THE RELATIONSHIP BETWEEN VASCULAR STRUCTURE, CONTRACTILE PROTEINS, VASCULAR REACTIVITY AND BLOOD PRESSURE IN ANIMAL MODELS OF HYPERTENSION

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by

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CHAPTER 2

METHODS

CHAPTER 3

Vascular Reactivity, Contractile Proteins and Blood Pressure Development in the Hypertensive WKY Rat.

CHAPTER 4

Vascular Reactivity, Contractile Proteins and Blood Pressure Development in the Spontaneously Hypertensive rat.

CHAPTER 5

Vascular Reactivity, Contractile Proteins and Blood Pressure After the Withdrawal of ACE Inhibitor Therapy in the SHR. Are the α_1-adrenoceptors Involved ?

Chapter 6

The Effect of Quinapril Treatment and its Withdrawal on Medial Thickness in the Aorta and Mesenteric Circulation of the SHR.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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PRESENTATIONS TO LEARNED SOCIETIES

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ABSTRACT

The principal aim of the studies presented in this thesis was to examine the relationship between vascular reactivity, contractile proteins and blood pressure development in the spontaneously hypertensive rat (SHR). In addition, the influence of angiotensin II on blood pressure and vascular structure and function was investigated.

In initial experiments hypertension was induced in the normotensive Wistar Kyoto rat (WKY), the most appropriate control for the SHR. Mineralocorticoid therapy (DOCA-salt) produced an increase and sustained elevation in systolic blood pressure in the normotensive WKY rat. The elevated blood pressure was associated with a marked increase in total 3-methylhistidine (a marker for contractile proteins), DNA and protein content. In contrast to the marked increase in contractile proteins, contractile responses of the perfused mesenteric preparation to vasoactive agents were similar in preparations from control WKY and hypertensive WKY rats. Moreover, the elevated blood pressure and increases in the total 3-methylhistidine, DNA and protein content were insensitive to angiotensin converting enzyme inhibitor (quinapril) treatment, α_1-adrenoceptor antagonist (doxazosin) treatment and calcium channel blockade (diltiazem). The data suggest that the elevation in blood pressure in the H-WKY does not mimic the characteristics of hypertension seen in the SHR in which vascular reactivity is augmented and sensitive to pharmacological treatments.

The perfused mesenteric preparations from SHRs demonstrated augmented reactivity to vasoactive agents when compared with preparations from WKY rats. However, the enhanced reactivity was not associated with increased total 3-methylhistidine, DNA and protein content in the mesenteric vasculature. ACE

inhibitor treatment of the SHR from 5 to 18 weeks of age prevented the development of hypertension and normalised contractile responses. Moreover, ACE inhibitor treatment reduced the total content of 3-methylhistidine, DNA and protein in the mesenteric vasculature. Both α_1-antagonist treatment and calcium blockade, although maintaining systolic blood pressure approximately 20 mmHg below that of untreated SHRs, were without influence on contractility and the biochemical parameters.

Cessation of ACE inhibitor therapy after 13 weeks of treatment for a period of 4 weeks resulted in both systolic blood pressure and vascular reactivity increasing but remaining lower than in untreated SHRs. In contrast, 3-methylhistidine, DNA and protein content of the mesenteric vasculature reverted to levels seen in vessels from untreated SHRs. In addition, co-administration of the α_1-adrenoceptor antagonist doxazosin with the ACE inhibitor and continuation of the α-adrenoceptor antagonist after withdrawal of the ACE inhibitor, prevented to a certain degree, the increases in 3-methylhistidine, DNA and protein content in the mesenteric vasculature observed after withdrawal of the ACE inhibitor, without preventing the increase in systolic blood pressure and augmented contractile responses . Although the data raise the possibility that inhibition of angiotensin II may influence growth of the mesenteric vasculature in this model the results also suggest that other process(es) involving angiotensin II, may influence structure and thereby contractility.

ABBREVIATIONS

The following abbreviations have been used throughout this thesis.

3EtH	3-ethylhistidine
3MH	3-methylhistidine
ACE	angiotensin converting enzyme
ANOVA	analysis of variance
AT_2	angiotensin II receptor
BSA	bovine serum albumin
cm, mm, nm, μm	length units (centimetres, millimetres, nanometres, micrometres)
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
EDRF	endothelial-derived relaxing factor
EGF	epidermal growth factor
FGF	fibroblast growth factor
Fig.	figure
g	gravity units
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
Hz	frequency units
IGF	insulin growth factor
KCl	potassium chloride
kg, g, mg, μg	weight units (kilogram, gram, milligram, microgram)
M, mM, μM, nM	concentration units (molar, millimolar, micromolar, nanomolar)
mA	current units (milliamps)
mmHg	millimetres of mercury
MOPS	3-morpholinopropanesulfonic acid
mRNA	messenger RNA
n	number of observations
NaCl	sodium chloride
NaOH	sodium hydroxide

NGF	nerve growth factor
NO	nitric oxide
°C	degrees Celsius
p	probability level
PBS	phosphate-buffered saline
PDGF	platelet-derived growth factor
RAS	renin-angiotensin system
rpm	revolutions per minute
s, ms, mins	time units (seconds, milliseconds, minutes)
s.c.	subcutaneous
s.e.m.	standard error of the mean
SHR	spontaneously hypertensive rat
SNS	sympathetic nervous system
TGF-ß	transforming growth factor beta
U	international units
WKY	Wistar-Kyoto rat

CHAPTER 1
INTRODUCTION

1.1 Human essential hypertension

1.1.1 Incidence and definition

In Australia, cardiovascular disease is the leading cause of death, being responsible for 43.3% of the total deaths in 1994 (National Heart Foundation, 1996). This represents more than one death from cardiovascular disease every ten minutes. One of the major predisposing risk factors for cardiovascular disease is high blood pressure. It has been reported that approximately 1,719,300 Australians between the ages of 20 and 69 years were hypertensive in 1995, ie they had a diastolic pressure of 95 mmHg or more or reported being on tablets for high blood pressure (National Heart Foundation, 1996). Although the proportion of persons with high blood pressure has fallen since the 1980s, probably due to improvement in the reliability of measurement and control of hypertension (for example through improved lifestyles and diet), it still remains a major health problem in populations not only in Australia but worldwide.

