated calcium sensitivity of SHR basilar resistance arteries were reduced in comparison with those from WKY (Winquist and Bohr, 1983). More recently, whole cell voltage clamp experiments on neonatal venous smooth muscle cells in primary culture suggest differences in potential operated calcium channels since peak Ca^{2+} currents on depolarisation were similar for SHR and WKY channels but those from WKY activated at more negative membrane potentials and those from SHR were activated for longer, allowing a greater total Ca^{2+} influx (Rusch and Hermsmeyer, 1988). If calcium channels from arterial smooth muscle behaved similarly then one would expect WKY to have a reduced K^+-stimulated calcium sensitivity since membrane potential should not be a limiting factor in a high $[K^+]$ medium and thus more Ca^{2+} should be available for the contraction process in the SHR arteries. Whilst it must be accepted that phenotypic changes might occur either in culture or following isolation of vascular smooth muscle cells, it is also important to note that smooth muscle characteristics can be studied under these circumstances in the absence of neural or endothelial influences. Using freshly isolated myocytes from tail arteries of SHR and WKY, it has also recently been shown that SHR myoctyes were more sensitive to noradrenaline and KCl induced contractions as determined by cell length changes (Bolzon and Cheung, 1989). In addition these cells had greater noradrenaline-stimulated and K^+-stimulated calcium sensitivities,

thus providing further evidence for intrinsic membrane abnormalities in SHR vascular myocytes. However, as described above, arterial preparations from SHR and WKY do not possess different K^+-stimulated calcium sensitivities. Therefore, it is possible that endothelial or neural influences might affect depolarisation induced contractions or alternatively the isolation procedure may have some influence on the membrane characteristics. Nevertheless the experimental data obtained from intact arteries suggests that generally in the SHR vasculature there is no enhanced sensitivity to membrane depolarisation stimuli.

In addition to potential operated calcium entry, receptor operated calcium influx should also be considered. In contrast to the depression of calcium sensitivity of noradrenaline activation in the SHR aorta, smaller conduit arteries or resistance-sized arteries display unchanged or increased noradrenaline-stimulated calcium sensitivity. The isolated hindquarters of adult SHR when compared with those from Wistar controls maintain noradrenaline induced contractions more effectively in low $[Ca^{2+}]$ perfusates (Folkow et al. 1977), while femoral (Mulvany et al. 1982; Nilsson and Mulvany, 1981) and mesenteric resistance arteries (Mulvany and Nyborg, 1980; Mulvany et al. 1981a and 1981b; Mulvany and Korsgaard, 1983; Nilsson and Mulvany, 1981; Nyborg and Mulvany, 1985) display leftward shifts in the noradrenaline-stimulated calcium dose response curves compared with those from WKY arteries. Calcium dose response curves in tail artery rings are similar (Mulvany et al. 1982) or increased (Aqel et al. 1986). Portal veins from SHR are also more sensitive

(Pegram and Ljung, 1981) while SPSHR femoral helical strips' noradrenaline stimulated calcium concentration response curves are not altered when compared with those from WKY controls (Soltis and Bohr, 1987). Bhalla et al. demonstrated that $^{45}Ca^{2+}$ influxes in caudal artery rings might resolve the apparent conflicting conclusions of unchanged K^+-stimulated calcium curves and enhanced noradrenaline-stimulated calcium curves in smaller arteries from SHR (Bhalla et al.1986). In this experiment calcium sensitivity was determined by the measurement of ^{45}Ca influx as opposed to tension development, and the authors reported that depolarisation-stimulated calcium sensitivity was similar for SHR and WKY preparations. However, if the arterial rings were stimulated through α_1 receptors by either noradrenaline or methoxamine, then greater ^{45}Ca fluxes were observed in SHR arteries. In this same study the possibility of the increased ^{45}Ca influx stimulated by α_1 activation being a result of different α_1 receptor forms or number was discounted by prazosin binding experiments.

Conclusions

Taking a general overview of agonist and calcium sensitivities in SHR it appears that a generalised increase in vascular sensitivity is not unequivocally associated with the hypertension although rarely do normotensive controls possess increased sensitivity in the smaller vessels or resistance arteries. Consequently the increased blood pressure in the SHR is likely to be a consequence of mechanisms other than a generally enhanced agonist sensitivity although it still remains at least a possible amplifying factor which acts in conjunction with those mechanisms

responsible for initiating the pressure rise. Noradrenaline is the most frequently investigated agonist and the general conclusion is that there is an increase in the SHR vascular smooth muscle cell noradrenaline sensitivity of isolated vessels or perfused limbs. However, this hypersensitivity is sometimes only revealed following elimination of neuronal noradrenaline reuptake by cocaine or by denervation, especially in the mesenteric circuit. In hindquarter perfusion preparations or isolated femoral resistance arteries, most evidence suggests that there is normal noradrenaline sensitivity in the SHR hindlimb. The lack of cocaine in the perfusion experiments described is probably not important since cocaine had little effect on the isolated resistance arteries (Mulvany et al. 1982). In mesenteric resistance arteries it has been demonstrated that noradrenaline has a greater affinity for alpha adrenoceptors in SHR than WKY which might explain the increased noradrenaline sensitivity observed in the mesenteric circuit (Nyborg and Bevan, 1988). An abnormal vascular handling of calcium in response to noradrenaline stimulation might also explain those cases of increased noradrenaline sensitivity observed in the SHR. Most studies investigating the noradrenaline stimulated calcium sensitivities of resistance arteries suggest an increase in this parameter, so it may be the underlying mechanism which explains the general increase in noradrenaline sensitivity. However, arteries in vivo are not normally in low calcium containing buffers and only under these circumstances is an increased calcium sensitivity observed; thus alterations in calcium sensitivity may simply be a marker for a general defect in the vascular smooth muscle membrane calcium handling. In 1974, Bohr

showed that carotid arteries from SHR were more responsive to non-physiological divalent cations than those from Sprague Dawley controls (Bohr, 1974), while more calcium was required to stabiliseKCl induced contractions of SHR femoral helical strips, also compared with Sprague Dawley controls (Holloway and Bohr, 1973; Hansen and Bohr, 1975), tail artery helical strips in comparison with WKY (Webb and Bohr, 1980) and basilar resistance arteries also compared with those from WKY (Winquist and Bohr, 1983). Jones has demonstrated that SHR vascular smooth muscle membranes are more labile than those from WKY since Na, K and Cl turnovers are enhanced. Removal of calcium from the medium increased K washout to a greater extent in SHR arteries. Therefore it was postulated that the SHR membranes had a reduced ability to retain calcium for membrane stabilisation (Jones, 1974). If the Na^+-K^+-ATPase of helical aortic strips is inhibited by ouabain or K^+-free media then SHR preparations contract more readily than those from WKY (Lamb et al. 1988). In this same study, the authors demonstrated that external calcium was required for these contractions. Thus abnormal calcium permeability might explain the observations. Readdition of calcium to Na^+-K^+-ATPase inhibited preparations in calcium free media revealed an increased calcium sensitivity of SHR preparations. The sensitivity increase was probably a result of reduced calcium binding in the SHR since if calcium depletion was stricter with EGTA to remove bound calcium then this eliminated the differences in calcium sensitivity. A reduced ability of SHR aortic membranes to bind calcium had been suggested previously (Zsoter et al. 1977). A decrease in calcium binding has also been observed in SHR erythrocyte membranes compared with those from

Wistars (Postnov et al. 1979) a finding also noted when erythrocytes from human essential hypertensives are compared with those from normotensive controls (Postnov et al. 1977; Postnov et al. 1979; Bing et al. 1986). Sensitivity increases in the SHR may also arise from a reduced ability to sequester calcium or extrude it from the myocytes. ^{45}Ca flux experiments suggest reduced calcium extrusion from SHR mesenteric artery plasma membrane preparations compared with WKY preparations as judged from reduced ATP dependent calcium accumulation into plasma membrane vesicles (Kwan et al. 1979). Venous plasma membrane preparations did not display such an abnormality, therefore decreased calcium extrusion may be a consequence of the raised pressure in the arterial circulation (Kwan and Daniel 1981). SHR aortic sarcoplasmic reticulum vesicles can accumulate less calcium than those from Sprague Dawley controls (Moore et al. 1975) or WKY (Webb and Bhalla, 1976). The SHR sarcoplasmic reticulum membrane may be leakier to calcium since SHR Ca^{2+}-ATPase activity was actually increased (Webb and Bhalla, 1976). Similarly, an aortic plasma membrane Ca^{2+}-ATPase activity increase has been noted in SHR compared with Wistar controls which might have been a compensatory response to the increased calcium permeability observed (Wei et al. 1976). Increased calcium permeability has also been noted in SHR aortic plasma membrane since removal of external calcium produced faster relaxation and a greater tension redevelopment upon calcium readdition in SHR compared with WKY controls (Noon et al. 1978).

In addition to increased calcium permeability and decreased sequestering ability in vascular smooth muscle cells from SHR,

it has also been suggested that there is a larger internal calcium pool available for contraction, at least in caudal arteries since noradrenaline or caffeine in calcium free media produced proportionally greater contractions in SHR preparations compared with WKY (Aqel et al. 1987). However a reduced calcium sequestration from the cytoplasm might also explain these particular observations.

However in spite of the evidence for altered membrane permeability and calcium handling described above, it is important to note that most evidence comes from aortic preparations where vascular reactivity studies would suggest that SHR preparations are not more sensitive to calcium. Therefore it remains conjectural that increased calcium sensitivity in the smaller arteries is a result of membrane abnormalities similar to those observed in the aorta.

Vascular Structure in Hypertension

There is no doubt that vascular structural remodelling occurs in sustained human essential hypertension. The total peripheral resistance is increased in such patients (Amery et al. 1967; Lund-Johansen, 1980) and the resistance increase is distributed throughout all organs of the body (London et al. 1984) albeit non-uniformly (Brody and Zimmerman, 1976). Even borderline hypertension is sufficient to induce structural thickening in the vasculature because it has been demonstrated that finger pulse volume is reduced (Zweifler and Nicholls, 1982). Data from forearm plethysmography show that essential hypertensive patients display an increased resistance to flow in the basal state

(Hulthen et al. 1984) and under conditions of maximal vasodilatation (Conway, 1963; Folkow et al. 1958). Hand plethysmography also reveals an increased minimal resistance (Sivertsson and Olander, 1968): consequently the resistance vasculature must possess narrowed lumens, possibly resulting from a thickened media encroaching upon the lumen. The resulting increase in media:lumen ratio results in steeper resistance curves and greater maximal resistance during noradrenaline infusion in the absence of differences in noradrenaline sensitivity between essential hypertensive patients and normotensive control subjects (Folkow et al. 1958; Sivertsson and Olander, 1968). Direct measurements of vascular morphology also revealed increased media:lumen ratios in essential hypertension. Short described increased media:lumen ratio in perfusion fixed mesenteric circuits of autopsy material (Short 1966a), although the increase was apparently due to a reduction in lumen diameter and no change in the medial cross sectional area of the media. He found that the smallest precapillary arteries showed no structural changes (Short 1966a,1966b) suggesting that the resistance to flow was situated upstream to protect the capillary circuit. One of the subjects studied had a stenosis of the superior mesenteric artery which probably protected the arteries distally from the increased blood pressure. In this particular case the resistance vasculature was structurally indistinguishable from that of previously normotensive subjects, thus providing evidence for the hypothesis that structural change is a phenomenon secondary to the blood pressure increase. Formalin-fixed renal arteries and arterioles have an increased media:lumen ratio in kidneys from essential hypertensive patients

and again the smallest arteries were not thickened, i.e. those with lumen diameters less than 100um (Furuyama, 1962). In this study the media:lumen ratio increase was apparently a result of an increased medial cross sectional area. Such an increase has also been observed in digital arteries which could account for the greater tension developments (Moulds, 1980) and more recently subcutaneous resistance arteries have been shown to have increased media thicknesses and reduced lumen diameters although the lumen difference failed to reach statistical significance (Aalkjaer et al.1987a). The increased tension that the arteries from the hypertensive patients could generate could be accounted for entirely by the increased media thickness (Aalkjaer et al. 1987a). However, the media cross sectional area was not significantly increased as had been observed by Short in 1966 - therefore it is possible that there is myocyte rearrangement around the lumen to account for thickening of the medial layer.

Also there have been suggestions that rarefaction of the resistance vasculature occurs in human essential hypertension. A reduced number of arteries in the lumen diameter range 60-200um has been observed (Short, 1958) and in the conjunctival circulation the number of small arteries is reduced (Harper et al. 1978); this is apparently a progressive phenomenon with time since in young borderline hypertensive patients there is a reduced capillary number but no difference in the small artery count in this particular bed (Sullivan et al. 1983).

The structural changes described in human essential hypertension are also observed in the vasculature of the SHR. The total

peripheral resistance is increased in comparison with control Wistar rats (Albrecht, 1974; Finch and Haeusler, 1974; Pfeffer and Frohlich, 1973) and the difference seems to develop with age (Albrecht, 1974; Finch and Haeusler, 1974). The total peripheral resistance is also increased compared with that in WKY (Cutiletta et al. 1978; Ferrone et al. 1979) although the increased resistance may vary in magnitude in different vascular beds (Ferrone et al. 1979). Renal hypertensive rats (Ferrone et al. 1979; Finch and Haeusler, 1974) and DOCA salt hypertensive rats (Finch and Haeusler, 1974) also demonstrate a raised total peripheral resistance so it is apparent that it is common to all forms of hypertension. When the resistance to flow afforded by individual vascular beds is studied, the overwhelming body of evidence suggests that the increased resistance is present throughout the body. Isolated hindquarter preparations from SHR exhibit greater perfusion pressures for given flow rates in comparison to Wistar controls (Finch and Haeusler, 1974; Folkow et al. 1970; Lais et al. 1974; Lais and Brody, 1978) and WKY controls (Sano and Tarazi, 1987; Folkow and Karlstrom, 1984; Mueller, 1983). Renal vascular resistance is also elevated in SPSHR compared with WKY and the difference increases with age (Berecek et al. 1980a), although in the SHR there is no apparent increase when compared with WKY at maximal vasodilatation but the resistance change produced by infused agonists was greater (Smeda et al. 1988a), which suggests that when subjected to vasoconstrictor influences, the SHR would have raised renal resistance likely to be due to an increased media:lumen ratio produced by an increase in media thickness but no lumen diameter reduction. However, when compared with Wistar rats the

SHR renal resistance is increased at maximal vasodilation (Gothberg et al. 1979). The coronary vascular resistance is increased in SHR in comparison with WKY (Friberg and Nordlander, 1986) as is the mesenteric circuit to WKY (Yamamoto and Cline, 1987) and Wistar rats (Haeusler and Haefly, 1970). Other hypertensive models also display increased resistance to flow within various vascular beds; for example, DOCA salt hypertension results in an increase in renal resistance in rats (Berecek et al. 1980b), and also in the pig hindlimb (Berecek and Bohr, 1977) and rat hindquarters (Finch and Haeusler, 1974; Vial et al. 1989a and 1989b). In addition renal hypertension in the rat raises resistance in the hindquarters (Lundgren et al. 1974) as it does in the rabbit (Wright et al. 1987).

Raised basal resistance is a consequence of reduced lumen diameter, probably resulting from medial thickening encroaching upon the lumen. Arterial rarefaction would also increase basal resistance but evidence from microplugging experiments suggest that it is unlikely that a reduced artery number could explain the differences in the resistance curves of SHR and Wistar perfused hindquarters (Hallback et al. 1976). This is because microplugging does not result in steeper curves nor higher maximal perfusion pressures as would media:lumen ratio increases in the resistance vasculature. Although the hypothesis does not receive a great deal of support, it is fitting to at least review the evidence for rarefaction.

Initially it was work on the cremaster circulation which drew attention to the possibility of a reduced number of small

arteries causing the increased resistance in the SHR. In comparison to the Wistar circulation, a reduced number of small arterioles was observed in the SHR cremaster bed which included a 50% reduction in the number of vessels less than 50 um internal diameter (Hutchins and Darnell, 1974). However, it was also noted that the lumen diameters of the SHR arterioles were greater, possibly as a compensatory mechanism to counter the raised resistance that the rarefaction would produce. However, the tissue was inspected while the rats were under the effects of anaesthetic and consequently there may also be different effects on the vessel tone between the strains. Later the same bed was investigated with WKY rats as the control animal. Initially there was thought to be no difference between SHR and WKY cremasteric arcades under conditions of maximal vasodilation (topical sodium nitroprusside plus denervation mediated by hypogastric nerve section) (Bohlen and Lobach, 1978). Shortly afterwards it became apparent that before denervation the SHR did display a rarefaction of the microcirculation and the nerve section then resulted in a greater number of arterioles opening to flow in the SHR to normalise the number of conducting arterioles (Bohlen, 1979). Thus it seems that only a functional rarefaction may exist in this bed. Abdominal skin arterioles were then shown to be reduced in number in the SHR (Haack et al. 1980) compared with samples from WKY rats but again the lumen diameter was increased; mesenteric circuit observations were similar (Henrich et al. 1978). When dilated the small arterioles of the spinotrapezius muscle had narrower lumens which was considered a likely cause of increased resistance in SHR, but the authors did discuss the possibility again of a functional rarefaction under normal

conditions because the smaller arterioles were only visible when the vasculature was relaxed (Engelson et al. 1986). Consequently, problems of visualisation do make it difficult to assess any degree of possible rarefaction. If indeed rarefication does occur, it would appear to develop with age and the hypertension in SHR. Functional rarefaction is not present at 4-5 weeks of age in the jejunal region of the gut wall but at 18-21 weeks a reduced arteriolar number is observed which is abolished following adenosine-induced vasodilatation (Bohlen, 1983). In contrast, skeletal muscle arteriolar rarefaction does appear due to the development of anatomical rarefaction, i.e. an actual loss of arterioles. This is based upon the observations of Prewitt et al. where rats were studied at three ages, 6-8 weeks, 12-14 and 16-18 weeks. At all three ages a reduced capillary density was observed in the gracilis muscle. At 12-14 weeks a rarefaction of the arteriolar circulation was observed whether or not denervation was performed but not following vasodilation (functional rarefication). At 16-18 weeks the arteriole number was reduced regardless of innervation state or vasodilation (anatomical rarefaction) (Prewitt et al. 1982). It is important to note that Bohlen observed reduced arteriolar numbers in rats aged approximately 18 weeks also (Bohlen, 1979; Bohlen and Lobach, 1978);therefore it may be suggested that rarefaction is a consequence of hypertension - or at least progresses with it - but it does not appear to be an early (i.e. initiating) factor in hypertension development.

Resistance Artery Structure

From the resistance curves produced in perfused preparations from

SHR and Wistar rats (e.g. Folkow et al. 1970) Folkow hypothesized that there was a reduced lumen diameter in the SHR resistance vasculature, and now there is much evidence to support this. For any given distending pressure, cannulated resistance arteries from the cerebral and mesenteric beds of SHR have narrowed lumens in comparison with WKY (Brayden et al. 1983) and basilar resistance arteries in SHR have smaller lumen diameters when unstretched - i.e. effectively under zero distending pressure (Winquist and Bohr, 1983). Myograph mounted resistance vessels from the femoral (Mulvany et al. 1982) and mesenteric beds (Mulvany et al. 1980) have reduced normalised lumen diameters when taken from SHR and compared with WKY. In the renal vasculature at maximal dilation there is no lumen difference as assessed from similar minimal resistances in isolated perfused kidneys (Smeda et al. 1988a); however, this finding is in contrast to the increased SHR renal resistance when compared with Wistar rats (Gothberg et al. 1979) and also when SPSHR are compared with WKY animals (Berecek et al. 1980a). There are reports of increased lumen diameters in SHR resistance arteries but there are instances where the measurement of the arterial dimensions had taken place at a greater distending pressure in the SHR. Henrich et al. reported greater lumen diameters in SHR mesenteric precapillary arterioles compared with those from Wistar rats (Henrich et al. 1978; Hertel et al. 1978) but the measurements were made in living animals during anaesthesia. However, mesenteric resistance arteries when perfusion fixed at maximal dilation and similar perfusion pressures did not reveal any lumen differences in the SHR (Lee et al. 1983) suggesting that SHR lumens may in fact be wider when distended by the

greater blood pressure in the hypertensive rat. Fixed SHR cutaneous arteries from the abdomen have wider lumens but this may have been a mechanism to counterbalance the circulatory effect of the apparent rarefaction in this bed (Haack et al. 1980). The microcirculations of the cremaster circuit (Wiegman et al. 1979) and spinotrapezius muscle (Engelson et al. 1986) had no alterations in lumen diameter or reduced diameters respectively, but it is possible that the vessels studied were in fact too small to be resistance arteries. This is also a possibility for the precapillary arterioles studied by Henrich et al. (1978) and (Hertel et al. (1978). When subject to greater distending pressures arterial lumen diameters are normalised in the microcirculation of the spinotrapezius muscle (Zweifach et al. 1981).

Overall, it seems that while there is conflicting evidence regarding microcirculation vascular dimensions in SHR, resistance sized arteries when studied under standardised conditions do have reduced lumen diameters.

In vivo, the vasculature is normally subject to vasoconstrictor stimuli; therefore differences which are found at maximal vasodilation - while revealing structural alterations and thus changed baselines from which vasoconstricting influences operate - do not provide a complete picture by themselves. The media:lumen ratio is the haemodynamically important parameter and structural alterations resulting in increased media:lumen ratios are responsible for the steeper activation curves with greater maximal pressor responses seen typically in Folkow's work (Folkow

et al. 1970). In other words, this structural change alters the background upon which vasoconstrictor influences operate, and for a given degree of smooth muscle activation (i.e. smooth muscle shortening), no increase in agonist sensitivity is required to produce an enhanced resistance change. Folkow has calculated that in the isolated perfused hindquarter preparation, the difference in resistance curves between SHR and Wistar could be entirely accounted for if the SHR resistance vessels possessed a 30% thicker media and a 7% narrower lumen (Folkow et al. 1970; Folkow, 1978). Evidence exists for this to be a good model since direct measurements of media thickness and lumen diameter in arteries mounted in a myograph reveal that in femoral resistance arteries from 14 week SHR have a 28% thicker media and a 21% lumen diameter reduction in comparison with WKY (Mulvany et al. 1982). Mesenteric resistance arteries from 5-6 month SHR have a 49% thicker media and 16% reduced lumen diameter (Mulvany et al. 1980). In 12 week SHR a 38% thicker media and 15% lumen diameter reduction are observed (Jespersen et al. 1985). Remarkably similar values are also noted for subcutaneous resistance arteries from essential hypertensive patients in comparison to normotensive controls where media thickness is increased by 22% and the lumen decreased by 8% (Aalkjaer et al. 1987a).

An increase in media thickness in the resistance vasculature appears to be a general feature of all vascular circuits in SHR. In addition to femoral and mesenteric vessel medial thickening described above for vessels in the myograph, the renal vasculature is hypertrophied (Smeda et al. 1988a), as are the carotid arteries (Eccleston-Joiner and Gray, 1988),

larger vessels of the cremasteric microcirculation - i.e. true resistance vessels with diameters greater than 130 um - (Bohlen and Lobach, 1978) and, in SPSHR, cerebral vessels (Hart et al. 1980). Even non-resistance vessels are thickened such as the aorta (Owens and Schwartz, 1982) portal vein (Greenberg et al. 1981) and pulmonary arteries (Greenberg et al. 1978). The nature of the hypertrophy differs between large conducting vessels and the resistance vessels. In the aorta, the increase in smooth muscle bulk appears to be a consequence of cell polyploidy and hypertrophy which develops with the hypertension (Owens 1987; Owens and Schwartz, 1982;). Thus these cellular abnormalities may be a result of the increased blood pressure since antihypertensive therapy by several means inhibits them albeit to differing degrees (Owens 1985; Owens 1987). Lee et al. have demonstrated that smaller mesenteric arteries thickened as a result of a hyperplasia of smooth muscle cells which was present early in the life of the SHR but the superior mesenteric artery thickened as a result of smooth muscle cell hypertrophy which developed with time (Lee et al. 1983) while intermediate vessels exhibited some hyperplasia and these cells hypertrophied with increasing age. Consequently, there seems to be a graded alteration in myocyte growth abnormality down the arterial tree - hypertrophy becomes of reduced significance in an inverse manner to myocyte hyperplasia. That there is hyperplasia of the myocytes in the resistance vasculature - or rather the mesenteric resistance vasculature - seems to be confirmed by the work of Mulvany and colleagues where the resistance vessels had an increased number of smooth muscle layers (Mulvany et al. 1978) and an increased nuclear count determined by means of a three-

dimensional disector (Mulvany et al. 1985). This myocyte proliferation may occur in the first week of life (Yang et al. 1989). In addition, hyperplasia is inferred from the work of Owens et al. where the increased smooth muscle content of mesenteric arteries could not be accounted for by an increase in cell size (Owens et al. 1988). Using the disector method the hypertrophy of mesenteric resistance vessels in 1-kidney 1-clip Goldblatt hypertensive rats is apparently due to myocyte hypertrophy. This cell growth was apparently load mediated since renal arcuate arteries which may be assumed to have been protected from the high pressure were not thickened (Korsgaard and Mulvany, 1988). Consequently the nature of the myocyte proliferative response varies depending upon the position in the vascular tree and the nature of the hypertension.

Load increases are thought to be a stimulus to myocyte growth, as exemplified by tissue culture experiments. An increased rate of DNA and protein synthesis is observed in rabbit ear artery rings when they are incubated under tension induced by small metal springs (Hume, 1980). Cells incubated on deformable meshes, undergoing cyclic deformation, show stimulated growth and cell division. For example, chicken embryo fibroblasts when incubated on a deformable mesh have a greater mitotic count when subjected to low frequency deformation (Curtis and Seehar, 1978). Cyclic stretching of rabbit aortic cells on an elastic membrane exhibit enhanced protein synthesis (Leung et al. 1977) and similarly a cyclic tension load on embryonic chick digital flexor tendons produce enhanced rates of protein and DNA synthesis (Slack et al. 1984). A cyclic deformation is not absolutely necessary since

feline cardiocytes grown on a deformable laminin surface and subjected to a 10% length stretch produce more RNA and protein which suggests that load in itself is sufficient to influence gene expression (Mann et al. 1989). Most recently a role has been ascribed to sodium influx in the transduction of the load signal, because a tension load on ferret papillary muscle results in an enhanced Na^+ influx and protein synthesis; the Na^+ influx may be the crucial signal since pharmacologic Na^+ influx alteration was also associated with parallel protein synthesis alterations (Kent et al. 1989). Isolated cardiocytes from SHR left ventricles are bigger than those of normotensive WKY and Fischer 344 rats (Bishop et al. 1979). This may not be a direct result of different neurohormonal influences since left ventricular cardiocytes were larger than those from right ventricles regardless of strain, suggesting that a load increase can directly influence the morphology of cells in the cardiovascular system in vivo. In support there were no differences in cardiocyte size from the right ventricles.

Studies on cultured myocytes from SHR and WKY show that cells originating from SHR proliferate faster in culture (Yamori et al. 1984), and this accelerated proliferation seems to be inherent since even after several passages SHR and SHRSP aortic derived myocytes grow faster than those from WKY (Yamori et al. 1981). However, the rats from whom the cells were obtained were approximately ten weeks old and therefore would have been hypertensive for about a month; therefore the possibility still exists that the cells had become 'programmed' for increased growth by exposure to an increased pressure load. A similar

situation might explain the results of faster growing myocytes from 20 week SHR compared with WKY (Resink et al. 1987). In a more recent study a role for EGF has been postulated since SHR-derived myocytes proliferated more rapidly than those from WKY. The SHR cells possessed twice as many EGF receptors and were more responsive to the addition of EGF with regard to increased internal pH, phosphoinositide hydrolysis and DNA synthesis (Scott-Burden et al. 1989).

However, tissue culture experiments are only of limited value since the behaviour of a cell isolated from the myriad neurohormonal influences which may otherwise influence it may be far removed from that normally occurring in vivo. It has been postulated that the vasculature of the SHR is more prone to hypertrophic changes than that of the WKY (Folkow, 1986). In other words, for a given pressure load medial development in the vascular tree will be accelerated either due to an abnormal pressure-structure relationship or due to factors independent of load. To this end, many studies have been performed where SHR are treated with antihypertensive therapy to normalise their blood pressure and the cardiovascular structural development is then studied in the absence of hypertension. Of course, it is immediately apparent that even this approach does not circumvent the problem of altered neurohormonal influence; a possible explanation for the inconsistency of experimental results more than likely reflects the fact that different therapeutic approaches will have various influences on the blood pressure homeostatic mechanisms that operate. However, accepting these limitations, the literature would suggest that the SHR vascular

system does indeed continue to hypertrophy in the absence of hypertension. In isolated perfused hindlimb preparations, the degree to which antihypertensive therapy can prevent the vascular structural change in SHR is determined by the resistance at maximal vasodilation and by the steepness and maxima of the resistance curves to infused agonists. When a variety of drugs were used it was shown that the minimal resistance was reduced and the noradrenaline dose response curves were shallower with reduced maxima (Weiss and Lundgren, 1978); the changes can be explained by an increased lumen diameter and decreased media:lumen ratio of the resistance vasculature. However, none of the treatments employed (propranolol, metoprolol, hydralazine, guanethidine plus hydralazine) maintained the SHR blood pressure at normal levels comparable with those of Wistar controls; consequently the data do not permit comparison of normotensive SHR and control resistance vasculature structures. Similarly, equal blood pressure reductions were brought about by hydralazine or captopril plus hydrochlorothiazide but in neither experiment was it reduced to normal WKY pressures (Sano and Tarazi, 1987). The pressure reductions resulted in reduced hindlimb resistance for the combination-treated but not hydralazine-treated rats. Thus different therapeutic means of reducing blood pressure do not result in similar structural regression. Unfortunately this study did not allow direct comparison of SHR resistance curves after treatment to normal WKY levels since blood pressures were not fully normalised, and, in addition, the treatment was begun at 17 weeks (when significant structural thickening would have developed), and continued for a further 12 weeks. It may be more difficult to reverse structural change than to prevent it.

Consequently the studies which may provide evidence for or against an enhanced vascular development in the SHR are those in which the blood pressure rise is avoided in the first instance by treatment from an early age and where the blood pressure is maintained at similar levels to that observed in WKY. Unfortunately it appears that the abilities of different drugs to control blood pressure do not match their ability to normalise SHR vascular structure and so in consequence it still remains premature to conclude that there is an enhanced drive for medial thickening and lumen encroachment in the SHR. Captopril appears to be successful in controlling pressure and structure: in myograph-mounted mesenteric resistance vessels from treated SHR no structural difference was noted when compared with those from control WKY (Freslon and Guidicelli, 1983) while in a later study only partial normalisation of media:lumen ratio was achieved as a result of an increase in lumen diameter as was the case with hydralazine (Christensen et al. 1989). Perindopril did normalise the media:lumen ratio though and this was due to an increase in lumen diameter and also importantly, a decrease in media thickness, both to WKY levels suggesting that perindopril at least could prevent changes in structure (Christensen et al. 1989). That different forms of treatment can have a variety of effects upon structure is highlighted by the fact that in the same study metoprolol resulted in no attenuation of structural development while isradipine could only partially normalise the media:lumen ratio by non-significant increases and decreases of lumen diameter and media thickness respectively. However, the interesting point to note from this investigation is that media volume was not affected by any of the treatments. Therefore the

smooth muscle mass was not reduced despite prevention of the blood pressure rise in SHR; thus a structural rearrangement of the cells around the lumen appeared to be the mechanism by which media thickness was reduced and lumen diameter correspondingly increased. Using a similar method, Jespersen et al. demonstrated that hydralazine treatment only increased lumen diameter with no media thickness change in mesenteric resistance vessels (Jespersen et al. 1985). Pinacidil (Jespersen et al. 1986a;1986b) verapamil and bepridil (Jespersen et al. 1986a) normalised blood pressure completely in SHR but mesenteric resistance vessel structure was not affected in any case. In the same two studies (Jespersen et al. 1986a;1986b) fixed samples of kidney, heart and lung could not provide evidence for attenuation of medial thickening. Indeed the majority of studies suggest that structural changes in the SHR vasculature are not dependent upon hypertension since only an incomplete attenuation of development occurs when the hypertension is prevented. Further examples of drugs that fail to ameliorate structure are metoprolol and felodipine; in combination on coronary vascular resistance (Friberg and Nordlander, 1986) felodipine on mesenteric resistance vessels (Mulvany et al. 1981a; Nyborg and Mulvany, 1985) and pre-and post-natal hydralazine treatment on perfusion fixed kidney vasculature (Smeda et al. 1988b). Hydralazine-mediated blood pressure reduction in older rats did not normalize the coronary vascular structure (Anderson et al. 1989). A combination of reserpine, hydralazine and chlorathiazide prevented the increase in SHR aortic smooth muscle mass by inhibiting cell hypertrophy (Owens, 1985) but as described previously, resistance vessel medial thickening is not a result of individual cell hypertrophy but

rather it is a result of cell hyperplasia. When human essential hypertensive patients are treated it seems that structure is not fully normalized in the subcutaneous resistance vessels when compared with normotensive controls (Aalkjaer et al. 1988), since media:lumen ratios were still greater. Also using the same vascular bed it was shown that there is a significant reduction in media:lumen ratio after treatment (Heagerty et al. 1988a) but not to the values published earlier (Aalkjaer et al. 1987a) for normotensive controls. It is important to note that these data do not suggest that human essential hypertension is associated with an abnormal blood pressure - vascular structure relationship, but rather they may reflect the fact that it could be more difficult to regress vascular hypertrophy than to prevent it.

If the SHR vasculature receives greater non-pressure dependent trophic stimuli then increased sympathetic activity is a strong candidate. Evidence for the sympathetic nervous system's influence on smooth muscle growth is provided by experiments where its influence is eliminated. Bevan demonstrated that following surgical denervation in rabbits (left superior cervical ganglionectomy) the smooth muscle cells in the ear artery on that side had reduced ^{3}H-thymidine uptake and therefore proliferated less rapidly than those in the innervated ear artery (Bevan , 1975). In a later publication Bevan demonstrated that there was a reduced smooth muscle bulk in the artery and a reduced media:lumen ratio (Bevan , 1984). Similarly, in the SPSHR, unilateral superior cervical ganglionectomy resulted in reduced wall:lumen ratio in cerebral vessels on that side (Hart et al. 1980). However, immunosympathectomy of SHR reduced the blood

pressure to that of normotensive Wistar rats but there was no apparent normalization of structural changes as deduced from noradrenaline dose response curves in isolated perfused hindquarter preparations; this led Folkow and co-workers to suggest that there was a genetic alteration in the blood pressure - vascular structure relationship in the SHR (Folkow et al. 1972). Similarly, the SHR systemic resistance was still maintained above that of WKY even when the blood pressure was normalized by nerve growth factor antiserum (Cutiletta et al. 1978). In vivo denervation in SHR also fails to influence mesenteric resistance vessel media:lumen ratios (Mulvany et al. 1981b; Nyborg et al. 1986) but these observations may have been a result of incomplete destruction of nerves in the SHR (Nyborg et al. 1986) or a more rapid nerve regeneration due to nerve growth factor release from the myocytes. In contrast however, in vivo denervation with nerve growth factor antiserum plus guanethidine for the first 4 weeks of life resulted in SHR blood pressures normalized to those of WKY; the nerve free mesenteric vasculature (confirmed by electron microscopy, fluorescence histochemistry and a lack of response to nerve stimulation) of SHR preparations had normalized lumen diameters and a reduced resistance. In addition, the denervation was associated with a reduction in the myocyte hypertrophy of the larger conducting arteries and the hyperplasia in the smaller arteries (Lee et al. 1987). For a more in depth discussion of the trophic interactions between smooth muscle cells and the sympathetic nervous system the review by Head (1989) is recommended. Such a discussion is not considered to be more than of a general interest in the present work, since the femoral resistance vasculature is poorly innervated (Mulvany

et al. 1982) and blood vessels in skeletal muscle lose their innervation when they enter the muscle (Fuxe and Sedvall, 1965). Consequently, in the present study where the effect of pressure reduction on the SHR vascular structure will be described for the femoral vessels, the direct influence of the sympathetic nervous system will be minimal, although of course not ignored.

Much of the data described above would suggest that the SHR vascular structure is either resistant to attenuation of structural change with treatment - whether the structural change is due to structural reorganisation or true medial growth. As previously described captopril successfully prevented medial hypertrophy of mesenteric resistance arteries and blood pressure development, (Freslon and Guidicelli, 1983) while a combination of reserpine, hydralazine and chlorothiazide from age 25 weeks to 48 weeks resulted in similar percentage reductions in blood pressure and media thickness of mesenteric resistance vessels of both SHR and WKY (Warshaw et al. 1980), a result which would not be expected if there was an abnormal blood pressure - vascular structure relationship in the SHR. In addition, Nordborg has shown that treatment with metoprolol and felodipine could prevent and reverse the media:lumen ratio increases observed in mesenteric arteries of SHR and SHRSP despite the fact that blood pressures were not fully normalised to those of WKY (Nordborg 1989).

The effect of antihypertensive therapy on femoral resistance vessel structure has not been determined directly. However, there are two studies on isolated perfused hindquarters which suggest

that there is no accelerated growth in the hindlimb vasculature (Folkow and Karlstrom 1984; Mueller 1983). In these two similar experiments it was noted that from 5 weeks and upwards the minimum resistance was elevated in SHR, but the difference between SHR and WKY was constant with time and thus no enhanced lumen encroachment appeared to have occurred in the SHR. The difference in the pressor responses to infused noradrenaline became greater with age suggesting greater medial thickening with time in the SHR as its blood pressure rose (and consequently resulting in greater media:lumen ratios). However, the difference in pressor response was not greater than the blood pressure difference which does not suggest any structural thickening in excess of that stimulated by the blood pressure difference. Therefore it is possible that the femoral vascular structure is not subject to excess non-pressure dependent mechanisms.

Aims of the Study

The major part of the experimental work in the present study concerns the structure pressure relationships in SHR and WKY femoral resistance vessels, and the work avoids the pitfalls of altered humoral influences which are encountered when drug therapy is used to alter blood pressure. This is achieved by inserting a mechanical barrier into the circulation - i.e. a physical means of pressure reduction. The barrier is a partially constricting silk ligature tied around the left external iliac artery. Consequently each rat studied has one normally perfused hindlimb and one perfused at a reduced pressure, and the two hindlimbs in each rat should be subject to the same circulating humoral influences. Further details are presented in the appropriate experimental introduction and methods. The ligature

experiment also permits comparison of vascular sensitivity to noradrenaline and noradrenaline stimulated calcium sensitivity in vessels from the same rat (i.e. with the same genetic and humoral background influences) to address the question of blood pressure influence on agonist sensitivity. In addition, two shorter studies have been described. Firstly, hypertension was induced in Wistar rats by chemical renal medullectomy by 2-bromoethylamine administration, and the effect of this tretment upon mesenteric resistance vessel structure was studied in addition to its effect upon noradrenaline sensitivity and noradrenaline-stimulated calcium sensitivity. Secondly, the vasopressin and vasopressin-stimulated calcium sensitivities in SHR and WKY mesenteric resistance vessels have been compared.

CHAPTER 2

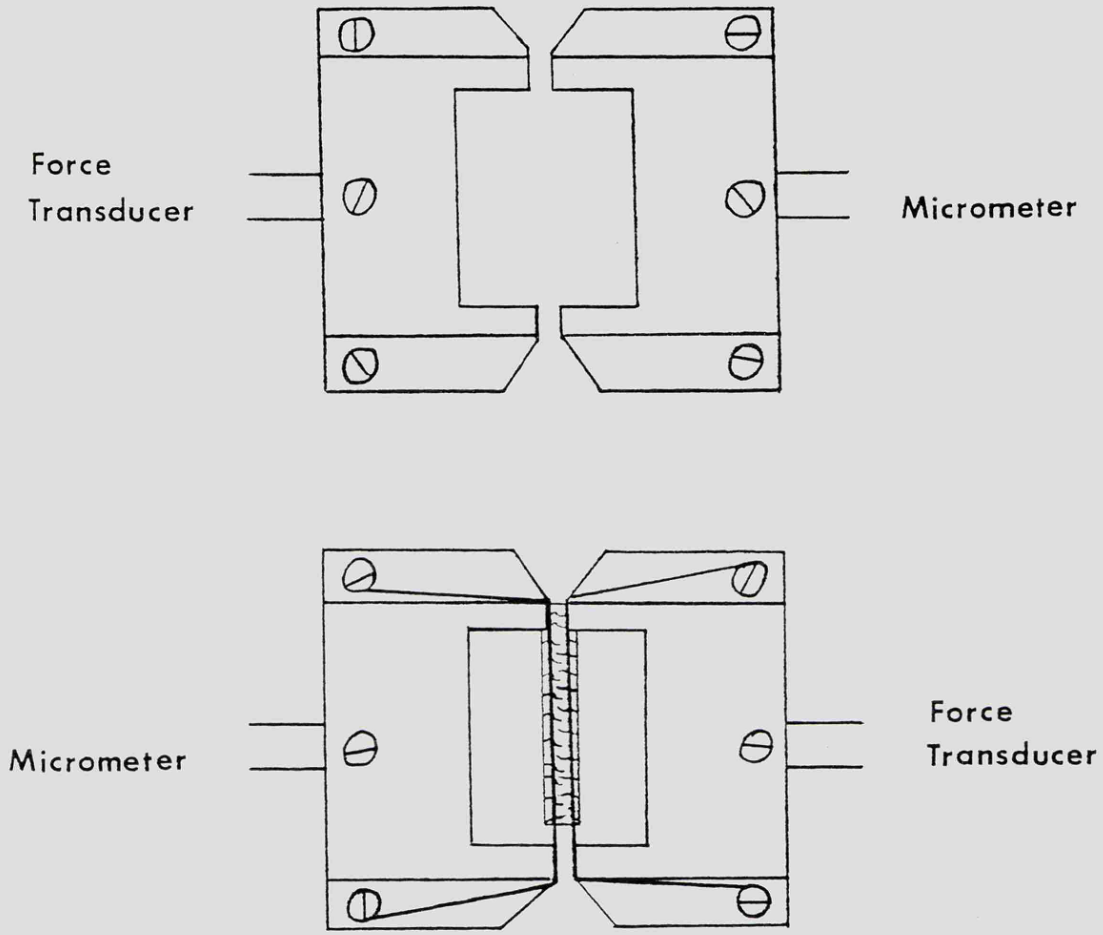
Methods and Equipment

The Myograph

The myograph was developed in the mid 1970s and originally described in 1977 (Mulvany and Halpern, 1977). The principle of the myograph is fairly straightforward but hypertension research has made huge gains from it since the small arteries which contribute to the resistance to blood flow - and hence hypertension - can be studied directly and under standardised conditions. The present generation of myographs now constructed permit the study of two arteries in parallel. The instrument consists of a 15 ml capacity bath in which is two separate pairs of mounting heads (Fig 3). One head of each pair is connected to a micrometer screw and is thus mobile while the other is attached to a force transducer by a connecting pin which passes through the side of the bath. Each head has two screws which are used to grip the wire upon which the artery is mounted. The bath is heated by a heating block either side which is warmed by circulating water. The bath has a gas inlet for bathing medium bubbling, and two drainage ports attached to a suction pump for bath emptying. The force transducers are fixed into the wall of the bath and are connected to a pre-amplifier. In the experiments described in this work the pre-amplifier signals are recorded using a Grass polygraph (Grass Instrument Co., Quincy, Mass., USA).

Vessels are threaded on to two wires, each of which is fixed by the screws on each mounting head. The wire is stainless steel and

FIGURE 3



Schematic illustration of the two sets of mounting heads in the myograph bath. Each set has one head connected to a force transducer and one to a micrometer screw. The lower set in this figure illustrates how the wires pass down the lumen of a vessel and are anchored by screws on the mounting heads.

is normally 40 um in diameter although 25 um or 30 um was sometimes required for the smallest arteries.

Morphology Measurements

In addition to the advantage of being able to measure tension developments in resistance arteries using the myograph, it is also possible to measure the thickness of the smooth muscle layer in the arterial wall. The myograph is designed with a small perspex window situated directly beneath the mounting heads. When the myograph is mounted on the stage of a light microscope the artery wall can be imaged using water immersion microscopy. A calibrated micrometer eyepiece is used to measure the thickness of the three wall layers, namely the adventitia, media and intima. The equipment used in the present study for morphological measurements of myograph mounted arteries was a micrometer eyepiece (X8, Zeiss), light microscope (model KHC, Olympus), and a salt water immersion lens (X25, Leitz-Wetzlar).

The medial thickness at l_o (i.e. the diameter to which the artery is set for the pharmacology experiments as described in the normalisation section) is the value that needs to be derived. This value, m_o, cannot be measured when the vessel is under tension since the wires tend to 'bite in' to the media in the region of contact with the artery wall when the artery is stretched. Consequently the wires are separated until the artery is just under tension and the media thickness is measured in addition to the gap between the wires. This allows calculation of the media volume and by assuming constant media volume (Carew et al. 1968) the medial thickness that the vessel would have when

stretched to l_o may be calculated.

Normalisation Procedure

In many vascular pharmacological experiments, arterial strips or rings are simply placed under a given tension load, e.g. '2g tension'. It is obvious that if all arteries in a study are treated thus, then smaller arteries would be under a proportionally greater stress than larger ones. The normalisation procedure used in connection with the myograph permits the setting of arteries to lumen diameters at which they develop maximum or near maximum active tension developments. The procedure is based upon the fact that vascular smooth muscle displays a 'dome shaped' active tension-length relationship. The force generating ability of the contractile elements increases with stretch up to an optimal stretch, after which further stretch reduces the active tension that may be produced and the passive tension increases sharply. The Law of Laplace can be applied to blood vessels since they are essentially distendable cylinders. Thus, from the relationship T=Pr where T is tension, P is pressure and r is the radius, then the effective pressure inside a cylindrical section of vessel can be calculated for any given internal circumference and wall tension. When applied to the myograph experiments, the changes in tension and internal circumference (as measured on the Grass polygraph and micrometer screw movement respectively) can be used to plot a tension-length curve for the vascular smooth muscle and thus the internal circumference of the vessel at an effective internal pressure of 100 mmHg (13.3 kPa) can be calculated. A curve fitting program is run on a programmable calculator (Hewlett Packard HP41 CV) as the

artery undergoes a series of stretches by turning the micrometer screw. When the vessel has been stretched to the point when the effective internal pressure is equal to or greater than 100 mmHg then the procedure is halted and the vessel relaxed while the calculator determines the effective internal diameter it would have when relaxed and under an effective transmural pressure of 100 mmHg. In addition, a micrometer reading is calculated to which the artery is set and thus set to any desired fraction of this diameter. If the artery is set to a diameter 90% of that it would have at 100 mmHg then the active tension development will be maximal or near maximal i.e. corresponding to the top of the dome shaped active tension-length curve.

Expressions:-

(i) Laplace's Law is described as $T = Pr$

(ii) L_{100} is the internal circumference when P is 100mmHg (i.e. when the vessel is relaxed and under an effective transmural pressure of 100 mmHg)

(iii) l_{100} is the effective diameter corresponding to L_{100} thus $l_{100} = L_{100}/\pi$

The term 'effective diameter' is used since the vessel is stretched out flat and is not cylindrical when on the wires.

(iv) l_o is the normalised effective diameter, such that $l_o = 0.9\ l_{100}$ when vessels are set to a diameter corresponding to 90% l_{100}.

ΔT is active tension, expressed as mN/mm segment length

ΔP is effective active pressure, expressed as kPa, and defined as $2000\ \Delta T/l_o$ where l_o is normalised lumen diameter in um.

ΔS is active media stress, expressed as kPa, and defined as

$1000\ \Delta T/m_o$.

Therefore arterial responses to activating stimuli can be calculated in three ways; ΔT, ΔP or ΔS. While ΔT and ΔP described the actual magnitude of the response, ΔS normalises the tension development to the media thickness of an artery, and thus describes the contractility of the smooth muscle.

Rejection Criteria

Arterial segments are rejected if $l_o > 300um$, or if $\Delta P < 13.3$ kPa in NAK, where NAK is the maximal activation solution and whose composition is described below in 'solutions'.

In the earlier myograph reports from Mulvany and Coworkers, l_o was defined as $l_o = 0.8\ l_{100}$, although all reports since 1980 define l_o as $l_o = 0.9\ l_{100}$.

Therefore when arteries are normalised the procedure derives the micrometer screw setting for any individual artery to be set to its l_o; the pitfall of standard tension loads is therefore avoided. When a vessel segment is set to l_o it is 'normalised'. Mesenteric arteries used in the present study were normalised assuming $l_o = 0.9\ l_{100}$. Mulvany has published experiments where femoral resistance arteries were set to $0.9\ l_{100}$ (Mulvany et al. 1982) but part of the present study was performed to confirm that this is the appropriate setting (Chapter 5).

Indirect Systolic Blood Pressure Measurement

Rats were placed in an ether box until they showed no response to a slight shake of the box. They were removed and placed on a

warmed pad. An inflatable cuff connected to an ordinary mercury sphygmomanometer was placed around the tail and distal to this a light source and photomultiplier cell connected to a plethysmograph was placed. An oscilloscope displayed the plethysmograph output. Pulse waves in the tail were visualised on the oscilloscope screen. The cuff was inflated until the waves were eliminated, and then the cuff was slowly deflated until the waves were just visible and the pressure recorded by the sphygmomanometer was noted as the indirect systolic blood pressure. For each rat the inflation-deflation procedure was repeated 2-3 times to confirm the recorded measurement.

Dissections

Rats were killed by stunning and cervical dislocation. Mesenteric resistance arteries were obtained from the mesenteric circuit which was exposed and excised after longitudinal incisions in the abdomen. Femoral resistance arterioles were obtained from hindlimbs which had been removed from the body to permit ease of handling. Dissections were performed in physiological saline solution (composition described below) using trabecular scissors and watchmaker forceps filed down further to permit finer handling.

Solutions

Vessels were dissected and normally held in the myograph in physiological saline solution, $PSS^{2.5}_{o}$, of the following composition (mM):-

NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.17, KH_2PO_4 1.18 K_2-EDTA 0.026, Glucose 5.5. $K\text{-}PSS^{2.5}_{o}$ is of the same composition

as $PSS^{2.5}_{0}$ but with equimolar substitution of KCl for NaCl. PSS^{0}_{0} is Ca-free PSS. $PSS^{0}_{0.1}$ is Ca-free with 0.1 mM EGTA, and PSS^{0}_{5} is Ca-free with 5mM EGTA. NAK is 10 uM noradrenaline in $KPSS^{2.5}_{0}$.

Drugs

Noradrenaline hydrochloride (Arterenol), cocaine hydrochloride, and [arginine] vasopressin (grade VI) were purchased from Sigma Chemical Company, UK. 2-bromoethylamine hydrobromide was purchased from Aldrich, UK. Heparin was purchased from Weddel Pharmaceuticals Ltd., Wrexham, UK.

Induction of Chemical Renal Medullectomy

Female Wistar rats weighing 170g-190g were used. 2-bromoethylamine hydrobromide (BEA) was administered as a single intra-peritoneal injected dose, 200mg BEA/kg body weight. The injected solution was made up as a 10% w/v solution in 150 mM NaCl. As controls, additional rats were injected with equivalent volumes of the saline vehicle. Animals were kept individually caged and given free access to standard chow and tap water. Three weeks after injection, the rats' indirect systolic blood pressures were measured using the tail cuff plethysmography method.

Chemical Renal Medullectomy Myograph Protocol

Rats were used for myograph experiments not less than one day and no more than three days after the blood pressure measurement, and were used singly for each myograph experiment. They were killed by stunning and cervical dislocation as previously described and two second-order artery branches were dissected and mounted in the myograph.

After bubbling with 5% CO_2 in O_2 and warming to 37°C for 30 minutes arterial morphology measurements were made and the segments then normalised. The standard start procedure used was three KPSS activations followed by one NAK activation. Activations were for 2 minutes each and full recovery was permitted between contractions. A noradrenaline concentration response relationship was then constructed (0.08 - 10 uM) with 2 minutes per concentration and the tension response at the end of each concentration application was used for calculation purposes. Next a noradrenaline-stimulated calcium concentration response relationship was constructed as previously described (Mulvany and Nyborg, 1980). Briefly, Ca depletion was effected by the following procedure:-

PSS^{o}_{5} for 15 seconds, 10 uM noradrenaline in $PSS^{o}_{0.1}$ for 2 minutes and PSS^{o}_{o} for 2 minutes. This sequence was performed three times, which is sufficient to abolish the response to noradrenaline in PSS^{o}_{o}. The calcium concentration in the bathing medium was then raised incrementally from 0 to 2.5 mM by bathing solution replacement. Each $[Ca^{2+}]$ was applied for 4 minutes and 10 uM noradrenaline was present for the latter 2 minutes of each 4 minute period. Tension responses at the end of each 4 minute period were used to calculate concentration response curves. For the two arteries from each rat a mean value for each measured parameter was calculated so that one value, for example, m_o, was obtained for each rat.

Statistical Analysis

Results were compared between strains using Student's unpaired t-test. Sensitivities to noradrenaline and calcium were calculated as pD_2 values where $pD_2 = -\log_{10}ED_{50}$ and ED_{50} values were

calculated in terms of molar concentrations. ED_{50} values were calculated using the HP41CV programmable calculator utilising a program which calculates ED_{50} by least squares analysis. Concentration response curves were compared using two-way analysis of variance. Results were considered significantly different if $P<0.05$.

Vasopressin Sensitivity Methods

Twelve week old SHR and WKY females were used. Rats were killed and second-order mesenteric arteries were dissected out as described previously. For each experiment an SHR and WKY rat were studied in parallel, one artery per rat, and arteries were alternated each day on the two transducers to compensate for changes in transducer sensitivity. Following warming to 37°C and bubbling with 5% CO_2 in O_2 the arterial segments were normalised and pre-treated by the following standard start procedure:- two NAK activations for 2 minutes each, one 2 mU/ml AVP activation (3 minutes), one 2mU/ml AVP in $KPSS^{2.5}_{o}$ (3 minutes) and a final NAK (2 minutes), where AVP is [arginine]-vasopressin. Arteries were allowed to relax completely between each of the activations. An AVP concentration response curve was then constructed. Increasing concentrations of AVP were applied, 3 minutes each concentration over a concentration range 0.06 - 2 mU/ml. Previous experience had shown that 2 mU/ml gives the maximum AVP response in these arteries. The response at the end of each 3 minute period was used for calculation purposes. Where oscillatory tension changes were observed, the mean tension for the last 20 seconds was used. An AVP-stimulated calcium dose response curve was then constructed as follows:- calcium was depleted from the arteries by a 15

second exposure to PSS^{0}_{5} , 2 minutes of 2mU/ml AVP in $PSS^{0}_{0.1}$ and then 2 minutes of PSS^{0}_{0} . This sequence was performed three times and is sufficient to reduce the agonist response in PSS^{0}_{0} to zero. The$[Ca^{2+}]$ in the bathing medium was then increased incrementally from 0 to 2.5 mM with each concentration applied for 6 minutes. 2mU/ml AVP was present for the latter 3 minutes of each 6 minute period. Tension responses at the end of each 6 minute period were used for calculation purposes. It became apparent that tachyphylaxis was a major problem, since the active tension responses remained small even when the $[Ca^{2+}]$ was 2.5 mM. Consequently in order to evaluate the AVP-stimulated Ca sensitivity, a second series of experiments were performed on a further set of arteries with a reduced exposure time to AVP. Arteries were dissected, mounted and normalised as before. The standard start was changed to three $KPSS^{2.5}_{0}$ activations and one NAK activation, 2 minutes each, with complete relaxation in between. The maximum response to AVP was then determined (2mU/ml) from a 3 minute activation. One hour later, calcium depletion was effected as previously described but with noradrenaline substituted for AVP. Preliminary experiments had shown that depletion of the noradrenaline-sensitive Ca pool could also eliminate AVP-induced contractions in PSS^{0}_{0} . Each pair of arteries was then subjected to a single $[Ca^{2+}]$ for 6 minutes with 2 mU/ml AVP present for the latter 3 minutes of the 6 minute period. The resulting AVP-induced contractile response was compared with the maximal AVP-induced contraction obtained previously, and the response expressed as a percentage of the maximum.