Hypertension has been divided into two phenotypic expressions:

(1) primary or essential hypertension where the cause is unknown. However, it is becoming clearer that essential hypertension results from an inherited predisposition whereby certain extrinsic or environmental factors modify the inherited susceptibility (Folkow, 1982).

(2) secondary hypertension where conditions such as renal artery stenosis and primary aldosteronism interfere with key components in cardiovascular control (Folkow, 1982).

More than 90% of hypertensive patients have essential hypertension.

Despite extensive investigation it still remains to be determined what the

initiating mechanism or mechanisms of hypertension might be. It has been postulated that a number of factors may be involved since the control of blood pressure and tissue perfusion involves a number of regulatory processes, which work in a interrelated manner and a disturbance in this equilibrium would result in hypertension as described in the past by Page (Fig. 1) (Khosla *et al* 1979).

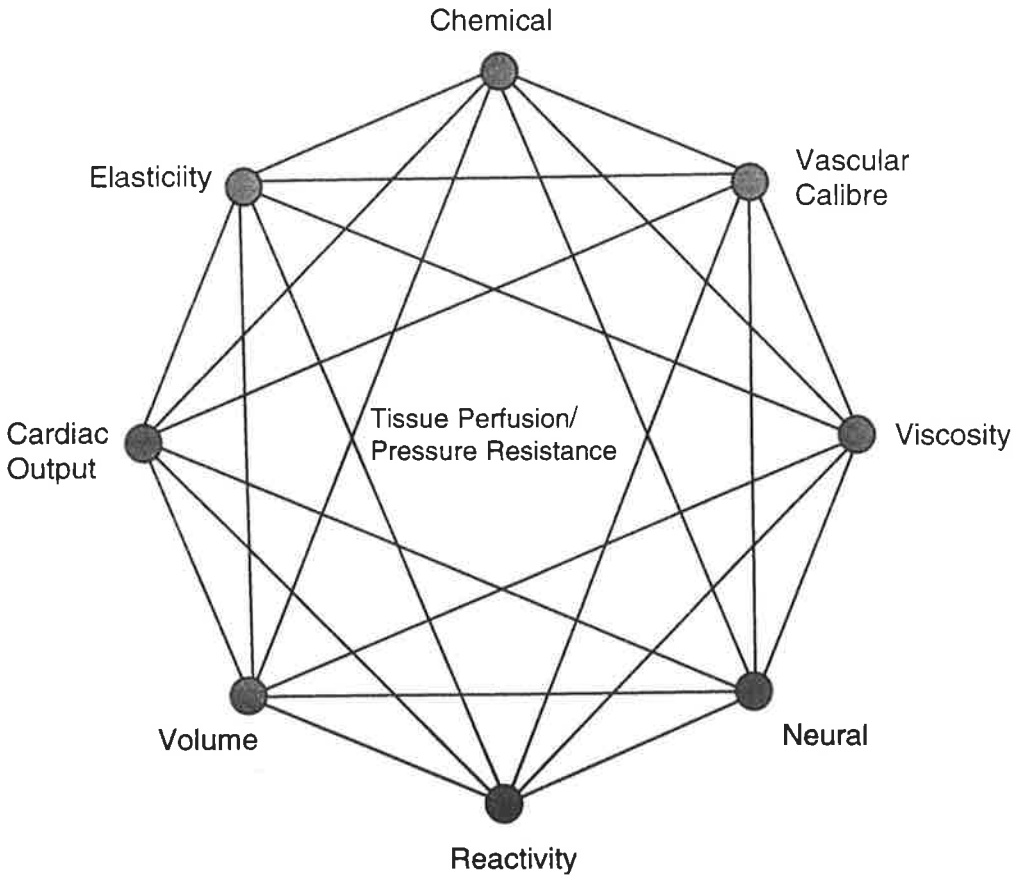


Fig. 1. Page's mosaic theory of hypertension.

1.1.2 Haemodynamics

In early essential hypertension, an elevated cardiac output and an increased heart rate are seen (Lund-Johansen, 1979; Korner, 1994). It has been suggested that these increases are due to an overactive sympathetic nervous system (Julius, 1990). During the early phase of hypertension the total peripheral resistance does not differ from that in normotensive controls (Lund-Johansen, 1979). However, as hypertension progresses there is a shift in the haemodynamic pattern and in the established phase of this disorder the cardiac output is normal, (a consequence of the stroke volume being normal or slightly reduced), and the peripheral resistance is increased (Lund-Johansen, 1979; Folkow, 1982).

1.1.3 Sympathetic nervous system

The sympathetic nervous system is one component upon which the control of blood pressure is dependent. It has multiple effects on a variety of organs and systems which directly or indirectly contribute to the regulation of blood pressure.

In human essential hypertension overactivity of the sympathetic nervous system has been observed. Studies have shown that haemodynamic measurements (ie cardiac output, heart rate and stroke volume) in patients at rest with borderline hypertension are increased and are returned to normal by autonomic cardiac blockade with propranolol and atropine (Julius & Esler, 1975) ie. indirectly demonstrating increased sympathetic activity.

Measurement of plasma levels of noradrenaline has been extensively used as an indirect index to quantify total sympathetic activity. The plasma concentration

of noradrenaline has been found to be elevated more predominantly in young patients with essential hypertension (ie individuals less than 40 years of age) when compared with their age-matched controls (Goldstein, 1983; Goldstein *et al* 1983; Esler *et al* 1986). For a more comprehensive analysis of the dysfunction of the sympathetic nervous system, a number of clinical methods have been developed to investigate regional sympathetic nervous system activity in essential hypertension. With the use of radiotracer methodology it has been shown that the rate of release of noradrenaline into plasma is elevated in hypertensive patients aged less than 40 years, but not in hypertensive patients aged 60 years and over, with a specific increase in both cardiac and renal noradrenaline spillover (Esler *et al* 1986).

Direct measurements of sympathetic nerve activity using microneurography have demonstrated the presence of increased muscle sympathetic nerve firing in both borderline and essential hypertension (Anderson *et al* 1989; Yamada *et al* 1989). However Yamada *et al* (1989) recorded increased activity of muscle nerve fibres of the tibial nerve not only in young (ie individuals 30 years or less) but in older patients with essential hypertension (ie individuals 51 years or older). The use of a noninvasive technique by which the investigators studied heart rate and arterial pressure variability as markers for sympathetic and vagal activity by power spectral analysis further supports enhanced sympathetic activity in essential hypertensives (Guzzetti *et al* 1988).

Most of the foregoing evidence indicates that enhanced sympathetic nerve activity may be an important initiating mechanism in the development of hypertension but not in patients with longstanding hypertension since both sympathetic activity and plasma noradrenaline levels appear to no longer be elevated. However, the mechanism or mechanisms involved still remain elusive.

1.1.4 Vascular Structure

As mentioned previously, established essential hypertension is characterised by an increase in total peripheral resistance which is believed to be associated with an altered vascular structure. Folkow postulated that the increase in peripheral resistance was attributed to growth of the resistance vessel wall such that it encroached on the lumen resulting in a reduced lumen diameter and an increase in media to lumen ratio (Folkow, 1982). Such structural changes would also be associated with an increase in resistance at maximal relaxation and in the responsiveness of blood vessels to vasoactive stimuli (Folkow, 1982). Indirect evidence for structural alterations in the resistance vasculature has been obtained from experiments which have demonstrated an increased resistance at maximal vasodilatation in the hand, calf (Sivertsson & Hansson, 1976) and forearm (Rosei *et al* 1995) of hypertensive patients. Clinical determinations of vascular distensibility and minimal vascular resistance in the forearm using a Xenon-133 washout technique have provided further *in vivo* evidence for structural alterations in the vasculature of essential hypertensives (Henriksen *et al* 1981).

Histological investigations of isolated resistance arteries have provided further evidence for an altered structure in small arteries. For example, Furuyama (1962) demonstrated medial hypertrophy in renal and superior mesenteric arteries from hypertensive patients. Short (1966), in postmortem studies fixed fully distended mesenteric and intestinal wall arteries and also demonstrated that the wall:lumen ratio was increased in arterioles in persons with essential hypertension.

Moreover, in vitro studies using the wire myograph technique have also provided further evidence for increased media:lumen ratios in small arteries isolated

from skin and subcutaneous biopsies in untreated essential hypertensive patients (Aalkjaer *et al* 1987; Rosei *et al* 1995). In these arteries the thickness of the media was greater by comparison with vessels from normotensive subjects but the lumen diameter had not significantly decreased (Aalkjaer *et al* 1987; Rosei *et al* 1995). The increased medial thickness was more prominent in larger vessels (about 200μm diameter) while vessels with a diameter approximately 100μm had no increase in medial thickness (Aalkjaer *et al* 1987). In contrast to the above studies, both Korsgaard *et al* (1993) and Short (1966) demonstrated a reduced lumen in resistance arteries from hypertensive patients. In conduit arteries, an increase in wall:lumen ratio with an internal diameter similar to that of normotensive subjects has also been observed (Weber *et al* 1996).

When examining the data there is a trend for the lumen diameters of vessels from hypertensive patients to be smaller although significance is not reached and since resistance to flow is proportional to the fourth power of the vessel diameter, a small change in the vessel radius could profoundly affect flow and produce a significant increase in resistance at maximal relaxation which is seen in haemodynamic studies (Triggle & Laher, 1985).

Although structural changes are evident in hypertensive resistance vessels it is important to note that a reduction in the number of microvessels (referred to as "rarefaction" of an arterial bed) would also contribute to an increase in the vascular resistance of a tissue. Support for the occurrence of rarefaction has been obtained from both postmortem arteriography and in vivo studies. Short (1958) showed a decreased vascularity in the intestinal wall in patients with hypertension. The conjunctival microcirculation has been investigated in vivo and it has been shown

that in established hypertension the arteriolar density and diameter of the vessels are reduced (Harper *et al* 1978).

1.1.5 Vascular responsiveness

As mentioned earlier, Folkow (1982) postulated that an increase in the wall:lumen ratio would result in an enhanced responsiveness of the vessel to vasoactive agents. However, altered intracellular changes or an altered receptor occupancy-response relationship can also contribute to increased vascular reactivity. The data available indicate a degree of controversy with regard to the responsiveness of resistance vessels from hypertensive patients to vasoactive agents. It has been demonstrated that the forearm vascular resistance response to high doses of intra-arterial noradrenaline or angiotensin II is increased in mildly hypertensive men compared with normotensive men (Egan *et al* 1988). Similar results have been obtained in patients with established essential hypertension in response to noradrenaline (Chang *et al* 1994). However, studies with isolated small resistance vessels from hypertensive patients have demonstrated either unaltered (Thulesius *et al* 1983; Falloon & Heagerty, 1994; Schiffrin *et al* 1994; Thybo *et al* 1995) or increased responses (Aalkjaer *et al* 1987; Angus *et al* 1992; Rosei *et al* 1995) to vasoactive agents when compared with those from normotensive subjects. Except in studies where endothelin-1 was used, the investigators demonstrated a depressed wall tension in small subcutaneous arteries from hypertensive patients (Schiffrin *et al* 1994).

Despite the discrepancies that exist with regard to whether responses are increased to vasoactive agents in resistance vessels there is agreement that there

is no difference in the sensitivity to various agonists in vessels from hypertensive patients, therefore indicating that smooth muscle function may not be altered in human essential hypertension.