Statistical Analyses

Results were compared as for those in the chemical renal medullectomy experiment, except that AVP sensitivities were calculated as pAVP where pAVP is defined as $-\log_{10} ED_{50}$ and ED_{50} was calculated in terms of mU/ml.

Determination of l_o for Femoral Resistance Arteries

Male SHR and WKY rats aged 11-13 weeks were used. Third order femoral arteries were dissected and mounted in the myograph. The bathing $PSS^{2.5}_{0}$ was bubbled with 5% CO_2 in O_2 and warmed to 37°C. The arterial segments were then normalised to l_o, where l_o was taken as 0.9 l_{100}, and then activated by a standard start procedure of two NAK contractions, one 10uM noradrenaline contraction, a $KPSS^{2.5}_{0}$ and a NAK activation. Each activating solution was applied for 2 minutes and complete relaxation was permitted between activations. Micrometer screw settings corresponding to l_o values of 0.6, 0.7, 0.8, 0.9, 1.0 and 1.1 l_{100} values were calculated using the normalisation program. Initially only a range of 0.6 - 1.0 l_{100} values were calculated but subsequently it became apparent that including 1.1 l_{100} would produce a fuller active tension-length relationship. Therefore the number of experiments at this setting is smaller. Experiments were performed using one or two arteries per rat and if rat availability was sufficient, then an SHR and a WKY artery were paired. l_o values were randomly set and two NAK activations were performed in order to determine the maximum tension response at each l_o setting. The procedure was continued until the arteries had been challenged at all the l_o settings. The mean of the two NAK responses was calculated and used to determine the mean

tension-length relationships plotted in figure 10.

It should be noted that the rats used had not been operated on. It was felt that a tension-length relationship need not be determined for arteries distal to an iliac artery constriction, since protected and unprotected arteries were to be studied at the same normalised setting - i.e. l_o would be the same fraction of l_{100} - so that morphological characteristics could be compared under standardised conditions. After all, standardisation is the major advantage of the myograph set up. However, in defence, it is important to note that in a previous study (Hansen et al. 1974), structural regression of the main femoral artery was accomplished by partial iliac artery occlusion and although tension responses were diminished, the tension length curves were not shifted in any direction in the arteries from the low pressure hindlimb compared with the normally perfused hindlimb.

Blood Pressure Protection Studies

Ligature Procedure

Five week old male SHR and WKY rats were prepared for surgery following determination of indirect systolic blood pressure. Under ether anaesthesia, the left side of the groin region was shaved and cleaned using 5% hibitane in 70% alcohol. A longitudinal incision approximately 1.5 cm in length was made in the skin layer and then the left external iliac artery was exposed following a 1 cm incision in the abdominal wall. The artery was separated from the vein and surrounding tissue by careful manipulation. A short length of 4/0 silk suture was passed under the artery and a short piece of stainless steel

wire, 0.4 mm in diameter and 5 mm long was laid next to the artery and on top of the silk. The silk was then used to tie the wire firmly to the artery thus temporarily preventing blood flow. The wire was then pulled out from the silk loop and blood flow returned. Consequently the artery was surrounded by an approximately 0.4 mm diameter loop which, at the time of placement, rested around the artery without blood flow restriction. The same piece of wire was used for all the ligature operations. Further groups of SHR and WKY rats underwent a sham operation which involved the tying of a loose loop around the left external iliac artery of approximately 1 cm internal circumference. This loop remained non-restrictive. For all operated rats, interrupted sutures were used to close the wound and a lignocaine smear (lignocaine hydrochloride gel BP 1%) was used as an analgesic.

Determination of Femoral Mean Arterial Pressure

Femoral mean arterial pressure (FMAP) was measured in sub-groups of SHR and WKY rats following cannulation of the femoral artery under ether anaesthesia. The femoral artery was exposed following a skin incision and separated from the vein and surrounding tissue by careful manipulation. 5 week rats were cannulated using PE10 tubing (internal diameter 0.28 mm, external diameter 0.61 mm) and only the left femoral artery was cannulated. 12 and 24 week rats underwent bilateral femoral cannulation using PE25 tubing (internal diameter 0.4 mm, external diameter 1.8 mm). The cannulae were filled with a sterile NaCl venous infusion solution (0.9%) containing 10 units per ml heparin. Cannulae were exteriorised at the nape and protected by a steel spring to prevent the rats from chewing the cannulae and self-exsanguinating.

Cannulae were connected to Satham P23hD pressure transducers and a Grass polygraph was used to record the blood pressure trace. Since bilateral femoral cannulation was performed, this allowed a direct comparison of FMAP in ligatured (protected) and unligated (unprotected) hindlimbs. Following the cannulation procedure the leg wound was closed using interrupted silk sutures. Rats were then placed unrestrained in perspex cages with free access to food and water. The top of the protecting steel spring was taped to the top of the cage. Arterial pressure traces were followed continuously but only the FMAP recorded during the 4-4½ hour post-operative period was used for calculation purposes, since the recorded pressure rose with time and stabilised by 4 hours. FMAP was calculated as diastolic pressure plus one-third pulse pressure. Following this recording period, rats were killed by an ether anaesthesia and cervical dislocation. Femoral resistance arteries were not taken from cannulated rats for the in vitro studies described below.

Femoral Resistance Artery Myograph Protocol

Third order branches of the left and right femoral arteries were dissected out and mounted in the myograph. The branching pattern was consistent between hindlimbs and between strains, therefore all experiments were conducted on equivalent arteries and there was no selection bias. The $PSS_0^{2.5}$ in the bath was warmed to 37°C and bubbled with 5% CO_2 in O_2 for at least 30 minutes, following which morphological measurements were made and the arteries normalised to l_o, where l_o was taken as $l_o = 0.9\ l_{100}$. As described in the Results Section, these arteries produce maximal or near maximal contractions at this l_o setting. The arteries were subjected to a standard start procedure of 2 NAK

activations, one 10 uM noradrenaline activation, a $KPSS_o^{2.5}$ activation and a further NAK. Each activating solution was applied for 2 minutes and complete relaxation was permitted between activations. Noradrenaline concentration response curves were then constructed (0.02 - 10 uM); each solution was applied for 2 minutes before replacement by the next noradrenaline concentration. Following washout of the highest dose, the arteries were allowed to relax completely and 5 minutes later 3uM cocaine was applied. This cocaine incubation lasted 10 minutes and then a second noradrenaline dose response curve was constructed in the presence of 3 uM cocaine. Maximum responses achieved in any noradrenaline dose were used for calculation purposes. Following washout, approximately 30 minutes elapsed before a noradrenaline-stimulated calcium concentration response curve was constructed exactly as described for the arteries in the chemical renal medullectomy experiment.

Noradrenaline, noradrenaline + cocaine and calcium pD_2 values were calculated from the recorded tension responses. The effect of cocaine on the noradrenaline sensitivity was calculated as the 'cocaine shift' where shift is defined as $pD_2^1 - pD_2^2$, and pD_2^1 and pD_2^2 are the noradrenaline pD_2 values in the absence and presence of cocaine respectively. Arteries from 5 week rats did not lend themselves to accurate active tension development measurements since these arteries tended to relax poorly, contract spontaneously and not maintain tension developments. Consequently after several 5 week rat myograph experiments the pharmacological investigation was terminated, and only the morphology of those arteries was measured.

Statistical Analysis

Data from ligatured and unligatured limbs were compared within strains by Student's paired t-test since measurements were made simultaneously. SHR unligatured limb data were compared with those from unligatured WKY by Student's unpaired t-test in order to determine strain differences in the absence of surgical intervention. Data from ligatured limbs from SHR were similarly compared to determine whether a reduced pressure load on SHR resistance arteries would produce vascular morphological and contractile responses similar to those observed in normally perfused WKY hindquarters. Where noradrenaline and calcium pD_2 values did not differ within or between strains dose response curves were compared using two way analysis of variance. In the few instances where data from both hindlimbs could not be obtained then the data from the successfully cannulated artery or mounted vessel were omitted from the analysis. A P value less than 5% was considered statistically significant. Data are presented as mean + SEM.

CHAPTER 3

Chemical Renal Medullectomy

Introduction

The classic experiments performed by Goldblatt (Goldblatt et al. 1934) served to demonstrate how simply the blood pressure of an animal may be altered by interference with the normal function of the kidney. The pressor effect of the stimulated renin-angiotensin system consequent to renal blood flow restriction can lead to a marked hypertension in rats, which may then be exaggerated as a result of load induced hypertrophy of the resistance vasculature.

In addition to the kidney's ability to raise blood pressure, it also seems that there are renal mechanisms for the lowering of blood pressure, including vasodepressor lipids synthesized in the renal medullary interstitial cells (Muirhead, 1980). Therefore hypertension may also be induced by inhibition of vasodepressor effects. The injection of 2-bromoethylamine hydrobromide (BEA) into rats has been shown to selectively damage the renal medulla (Murray et al. 1972) and in consequence induce hypertension in rats (Bing et al. 1983; Taverner et al. 1984). The blood pressure increase appears not to be attributable to sodium retention or pressor effects of an activated renin-angiotensin system since previous work in this department has demonstrated that the rats possess normal or low plasma renin concentration (Bing et al. 1983; Taverner et al. 1984). Muirhead has demonstrated that when one kidney one clip hypertensive rats are unclipped then there follows an immediate blood pressure fall despite the undoubted

increase in vascular structure which would have occurred after 3-6 months of hypertension (Muirhead et al. 1985). Chemical papillectomy by BEA prevented the rapid blood pressure fall, and the authors suggested that an antihypertensive neutral renomedullary lipid is the main putative hormone responsible. Also it is of interest that treatment with captopril before unclipping led to lipid granule accumulation in the renomedullary interstitial cells, and then the pressure drop following unclipping only took 20 minutes rather than three hours. The cells degranulated on unclipping (Muirhead et al. 1985). Chemical renal medullectomy also attenuates the blood pressure fall following reversal of two-kidney, one clip hypertension in rats (Bing et al. 1981).

Hypertension of all forms seems associated with structural change of the resistance vasculature, for example, mesenteric arteries in the renal hypertensive rat (Korsgaard and Mulvany, 1988) and in SHR (Mulvany et al. 1978); subcutaneous arteries in human essential hypertension (Aalkjaer et al. 1987a) and femoral and renal arteries in the DOCA salt hypertensive rat (Vial et al. 1989a,1989b). Structural changes have not been recorded in hypertension induced by chemical renal medullectomy and consequently the myograph has been used to measure the morphology of mesenteric resistance vessels of BEA - medullectomized rats compared with controls. In addition the noradrenaline sensitivity and noradrenaline-stimulated calcium sensitivity has been examined in this model of hypertension in the present study. In previous experiments in this department (Russell et al. 1986) it has been shown that bolus injections of noradrenaline resulted in

reduced pressor responses in the medullectomized rats so it was of interest to study noradrenaline induced contractions of isolated resistance vessels in this model of hypertension. The methodology employed in this study has been described in the methods section.

Results

Of the 33 rats treated with BEA, there were 18 survivors including 12 that showed some degree of renal medullary necrosis. This survival rate was consistent with previous experience in this laboratory. Although the condition of the renal medulla was not investigated, they would normally show severe medullary ablation. The 12 surviving medullectomized rats were compared with 12 saline injected controls and the results are listed in Table 1. There were no arteries which met the rejection criteria. From Table 1 it can be seen that the BEA treated rats had significantly greater blood pressures ($P < 0.02$) and thus a mild hypertension. The normalised media thickness, m_o and the media volumes of the mesenteric resistance arteries from the BEA-treated rats were increased by 7% but the difference was not significant. Lumen diameters, l_o, were effectively unchanged and media:lumen ratios were increased by 6% but again not significantly so. The contractile ability of the vessels was unchanged as measured by ΔT, ΔS or ΔP values; likewise there was no difference in the noradrenaline-stimulated calcium sensitivities. However, the noradrenaline sensitivity of mesenteric resistance arteries from BEA-treated rats was significantly reduced ($P < 0.05$), and the noradrenaline dose

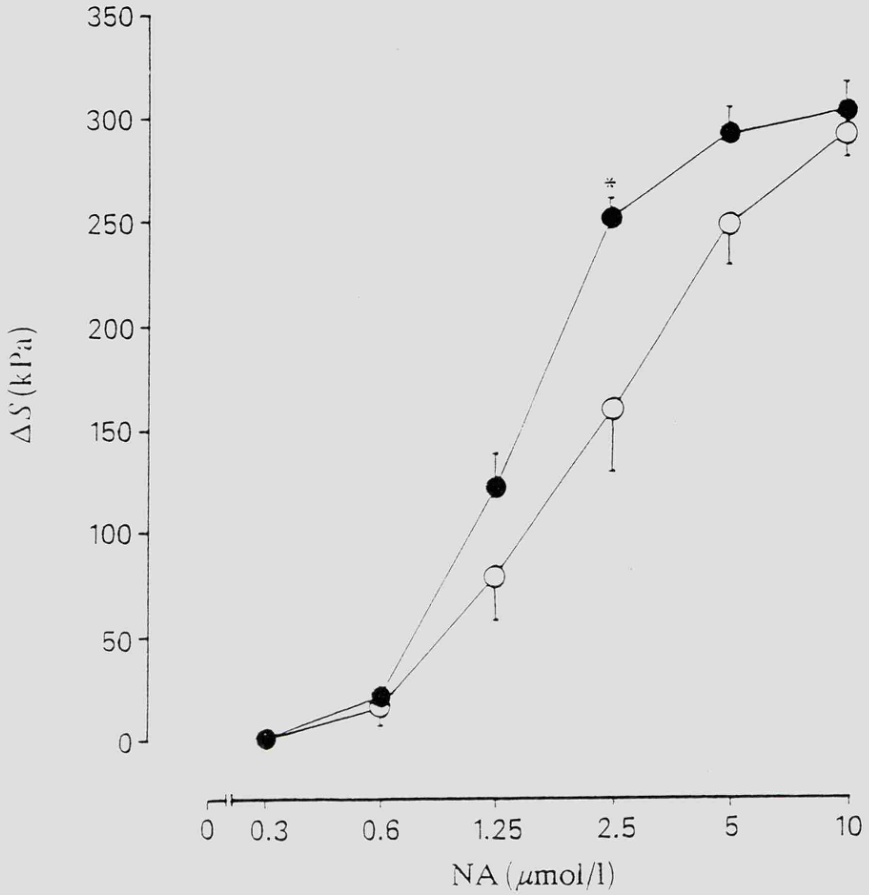
TABLE 1

The indirect systolic pressures and weights of BEA-medullectomised rats and controls and the structure and reactivity of the mesenteric resistance arteries.

	BEA (n = 12)	Controls (n = 12)
media thickness, m_o um	10.11 ± 0.56	9.43 ± 0.50
lumen diameter, l_o um	195 ± 6	193 ± 5
media:lumen ratio x100	5.25 ± 0.27	4.95 ± 0.28
media volume um^3/um	6475 ± 528	6044 ± 395
maximal active tension ΔT, mN/mm	3.26 ± 0.25	3.22 ± 0.17
Effective active pressure ΔP, kPa	33.19 ± 1.74	33.23 ± 1.42
Maximal media stress ΔS, kPa	325 ± 13	345 ± 16
NA pD_2	5.693 ± 0.078*	5.869 ± 0.031
Ca pD_2	4.025 ± 0.052	4.127 ± 0.140
Indirect systolic blood pressure, mmHg	123 ± 3.5**	112 ± 2.4
Body weight g	218 ± 5	217 ± 6

BEA is 2-bromoethylamine:ΔT, ΔP and ΔS are responses to 10uM noradrenaline (NA) in KPSS, *$P<0.05$, **$P<0.02$.
Results expressed as mean ± standard error.

FIGURE 4

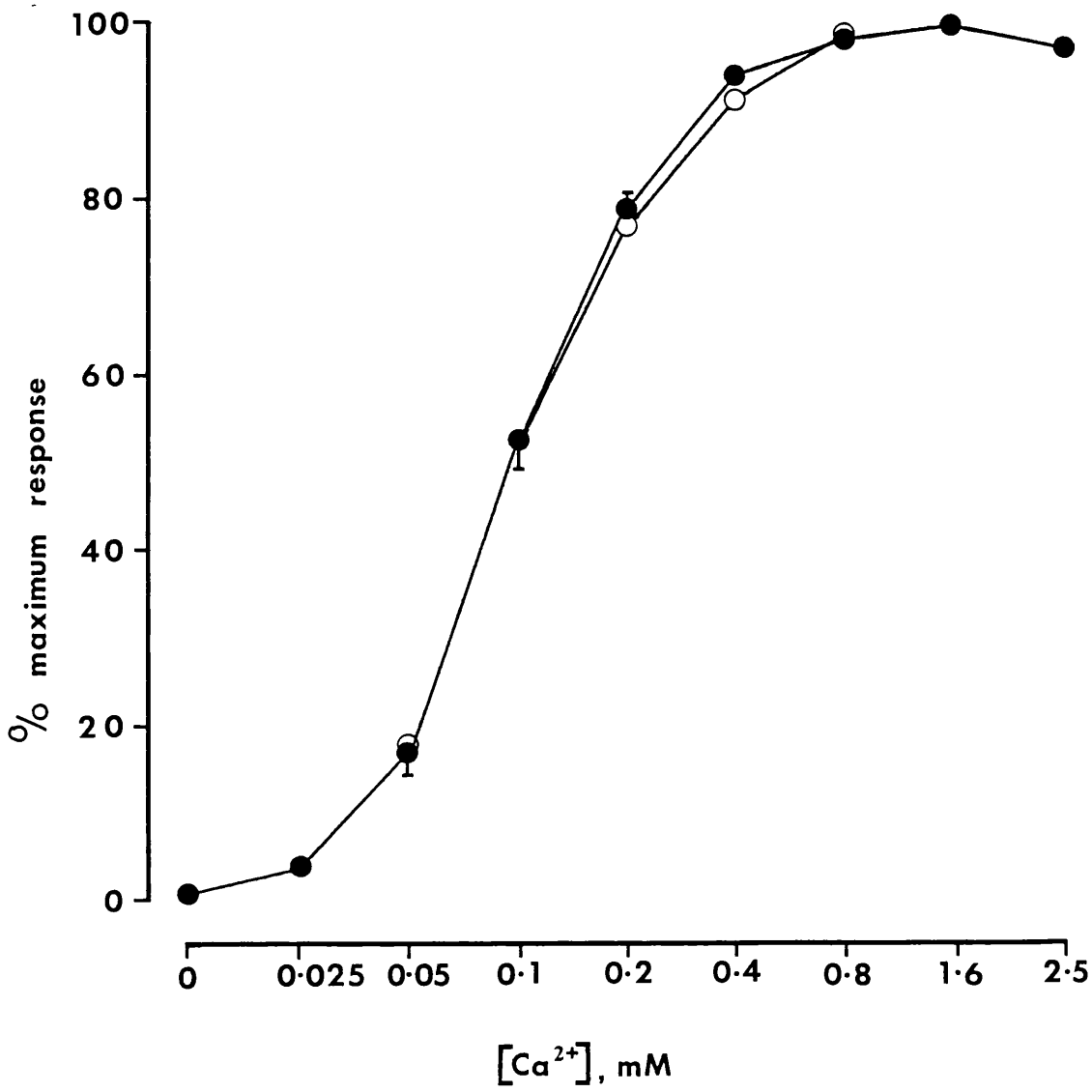


Noradrenaline (NA) concentration response curves for mesenteric resistance arteries from BEA medullectomized rats (○, n = 12) and saline injected controls (●, n = 12). Points were calculated using the mean response of two arteries from each rat. Bars indicate + or - standard errors. Comparison of the curves by 2 way ANOVA gave $P < 0.01$. * indicates $P < 0.01$ for the response of 2.5 umol/l by Student's t-test

response curves (Fig 4) were significantly different ($P<0.01$). Noradrenaline-stimulated calcium dose response curves are shown in Figure 5 and do not differ significantly.

Thus, three weeks after BEA injection, the only significant change detected in the mesenteric resistance vasculature is a significant reduction in noradrenaline sensitivity. However, it is considered that the small but statistically insignificant increases in media thickness, media volume and media thickness:lumen diameter ratios do in fact indicate some degree of structural remodelling - a longer period of hypertension would probably result in statistically significant increases in these parameters.

FIGURE 5



Normalized calcium concentration response curves for mesenteric resistance arteries from BEA medullectomized rats (○,n = 12) and saline injected controls (●,n = 12). Bars indicate + or - standard errors. Where bars are absent they are contained within the symbol. The curves are not significantly different by 2 way ANOVA comparison.

CHAPTER 4

Vasopressin Sensitivity in SHR

Introduction

The SHR vasculature appears to be generally either more or equally sensitive to applied agonists at least in the case of the resistance vessels (Chapter 1). However, there is a relative lack of data concerning vasopressin sensitivity in comparison to the numerous studies on noradrenaline. Consequently this study was performed to investigate the vasopressin sensitivity in isolated mesenteric resistance vessels from SHR and WKY. As reviewed previously (Johnston, 1985) vasopressin acts in concert with the sympathetic nervous system and renin-angiotensin system as an integrated neurohormonal system in blood pressure control, although its involvement in blood pressure control is likely to be its influence on plasma volume (via V_2 receptors) rather than its vasoconstrictor effect (via V_1 receptors) even though it is one of the most potent vasoconstrictors known. Still it is of interest to investigate the relative sensitivities of the SHR and WKY vasculatures to this agonist. Previously it has been shown that perfused kidneys of SHRSP are more sensitive to vasopressin infusion than those of WKY (Berecek, 1980a) while the isolated perfused hindquarter preparations of SHR and WKY are equally sensitive to the vasoconstrictor effects of vasopressin (Jandhyala et al. 1980). Interestingly it has been demonstrated that SHR plasma vasopressin levels are higher than in WKY and correlated to blood pressure in the SHR; therefore it seems that vasopressin plays an important vasopressor role especially since infused vasopressin in WKY was 1000 times less effective at increasing the blood pressure (Mohring et al. 1978) when at SHR

plasma levels. More recently there has been evidence produced that endogenous vasopressin contributes to the hypertension in SHR since the hypertension was significantly attenuated in SHR when the rats were infused with a vasopressin antagonist (Sladek et al. 1988). However, the mechanism by which it does contribute remains unclear since a specific V_1 antagonist did not attenuate the pressure excess while the antagonist used in the study has V_1 and V_2 effects but the V_2 antagonism was incomplete. Nevertheless it seems that there exists a role for vasopressin in SHR hypertension. The methodology used in this present study is described in the methods section.

Results

This study utilized a total of 68 rats with 34 each of SHR and WKY. The mean blood pressures and weights of these animals is presented in Table 2. At the time this study was performed I did not possess a Home Office Licence and I was therefore dependent upon the blood pressure measurements being made by another licence holder and unfortunately he was unable to perform the blood pressure measurement procedure on all the rats. Thus, 5 SHR and 6 WKY rats did not undergo blood pressure measurements. Of the 68 arteries mounted in the myograph (34 from either strain) only one met a rejection criterion; this was a WKY artery and the normalized lumen diameter exceeded 300um. The lumen diameters and sensitivity data are also presented in Table 2. SHR arteries had significantly narrower lumens ($P<0.01$) but AVP and AVP-stimulated calcium sensitivities were not different between the strains. AVP concentration response curves are presented in Figure 6. Tension responses were greater in the SHR arteries ($P<0.05$ at 2mUnits/ml) probably as a result of increased media

TABLE 2

The indirect systolic pressures and weights of SHR and WKY controls and lumen diameters and reactivity data of mesenteric resistance arteries

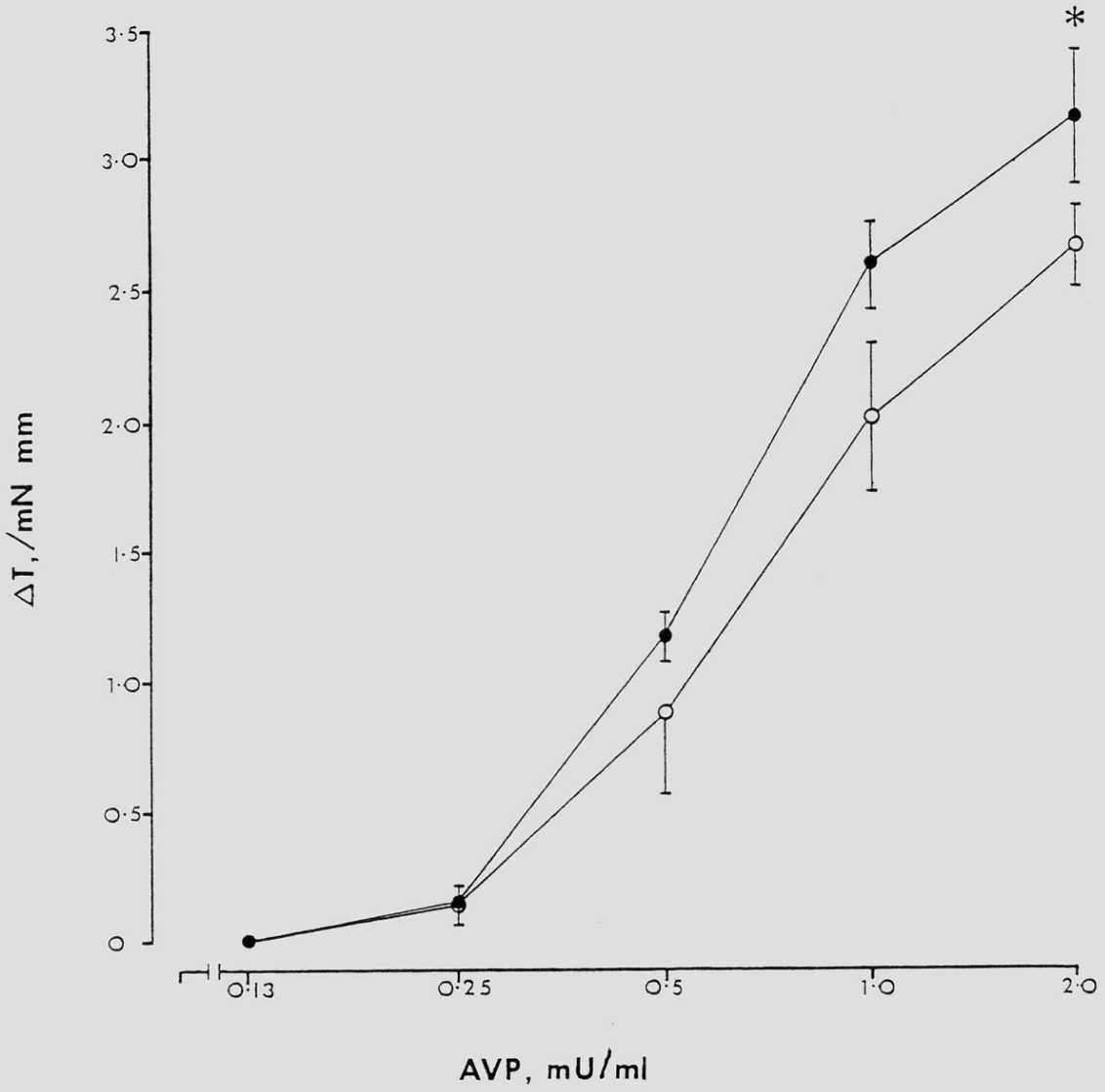
	SHR	WKY
Indirect systolic blood pressure, mmHg	140 ± 2 (29)**	106 ± 1 (28)
Body weight, g	203 ± 2 (29)**	191 ± 2 (28)
lumen diameter, l_o um	211 ± 4 (34)*	229 ± 5 (33)
pAVP	0.213 ± 0.029 (10)	0.147 ± 0.062 (9)
pCa	3.669 ± 0.054 (7)	3.613 ± 0.107 (6)
ΔT (NAK) mN/mm	4.23 ± 0.13 (34)**	3.22 ± 0.11 (33)
ΔT (AVP) mN/mm	3.46 ± 0.09 (34)**	2.69 ± 0.10 (33)
ΔP (NAK), kPa	40.14 ± 1.02 (34)**	28.17 ± 0.80 (33)
ΔP (AVP), kPa	32.97 ± 0.97 (34)**	23.81 ± 0.93 (33)

ΔT and ΔP are maximal active tension and effective active pressure, respectively. pAVP is $-\log_{10} ED_{50}$ (mU/ml). pCa is vasopressin-stimulated calcium sensitivity and defined as $-\log_{10} ED_{50}$ (M).