Table 1.1. summarises the features of human essential hypertension that have been discussed above.

1.1.6 Antihypertensive treatment

The primary aim of antihypertensive therapy is to reduce blood pressure. This aim can be achieved by the currently available range of antihypertensive drugs, whatever their mechanism of action. Normalisation of blood pressure alone may not be sufficient to prevent mortality and morbidity related to cardiovascular disease associated with hypertension and regression of altered vascular structure is required for therapy to be beneficial in the long term. In this regard, it has been shown indirectly that a number of antihypertensive agents may cause regression of an altered structure. Sivertsson and Hansson (1976) demonstrated that after five years of antihypertensive therapy the difference in vascular resistance between patients and control subjects had decreased significantly. Shorter treatment periods with the alpha receptor antagonist doxazosin (6 week treatment period) (Mozzato *et al* 1989), the angiotensin converting enzyme inhibitor cilazapril (20 weeks) (Kiowski *et al* 1996) and the calcium antagonist nitrendipine (six months) (Agabiti-Rosei *et al* 1991) have been shown to significantly reduce either minimal forearm or calf vascular resistance in hypertensive patients.

In vitro studies have also demonstrated the regression of structural changes in resistance vessels from hypertensive patients after antihypertensive therapy.

Table 1.1. Features of human essential hypertension.

Altered haemodynamics	Early phase - ↑ cardiac output & ↑ heart rate Established phase - ↑ peripheral resistance - normal cardiac output
Altered sympathetic nervous system	↑ plasma noradrenaline ↑ sympathetic activity
Altered vascular structure	↑ peripheral resistance at maximal relaxation Histology → ↑ media:lumen ratio in small resistance vessels → ↑ media:lumen ratio in conduit vessels
Function	Both unaltered and increased responses to vasoactive agents in isolated small resistance vessels. ←→ in sensitivity

Schiffrin and co-workers (1994) found that resistance arteries dissected from subcutaneous gluteal biopsies obtained from patients treated for one year with the angiotensin converting enzyme inhibitor cilazapril showed a reduced media:lumen ratio by comparison with the media:lumen ratio before treatment. However, the media:lumen ratio was still slightly, but significantly, greater than the media:lumen ratio of resistance vessels from normotensive control subjects. In contrast, in arteries from patients treated with the ß-blocker atenolol the media:lumen ratio was not significantly changed after one year of treatment. Active wall tension responses to endothelin-1 were depressed in vessels from hypertensive patients and were normalised in cilazapril-treated but not in atenolol-treated patients. Similar results were obtained by Thybo and co-workers (1995) in a study where they used a different angiotensin converting enzyme inhibitor (perindopril) and the same ß-blocker, atenolol. In concurrence with the above studies, angiotensin converting enzyme inhibitors also caused regression of the intima-media thickness of large arteries in hypertensive patients (Mayet *et al* 1995). However, upon withdrawal of antihypertensive agents (including angiotensin converting enzyme inhibitors) hypertension redevelops suggesting that even after long term treatment regression of cardiovascular hypertrophy is not complete and that full regression of the structural changes may be required to maintain a normal blood pressure (Korner, 1994).

Although not discussed above, it is important to mention that humoral factors such as angiotensin II, kinins and atrial natriuretic peptide as well as altered cell membrane transport and genetic abnormalities, have also been identified as underlying factors in hypertension.

1.2 Genetic rat model of hypertension

Human studies investigating essential hypertension have provided valuable information concerning the disorder. However, limitations of human studies have made it difficult to determine the relationship that exists between blood pressure and the vascular alterations seen in hypertension. Therefore, the development and use of a number of animal models have become important tools in investigating the aetiology of hypertension. The different genetic rat strains that exist include the spontaneously hypertensive rat (SHR), Dahl salt-sensitive and salt-resistant rat, the Milan hypertensive strain, the Sabra hypertensive rat, the Lyon hypertensive strain and the New Zealand genetic strain. The causal mechanisms responsible for the increase in blood pressure differ between the various strains. Considering that essential hypertension is multifactorial and that various subsets (each characterised by different pathophysiological mechanisms) may exist, the use of different genetic models may help determine common links (Trippodo & Frohlich, 1981). Of the genetic strains mentioned, the SHR has been the most extensively used. A number of reservations have been expressed regarding the use of genetically hypertensive rats (eg the expressions of genetically determined hypertension are unlikely to be identical in humans and rats); however, several similarities exist between essential hypertension in humans and hypertension in the SHR (Trippodo & Frohlich, 1981).

In addition to genetic rat models of hypertension, secondary or experimental rat models of hypertension (whereby hypertension can be induced by a variety of methods) are also used. Although in these models the initiating stimulus is defined, their use has provided information to suggest how genetic predisposing elements

may affect the cardiovascular system (Schenk & McNeil, 1992). However, the main focus in this discussion will be on factors involved in the most commonly used genetic rat model of hypertension, the SHR.

1.2.2 Haemodynamics

In young SHRs, an elevated cardiac output and an increased heart rate have been observed (Nordlander, 1988). With the progression of hypertension in the SHR there is a shift towards an increased total peripheral resistance and a normal cardiac output (Nordlander, 1988; Trippodo & Frohlich, 1981).

1.2.3 Sympathetic nervous system

As with human essential hypertension, the sympathetic nervous system has also been implicated in the development of hypertension in the SHR. Evidence for the role of the sympathetic nervous system in increasing blood pressure has been demonstrated by Lee *et al* (1987). These investigators showed that neonatal sympathectomy of the SHR using guanethidine and antiserum to nerve growth factor (NGF) during the first four weeks after birth prevented the development of hypertension (Lee *et al* 1987). Similarly, induction of hypertension by either DOCA-salt treatment or by constriction of one renal artery with contralateral nephrectomy can be prevented after injection of Sprague Dawley rats with antiserum to nerve growth factor (a procedure that leads to intensive destruction of the peripheral sympathetic system) (Ayitey-Smith and Varma, 1970).

Numerous studies have suggested both qualitative and quantitative

differences in the sympathetic innervation of blood vessels in the SHR when compared with its normotensive WKY control. Morphometric studies have shown enhanced sympathetic innervation in mesenteric arteries (Lee *et al* 1983; Lee. 1985), caudal arteries (Cassis *et al* 1985) and cerebral arteries (Lee & Saito, 1984) from SHRs in comparison with the corresponding vessels from WKY rats. Histochemically, it has been demonstrated that the extent of noradrenaline fluorescent fibres in jejunal small arteries and arterioles (Haebare *et al* 1968: Ichijima, 1969), choroidal arteries (50-15μm) and small coronary arteries (Haebare *et al* 1968) from SHRs was greater than those of WKYs from 40 days of age. Both morphometrically and histochemically it has been shown that the nerves are located in the adventitial layer or in close proximity to the external border of the media but are not seen in the medial layer (Ichijima, 1969; Cassis *et al* 1985; Lee, 1985).

Noradrenaline concentrations in a number of vascular and non-vascular tissues from SHRs are elevated when compared with levels in tissues from age-matched normotensive WKY controls. The range of tissues that have been examined include caudal artery (Cassis *et al* 1985), mesenteric artery, kidney. aorta, vas deferens (Head *et al* 1985) and cerebral arteries (Lee & Saito, 1984) . Moreover, Donohue *et al* (1988) demonstrated that the increase in noradrenaline concentration in mesenteric arteries occurs immediately after birth. In contrast to the mesenteric arteries, the increased noradrenaline content in the caudal artery and aorta follows the development of hypertension (Donohue *et al* 1988).

In vitro studies using both perfused and ring preparations have demonstrated, in the presence of cocaine, that SHR preparations are hypersensitive to noradrenaline revealing enhanced noradrenaline uptake reflecting an increased degree of innervation (Mulvany *et al* 1980; Cassis *et al* 1985). In addition to

hypernoradrenergic innervation, sympathetic nerve activity is also enhanced in the SHR (Iriuchijima, 1973; Judy *et al* 1976; Lundin *et al* 1984).

The precise mechanism involved in the increased sympathetic innervation in the SHR is not clear but NGF is believed to contribute to this phenomenon. It is agreed that NGF is required for the growth and maintenance of sympathetic nerves in the periphery. A positive correlation exists between levels of NGF and the density of sympathetic innervation of target tissues (Korsching & Theonen, 1983). Elevated levels of NGF mRNA are found in mesenteric arteries in the SHR as early as 2 days of age which is consistent with the enhanced sympathetic innervation seen at the same age (Donohue *et al* 1988; Falckh *et al* 1992). At 12 weeks of age the NGF content of the mesenteric artery, spleen and sciatic nerve of SHRs is similar to WKY levels suggesting that once the process of sympathetic innervation is complete NGF is required only to maintain the nerves (Ueyama *et al* 1992). Collectively there is strong evidence supporting the involvement of an enhanced sympathetic nervous system in the development of hypertension in the SHR.

1.2.4 Vascular structure

The arterial wall is divided into three anatomical zones; the intima, the media and the adventitia, each having morphological and functional differences which are influenced by hypertension.

1.2.4.1 Intima

The vascular endothelium is recognised as a regulatory organ that plays an important role in cardiovascular function. The endothelium modulates smooth

muscle tone, cell growth and proliferation, participates in inflammatory and immune responses and mediates hemostasis in the vessel wall (Rubanyi, 1993). In hypertension, the intima undergoes morphological changes which include bulging of the endothelium into the lumen and thickening of the subendothelial space (Clozel *et al* 1990; Dusting, 1995). Endothelial cells undergo changes in shape, they increase in height and in number and when damaged, they also produce large amounts of growth factors (Schwartz & Benditt, 1977; Chobanian, 1990; Bobik & Campbell, 1993). In addition, changes in the morphology of the intima in hypertension is accompanied by increased permeability to a number of substances such as lipoprotein, ferritin, albumin and horseradish peroxidase (Limas *et al* 1980: Chobanian, 1990; Bobik & Campbell, 1993; Tedgui, 1996). Functionally the endothelium is also altered during hypertension. Endothelium-dependent relaxation in vessels from SHR is attenuated. It has been suggested that this is not due to a defective release of endothelium derived relaxing factor (EDRF)/nitric oxide (NO) (Sawada *et al* 1994) but to the simultaneous increased release of a contracting factor (Vanhoutte, 1989). It can therefore be speculated that endothelial dysfunction in hypertension may play a role in the pathophysiology of vascular structure.

1.2.4.2 Media

The medial layer of an artery consists of smooth muscle cells which are critical for its function. Vascular smooth muscle cells have at least three functions : (1) contraction and relaxation of the artery (2) releasing proteins from the extracellular matrix and (3) growth of the artery (Michel *et al* 1990). These functions are not only involved in the normal physiological development of arteries but also in the pathogenesis of disease of the arterial wall such as is seen in hypertension.

Abnormalities of cardiovascular haemodynamics which have been identified in hypertensive animals are similar to those seen in humans with essential hypertension for example total peripheral resistance (under complete resting conditions) is increased (Lundgren, 1975). Consistent with the observations in human essential hypertension, the differences in resistance between hypertensive and normotensive animals is also believed to be associated with structural alterations in the peripheral vasculature (Webb & Bohr, 1981). It is generally agreed that the structural changes are due to an increase in vessel wall thickness and smooth muscle mass.