*$P < 0.01$, **$P < 0.001$ SHR vs WKY.

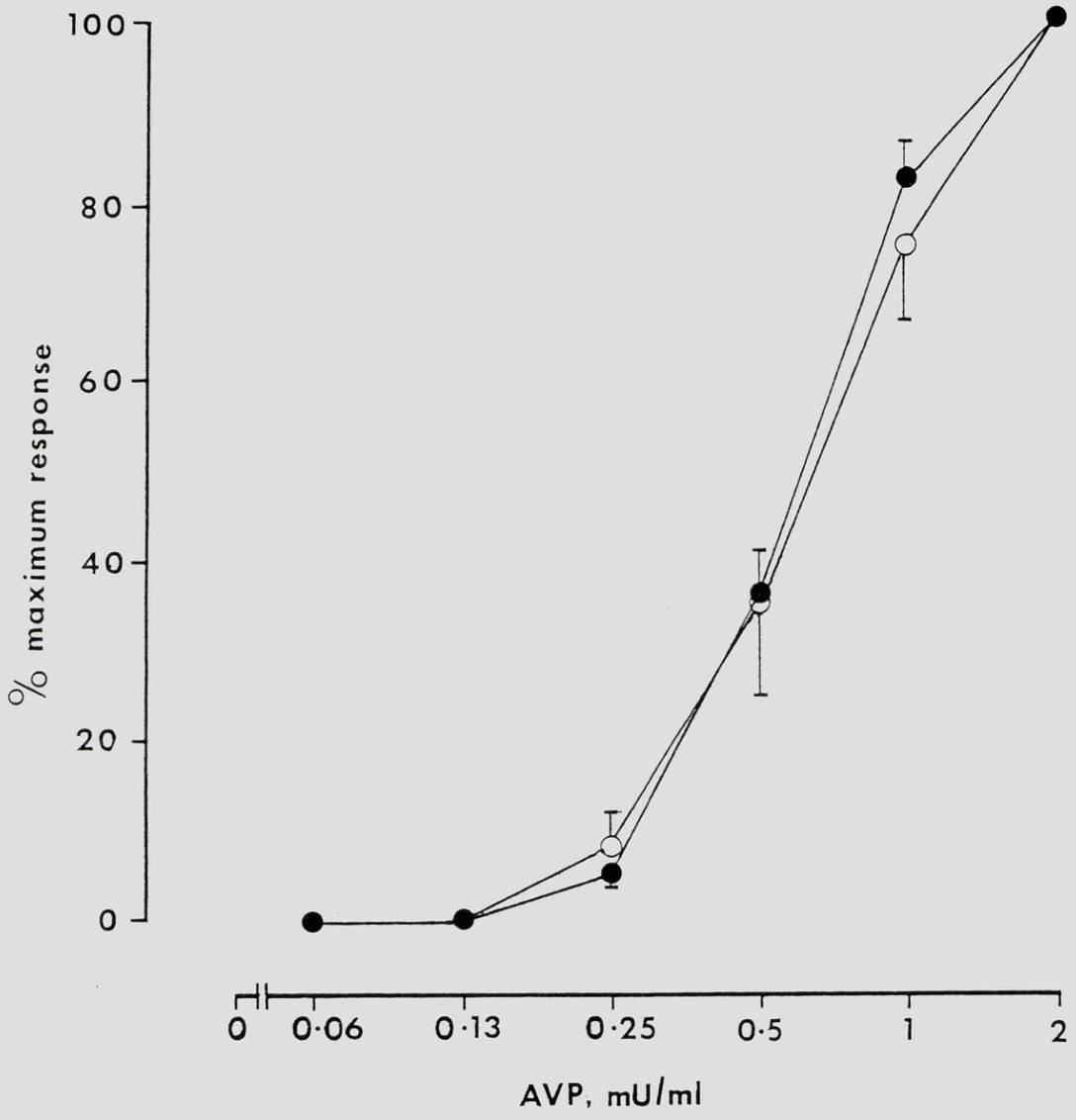
Results are expressed as mean ± standard error (n)

FIGURE 6



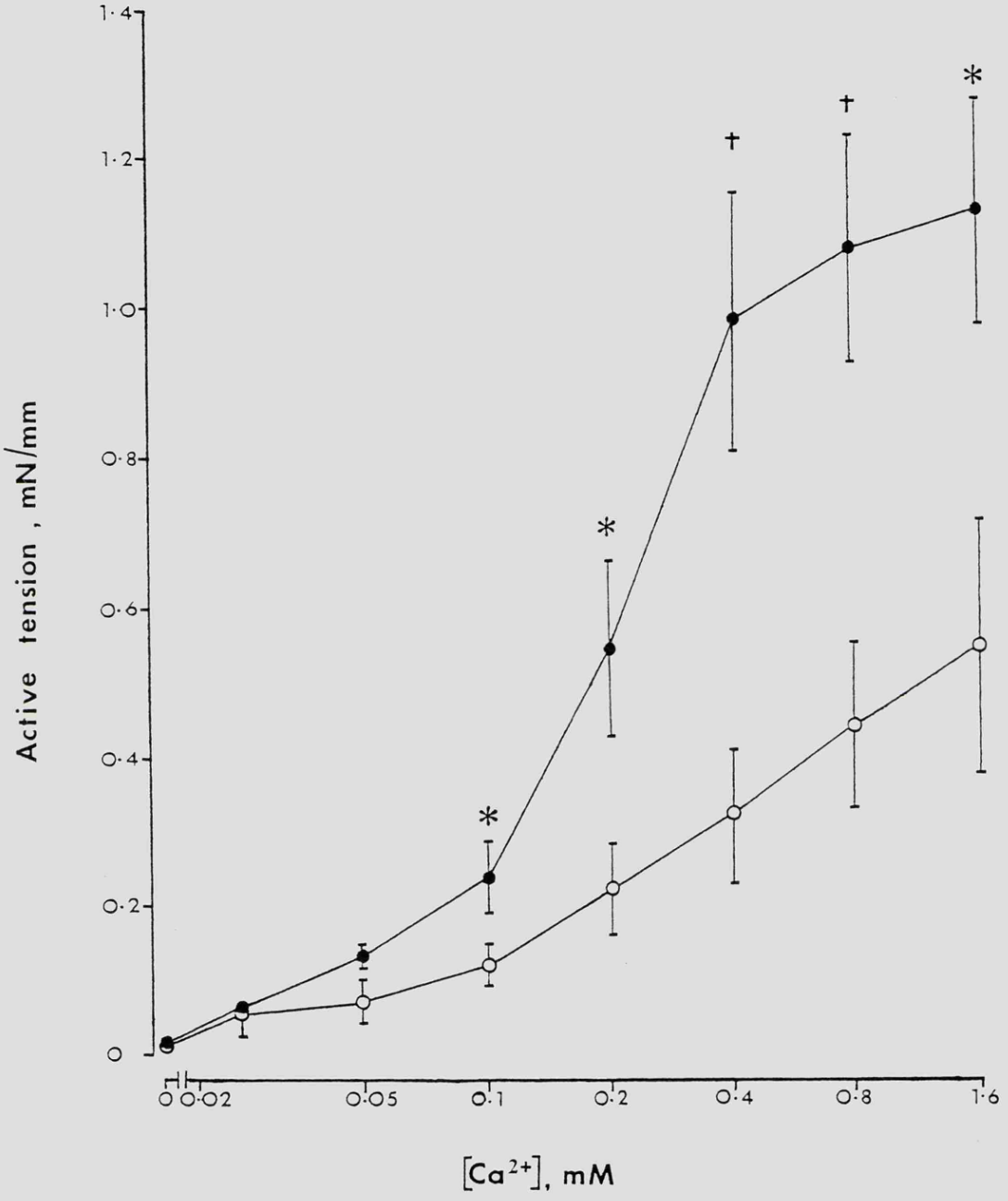
Arginine vasopressin (AVP) concentration response curves for mesenteric resistance arteries from SHR (● ,n = 10) and WKY rats (○ ,n = 10). Bars indicate ± standard errors. Overlapping bars omitted for clarity.
* indicates that SHR arteries produce greater active tensions at 2 mU/ml AVP, $P < 0.05$ Student's t-test.

FIGURE 7



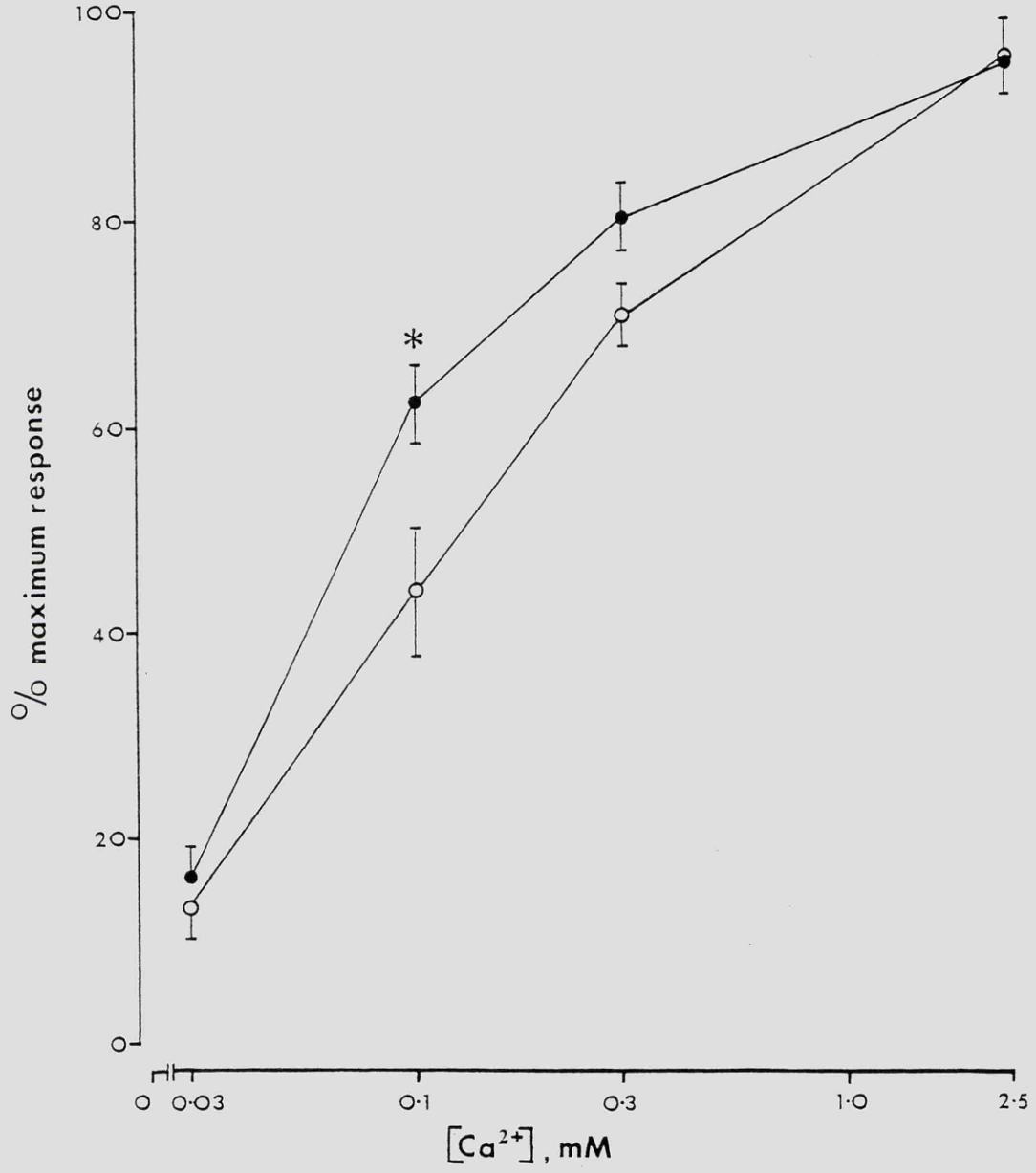
AVP concentration response relationship of Figure 6 replotted as % maximum responses. The curves are not significantly different by 2 way ANOVA comparison. Bars indicate + or - standard errors. ● SHR, ○ WKY.

FIGURE 8



AVP stimulated calcium concentration response curves of SHR mesenteric resistance arteries (● ,n = 7) and those of WKY (○ ,n = 6). Bars indicate ± standard errors. Comparison of tension responses by Student's t-test gave * $P < 0.05$, † $P < 0.01$.

FIGURE 9



Normalized AVP stimulated calcium concentration response curves following noradrenaline-mediated calcium depletion of mesenteric arteries from SHR (●) and WKY (○). Points represent the % recovery of the maximal AVP response. 6 arteries per point. Bars indicate ± standard error. 2 way ANOVA of curves gave $P < 0.001$. * indicates $P \leq 0.05$ at 0.1mM Calcium by Student's t-test

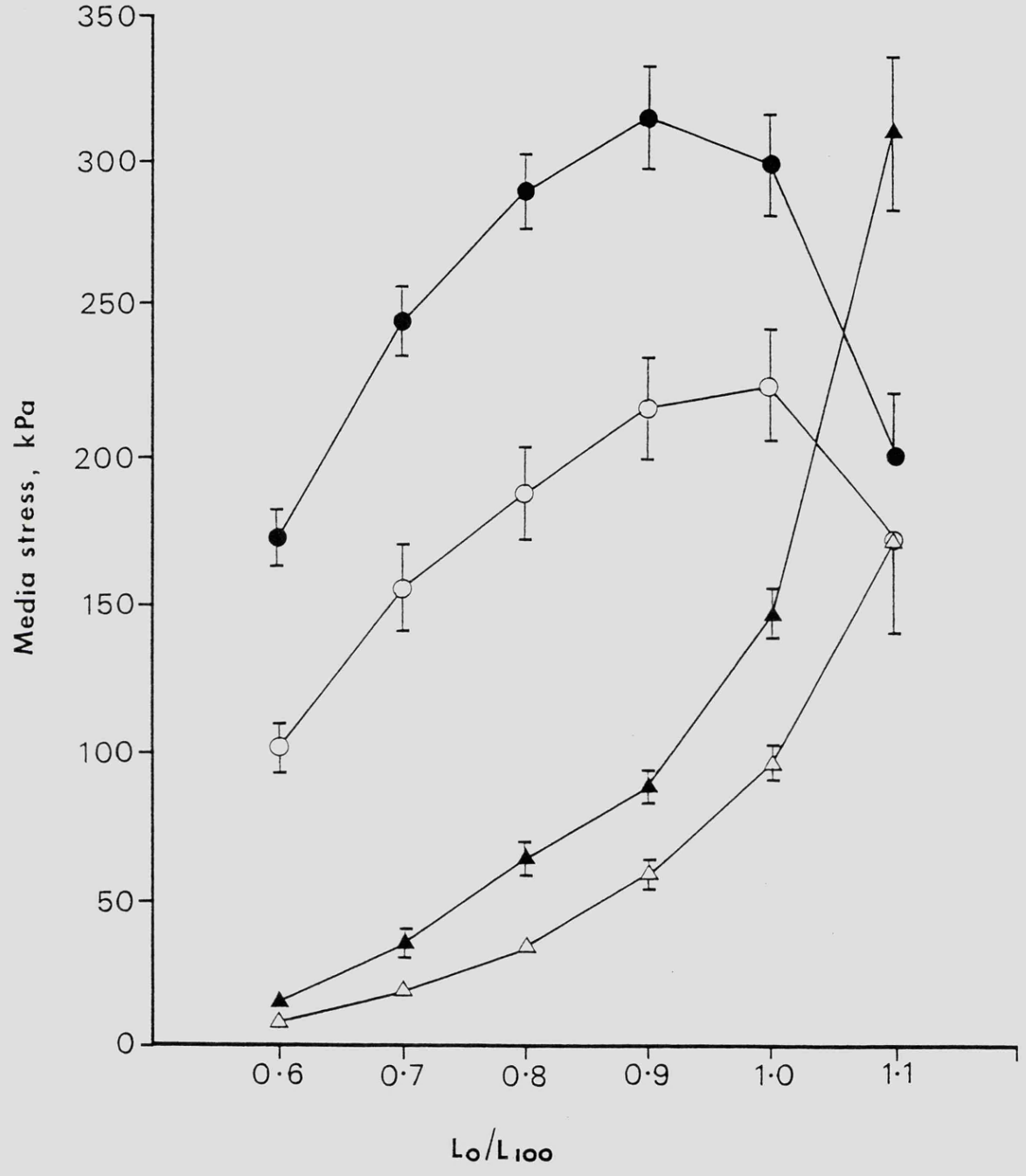
thickness in the SHR arteries although this measurement was not made. Consequently normalized concentration response curves, plotted as % of the maximum response, are presented in Figure 7. The curves are not significantly different. AVP-stimulated calcium concentration response curves are presented in Figure 8. The SHR arteries produced greater tension responses but the actual magnitudes of the responses were small in both strains (compare with Figure 6) thus tachphylaxis appeared to have occurred. For this reason the second form of calcium concentration response curve was performed as described in the methods section. This second means of determining the AVP-stimulated calcium sensitivity revealed that SHR arteries were able to produce greater normalized responses in low [Ca^{2+}] media and were therefore more sensitive. The two curves were significantly different ($P < 0.001$) when compared with 2-way ANOVA (Figure 9). In all experiments SHR arteries displayed oscillatory tension changes, whilst only two of thirty-three WKY arteries did so in activating AVP concentrations.

CHAPTER 5

Determination of l_o in Femoral Resistance Arteries

The tension-length relationships determined for SHR and WKY arteries are presented in Figure 10. The WKY results were obtained from 15 arteries from 9 rats, while the SHR results represent the findings for 14 arteries from 10 rats. The results are plotted as media stress (ΔS) vs l_o/l_{100} to take into account the increased media thickness of the SHR arteries. From this figure it can be seen that contractile responses for WKY femoral arteries are greater at 90% l_{100}, while those for SHR are near maximal at this setting. In consequence, all subsequent femoral artery experiments were performed at this setting, i.e. l_o for femoral arteries was taken as 0.9 l_{100}. The SHR ΔS values were reduced in comparison to those from WKY, a result observed in the later blood pressure protection experiments. The lumen diameters of the SHR arteries tended to be smaller than WKY and therefore the mounting procedure may have been relatively more traumatic for the SHR arteries, especially since these experiments were among the first femoral experiments performed. (Alternatively it may be that the increased media thickness in SHR femoral arteries is resultant upon an increase in extracellular components).

FIGURE 10



The relationship between maximal active response (NAK activation) and lumen circumference of femoral resistance arteries from SHR (○,n = 14) and WKY (●,n = 15). L_o/L_{100} indicates the internal circumference expressed as a fraction of the circumference arteries would have when relaxed and under a transmural pressure of 100 mmHg. Also included in this figure are the passive stress developments at each lumen circumference for WKY (▲) and SHR (△). Where no bars are indicated the standard error bars are contained within the symbol (or omitted as an overlapping bar in the case of passive media stress for SHR arteries at L_o/L_{100} = 1.1).

CHAPTER 6

Blood Pressure Protection Studies

Introduction

As described in the general introduction, the hypertension observed in the SHR has been partly or completely normalized by many forms of treatment, including drug therapy and chemical - or immuno-sympathectomy. The vascular structure in the treated rats generally does not normalize to that measured in WKY control rats, suggesting that there is an abnormal blood pressure - vascular structure relationship in SHR such that blood pressures in the normal range can induce medial thickening and increase media:lumen ratios; alternatively there may be altered neural or humoral factors which possess trophic capabilities. That hypertrophy may occur in rat normotensive vascular beds is amply demonstrated in the coarctation model of hypertension. This model involves the partial constriction of the abdominal aorta either between or proximal to the origins of the two renal arteries. The subnormally perfused kidneys initiate hypertension in the vasculature proximal to the constriction, but distal to the constriction the vasculature does not suffer the increased load since the resistance of the ligature or clip on the aorta is sufficient to prevent a pressure load increase. It has been demonstrated in rabbits that coarctation results in wall thickness increases in vessels proximal to the coarctation (ear, basilar and radial) that are proportional to the blood pressure increases (Bevan et al. 1975) and this is associated with increased mitotic counts as shown by ^{3}H-Thymidine incorporation studies (Bevan 1976; Bevan et al. 1976). Proximal to the

coarctation vessels had increased media thicknesses and reduced lumen diameters (Bevan 1976) while distally the blood pressure and vascular structure was normal (Bevan et al. 1975; Bevan 1976). Apparently in the rabbit at least the increase in mitotic count may only be a short term response to the rapid pressure rise since the nuclear labelling excess becomes lost (Bevan et al. 1980). In rats an increased DNA synthesis and hyperplasia is observed in the aorta above but not below the coarctation (Owens and Reidy, 1985) although previously Overbeck demonstrated that aortic thickening could occur above and below the constriction; sympathectomy could only inhibit the thickening below (Overbeck, 1979). There therefore may be a role for the sympathetic nerevous system in coarctation hypertrophy. Wall thickening in normotensive beds in coarctation hypertension is also observed in femoral arteries and smaller renal arterioles (Liu et al. 1988). Increased wall:lumen ratios are also observed in the small vessels of the cremaster circulation (Plunkett and Overbeck, 1985) which is exposed to a normal blood pressure below the coarctation and this structural change also happens in sympathectomized rats (Plunkett and Overbeck, 1988) suggesting that circulating factors may indeed influence vascular structure. Circulating substances may explain the hypertrophy of portal veins and pulmonary arteries of SHR which are not exposed to hypertension (Greenberg et al. 1978). Greenberg has also demonstrated that SHR and WKY parabiosis will result in WKY portal vein hypertrophy, therefore a circulating factor from SHR can initiate hypertrophy in WKY or a factor is released in WKY as a result of the blood pressure rise observed in the parabiotic WKY (Greenberg et al. 1981). More recently it has been shown that

cross circulation of SHR and WKY rats results in an increased pressure in WKY which can be prevented if SHR are pre-treated with a digoxin antibody prior to cross-circulation (Zidek et al. 1989). Thus the possibility exists that a putative circulating factor is an inhibitor of the Na-K-ATPase. Other possible circulating factors are vasoconstrictor agonists since noradrenaline (McDermot and Morgan, 1989; Blaes and Boissel, 1983; Yamori et al. 1984) and vasopressin and angiotensin II (Campbell-Boswell and Robertson, 1981; Geisterfer and Owens, 1989; Geisterfer et al. 1988) can stimulate myocyte growth or proliferation in culture. SHR hearts may secrete a growth factor since addition of a supernatant of SHR myocardial homogenate can increase protein synthesis in cultured Sprague Dawley heart cells (Shen et al. 1987). One interesting study suggests that vascular structure is dependent on blood pressure or humoral influences of the host rather than the smooth muscle cell genetic make up (Pang and Scott, 1985). In this study jejunal arteries were inserted as shunts between femoral arteries and veins and regardless of source (i.e. either WKY or SHR) the host determined the shunt structure such that arteries inserted into SHR displayed media thickness increases and lumen diameter reductions. It may be that host sympathetic innervation mediated the control of the structure since there is further evidence from cross transplantation experiments that host innervation is required to produce host-like vessel properties. SHR caudal arteries were more sensitive to noradrenaline than WKY and the membrane potential was less negative. When caudal artery segments were transplanted into the anteior eye chamber, the segments assumed the membrane properties of the host but not if the eye

had been subject to sympathetic denervation (Abel and Hermsmeyer, 1981). Thus host innervation was required for transmission of host membrane properties.

The application of a constricting ligature can be used in other circumstances to alter blood pressure distally in a manner which does not induce hypertension as happens in coarctation hypertension. In 1968 it was shown that ligaturing the vasculature supplying the hindlimb of the cat resulted in a maintained reduced blood pressure in that 'protected' limb, and when the resistance vasculature was activated either by nerve stimulation or noradrenaline infusion then the protected limb produced shallower resistance curves in comparison to the control hindlimb, indicative of reduced media:lumen ratios (Folkow and Sivertsson, 1968). Likewise, a partially constricting ligature around the femoral artery of the pig prevents the hindlimb resistance increase associated with DOCA salt hypertension in the animal (Berecek and Bohr, 1977). Again, a ligature of the aorta but below the kidneys results in reduced minimal resistances and shallower resistance curves in SHR and Wistar rats (Folkow et al. 1971) demonstrating that a physical means of blood pressure reduction in SHR may produce structural regression even if therapeutic means do not. This is the approach that has been employed in the present study, i.e. the relationship between resistance vessel structure and perfusion pressure has been investigated by placing a partially constricting ligature in the circulation to protect the resistance vessels distally from the rise in blood pressure that occurs in the SHR with age. The method is essentially similar to that previously employed (Hansen

and Bohr, 1975) whereby the femoral vasculature is subjected to reduced pressure by unilateral iliac artery partial constriction, but refined similarly to that described more recently (Field and Soltis, 1985) whereby the external iliac artery occlusion was effected by the placement of a standard diameter loop around the artery. The constant ligature diameter was produced by tying a small length of stainless steel wire to the artery and then the wire was pulled out from the tie (as described in the methods) leaving a loop with approximately the same diameter as the wire. The important point of this procedure is that vessels distal to the constriction should be exposed to the same circulating influences as those exposed to the raised pressure in the contralateral limb.

Results

1.Blood Pressure and Arterial Morphology

(i) 5 weeks

Indirect systolic blood pressures were not different between strains (Table 3). However, FMAP was significantly higher in SHR (Table 4). Some of this difference may be due to the slightly smaller WKY rats which tolerated the cannulation procedure less well than SHR. There was no significant difference in any of the morphological measurements made on arteries at this age when comparing SHR with WKY (Table 5). Whilst it was possible to mount the small arteries taken from these rats at 5 weeks of age and subsequently to make measurements of morphology, it was not possible to undertake pharmacological studies due to the development of spontaneous contractions in many of the arteries.

TABLE 3

Indirect systolic blood pressures and body weights of rats used in the ligature study

Age (weeks)	Blood Pressure(mmHg)		Body weight(g)	
	SHR	WKY	SHR	WKY
5	109 ± 5 (16)	109 ± 4 (16)	74 ± 3 (16)	66 ± 4 (16)
12	161 ± 3 (22)*	145 ± 4 (19)	282 ± 4 (22)**	246 ± 6 (19)
24	200 ± 3 (16)**	138 ± 4 (19)	412 ± 8 (16)	394 ± 7 (19)

Data does not include measurements of sham operated animals.
*$p < 0.002$, **$p < 0.001$ SHR vs WKY
Results expressed as mean ± standard error (n)

BLE 4

moral mean arterial pressures in ligatured and unligatured hindlimbs

	SHR		WKY	
	Ligatured	Unligatured	Ligatured	Unligatured
weeks	-	94 ± 9 (4)†	-	65 ± 2 (4)
weeks	94 ± 8 (7)**	145 ± 8 (7)††	61 ± 5 (7)**	89 ± 4 (7)‡
weeks	125 ± 3 (5)*	163 ± 6 (5)††	90 ± 6 (5)**	123 ± 3 (5)‡

≤0.01, **p≤0.005. Ligatured vs Unligatured within a strain

≤0.002, ††p = 0 SHR unligatured vs WKY unligatured

dicates WKY unligatured is not different to SHR ligatured

sults expressed as mean ± standard error (n)

TABLE 5

Morphological characteristics of femoral resistance arteries from 5 week SHR and WKY rats

	SHR	WKY
media thickness, m_o um	7.87 ± 0.38	7.13 ± 0.36
lumen diameter, l_o um	96 ± 5	102 ± 4
media:lumen ratio x100	8.65 ± 0.53	7.53 ± 0.59
media volume um^3/um	2567 ± 198	2350 ± 118

Results expressed as mean ± standard error, n = 12 for all values.
SHR characteristics not significantly different to WKY for any parameter.
Calculations are based on mean value per rat (one artery from each hindlimb).

TABLE 6 Noradrenaline sensitivities, cocaine shifts and calcium sensitivities of femoral resistance arteries

	12 weeks				24 weeks			
	SHR		WKY		SHR		WKY	
	Unlig	Lig	Unlig	Lig	Unlig	Lig	Unlig	Lig
NA pD_2	5.962 †† ±0.052 (13)	6.651 ** ‡ ±0.148 (13)	6.756 ±0.110 (11)	6.883 ±0.165 (11)	6.135 † ±0.088 (12)	6.161 § ±0.091 (12)	6.636 ±0.108 (12)	6.613 ±0.129 (12)
NA(+cocaine) pD_2	6.269 †† ±0.090 (13)	6.766 * ‡ ±0.160 (13)	6.914 ±0.146 (11)	6.778 ±0.177 (11)	6.150 †† ±0.100 (12)	6.236 § ±0.102 (12)	6.863 ‖ ±0.103 (9)	6.647 ±0.141 (9)
Shift	-0.307 ¶‖ ±0.084 (13)	-0.115 ‡ ±0.144 (13)	-0.157 ±0.103 (11)	0.105 ±0.201 (11)	-0.015 ±0.049 (12)	-0.076 ±0.058 (12)	-0.237 ¶‖ ±0.115 (9)	-0.020 ±0.147 (9)
Ca pD_2	3.891 † ‖ ±0.037 (12)	4.003 § ±0.055 (13)	3.719 ±0.078 (11)	3.807 ±0.039 (11)	3.718 ‖ ±0.057 (11)	3.835 ‡ ±0.067 (11)	3.718 ±0.052 (10)	3.697 ±0.058 (10)

Unlig and Lig indicate arteries from unligatured and ligatured hindlimbs respectively
Results expressed as mean ± standard error (n)
*$P<0.05$, **$P<0.001$ Lig vs Unlig within strain by pD_2 comparison.
†$P<0.05$, ††$P<0.001$ SHR Unlig vs WKY Unlig by pD_2 comparison.
‡ Indicates SHR Lig is not different to WKY Unlig.
§ $P<0.01$ SHR Lig vs WKY Unlig by pD_2 comparison.
‖ $P<0.05$ within strain by ANOVA.
¶‖ $P<0.001$ cocaine enhancement of noradrenaline sensitivity by ANOVA.

TABLE 7 Maximal contractile properties of femoral resistance arteries

	12 weeks				24 weeks			
	SHR		WKY		SHR		WKY	
	Unlig	Lig	Unlig	Lig	Unlig	Lig	Unlig	Lig
(n)	(15)	(15)	(11)	(11)	(12)	(12)	(13)	(13)
Active tension ΔT,mN/mm	4.16* ±0.28	3.31 ≠ ±0.21	3.65** ±0.16	2.17 ±0.14	3.88 ±0.35	3.45 ≠ ±0.24	3.57* ±0.32	2.49 ±0.20
Active media stress ΔS,kPa	315 ± 24	342 ≠ ± 26	363 ± 19	305 ± 21	241 ± 31	285 ≠ ± 31	332 ± 35	315 ± 33
Effective active pressure ΔP,kPa	44.5** † ±2.4	34.6 ≠ ±2.0	36.6** ±1.3	20.1 ±1.5	45.6* †† ±2.7	36.5 ≠ ±2.3	35.1** ±2.4	21.7 ±1.3

Unlig and Lig indicate arteries taken from unligatured and ligatured hindlimbs respectively.
*$P<0.05$, **$P<0.001$ Lig vs Unlig within strain.
†$P<0.05$, ††$P<0.001$ SHR unlig vs WKY unlig.
≠ indicates SHR lig is not different to WKY unlig.
Results expressed as mean ± standard error.

(ii) 12 weeks

FMAP was greater in SHR unligatured hindlimbs when compared with WKY ($P<0.001$, Table 4,Figure 11) and reduced by the ligature in both strains ($P<0.005$ for SHR and WKY, Table 4,Figure 11). The FMAP in the ligatured limb of the SHR was similar to that observed in the normally perfused WKY hindlimb. Morphological measurements revealed that SHR femoral resistance arteries normally display an increased media thickness, media volume and media thickness:lumen diameter ratio. All these parameters were statistically significantly different when compared with WKY arteries (Figures 12-14). However lumen diameter was not different between the two strains (Figure 15). When exposed to a reduced pressure resistance arteries from both strains developed significantly less media thickness, reduced media volume and media:lumen ratio (Figures 12-14). The lumen diameter also increased in both rat strains but only reached statistical significance in WKY (Figure 15). The FMAP was similar in SHR ligatured and WKY unligatured femoral arteries (Table 4,Figure 11), and the morphology from the arteries from the SHR protected limb and the WKY unprotected limb was indistinguishable (Figures 12-15).

(iii)24 weeks

The pattern of FMAP was similar to that seen at 12 weeks of age; on the unligatured side hindlimb pressures remained significantly higher in SHR compared with WKY ($P<0.001$, Table 4,Figure 11), and in both strains the pressures in the unligatured side were higher than in the protected limb (SHR $P<0.01$, WKY $P<0.005$, Table 4,Figure 11). Again in the SHR

the FMAP on the ligatured side was not different from that observed on the unprotected side in the WKY. Measurements of morphology also revealed a similar pattern to that seen at 12 weeks (Figures 12-15): media thickness, media:lumen ratio and media volume were greater in SHR unprotected arteries in comparison with WKY, whilst lumen diameter was significantly reduced in SHR (Figure 15). Arteries protected from the developing pressure failed to display normal medial development in both strains. Resistance arteries distal to the ligature did not have significantly larger luminal diameters (Figure 15). Again at this age in the presence of similar perfusing pressures the morphological parameters from arteries distal to the SHR ligature did not differ from those observed in the unprotected WKY hindlimb (Figures 12-15).

2. Noradrenaline Sensitivity

(i) 12 weeks

Sensitivity to noradrenaline was not altered by the application of the ligature in the WKY; pD_2 values being similar in arteries from both hindlimbs (Table 6, Figure 16). In the presence of cocaine this pattern was not altered in WKY indicating that the silk tie had not altered the functional activity of the nervous supply to the vasculature (Table 6,Figure 17). In arteries from SHR hindlimbs, there was reduced sensitivity to noradrenaline on the unligatured side; the protected side displayed a sensitivity to noradrenaline that was identical to that seen in the WKY arteries (Table 6; Figure 16). Noradrenaline dose response curves in the presence and absence of cocaine were not

different for any arteries except those from the unprotected SHR hindlimb (Table 6, Figure 17); Nevertheless, these unprotected arteries were less sensitive to noradrenaline than the other three artery groups in cocaine (Figure 17). Again therefore the sensitivity to noradrenaline of SHR femoral resistance arteries distal to the ligature was similar to that observed in arteries from the unprotected bed of the normotensive WKY rats (Figure 17).

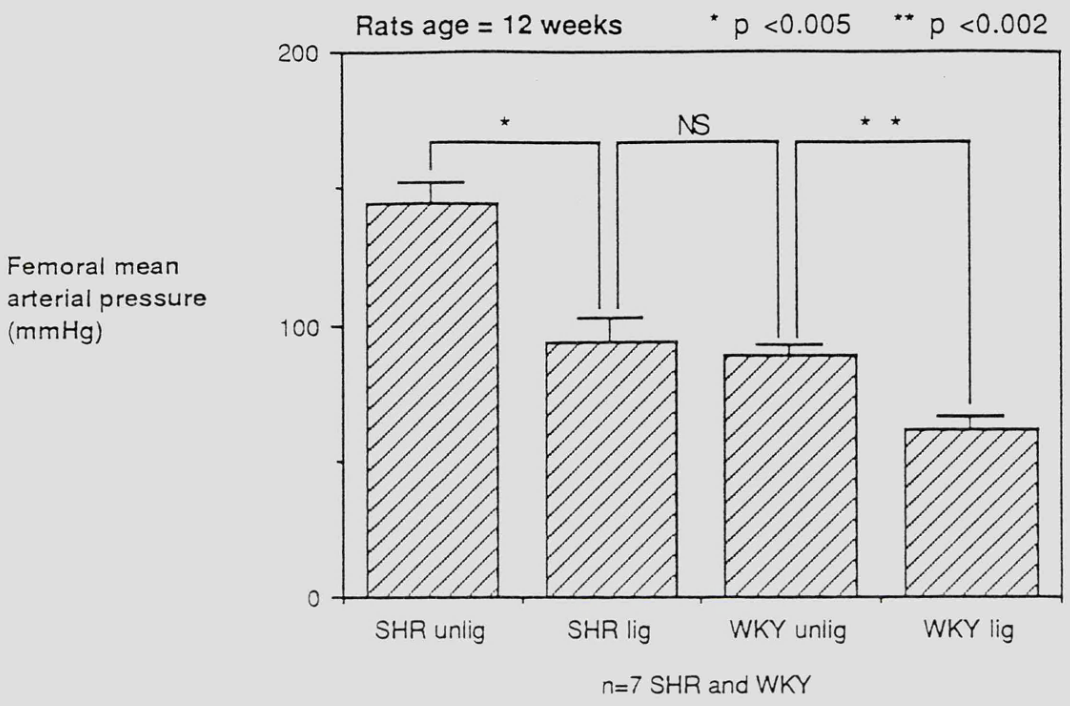
(ii) 24 weeks

In contrast to data from rats aged 12 weeks, resistance arteries from both protected and unprotected limbs in SHR were less sensitive to noradrenaline in comparison with WKY. The ligature had no effect on noradrenaline sensitivity in either strain (Table 6, Figure 18). SHR vessels continued to be less sensitive to noradrenaline compared with those from WKY in the presence of cocaine (Table 6,Figure 19). The only nordrenaline dose response curve to be significantly enhanced by cocaine was that from WKY unligatured arteries ($P<0.001$ using ANOVA, Table 6), indicating that noradrenaline re-uptake in SHR vessels at this age must be small. This enhancement in WKY unprotected arteries resulted in unprotected arteries being marginally more sensitive than protected arteries ($P<0.05$ ANOVA, Table 6).

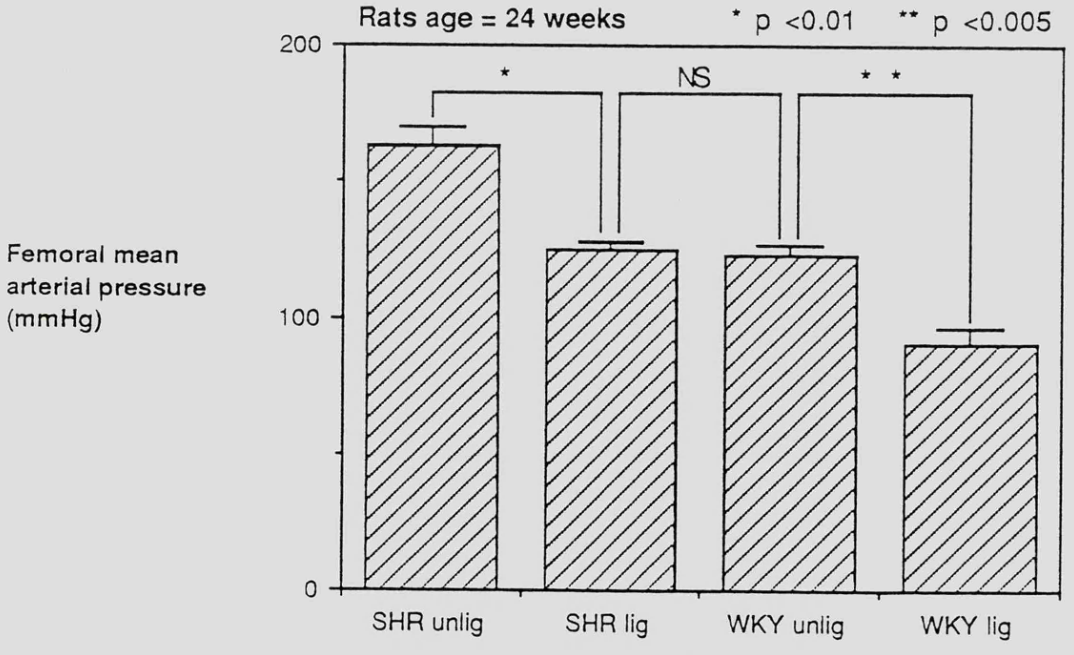
3. Calcium Sensitivity

Calcium sensitivity was increased in unprotected arteries from 12 weeks SHR compared with those from WKY, the difference being just significant ($p = 0.047$,Table 6). At 24 weeks, calcium sensitivities of these arteries were identical (Table 6).