Both histological and *in vitro* studies using the myograph technique have shown that in the mesenteric, cerebral, renal, coronary and femoral vascular beds of SHRs the medial thickness is increased, compared with the corresponding vessels from WKYs (Nordborg & Johansson, 1979; Mulvany *et al* 1980; Lee *et al* 1983; Nordborg *et al* 1983; Klepzig *et al* 1987; Smeda *et al* 1988; Bund *et al* 1991). Increased medial thickness in resistance vessels has not only been observed in genetic rat models of hypertension such as the SHR and Ren-2 transgenic rat (Thybo *et al* 1992) but also in secondary models of hypertension such as DOCA-salt (Schiffrin, 1992), renal (Deng & Schiffrin, 1991) and angiotensin II infused WKY hypertensive rats (Boonen *et al* 1993).

Although there is general agreement that medial thickness increases in hypertensive rats there are discrepancies in the literature as to whether the lumen diameter of resistance vessels changes in the developmental or established phase of hypertension in the SHRs. Studies have shown either no change (Lee *et al* 1983; Smeda *et al* 1988; Bund *et al* 1991) or a decrease in lumen diameter (Mulvany *et al* 1980; Nordborg *et al* 1983), not only in resistance vessels from different vascular

beds (which would suggest that different vascular beds respond differently), but also in vessels from the same bed.

In the SHR, structural changes have been observed in the cerebral vessels (radius >80μm), mesenteric arteries (radius between 20 to 99 μm) and renal arteries (50 to 149 μm in diameter) as early as 15 days of age (Nordborg *et al* 1983; Nordborg & Johansson, 1979). It has been demonstrated that there was an increase in the medial thickness of large and small mesenteric and renal resistance arteries from SHRs at 3-4 weeks of age (Lee, 1985; Smeda *et al* 1988) which is also consistent with the findings of other investigators (Morton *et al* 1990). In contrast to these studies it has been demonstrated that third order branches distal to the femoral artery from 5 week old SHRs were not significantly different in medial thickness or medial cross-sectional area when compared with vessels from WKYs, although a difference was seen at 12 weeks of age (Bund *et al* 1991). Similarly, in renal arteries of 30-50μm in diameter from SHRs significant increases in wall thickness were only observed at 10 weeks of age when compared to arteries from normotensive controls (Limas *et al* 1980).

In most investigations the conduit arteries such as aorta, carotid artery, superior mesenteric artery and the main renal artery demonstrated an increase in wall thickness (Limas *et al* 1980; Owens & Schwartz, 1982; Lee *et al* 1983; Eccleston-Joyner & Gray, 1988; Smeda *et al* 1988). Increase in medial thickness of the aorta and carotid artery have been observed in the SHR foetus (22 day gestation) with luminal diameters similar to that of vessels from WKY foetuses (Eccleston-Joyner & Gray, 1988). Similar results have also been obtained in the carotid artery at birth where the lumen size was similar in both WKY and SHR but medial thickness was greater in the SHR as was the wall to radius ratio (Gray,

1982). Contrary to the above studies, other investigators have shown that medial and vascular wall thickness in the aorta are not increased at 4-5 weeks of age (Limas *et al* 1980). However, by 10 weeks of age and older the aortic media in SHRs is significantly thicker than that in WKYs (Limas *et al* 1980). With the superior mesenteric artery, the medial thickness and media:lumen ratio has been shown not to be increased in 10 week old SHRs but has increased by 28 weeks of age (Lee *et al* 1983). On the other hand, Pang and Scott (1981) demonstrated no significant difference in medial thickness and lumen diameter of the abdominal aorta and renal artery of SHR ranging from two to eighteen weeks of age when compared with normotensive control vessels.

Regardless of whether observations are made on resistance vessels or conduit vessels, discrepancies exist between studies. It is unclear why these exist but differences in the method of vessel preparation, differences in SHR characteristics or differences in control strains may influence the outcome of morphological studies.

Two mechanisms have been proposed to be involved in the enhanced smooth muscle mass thickening : (1) cellular growth or (2) remodeling. Vascular smooth muscle cells display two distinct growth responses; an increase in smooth muscle cell size (hypertrophy) or an increase in smooth muscle cell number (hyperplasia). The cellular basis of the increase in medial thickness varies between different resistance vessels. Lee (1985) suggested that in both large and small mesenteric arteries the increase in media cross-sectional area in young SHRs (3-4 weeks of age) was due to hyperplasia. However, at the established phase the further increase in the medial cross-sectional area seen in the larger mesenteric arteries was probably due to hypertrophy, whereas in the small mesenteric arteries

only hyperplasia occurred (Lee *et al* 1983). Both Mulvany *et al* 1985 and Owens *et al* 1988 have also demonstrated hyperplasia of smooth muscle cells of the media in mesenteric resistance vessels in adult SHRs.

In contrast to the resistance vessels, the increase in medial thickness seen in conduit vessels such as the aorta and superior mesenteric artery is associated with a predominance of smooth muscle cell hypertrophy and an increase in cellular deoxyribonucleic acid (DNA) content due to polyploidy (Owens *et al* 1981; Owens & Schwartz, 1982; Black *et al* 1988).

The second mechanism that has been associated with an increase in the media is remodeling. Remodeling has been defined as the rearrangement of cellular or extracellular components in the vessel wall resulting in a decrease in the external diameter and encroachment on the vascular lumen without change in the total amount of the vessel components (Baumbach & Heistad, 1989). Thybo *et al* (1992) have demonstrated that third order mesenteric resistance arteries from Ren-2 transgenic rats have increased medial thickness and a decrease in lumen diameter, but that the cross-sectional area of the media is not altered when compared with the normotensive control, suggesting that remodeling has occurred without any growth of smooth muscle cells. In cerebral arterioles from SHRs, Baumbach and Heistad (1989) demonstrated a decrease in external diameter but an increase in cross-sectional area of the media, suggesting that remodeling occurred with a small degree of growth (24%). Furthermore Heagerty *et al* (1993) using a "remodeling index" (defined by Baumbach and Heistad as "the proportion of change in lumen that can be ascribed to remodeling") and a "growth index" (defined as "the percentage change in wall cross-sectional area") demonstrated that both remodeling and growth occur in resistance vessels from genetic and secondary rat

models of hypertension and human hypertensives.