FIGURE 11

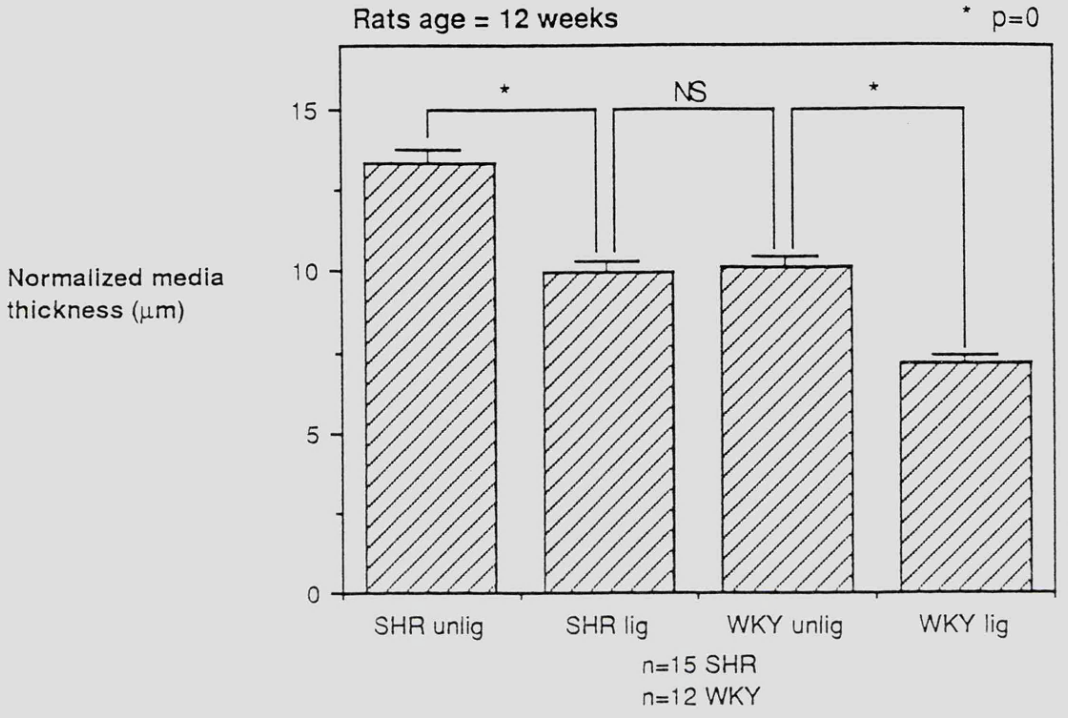


SHR unlig vs WKY unlig $p < 0.001$

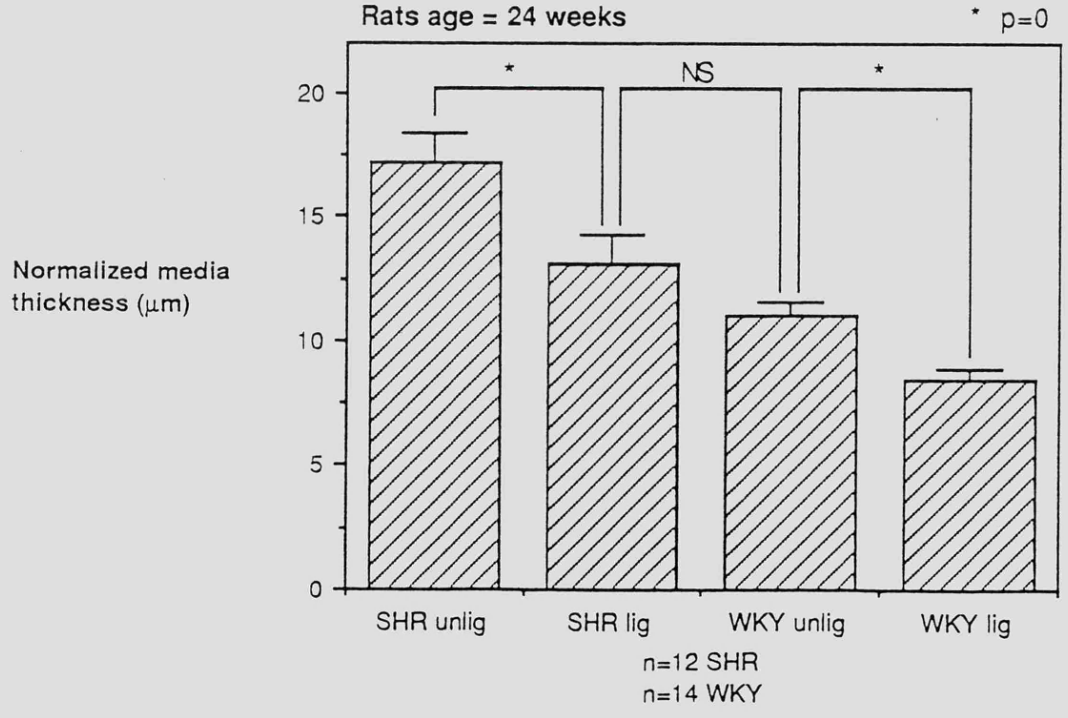


SHR unlig vs WKY unlig $P < 0.001$

FIGURE 12

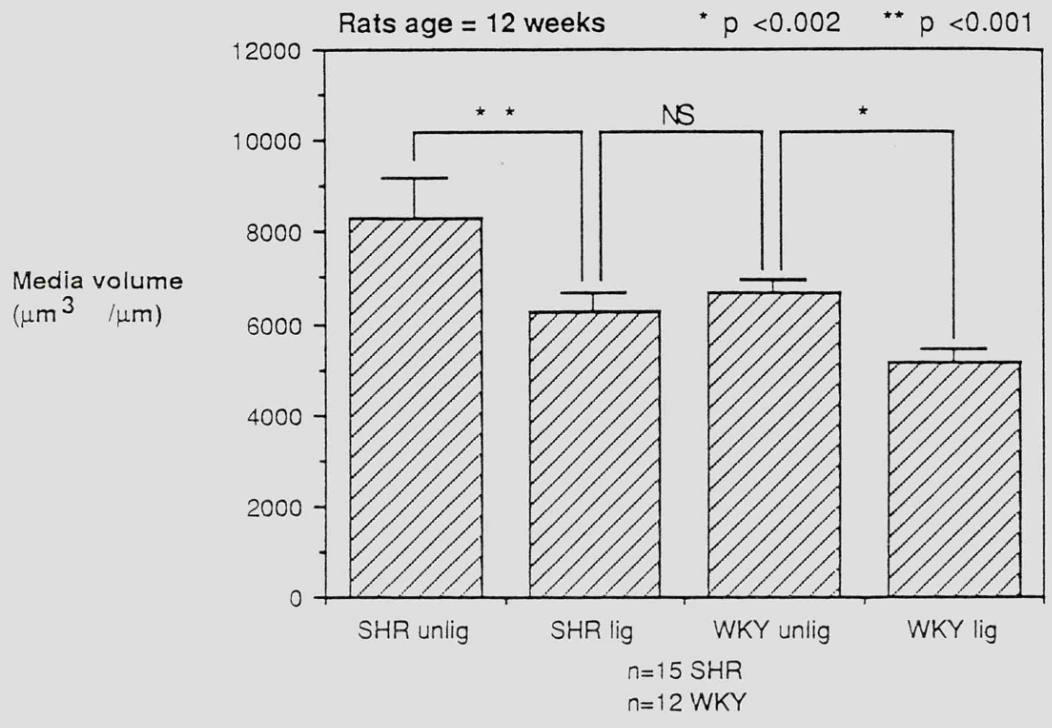


SHR unlig vs WKY unlig P< 0.001

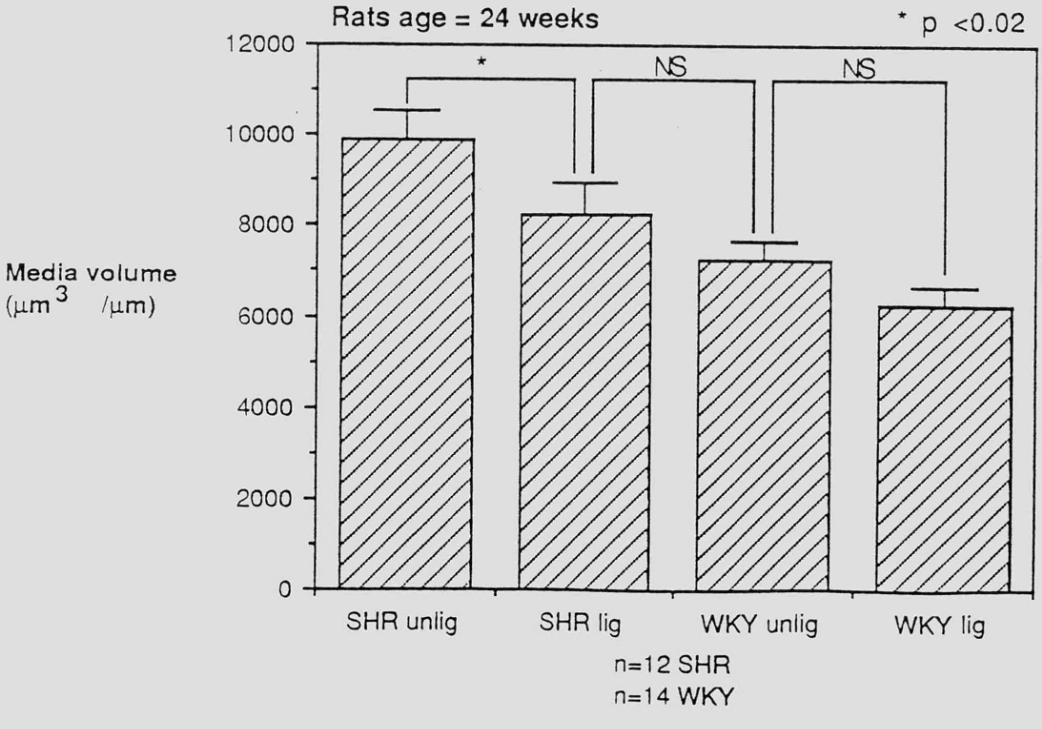


SHR unlig vs WKY unlig P < 0.001

FIGURE 13

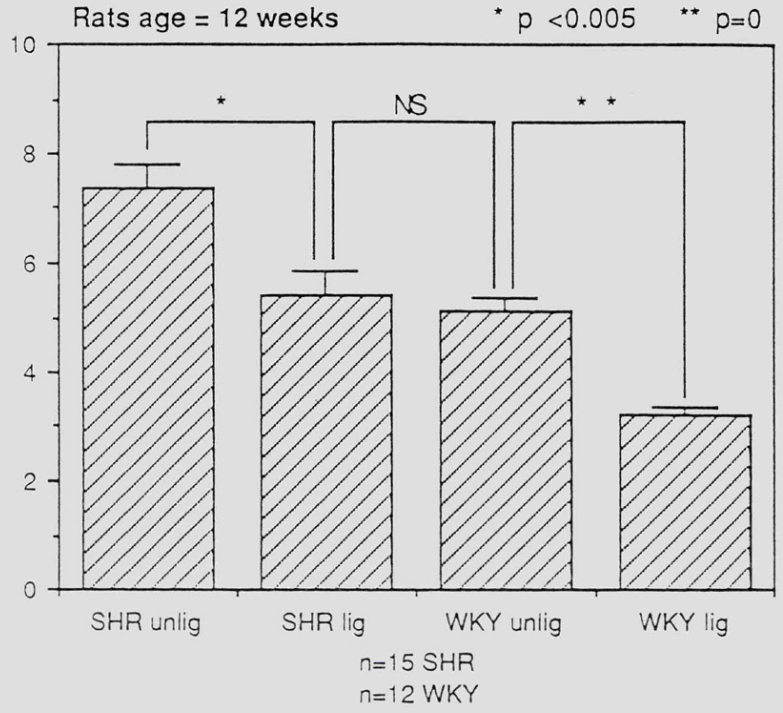


SHR unlig vs WKY unlig P< 0.001

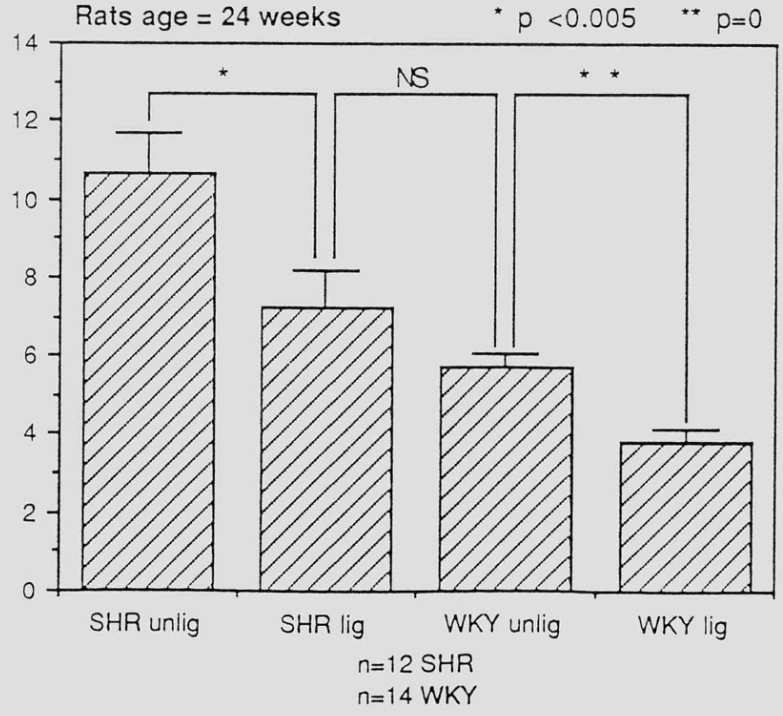


SHR unlig vs WKY unlig P<0.01

FIGURE 14

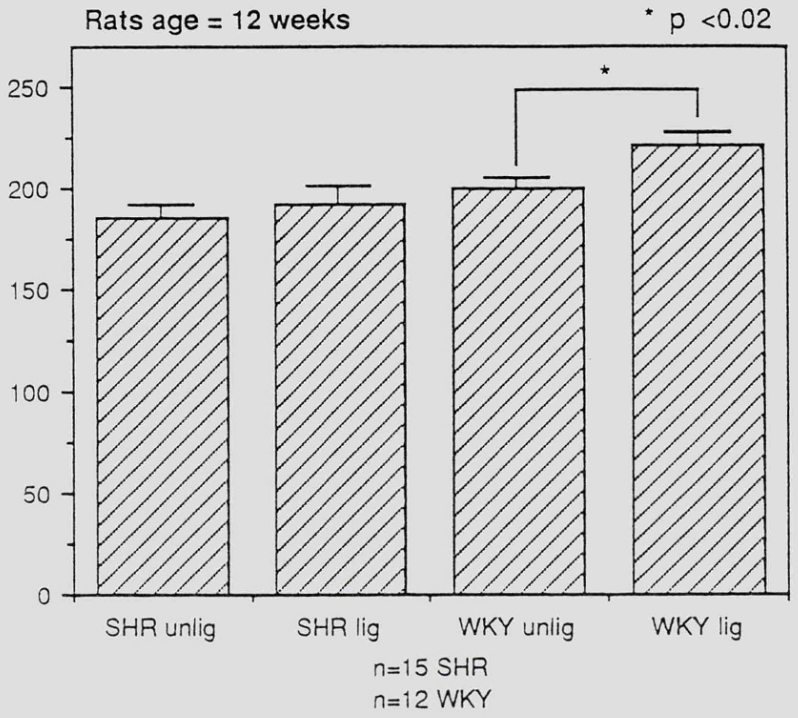


SHR unlig vs WKY unlig P < 0.001

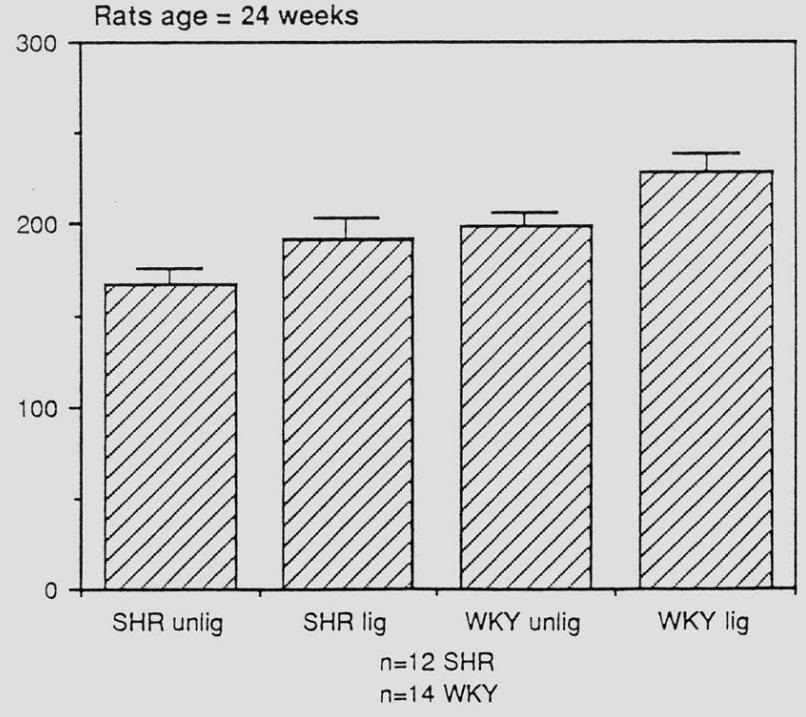


SHR unlig vs WKY unlig P < 0.001

FIGURE 15

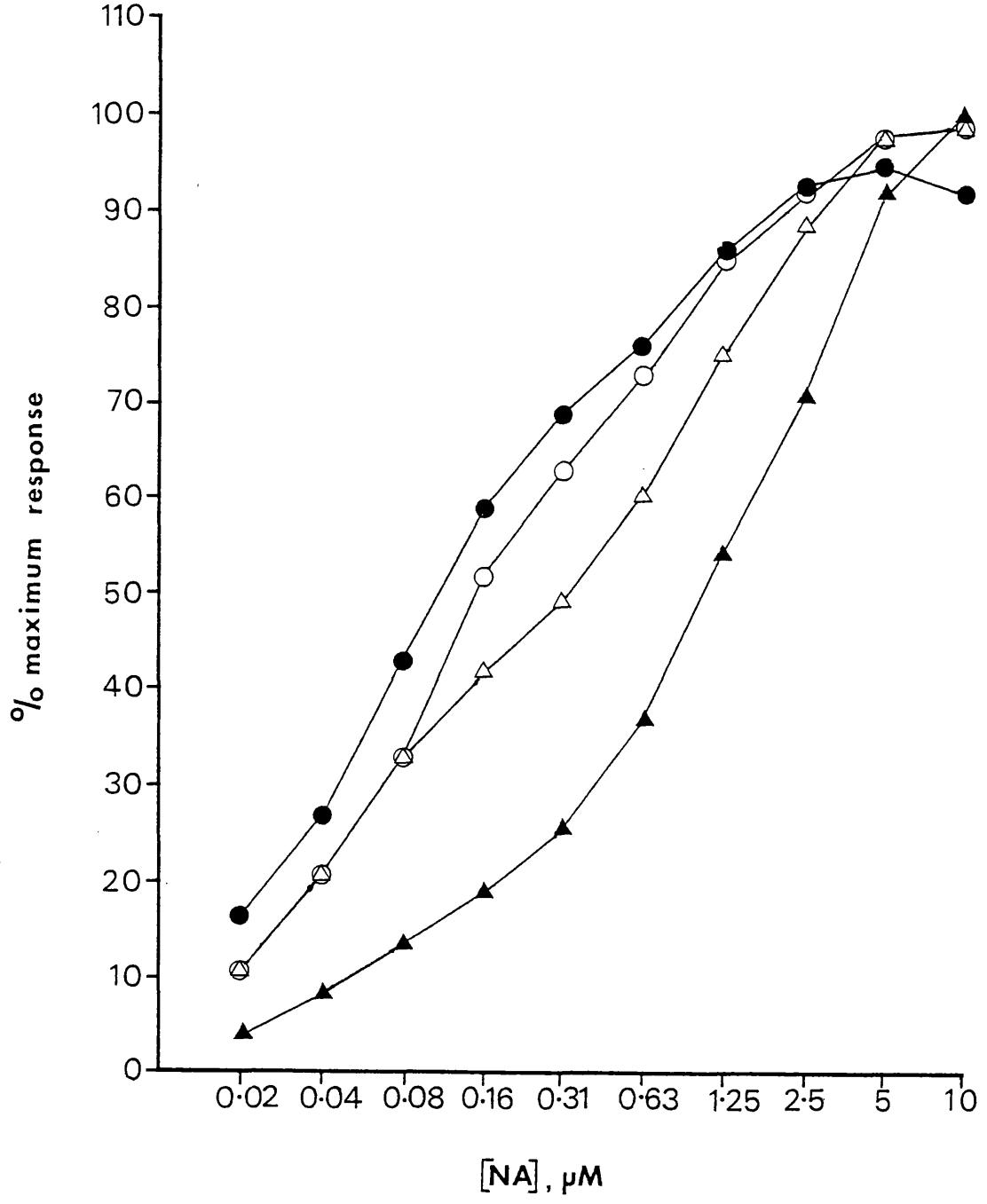


SHR unlig vs WKY unlig Not significantly different



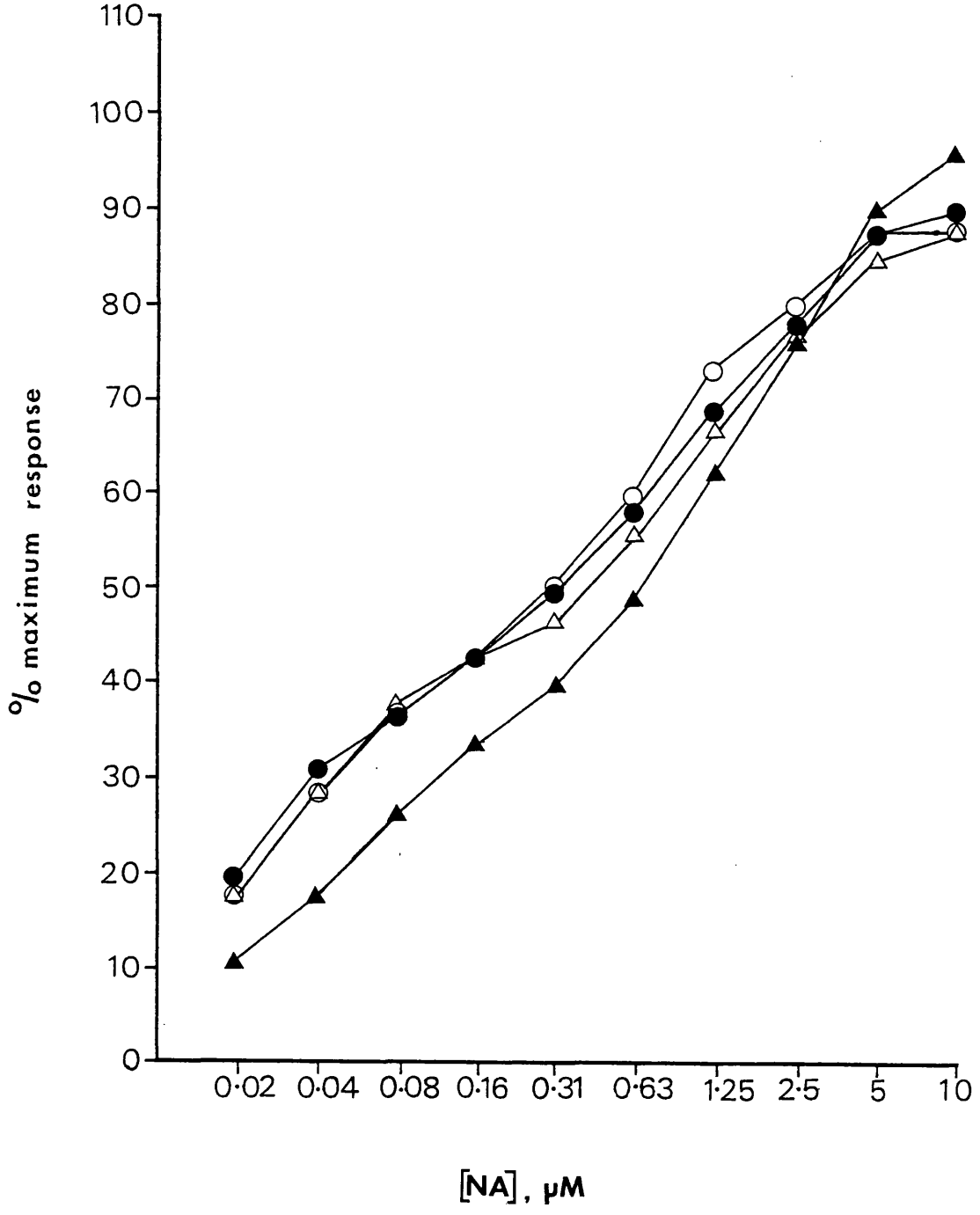
SHR unlig vs WKY unlig $P < 0.05$

FIGURE 16



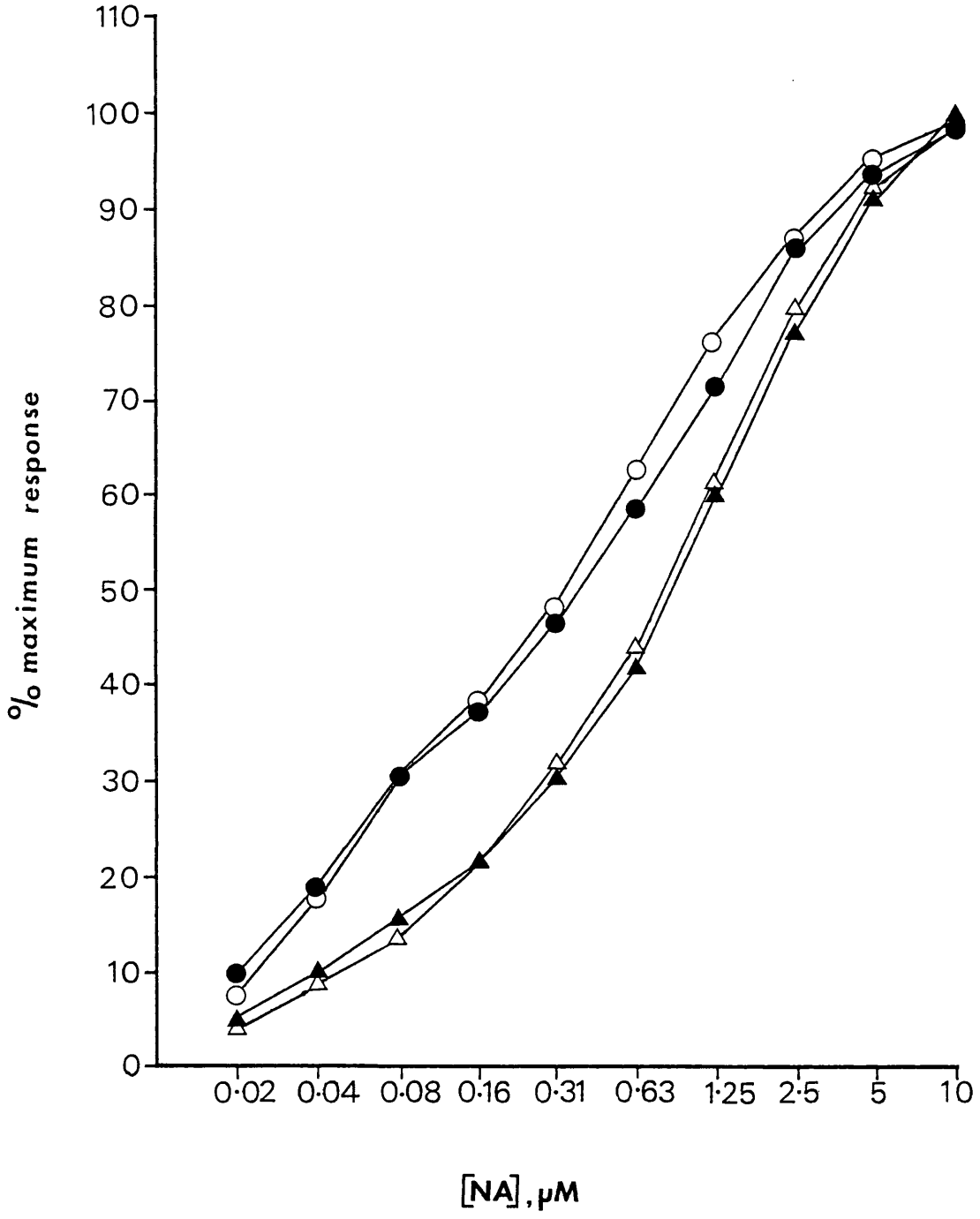
Normalized noradrenaline (NA) concentration response curves for femoral resistance arteries from 12 week rats. ▲ SHR unligatured, △ SHR ligatured, ○ WKY unligatured, ● WKY ligatured. Points represent % maximum response to noradrenaline. Error bars omitted for clarity (max 7.2%, typically 3-5%). n = 13 for SHR, n = 11 for WKY. For statistical comparison, see Table 6.

FIGURE 17



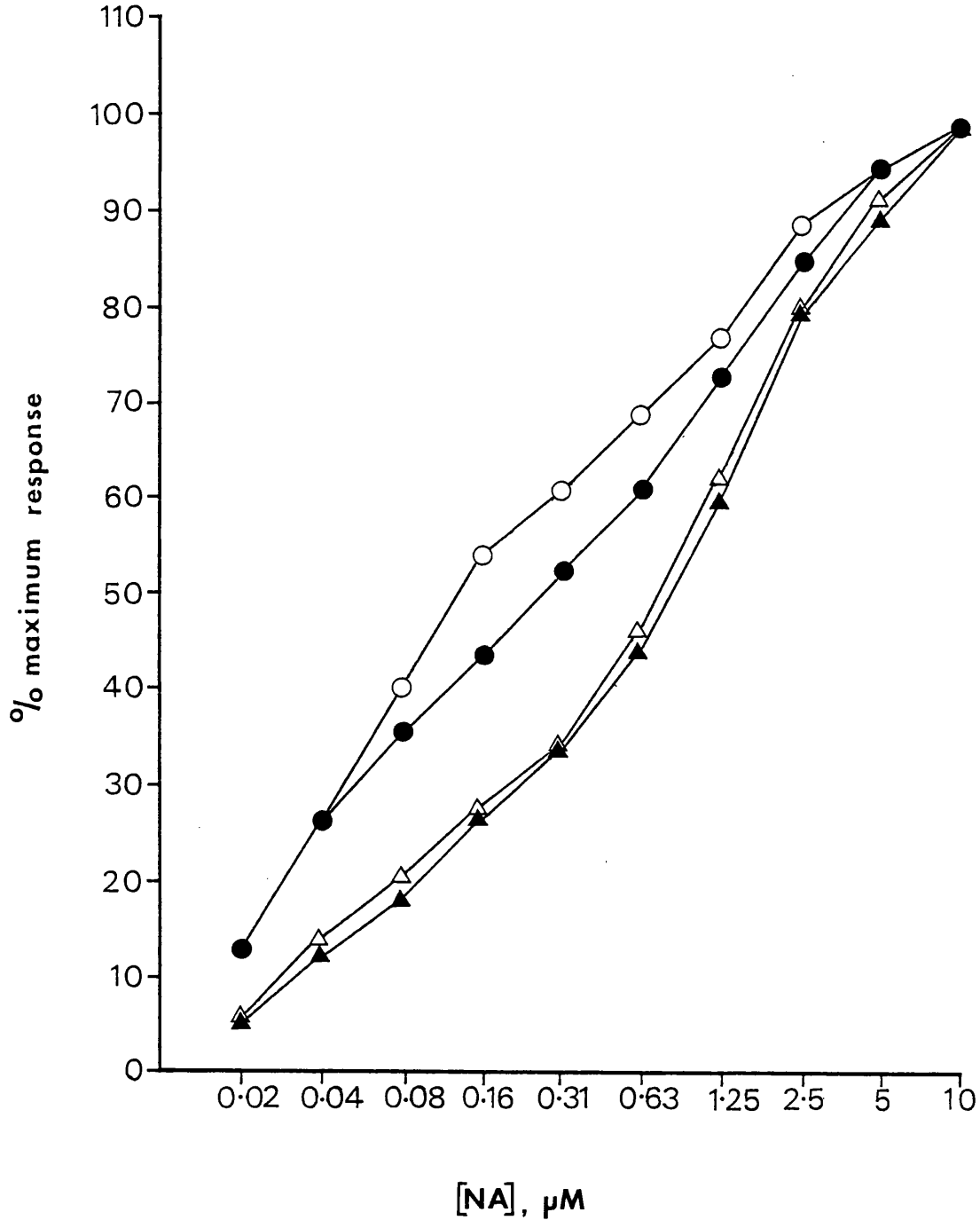
Normalized noradrenaline (NA) concentration response curves for femoral resistance arteries from 12 week rats in the presence of 3 um cocaine. Points represent % of the maximum noradrenaline response in the abense of cocaine. Symbols as for Figure 16. Error bars omitted for clarity (max 6.2%, typically 3-5%). n = 13 SHR, n = 11 WKY.
For statistical comparisons see Table 6.

FIGURE 18



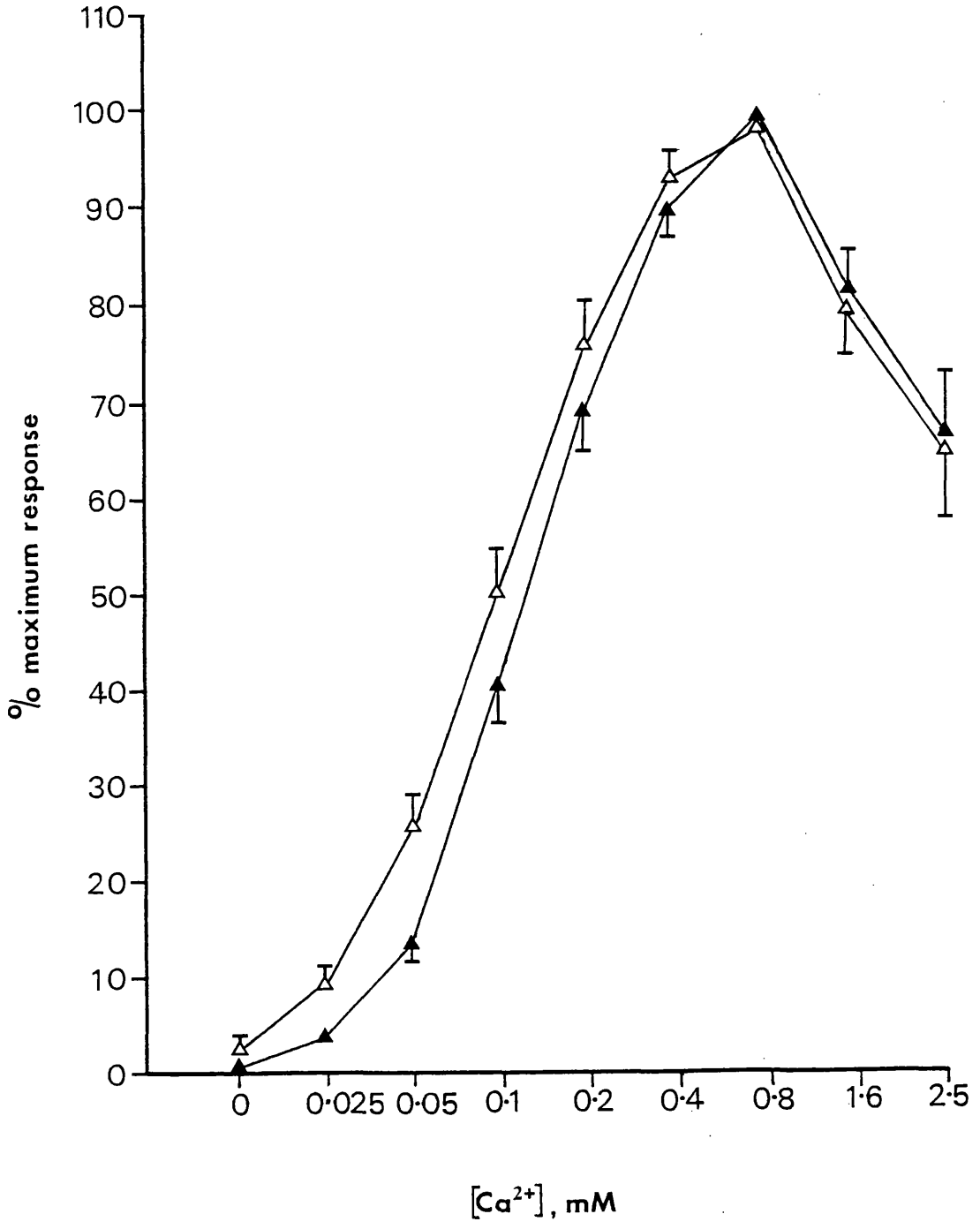
Normalized noradrenaline concentration response curves for femoral resistance arteries from 24 week rats. Symbols as for Figure 16. Error bars omitted for clarity (max 6.7%, typically 2-5%). n = 12 for SHR and WKY. For statistical comparisons see Table 6.

FIGURE 19



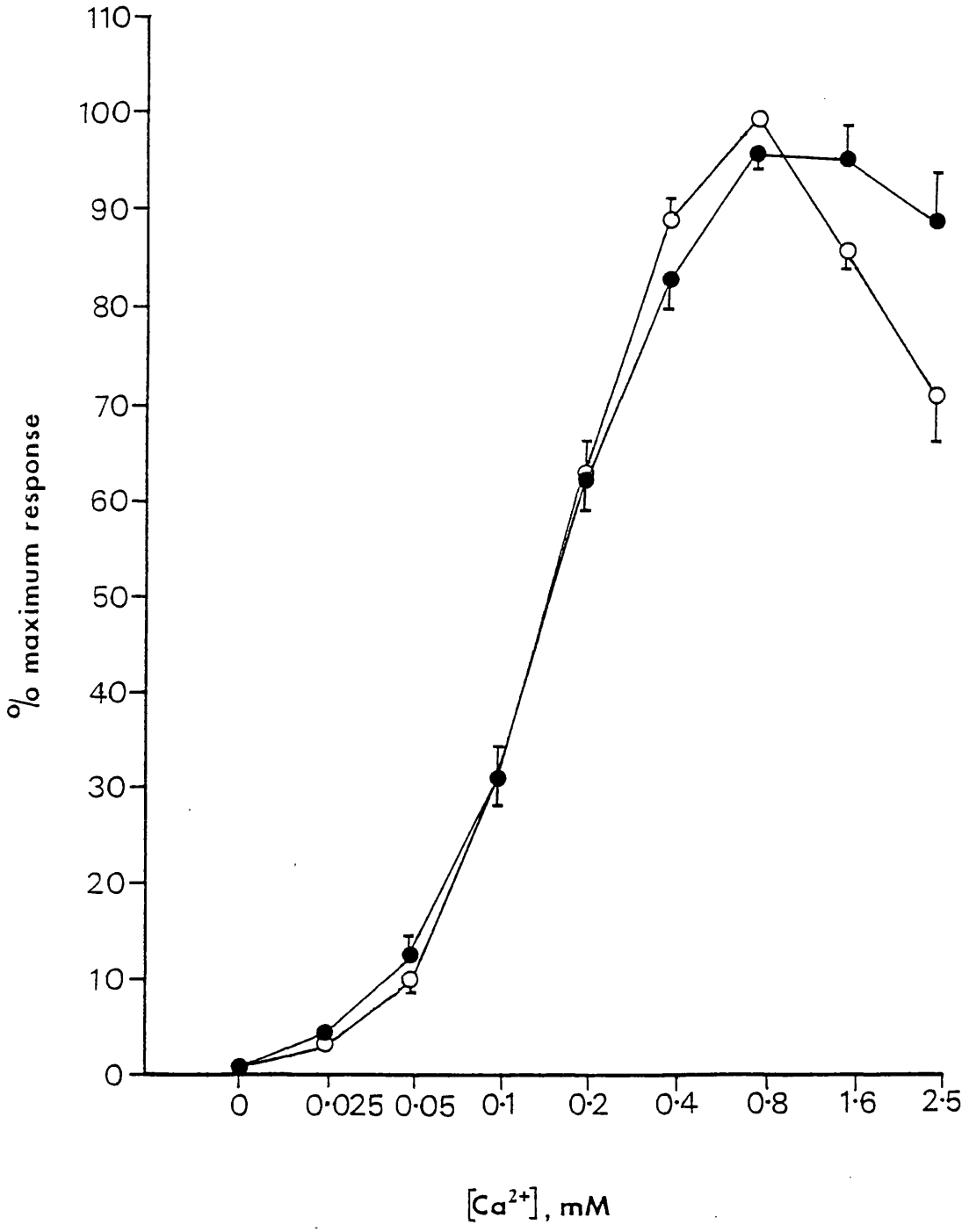
Normalized noradrenaline concentration response curves for femoral resistance arteries from 24 week rats in the presence of 3uM cocaine. Points represent % of the maximum noradrenaline response in the absence of cocaine. Symbols as for Figure 16. Error bars omitted for clarity (max 5.7%, typically 3-5%). n = 12 SHR, n = 9 WKY.
For statistical comparisons see Table 6.