These findings indicate that both growth and remodeling occur but it is the relative contributions of the two mechanisms that varies between blood vessels and forms of hypertension.

1.2.4.3 Extracellular Matrix

The extracellular matrix components are believed to exert a variety of important influences on smooth muscle cell function (including growth and migration) and also entrap growth factors (Scott-Burden *et al* 1992). Components of the extracellular matrix interact with the paracrine or autocrine modulators and also directly with smooth muscle cells via integrin receptors (Hahn *et al* 1993). The direct interaction of the extracellular matrix with the smooth muscle appears to modulate phenotypic differentiation and intracellular signalling of vascular smooth muscle cells (Hahn *et al* 1993).

Hypertension has also been implicated in influencing the extracellular matrix but there are discrepancies as to what changes occur. Increases in collagen synthesis have been demonstrated, since prolyl hydroxylase activity (a marker for collagen biosynthesis) is increased in the aorta and mesenteric artery of DOCA-salt rats and adult SHRs (Ooshima *et al* 1975; Iwatsuki *et al* 1977). In vitro studies have shown an increase in incorporation of ^{14}C-labelled proline into collagenase-digestible protein also indicating an increase in collagen biosynthesis in arterial tissue of DOCA-salt rats and SHRs (Ooshima *et al* 1975; Iwatsuki *et al* 1977). Similarly, it has been shown that both elastin and collagen levels are increased in the left coronary of SHRs (Anversa *et al* 1984). The increase in both collagen and

elastin levels in the aorta of SHRs has been seen as early as 42 days of age. It has also been suggested that the increase in collagen synthesis seems to be pressure related since similar increases in collagen synthesis are not seen in the vena cava or the mesenteric vein in hypertension (Iwatsuki *et al* 1977). In contrast, Lee *et al* (1983) demonstrated that in the superior mesenteric artery and large and small mesenteric resistance vessels there was a significant increase in elastin but not in collagen in 10-12 week old SHRs. Brayden et al (1983) observed not only no difference of collagen levels but also elastin levels in mesenteric vessels from SHRs by comparison with vessels from WKY rats.

1.2.5 Factors implicated in altering vascular structure

The underlying process or processes involved in growth or remodeling of the vessel wall are unclear. Nevertheless, a large number of substances have been reported to be involved in modifying smooth muscle cell growth or function *in vitro*, including growth factors, hormones, neurotransmitters and eicosanoids (Schwartz & Liaw, 1993).

Growth factors such as platelet derived growth factor (PDGF), epidermal growth factors (EGF), insulin growth factors (IGF), transforming growth factor-ß (TGF-ß) or fibroblast growth factors (FGF) have been shown to stimulate the growth of quiescent smooth muscle cells and endothelial cells in culture (Schwartz *et al* 1990; Bobik & Campbell, 1993). Furthermore, growth factors have been shown to be produced by endothelial and smooth muscle cells in addition to blood borne cells. Therefore growth factors can act in a paracrine or autocrine fashion (Dzau & Gibbons, 1991; Scott-Burden *et al* 1992). In addition to their mitogenic properties,

growth factors have a number of other actions. For example, they can increase synthesis of extracellular matrix proteins and other proteins, regulate cell differentiation, act as chemotactic agents and increase random migration of endothelial and smooth muscle cells (Scott-Burden *et al* 1992; Bobik & Campbell, 1993).

Vasoconstrictor agonists such as catecholamines, angiotensin II and endothelin have been implicated as growth promoters.

It is generally accepted that angiotensin II is produced locally in vascular tissue in addition to it being found in the circulation (Dzau, 1988). Angiotensin II is believed to be a bifunctional growth modulator capable of inducing hypertrophy or hyperplasia of vascular smooth muscle cells. It has been demonstrated that in serum-free media angiotensin II induces hypertrophy but in media containing serum, hyperplasia occurs, suggesting that an interaction between angiotensin II and growth factors is required for hyperplasia to take place (Bunkenburg *et al* 1992; Scott-Burden *et al* 1992). Other studies have also demonstrated angiotensin II to be a potent hypertrophic agent in cultured rat aortic smooth muscle cells (Turla *et al* 1991; Berk & Rao, 1993) with widespread but also selective increases in the content of many proteins (Turla *et al* 1991). In addition, it has been shown that angiotensin II induces expression of proto-oncogenes c-fos (Natfilan *et al* 1989(b); Berk & Rao, 1993) and c-myc (Natfilan *et al* 1989(a); Berk & Rao, 1993) in cultured quiescent rat aortic smooth muscle cells. Angiotensin II has also been shown to stimulate synthesis of PDGF A-chain (Natfilan *et al* 1989(a); Berk & Rao, 1993) and TGF-ß mRNA (Gibbons *et al* 1992) and the release of PDGF A-chain (Natfilan *et al* 1989; Berk & Rao, 1993) and promote the conversion of latent TGF-ß to its biologically active form (Gibbons *et al* 1992).

Catecholamines have also been demonstrated to stimulate proliferation of aortic smooth muscle cells in the presence of serum (Blaes & Boissel, 1983; Chen *et al* 1995). Adrenaline alone cannot initiate proliferation suggesting that the presence of serum, and consequently growth factors, are needed to induce the potentiating effect of adrenaline (Blaes & Boissel, 1983). Furthermore, stimulation of the α_1-adrenoceptor, both in cell culture and in the intact aorta maintained in organ culture under tension leads to increases in the amount of RNA, protein and sarcomeric α-actin mRNA while not affecting cytoskeletal ß-actin mRNA (Chen *et al* 1995(a)). In addition, it was demonstrated that α_{1B}-adrenoceptors possibly induce hypertrophy and that stimulation of α_{1A}/α_{1D} adrenoceptors attenuates the growth response (Chen *et al* 1995(a)). In vivo it has been shown that sympathetic denervation (Bevan, 1975) and chemical-induced denervation (Lee *et al* 1987) are associated with a decrease in smooth muscle cell proliferation in arteries, indicating a trophic effect of the sympathetic nervous system on blood vessels.