FIGURE 20



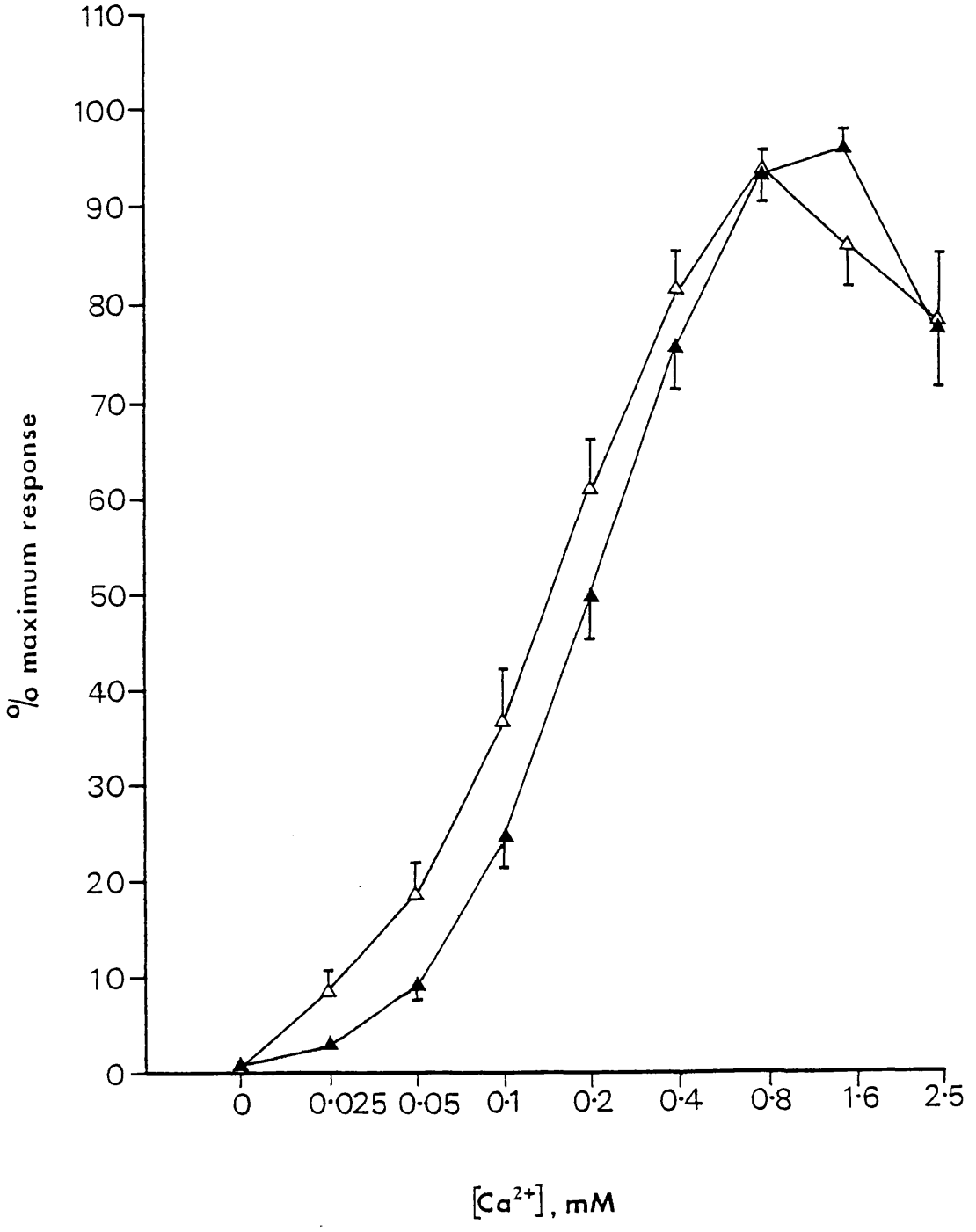
Normalized calcium concentration response curves for femoral resistance arteries from 12 week SHR unligatured (▲) and ligatured (△) hindlimbs. n = 13. Bars indicate + or - standard error. 2 way ANOVA on curves gave $P<0.05$.

FIGURE 21



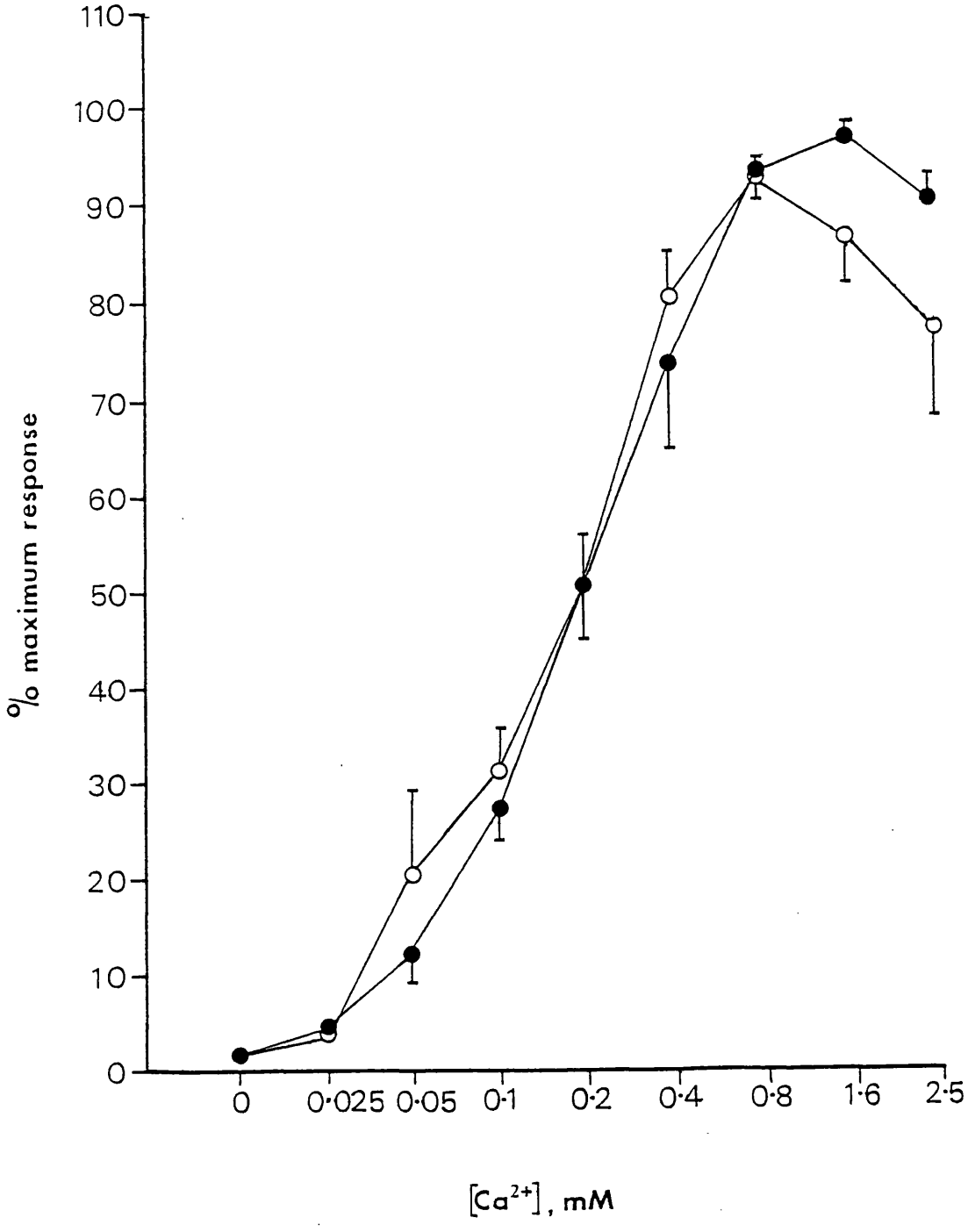
Normalized calcium concentration response curves for femoral resistance arteries from 12 week WKY unligatured (○) and ligatured (●) hindlimbs. n = 11. Bars indicate + or - standard error. Curves are not significantly different (2 way ANOVA).

FIGURE 22



Normalized calcium concentration response curves for femoral resistance arteries from 24 week SHR unligatured (▲) and ligatured (△) hindlimbs. n = 11. Bars indicate + or - standard error. 2 way ANOVA on curves gave $P<0.05$.

FIGURE 23



Normalized calcium concentration response curves for femoral resistance arteries from 24 week WKY unligatured (△) and ligatured (▲) hindlimbs. n = 10. Bars indicate + or - standard error. Curves are not significantly different (2 way ANOVA).

There was an enhanced sensitivity in protected SHR arteries compared with their unprotected controls at both 12 and 24 weeks ($P<0.05$ by 2-way ANOVA, Table 6). Calcium dose response curves are presented in Figures 20-23.

4. Vascular Contractility

(i) 12 weeks

Active media stress, ΔS, was neither significantly different between strains nor significantly affected by the ligature in either strain (Table 7). Effective active pressures, ΔP, were higher in SHR and reduced by the ligature in both strains (Table 7). The ligature resulted in reduced maximum ΔT values in each strain (Table 7). However, despite the greater medial volume in arteries from the SHR unprotected hindlimb, in comparison with WKY unligatured limbs, these arteries did not produce significantly greater maximum tension developments (Table 7). This was probably a consequence of the non-significant greater media stress displayed by unprotected arteries from WKY rats. However, taken as a whole, it appears that arterial ΔT and ΔP values are largely related to perfusion pressure.

(ii) 24 weeks

At this age, ΔP was greater in SHR arteries and reduced in both strains by the ligature (Table 7). The calculated media stresses, ΔS, were neither significantly altered by the ligature nor different between strains (Table 7). The 18% higher ΔS of SHR protected arteries compared with the SHR unprotected arteries and the 38% greater ΔS of WKY unprotected arteries compared with the SHR unprotected arteries, explains the failure of unprotected SHR arteries

to generate significantly greater active tension in comparison with SHR protected and WKY unprotected arteries (Table 7), although numerically the ΔT pattern mirrored that of the perfusion pressures, as was the case for 12 week old animals.

5. Sham Operated Animals

Table 8 describes the FMAP of 3 sham operated animals of either strain. Since only 3 were studied, statistical comparison is not appropriate but it can be seen that the loose ligature does not reduce the perfusion pressure of the sham operated side. The results obtained for the SHR do appear to be low, but this does not affect the conclusion that a large silk loop has no resistricting influence on blood pressure. Table 9 describes the morphology of the arteries at 12 weeks in the sham experiment. The loose ligature does not significantly alter any parameter and SHR arteries have a greater media thickness, media volume and media:lumen ratio. Table 10 presents the sensitivity data and again no parameter is affected significantly by the sham operation. Therefore there are no intrinsic differences between left and right hindlimbs in the absence of a constricting ligature. SHR arteries were less sensitive to noradrenaline in the presence and absence of cocaine as was noted in unprotected arteries of SHR compared with those from WKY in the constricting ligature experiment. The data in Table 10 also shows that SHR arteries from the non-manipulated hindlimb in the sham group did not display any significant effect of cocaine on the noradrenaline sensitivity in contrast to animals that had been subject to a constricting iliac ligature. It is possible that this was because the sham group SHR were less hypertensive. However, the magnitudes of the cocaine shifts continued to be

TABLE 8

Femoral mean arterial pressures (mmHg) in sham operated rats

	WKY		SHR	
Rat No.	Unlig	Lig	Unlig	Lig
1	90	78	114	128
2	97	97	130	122
3	93	92	111	118
Mean	93	89	118	123

small. Also in agreement with the experimental group, the sham operated SHR arteries proved significantly more sensitive to calcium. An inspection of the data from Tables 6 and 10 would suggest that the WKY sham operated animals were less sensitive to calcium than those in the main experimental group. It may be of considerable consequence that the sham operated group were not studied in parallel with the experimental group. Consequently there may have been an inherent difference in the phenotype of the rats used for the sham group to produce the different results. Alternatively, it may be that the pharmacological properties of unprotected WKY arteries in the experimental group were somehow altered by the presence of the ligature in the contralateral limb. Nevertheless, calcium sensitivy remained reduced in the WKY normally perfused arteries compared with those from SHR unprotected hindlimbs.

TABLE 9

Morphological characteristics of femoral resistance arteries from 12 week sham operated rats

	SHR		WKY	
	Unlig	Lig	Unlig	Lig
m_o(um)	12.74** ±0.68	12.30 ±0.43	9.96 ±0.32	10.02 ±0.34
l_o (um)	187 ±10	183 ± 6	193 ± 7	190 ± 7
m_o /l x100	7.48* ±1.12	6.91 ±0.44	5.24 ±0.25	5.36 ±0.28
media volume (um^3/um)	7847* ±464	7474 ±295	6363 ±335	6340 ±382

n = 11 SHR, n = 13 WKY, All parameters NS within strain

*P<0.05 SHR unlig-WKY unlig
**P<0.001 SHR unlig-WKY unlig

Results expressed as mean ± standard error

TABLE 10

Noradrenaline sensitivities, cocaine shifts and calcium sensitivities of femoral resistance arteries from 12 week sham operated rats

	SHR		WKY	
	Unlig	Lig	Unlig	Lig
NA pD_2	5.972* ±0.134	6.026 ±0.096	6.472 ±0.129	6.346 ±0.098
NA(+COC) pD_2	6.043* ±0.166	6.175 ±0.116	6.556 ±0.169	6.474 ±0.148
Shift	-0.069 ±0.058	-0.145 ±0.036	-0.075 ±0.123	-0.167 ±0.088
Ca pD_2	3.882* ±0.040	3.901 ±0.033	3.609 ±0.108	3.553 ±0.112

n = 10 or 11 SHR, n = 12 or 13 WKY.
All parameters NS within strain
* $P < 0.05$ SHR unlig - WKY unlig
Results expressed as mean ± standard error

TABLE 11

Maximal contractile properties of femoral resistance arteries from sham operated rats

	SHR		WKY	
	Unlig	Lig	Unlig	Lig
Active tension ΔT, mN/mm	4.38* ±0.32	3.88 ±0.18	3.44 ±0.19	3.26 ±0.24
Active media stress, ΔP, kPa	358 ±36	320 ±20	348 ±20	327 ±23
Effective active pressure ΔP, kPa	46.7** ±1.9	42.8 ±1.5	35.6 ±1.3	34.0 ±1.7

n=11 SHR, n=13 WKY All parameters NS within strains
* $P<0.05$ SHR unlig - WKY unlig
** $P<0.001$ SHR unlig - WKY unlig

Results expressesd as mean ± standard error

CHAPTER 7

DISCUSSION

1. Chemical Renal Medullectomy

The present study has confirmed that chemical renal medullectomy is associated with a raised blood pressure consistent with previous experience from this laboratory (Bing et al. 1983; Taverner et al. 1984). The blood pressure increase was small but nevertheless significant; mean indirect systolic blood pressures were increased by approximately 10%. Raised blood pressures are classically associated with thickening of the resistance vasculature media and a raised resistance to blood flow (Folkow, 1984). In the present experiment no significant structural changes were observed although it seemed as if medial growth might have been set in train since media thickness and media volume of the mesenteric resistance arteries were increased by 7%. It is therefore possible that the error involved in the morphological measurements was too great to produce a significantly different result with the number of rats used. More likely however is the possibility that structural thickening of the arteries in response to the increased bood pressure lags somewhat behind the pressure rise. While three weeks post injection a mild hypertension can be demonstrated, this time span may be insufficient for the detection of medial thickening. Indeed, Russell et al. reported an increased peripheral resistance 6 weeks after BEA administration indicative of structural change in the resistance vasculature (Russell et al. 1986). Further experiments are thus required with a longer duration to determine whether this model of hypertension does in fact stimulate vascular remodelling. In addition, since blood pressures were only measured at the end of a three week period, it is not known for how long the medullectomised rats had been hypertensive and therefore the lack of any significant structural

change in the mesenteric resistance vasculature of the medullectomised rats may not be attributable to the small pressure rise, but rather to a short hypertensive period. Maximal contractile responses were not significantly altered in the medullectomised group which lends support to the contention that structural thickening had not occurred to any real extent.

Although significant structural abnormalities could not be detected in the mesenteric resistance vasculature, there does appear to be a marked reduction in the noradrenaline sensitivity. Calcium sensitivities were unchanged; therefore there is no role for an alteration in the sensitivity to calcium in this bed in the suppression of the noradrenaline response. Thus some alternative persistent functional change had occurred. Previous experiments in this laboratory (Russell et al. 1986) have demonstrated a reduced pressor response to bolus noradrenaline injections in medullectomized conscious rats. If the reduction was due to a circulating factor then this must become tightly bound to the arteries since there is an abnormal noradrenaline response in the myograph-mounted arteries which undergo repeated washings with synthetic media. In the conscious rats described above it seems that there may have been a role for vasopressin modulation of the noradrenaline response since the V_1 receptor antagonist d $(CH_2)5$ Tyr (Me) AVP could reverse the noradrenaline response suppression. In the in vitro experiments described in the present study it seems unlikely that vasopressin would still be present to modulate the noradrenaline responses of the resistance arteries unless there are sufficient stores within the vessels despite continued incubation and washing in vasopressin-

free media. It is possible that the renomedullary vasodepressor lipids are still in contact with the smooth muscles and thus result in the suppression of noradrenaline sensitivity - indeed α receptor antagonist activity has been ascribed to a short acting polar lipid (Smith et al. 1981). However, since the renal medulla has been damaged it does not seem likely that vasodepressor lipids are involved in the reduction of the noradrenaline sensitivity; i.e. when intact the medulla may secrete vasodepressor factors to maintain a blood pressure lowering influence. But, when damaged, this influence is lost and hypertension results. Since the factor is lost then it cannot be that factor that is the mechanism by which noradrenaline sensitivity is reduced. The mechanism remains unclear therefore. An increased neuronal amine uptake would result in a reduced noradrenaline sensitivity as observed in the present study. However, noradrenaline dose response curves were not constructed in the presence of an inhibitor of this re-uptake such as cocaine. It therefore remains speculation that a presynaptic abnormality may play a role in the suppressed noradrenaline sensitivity.

In conclusion, therefore, the present study has confirmed that BEA induced chemical renal medullectomy is associated with a raised blood pressure in Wistar rats. However in the time period used there were no significant structural alterations in the mesenteric resistance vasculature. It has been demonstrated that the hypertension is associated with a reduction in the noradrenaline sensitivity of mesenteric resistance arteries which may be a compensatory mechanism induced to counter the

hypertension. The noradrenaline sensitivity suppression is not associated with a reduced sensitivity to activator calcium.

2. Vasopressin Sensitivity in the SHR

The present study indicates that the vasopressin sensitivity of mesenteric resistance arteries from the SHR is normal when compared with that of Wistar Kyoto controls in a normal physiological saline solution. There is a paucity of data concerning vasopressin sensitivity in this model with which to compare the data described in this experiment, although it has been demonstrated that at 2 weeks of age the mesenteric resistance arteries of the SHR have an increased AVP sensitivity when compared with those from WKY (Gray and Demey, 1985). Isolated perfused kidneys of the SPSHR are more sensitive to vasopressin (Berecek et al. 1980a) while SHR isolated perfused hindquarters preparations do not exhibit an abnormal vasopressin sensitivity when compared with WKY controls (Jandhyala et al. 1980). Basilar artery ring preparations from SPSHR and WKY also had similar ED_{50}s but the hypertensive rat arteries developed greater tension productions which were not accounted for by any medial thickening since responses were normalised to a maximal K^+-induced contraction (Hermsmeyer and Rusch, 1984). It therefore appears that much like many other agonists, the SHR (or SPSHR) vasculature is either equally or more responsive to vasopressin. Interestingly, it seems that vasopressin's agonist effect is modulated by presynaptic vasoactive agents since destruction of the nerve endings in vitro by 6-OHDA resulted in a three-fold increase in vasopressin sensitivity in the basilar arteries from both strains (Hermsmeyer and Rusch, 1984). Since ED_{50}s were

similar between strains before and after denervation then the myocytes' sensitivities must have been similar and, moreover, not under differential presynaptic control. However, the role of the endothelium in the contraction of arterial segments cannot be ignored since at least in canine cerebral and coronary arteries vasopressin induces relaxation via V_1 receptors on the endothelium (Vanhoutte et al. 1984). In addition, it has been demonstrated that AVP-induced pressor responses in the perfused Wistar mesenteric circuit are endothelium-modulated since pressor response and sensitivity were increased after de-endothelialization. The vasopressin constrictor influence is modulated by simultaneous endothelium derived relaxing factor (Randall, 1988), but the role of the endothelium was not investigated in the present study. A further consideration regarding AVP-mediated contractions in vascular preparations is that of tachyphylaxis. Preliminary experiments on the mesenteric arteries in this laboratory revealed that repeated AVP applications resulted in progressively smaller contractile responses. Tachyphylaxis has also been noted in canine basilar arteries (Vanhoutte et al. 1984). Thus the dose response curves may have been influenced by a progressive desensitization to the agonist properties of vasopressin.

It has been well documented that the calcium sensitivity of the noradrenaline response in mesenteric resistance arteries is increased in the SHR (e.g. Mulvany and Nyborg, 1980), while that of K^+-induced contractions is normal (Mulvany and Nyborg, 1980). When the same protocol used by these workers was used in the present study to determine the AVP-stimulated calcium

sensitivity, it was found that the magnitude of the vasopressin-induced contractions were much reduced even when calcium had been fully restored to the bathing medium. Thus, although the SHR mesenteric arteries appeared to be more responsive to AVP in most calcium concentrations (Figure 8) the likelihood was that there was no sensitivity increase as such; rather the SHR arteries might have been less prone to tachyphylaxis induced by the repeated vasopressin applications. For this reason, the calcium sensitivity was determined by an alternative approach as described in the methods section. By reducing the total exposure time to the agonist and by leaving an hour between AVP-induced contractions then tachyphylaxis was avoided as can be seen by the near full recovery of the contractile response in 2.5mM Ca^{2+} (Figure 9). Therefore it does seem that the mesenteric resistance arteries of the SHR were indeed more sensitive to calcium. Tachyphylaxis to angiotensin II has also been described for a variety of arteries in the rat (Juul et al. 1987) which could be overcome by inducing a submaximal tone by a variety of other agonists. It would be of interest therefore to repeat the comparisons made in the present work in the presence of a small pre-constriction by K^+ for example.

Why the SHR should be more sensitive to calcium in vascular contractile properties is debatable. It may be that there is a reduced ability to control cytoplasmic [Ca^{2+}] leading to an increased availability of Ca^{2+} for the myocyte contractile apparatus. Indeed, vascular preparations from the SHR show reduced Ca^{2+} extrusion from the plasma membrane of mesenteric arteries (Kwan et al. 1979) and erythrocytes (Devynck et al.

·1981) for example, and an increased Ca^{2+} permeability in the aorta (Noon et al. 1978). In cultured myocytes derived from the aorta, vasopressin has been shown to induce a greater rise in cytoplasmic calcium concentrations in those cells from SHR compared with WKY-derived cells (Nabika et al. 1985) which might correlate to a greater contractile response if the cultured cells behaved similarly in the arterial wall. Calcium sequestration to internal stores may also be impaired (Moore et al. 1975; Orlov and Postnov, 1980; Webb and Bhalla, 1976). Another possibility is that there may be a reduced calcium binding to stabilise the myocyte membranes and thus when $[Ca^{2+}]$ is limited then the myocyte membranes from SHR may be more labile (Holloway and Bohr, 1973).

In the present experiment, oscillatory tension changes were noted in SHR arteries but normally not in those from WKY, thus revealing another abnormality in the vasopressin response of the SHR resistance arteries. The frequency of the oscillations was approximately 10-12 cycles per minute and represented significant proportions of the tension developments in some arteries. It is interesting to compare this with the observations of Hermsmeyer and Rusch (1984) where these authors observed oscillatory activity in the WKY basilar arteries (but not those from SHR) albeit at much reduced frequencies of 1-3 cycles per minute. Thus it seems that there exists a marked variation in vascular responses to vasopressin depending upon the point in the circulation from which the arteries are obtained. However, it should be noted that Hermsmeyer and Rusch used [Lysine] vasopressin rather than [Arginine] vasopressin which is a

possible explanation for their observations. SPSHR tail artery strips have been shown to produce tension oscillations upon activation by noradrenaline while those from WKY did not; the tension cycling depended upon the noradrenaline, calcium and potassium concentrations (Lamb et al. 1985). A single gene locus might control this activity (Bruner et al. 1986). Although in the present study the mechanism(s) behind the oscillatory contractions was not investigated, changes in potassium conductance and therefore K^+-activated Ca^{2+} fluxes were implicated (Hermsmeyer and Rusch, 1984; Lamb et al. 1985).

In summary therefore it has been shown that mesenteric resistance arteries from SHR display normal AVP sensitivities but possess an increased AVP-stimulated calcium sensitivity. In addition SHR arteries displayed oscillatory tension developments whereas WKY arteries generally did not. Therefore this study provides further evidence that agonist responses of SHR vascular preparations are abnormal when compared with those of WKY; it is possible that these changes might play a role in the aetiology of hypertension in the SHR.

3. Blood Pressure Protection Study

This experiment has investigated the effects of protecting the vasculature of one limb of a rat from the rise in blood pressure occurring in the animal as it ages; studies have been performed on Wistar Kyoto and spontaneously hypertensive rats and the myograph has been used to measure arterial morphology and contractility under standardised conditions.

Direct measurements of femoral mean arterial pressure indicated that at 5 weeks of age the femoral bed was perfused at 45% higher pressure in SHR; however the WKY rats appeared to tolerate the cannulation procedure less well and in consequence it is believed that WKY FMAP was under-estimated by this technique. Indeed indirect systolic blood pressure measurements suggested no difference in blood pressure at 5 weeks of age and, moreover, femoral resistance artery morphology did not differ between the two strains at this age. At 12 and 24 weeks the FMAP on the unprotected side was significantly greater in SHR and at both these time points the FMAP was significantly attenuated by the partially constricting ligature in both strains. Moreover at both these ages the protected hindlimb of SHR had the same FMAP as that recorded in the unprotected WKY hindlimb. Therefore there was a 'normotensive' vascular bed in the genetically hypertension-prone rat strain, which permitted a comparison of resistance vessel morphological and functional activity from animals of different genotype but under similar pressure. However, there is an important caveat concerning these FMAP values. Since cannulation of the femoral arteries involved complete occlusion then it is possible that a falsely high blood pressure measurement would be obtained distal to the ligature, as a result of a decreased flow through the ligatured site and the resultant decreased blood pressure gradient, i.e. a reduced flow would give an overestimate of the blood pressure in the ligatured hindlimb, and the FMAP of the SHR protected limb may in

fact be lower than measured and not similar to that observed in unprotected WKY hindlimbs. This should be borne in mind when FMAP values are discussed.

In previous studies attenuation of the blood pressure rise in SHR by antihypertensive therapy has not necessarily resulted in a reduction of the cardiovascular hypertrophy normally found in the untreted SHR as described in detail in the general introduction. Thus the use of drugs has provided conflicting evidence with regard to the relationship between blood pressure and cardiovascular structural changes in SHR. In the present study it has been demonstrated that by applying a ligature to an artery into which the artery grows and consequently gradually compressing the artery wall and resulting in a normotensive vascular bed, the SHR resistance vascular becomes structurally

indistinct from an unprotected WKY vascular bed. However, it should be pointed out that at 24 weeks of age the protected SHR femoral resistance vessels did display an 18% greater media thickness and a 27% greater media:lumen ratio when compared with equivalent arteries from the unprotected WKY bed; neither of these parameters was statistically significantly different. Therefore, it appears that when the SHR femoral vasculature is exposed to normal pressure it assumes the morphological characteristics of a 'normotensive' femoral bed. This is in contrast to those reports where the vasculature of the SHR developed as if exposed to high pressures despite the blood pressure being maintained at normotensive pressures. Also, as discussed in more detail in the general introduction, it does not appear that the femoral vasculature thickens to any greater extent than can be accounted for by the blood pressure rise judging by perfusion pressure increases upon activation (Folkow and Karlstrom, 1984; Mueller, 1983); i.e. the ΔP values from SHR preparations were increased in proportion to the blood pressure increases. However, the same data might suggest that a greater growth does occur and blood pressure rises concomitantly. The technique used in the present study would have revealed structural changes in the protected arteries if structural alterations occurred in the absence of blood pressure increases. In other words, if an abnormal growth were the stimulus for an increased blood pressure then protected SHR arteries ought to have displayed enhanced structural development compared with normally perfused WKY arteries.

The apparent inconsistency of the present data compared with that

of many studies which suggest that only incomplete or non-existent structural development attenuation occurs may not only arise from the vascular bed studied, but also by the fact that a mechanical barrier was inserted into the circulation rather than a therapeutic approach to blood pressure control.

As described in the methods section the structures of the resistance arteries were compared using the calculated values for media thicknesses and lumen diameters where luminal diameters correspond to 90% of l_{100} since maximal tension responses are obtained at this lumen setting. Since media thickness at l_0 will depend upon this value then an inappropriate l_0 setting might distort the structure pattern in the groups. For example, a high l_0 would serve to decrease m_0 and $m_0:l_0$ ratio. However media volume is not altered by the degree of stretch (Carew et al. 1968) and Figure 13 illustrates that media volume is different between the strains and affected by the ligature in a similar fashion to m_0 (Figure 12) at 12 weeks. In consequence the structure conclusions based upon m_0 and $m_0:l_0$ are not drawn from data confounded by inappropriate settings of the lumen dimensions at this age. Thus a reduction in calculated media thickness by the ligature does represent a true reduction in the smooth muscle mass of the vascular wall. It has been demonstrated previously that iliac occlusion and a resultant 50% decrease in saphenous arterial pressure for 2-3 weeks can reduce femoral artery medial cross sectional area in rats (Hansen et al. 1974). However, it should be noted that of the rats used (SHR, Sprague-Dawley and DOCA hypertensive Sprague-Dawley), only a significant reduction was achieved in the normotensive rats. But, it must

also be noted that group sizes were small (n=6) and the authors investigated reversal of structural development rather than attenuation of growth, since the SHR were hypertensive before the occlusion was made and the pressure reduction was only applied for 2-3 weeks before morphological measurements were made. Consequently, it is likely that there was insufficient time for significant medial atrophy.

At 24 weeks in the present study the media volume was not significantly reduced by the ligature in WKY despite significant falls in m_0 and $m_0:l_0$. The pattern of the results matches that for m_0 and $m_0:l_0$ and so the large errors observed might have obscured a change. Alternatively, there may be remodelling of myocytes around the larger lumen so that a significant m_0 decrease may occur without the need to postulate a significant media volume decrease: the increase in lumen diameter in arteries distal to the ligature in WKY narrowly failed to reach statistical significance at 24 weeks ($p=0.052$) when compared with the unprotected side. A further possibility is that the lumen diameters in vivo may not be greater in those from ligatured limbs. As described above over estimates of lumen diameter will underestimate $m_0:l_0$ ratio. In consequence the differences in m_0 and $m_0:l_0$ may be less marked in vivo between protected and unprotected resistance arteries in 24 week WKY rats.

A number of previous studies using mesenteric resistance arteries have reported increased sensitivity to exogenously applied noradrenaline in SHR provided neuronal amine uptake mechanisms are blocked (for example, Mulvany et al. 1980) or the tissues are

chemically denervated (Whall et al. 1980). Similar observations have been made in tail arteries (Webb et al. 1981). However, this has not been the case when femoral resistance arteries (unchanged) or caudal artery rings (reduced) have been studied (Mulvany et al. 1982). In the present study noradrenaline sensitivity was decreased in unprotected femoral resistance arteries from SHR, both in the presence and absence of cocaine, when compared with unprotected WKY arteries. This may be an adaptive process to compensate for the medial thickening which inevitably leads to enhanced reactivity. There is support for this contention: at 12 weeks of age the vessels from protected SHR hindlimbs displayed similar morphological features and noradrenaline sensitivity as those from unprotected hindlimbs of WKY exposed to the same pressure. However, at 24 weeks of age both protected and exposed arteries from SHR were less sensitive to noradrenaline than those from WKY. The data at 12 weeks of age may be compared with those reported by Field and Soltis (1985) where a similar iliac ligature was employed. In that study femoral artery rings from SHR were more sensitive to noradrenaline than WKY arterioles. However, the ligature brought about a reduction in noradrenaline sensitivity such that the protected arteries from SHR displayed similar sensitivities to WKY. Therefore, in both this and the current study the placing of a ligature in SHR caused protected arteries downstream to display noradrenaline sensitivity similar to that seen in the normotensive rat strain. The fact that the present study utilised resistance arteries rather than main femoral artery rings might explain the apparent different direction of sensitivity change. However, it is nevertheless of great interest that both studies

reveal a normalization of the SHR femoral vasculature noradrenaline sensitivity.

The sensitivity of the rat resistance vasculature to noradrenaline is influenced by the degree of stretch such that sensitivity increases with increasing tension (Nilsson and Sjoblom, 1985). If the arteries from the ligatured limb were at proportionally greater resting tensions at their normalised diameters compared with those from the unligatured limb then this might be a possible explanation for the greater noradrenaline sensitivity observed in the protected arteries of the SHR compared with the control unprotected arteries. Likewise, a similar case could be made for the greater noradrenaline sensitivity observed in WKY unprotected arteries when compared with those from the SHR unligatured hindlimb. Further experiments involving sensitivity changes with tension would be required to test this possibility.

Distal to a constriction there is a reduction in reactivity to infused noradrenaline in hindlimbs in the rat following aortic constriction (Folkow et al. 1971) and in the cat subsequent to femoral artery occlusion (Folkow and Sivertsson, 1968). This is likely to be a consequence of a change in the arterial morphology since the sensitivity was unchanged in both cases. In addition, isolated main femoral arteries from WKY distal to a ligature on the iliac artery demonstrated no alteration in noradrenaline sensitivity although in SHR femoral arteries from the unprotected side were more sensitive than those from the ligatured limb (Field and Soltis, 1985). It therefore appears that blood

pressure variations do not change agonist sensitivities in general when a partially constricting ligature is used. In support, the sensitivities of femoral helical strips to a variety of agonists were not altered when rats underwent unilateral external iliac partial occlusion and strips from protected and unprotected hindlimbs were compared (Hansen and Bohr, 1975); therefore where hypertensive rats displayed altered sensitivities in comparison with controls (SHR and DOCA hypertension) these differences were maintained in the low pressure system and were therefore intrinsic to the models and not consequent to increased perfusion pressures.

Following antihypertensive therapy noradrenaline sensitivity may increase in the SHR mesenteric resistance vasculature (Jespersen et al. 1985) although the hydralazine treatment only increased the noradrenaline sensitivity in the presence of cocaine. In the absence of cocaine there was no sensitivity change induced by the therapy. It would therefore appear that the myocytes were hypersensitive but a pre-synaptic modulation of noradrenaline availability must have been initiated. Treatment with felodipine induced noradrenaline sensitivity increases in the presence and absence of cocaine (Nyborg and Mulvany, 1985) while no change was reported for felodipine treatment in an earlier study by the same group (Mulvany et al. 1981a). Noradrenaline sensitivity of the WKY mesenteric vasculature was unchanged (Jespersen et al. 1985, Mulvany et al. 1981a) or increased in the absence and presence of cocaine (Nyborg and Mulvany, 1985) in these studies. Therefore therapeutic mechanisms for blood pressure control do not have consistent influences upon vascular noradrenaline sensitivity -

at least as regards the mesenteric circuit.

The data from the present study indicate that cocaine only increases sensitivity to noradrenaline to a minor extent in the femoral resistance vasculature. Either this could be due to a small innervation of the arteries studied or the presence of underactive amine uptake mechanisms. The only shift in noradrenaline sensitivity in the presence of cocaine that attained statistical significance by Student's paired t test of noradrenaline pD_2 and noradrenaline + cocaine pD_2 values was in SHR unprotected arteries at 12 weeks ($P<0.005$). Two way analysis of variance comparisons of dose response curves in the presence and absence of cocaine revealed that in addition to the 12 week SHR unprotected arteries only 24 week WKY unprotected arteries displayed enhanced noradrenaline sensitivity with cocaine. Consequently within and between strain comparisons were not considered appropriate since the cocaine shifts were small and/or non-significant. It has previously been demonstrated that femoral resistance arteries have small cocaine-induced shifts in noradrenaline sensitivity and this was ascribed to the paucity of the sympathetic innervation in these arteries (Mulvany et al. 1982). Therefore it is unlikely that the structural attenuation seen in our study was due to an impairment of innervation.

The calcium sensitivity of the noradrenaline response has been reported to be increased in isolated resistance arteries from the mesenteric arcade (for example, Mulvany and Nyborg, 1980; Nyborg and Mulvany, 1985), femoral resistance arteries (Mulvany et al. 1982; Nilsson and Mulvany, 1981) and in the portal vein (Pegram

and Ljung, 1981). However, in the aorta (Pedersen et al. 1978) the SHR possesses reduced calcium sensitivity in comparison to Wistar controls. Treatment with felodipine increased sensitivity to calcium in both SHR and WKY mesenteric arteries (Nyborg and Mulvany, 1985) although generally blood pressure reduction does not appear to affect the calcium sensitivity of these arteries (Jespersen et al. 1985; Mulvany et al. 1981a). In one study involving iliac ligature (Field and Soltis, 1985) it was concluded that in unprotected SHR femoral arteries the sensitivity to calcium was increased. However, strictly speaking, an increased calcium sensitivity would imply an enhanced ability to maintain a contractile response in the presence of media with low calcium concentrations: in their study all arteries displayed similar contractile responses when challenged in low [Ca^{2+}] media. Whether there exists a strain difference in the femoral resistance arteries used in the present work unfortunately cannot be resolved beyond all doubt. As described in the results, it appears that from the data obtained from the sham operated group then SHR femoral resistance arteries are indeed more sensitive than those from WKY rats. However, comparison of control hindlimb arteries in the proper experimental group (i.e. unligatured sides) only just yielded a significant difference in calcium sensitivities between the two strains at 12 weeks. This is probably a consequence of the different batches of rats used as described in the Results Section. However, at both ages there was an increased noradrenaline-stimulated calcium sensitivitiy in SHR arteries on the protected side. If it is concluded that there is an increased calcium sensitivity in SHR femoral resistance arteries - at least in the 12 week rats - then the increase is

not due to the increased perfusion pressure since arteries distal to the ligature were more sensitive at both 12 and 24 weeks when compared with the unprotected side in the SHR.

The possible effects of flow and flow changes induced by the ligature upon the structure of the femoral resistance arteries investigated in the present study has thus far not been considered. When chronic decreases in blood flow in the left carotid artery of the rabbit were induced by a ligature, diameter reductions were observed compared with the right carotid artery. This diameter change was endothelium dependent since removal of the endothelium by detergent or mechanical means inhibited this response (Langille and O'Donnell, 1986). Similar observations have been made in the rat where a flow reduction in one carotid artery resulted in a narrower lumen (Guyton and Hartley, 1985). Interestingly in this latter study in association with the lumen reduction, there was a medial area reduction sufficient to maintain the media thickness. While a possible flow mediated structural response in the rat femoral vasculature cannot be discounted, such a response remains an untested possibility since flow measurements were not made. Another possibility to consider is turbulent flow which might be initiated by the partial constriction. However, the site from which the arteries were taken in the present study is way downstream from the ligature site and therefore ought not to be directly affected by turbulence upstream although factors released from the turbulence site might conceivably influence arterial morphology and reactivity. Nonetheless, in the light of the fact that ligatured hindlimbs apparently developed as normal then it is unlikely that

a marked flow reduction had occurred. Indeed the pattern of the structural differences in the vasculature between strains and between ligatured and unligatured hindlimbs matched the blood pressure pattern observed in the cannulated subgroup of rats, therefore suggesting that the perfusion pressure is the major determinant of the femoral resistance vascular structure.

In summary, it has been demonstrated in these blood pressure protection studies that femoral resistance arteries from both SHR and WKY rats structurally adjust dependent upon the pressure to which they are exposed. This experiment does not provide support for the hypothesis that the SHR possesses a load-independent propensity for increased smooth muscle proliferation. In addition the SHR resistance arteries displayed reduced sensitivity to noradrenaline in contrast to the normally observed increase. The effect of cocaine on noradrenaline sensitivities in the femoral arteries was small and therefore an increased neuronal amine uptake mechanism does not account for the suppressed noradrenaline sensitivity in the absence of cocaine. Blood pressure protection normalised noradrenaline sensitivity at 12 weeks, but not at 24 weeks, in SHR femoral resistance arteries. From data obtained in the ligature and sham operation experiments it is suggested that, at least at 12 weeks of age, there is an increased noradrenaline-stimulated calcium sensitivity in the SHR femoral resistance vasculature in agreement with the trend in the literature, even though the calcium sensitivity of the WKY groups was not consistent. Calcium sensitivity could be increased in the SHR by perfusion pressure protection but not in WKY preparations.

CHAPTER 8

GENERAL DISCUSSION

The experiments described in this work have been performed in order to further elucidate the structural and functional changes associated with the resistance vasculature in an experimental (chemical renal medullectomy) and genetic (spontaneous hypertension) model of rat hypertension.

With regard to structure it is beyond doubt that hypertension is associated with an increased media thickness and media:lumen ratio in the resistance vasculature of human essential hypertensive patients and spontaneously hypertensive rats as reviewed in the Introduction. What is not clear is which abnormality is the stimulus for the other; in other words there is a 'chicken-and-egg' situation for structural abnormalities and raised blood pressure. Age related studies have suggested that in the SHR and in human essential hypertension structural changes are present early in life but then so are raised blood pressures. For example, in the human case, offspring of essential hypertensives had marginally increased blood pressures and resistance artery media:lumen ratios (Aalkjaer et al. 1987b), and in the SHR it has been demonstrated that even at 1 day the rats have a raised blood pressure and an increased left ventricular: body weight ratio (Gay and DeMey, 1985), and at 15 days the slight blood pressure excess is also associated with a generalised increased in media:lumen ratio of the resistance arteries (Nordborg and Johansson, 1979).

Experimental models are required in order to investigate

structural changes secondary to a blood pressure rise. DOCA hypertension in the rat (Vial et al. 1989a, 1989b) is associated with structural refashioning as is hypertension induced by renal artery clipping (Mulvany and Korsgaard, 1983). Direct measurements of small artery dimensions have not previously been made in the 2-bromoethylamine-induced chemical renal medullectomy rat model of hypertension, but it has been demonstrated that this model is associated with an increased peripheral resistance (Russell et al. 1986), indicative of a remodelling of the resistance vasculature. Consequently, direct measurements have now been made as described in this work and have been recently published (Bund et al. 1989). The chemical renal medullectomy was associated with a small but significant rise in blood pressure. The indirect systolic pressure was on average raised by 10%. Mesenteric resistance artery medial thicknesses, media volumes and media:lumen ratios were increased by 7%, 7% and 6% respectively but the increases were not statistically different. However, this might reflect the fact that the pressure rise was small and, since no information on the rate of rise was gained, the rats may have been hypertensive only for a short period. Indeed, the rats used in the study by Russell et al. (1986) were studied six weeks after injection of 2-bromoethylamine and the blood pressure rise was approximately twice that observed in the present study. The greater blood pressure over a longer period would more than likely explain the structural change of the resistance vasculature as determined by the increase in peripheral resistance. It is considered highly likely that a longer treatment would provide evidence for significant myocyte growth in this experimental model. With regard to the SHR, there is a

general opinion that myocyte development is not closely associated with blood pressure since blood pressure treatment regularly does not fully normalise the medial development and, in addition, cultured SHR myocytes proliferate more rapidly. Therefore it is often suggested that in this rat the myocytes have a reinforced growth response for a given blood pressure. In the experiments described in the present work concerning the SHR femoral vasculature, it has been shown that at 12 and 24 weeks of age the increased femoral mean arterial pressure is associated with equivalent increases in the medial layer of the femoral resistance arteries. The reduced pressures distal to a partially constricting ligature were associated with proportionally attenuated medial development, suggesting a close relationship between vascular structure and blood pressure in the SHR and WKY controls. Most importantly, when the SHR femoral resistance vasculature was exposed to normal blood pressures - i.e. those noted in unligatured WKY hindlimbs - then normal structural development was observed. If this is the case then how is the postulated self-reinforcing relationship between raised blood pressure and structure initiated and continued during the life span of the rat ? It is possible that a genetic alteration in the blood pressure - vascular structure relationship is present at an early age to initiate the positive feedback loop; once this loop is set in train the initiating factor may be lost entirely or camouflaged by the myriad other influences upon smooth muscle growth. Considering the femoral vasculature as studied by means of hindquarter resistance experiments, then a similar pattern emerges as described for the present myograph experiments. Folkow has shown that in the older rats the blood pressure excess in SHR

is roughly equivalent to the minimal resistance increase, calculated wall:lumen ratio and maximal pressor responses but in the young (6 weeks) SHR these parameters were increased to a much greater extent than the mean arterial pressure (Folkow and Karlstrom, 1984). Similar observations have been made by Mueller who noted that hindquarter resistances were greater in SHR before an appreciable blood pressure rise at 5 weeks and subsequent parallel increases in minimal vascular resistance and maximal pressor response with age in SHR and WKY (Mueller, 1983). It could be the case that early abnormal medial development and the subsequent resistance increase stimulates the development of hypertension and the pressure rise stimulates further myocyte growth. Folkow has suggested that this may be the case in SHR and human essential hypertension (Folkow, 1958;1987). In the 5 week rats in the present myograph study, there may have been a similar situation. Indirect systolic blood pressures were normal but there was a tendency toward an enhanced medial development although the differences did not reach statistical significance ($P = 0.17$ for media thickness and media:lumen ratio comparisons). However, it must also be noted that direct determination of FMAP in 5 week rats revealed a greater pressure in SHR but, as previously discussed, it is thought that the smaller WKY rats tolerated the cannulation procedure less well. If there is an enhanced myocyte growth and/or proliferation in the SHR then it may be possible to find an abnormal cellular signalling mechanism in this strain of hypertensive rat. One second messenger system which has been the focus of much attention is the inositol phosphate system (Berridge and Irvine, 1989; Heagerty and Ollerenshaw, 1987). Experiments have shown that in coarctation

hypertension there is an enhanced turnover of phosphatidyl inositol in aortic segments proximal to the coarctation but not below (Ollerenshaw et al. 1988) which persists in vitro. A similar experiment showed that this model of hypertension is associated with hyperplasia of the aortic myocytes exposed to the hypertension as opposed to the hypertrophy observed by Ollerenshaw et al. (Owens and Reidy, 1985). However, it was also noted that endothelial injury might also have occurred (Owens and Reidy, 1985) and thus the high angiotensin II levels associated with this model of hypertension might have been a stimulus for the enhanced inositol phosphate turnover (as a result of greater access to the myocytes) observed in the experiments of Ollerenshaw et al. rather than an increased load stimulating the turnover and myocyte growth. In the SHR aorta it has been demonstrated that there is an enhanced basal turnover of phosphatidyl inositol in 5 week animals which is absent in older animals of 19 weeks (Heagerty et al. 1986). It might therefore be possible for the abnormal phosphatidyl inositol metabolism to be a stimulus for growth early in the life of spontaneously hypertensive rats - however in small skin arteries (Durkin et al. 1988; 1990) or mesenteric resistance arteries (AM Heagerty, personal communication) no such enhancement was observed at either 5 or 12 weeks, although noradrenaline stimulated inositol phosphate production was enhanced in SHR skin arteries (Durkin et al. 1990). In addition, at 24 weeks of age there was no measurable difference in femoral arteries inositol phosphate production between SHR and WKY preparations, and the significant blood pressure reduction induced by the ligature procedure described in the present work had no influence on this production

(Durkin et al. 1988). Thus while it may seem that abnormal phosphatidyl inositol hydrolysis occurs in the aorta of young SHR, it has yet to be determined whether such an abnormality occurs in the resistance vasculature of the SHR leading to smooth muscle growth, either through inositol trisphosphate production or diacylglycerol production, the latter molecule being the second product of phosphatidyl inositol hydrolysis by phospholipase C.

Intracellular pH (pHi) is an important moderator of many cellular processes, possibly including cell growth. Therefore it is possible that SHR myocytes might possess a more alkaline pHi as the putative signal for enhanced growth. In a recent study conducted in this laboratory it has been shown that at 5 weeks of age the mesenteric resistance vasculature from SHR does indeed have a more alkaline pHi in comparison to WKY controls and, moreover this abnormality is no longer present at 12 weeks of age (Izzard et al. 1989). The cytoplasmic pH increase at 5 weeks might be a result of increased Na^+/H^+ antiport activity (Izzard and Heagerty, 1990).

In addition to abnormal cellular processes there may also be abnormal extraceullar stimuli to myocyte growth and proliferation. A prime candidate as a source of such stimuli is the sympathetic nervous system. In a recent review (Head, 1989) much evidence has been described which suggests a greater innervation of periphral beds in the SHR in young rats before significant hypertension has been established as well as in mature SHR. Denervation can markedly influence arterial myocyte

growth and it is therefore possible that the ligature procedure might compromise sympathetic activity distal to the constriction. Considering the histofluorescence examination conducted by Mulvany and colleagues (Mulvany et al. 1982) and the small cocaine shifts observed in those femoral resistance arteries and those in the present study, it seems that the femoral resistance arteries have only a limited innervation and consequently it is felt unlikely that sympathetic innervation has any major influence upon the vascular media in this bed. Consequently the ligature procedure should have no significant effect upon the structure of the resistance arteries through inhibition of any nerve mediated influence. At 12 weeks in the SHR the ligature did have a slight but significant influence on the cocaine effect while at 24 weeks it did not. It was at 24 weeks when it seemed that there was a trend toward a greater medial content in the protected SHR arteries compared with unprotected WKY arteries. Consequently it is unlikely that changes in sympathetic activity mediate significant differential influences upon medial growth either between strains or between protected and unprotected beds.

A further criticism of the ligature procedure to reduce blood pressure is its possible effects upon flow. Flow reductions may influence vascular structure (Chapter 7). If changes in flow were the stimulus for arterial remodelling rather than pressure changes then there exists a possibility for such a mechanism in the present study. However, in defence of our conclusions the magnitude of the FMAP difference between protected and unprotected femoral arteries closely matches that of the medial volume changes measured. The lumen reductions reported in the

carotid arteries above were of much smaller magnitudes than the flow rate reductions. In addition, it was my impression that there was no reduction in hindlimb size distal to the ligature although hindlimb weights were not determined. Therefore it is believed that flow restriction was not sufficient to inhibit tissue growth.

Arterial reactivity is the contractile response elicited by an agonist and is contributed to by the smooth muscle contractility (i.e. force generating ability of the smooth muscle, ΔS), sensitivity, volume and arrangement (i.e. how the myocytes are organised to determine the lumen diameter). Therefore structural regression or attenuation as a consequence of reduced blood pressure is not in itself an indicator of reduced reactivity. Indeed it is possible that changes in one component of reactivity might be compensated for by adjustment of another. In a recent report (Smeda et al. 1988a) the increased reactivity in pre-hypertensive SHR isolated perfused kidneys may have been at least partly compensated for by a decreased noradrenaline sensitivity. Hypertension induced by chemical renal medullectomy is associated with a reduced noradrenaline sensitivity in mesenteric resistance arteries before significant medial growth occurs (Bund et al. 1989). There were no significant differences in media stress between femoral resistance arteries either between strains or between protected and unprotected arteries. Therefore any differences in active tension or active pressure must be a consequence of altered arterial geometric design. Maximal active tension productions of unprotected SHR arteries were not significantly greater than those from WKY but the ligation

procedure resulted in significant reductions in both strains at 12 weeks and WKY at 24 weeks. It might appear at first sight then that maximal contractile properties are not influenced as significantly as arterial structure by blood pressure differences within or between strains. However, effective active pressures are significantly greater in SHR and reduced significantly by the ligature in both strains. Therefore the trend for smaller lumen diameters in unprotected arteries is sufficient to produce a pattern of significance for maximal contractile response as is noted for blood pressure. While no sensitivity data could be obtained for 5 week rats in this study, there is evidence from the 12 week animals that noradrenaline sensitivity is reduced in SHR femoral resistance arteries possibly as a compensation for the medial hypertrophy. The depression of sensitivity was not an age-related intrinsic mechanism since attenuation of the pressure rise distal to the ligature resulted in a noradrenaline sensitivity comparable to that of WKY controls in addition to attenuation of medial growth. The apparent compensation for increased media by reduced noradrenaline sensitivity was also observed at 24 weeks but at this age blood pressure normalisation was not associated with enhanced noradrenaline sensitivity in the SHR. There is conflicting data regarding noradrenaline sensitivity in the femoral vasculature of SHR and WKY rats; the literature indicates that sensitivity may be unchanged or increased in the SHR. Pressure reduction by aortic constriction did not influence sensitivity (Folkow et al. 1971), while using an iliac ligature local pressure reduction decreases noradrenaline sensitivity in SHR main femoral arteries to that of WKY femoral arteries (Field and Soltis, 1985). The disparities

may reflect the different techniques used, differences in WKY genetic backgrounds (Kurtz et al. 1989) or age (Adams et al. 1989). The finding that noradrenaline sensitivity was reduced in the SHR preparations goes somewhat against the mainstream opinion. It may be that arteries from an alternative site in the femoral bed may have yielded less surprising sensitivity data. As reviewed in detail in the Introduction Section, there are many reports of an increased calcium sensitivity in SHR resistance arteries which may be a consequence of a host of mechanisms including membrane permeability and potential, calcium extrusion or sequestration and activator calcium availability.

The data in this work suggest that there is an increased noradrenaline-stimulated calcium sensitivity in the femoral resistance vasculature. Increased calcium sensitivity is not just a feature of noradrenaline activation since vasopressin-stimulated calcium sensitivity is also described in this work in the SHR mesenteric resistance vasculature, and has been published (Bund et al. 1988). How blood pressure and calcium sensitivity are related is however unclear. The increased calcium sensitivity in the femoral bed of the SHR is not apparently a consequence of raised blood pressure since prevention of the blood pressure rise by the ligature served to increase sensitivity further in this strain. It is possible then, that the SHR femoral vascular smooth muscle has an intrinsically increased calcium sensitivity which is at least partly reduced as a consequence of the increased pressure load. At 24 weeks the raised blood pressure was associated with normal calcium sensitivity. However, with no sham data available for 24 week rats there is no proper calcium sensitivity baselines with which to compare the ligatured rats' femoral artery calcium sensitivities.

CHAPTER 9

SUMMARY AND CONCLUSIONS

Summary

1. Chemical renal medullectomy is associated with mild hypertension and reduced noradrenaline sensitivity in the mesenteric resistance arteries, although over the time span of this experiment no significant structural abnormalities developed.
2. The vasopressin sensitivity of mesenteric resistance arteries is normal in the SHR but vasopressin-stimulated calcium sensitivity is enhanced in comparison to WKY normotensive controls.
3. The sensitivities to noradrenaline and calcium of SHR femoral resistance arteries are influenced by blood pressure to a much greater extent than those from WKY rats, since SHR arteries displayed a reduced noradrenaline sensitivity which could be prevented by attenuation of the blood pressure rise at 12 weeks. Calcium sensitivity is enhanced in the SHR preprations at 12 weeks, and when the blood pressure increase is avoided, calcium sensitivity in the SHR arteries is enhanced at both 12 and 24 weeks.
4. The structure of the femoral resistance arteries in SHR and WKY rats is mainly dependent upon the femoral perfusion pressure since attenuation of the perfusion pressure rise with age leads to paralleled attenuation in structural development. The SHR arteries are not subject to enhanced growth influences in comparison with those from WKY (apart from raised blood pressure) since normalisation of the blood pressure in SHR femoral beds results in SHR resistance arteries structurally indistinguishable from those derived from WKY subject to similar blood pressures.

Conclusions

The experiments I have described have shown that changes in structure and reactivity of resistance arteries are present in both the spontaneously hypertensive and chemical renal medullectomy rat models of hypertension. I have not proposed that any altered vascular properties observed are causes of hypertension, rather that the hypertensive state is associated with abnormalities of the resistance vasculature. The abnormalities observed in the spontaneously hypertensive rat to a large extent could be prevented by attenuation of the pressure load without changing neurohormonal influences, suggesting that blood pressure has a direct influence on structure and reactivity. If it is accepted that the SHR is the best animal model available for essential hypertension in man then these experiments have shown that, if blood pressure is controlled adequately, cardiovascular abnormalities may be at least attenuated and, hopefully, the morbidity resultant upon cardiovascular lesions reduced in consequence. As quoted by Folkow 'to understand a science, one must know its history'; all we can still understand is that hypertension remains an 'irritatingly elusive disorder of regulation'. While we may be able to counter the effects of the disease we can only wonder if we shall ever be able to conquer it.

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