Growth inhibitors have also been demonstrated to be produced by endothelial and smooth muscle cells. These include EDRF/NO, heparin sulphate and TGF-ß (which acts as both inhibitor and promoter) (Dzau & Gibbons, 1991).

In addition to the possible influence of growth factors on smooth muscle growth and remodeling, smooth muscle cells from SHRs are characteristically different from those of WKY rats. It has been demonstrated that the proliferation rate of cells from SHR in response to serum was significantly higher than that of cells from normotensive WKY (Hamet *et al* 1988; Hadrava *et al* 1989; Saltis *et al* 1992; Saltis *et al* 1993). Vascular smooth muscle cells from SHR also attain quiescence at higher densities than vascular smooth muscle cells from WKY rats (Hadrava *et al* 1989; Saltis *et al* 1993). However, this growth characteristic of

vascular smooth muscle cells seems to depend on the nature of the mitogenic signal, since Saltis *et al* (1993) demonstrated that EGF and PDGF cause smooth muscle cells from SHRs to replicate to a higher density before attaining quiescence than vascular smooth muscle cells from WKY rats, whereas the opposite was true in the presence of b-FGF. It has been demonstrated that expression of proto-oncogene products c-myc and c-fos was increased in cultured vascular smooth muscle cells from SHRs in response to PDGF and EGF suggesting their involvement in the exaggerated growth response of vascular smooth muscle cells in SHRs (Hamet *et al* 1988). Increased genetic expression of growth factors has also been observed in aortae of hypertensive rats. For example in DOCA-salt treated rats genetic expression of TGF-ß is increased and in SHRs both TGF-ß and PDGF A- and B- chain gene expression are increased (Negoro *et al* 1992). Genetically inherited defects in intracellular signalling mechanisms may also exist in vascular smooth muscle cells from SHRs (Saltis *et al* 1992; Saltis & Bobik, 1995)

Mechanical force related to pressure and/or flow have also been shown to regulate growth. Bardy *et al* (1995) using the rabbit aortic vessel wall in an organ culture system demonstrated that pressure per se is capable of enhancing total protein synthesis in the presence of serum but has no effect on DNA synthesis. Flow increases DNA synthesis and stimulates total protein synthesis regardless of the pressure or the absence of serum (Bardy *et al* 1995). Mechanical stress related to flow has also been shown to alter the morphology of endothelial cells, to induce the production of growth factors (i.e PDGF-B, bFGF and TGF-ß) and vasoactive substances (ie PGI_2 , EDRF and endothelin-1) by bovine aortic endothelial cells (Malek & Izumo, 1994).

It appears that a proper balance between the actions of growth modulators

and inhibitors is important for the maintenance of the vascular wall structure and function (Fyhrquist *et al* 1995). Therefore, any disruption in this balance such as mechanical overload and/or changes in growth characteristics inherent in vascular smooth muscle cells could lead to the structural and functional alterations seen in blood vessels from SHRs.

1.2.6 Rarefaction

Rarefaction refers to the phenomenon of a reduced number of microvessels. This would result in an increased peripheral resistance. It has been proposed that arteriolar rarefaction is a mechanism for blood flow regulation so that the tissue is not being overperfused (Struyker-Boudier *et al* 1992). The role of rarefaction in increasing peripheral resistance has been noted in the mesenteric artery (Henrich *et al* 1978), ciliary processes and choroid in the eye, coronary circulation (Struyker-Boudier *et al* 1992), and cremaster muscle (Hutchins & Darnell, 1974) of genetic rat models of hypertension and in vascular beds of rats with experimental secondary hypertension (Prewitt *et al* 1984; Struyker-Boudier *et al* 1992).

Prewitt *et al* (1982) demonstrated that the density of small arterioles and capillaries of the gracilis muscle was not significantly different between SHRs and WKYs at 6-8 weeks of age. By 12-14 weeks of age the vessels were present but constricted, a state of functional rarefaction, and finally, at 16-18 weeks of age there was a reduced number of vessels due to anatomical rarefaction. However, there does seem to be inconsistencies in the literature concerning the age period at which rarefaction occurs as there have been studies showing rarefaction occurring at an early age, 4-6 weeks and 5-7 weeks (Struyker-Boudier *et al* 1992).

Although rarefaction appears to play a role in hypertension, from the literature available it is difficult to determine whether it is involved in its development or maintenance.

1.2.7 Vascular responsiveness

Altered vascular contractility is another characteristic of hypertension. This alteration of vascular responsiveness (ie. the ability of the preparation to respond to an agonist) may be due to either structural or functional differences or both (Folkow & Karlstrom, 1987). Structural alterations of the vessel wall result in an increase in slope and maximum of concentration-response curves to unrelated agonists without changing the median effective concentration or threshold (Kong, *et al* 1991). Functional changes lead to the horizontal displacement of concentration-response curves without changes in slope or maximum (Kong, *et al* 1991).

Lais and Brody (1978) have reported vasoconstrictor hyperresponsiveness in the hindquarter vasculature of 3 week old SHR. Similarly, in the adult SHR, an increased vasoconstrictor responsiveness has been observed in a number of perfused vascular beds such as the mesenteric (Haeusler & Haefely, 1970; Bhattacharya *et al* 1977; Ekas & Lokhandwala, 1981; Longhurst *et al* 1986; Inoue *et al* 1990), renal (Collis & Vanhoutte, 1977; Smeda *et al* 1988) and hindquarter (Bhattacharya *et al* 1977; Folkow, 1979) following administration of a variety of agents such as noradrenaline, vasopressin, potassium chloride, barium chloride and angiotensin II. Both higher maximal responses and a steeper slope of the concentration-response curve have been shown in the majority of studies. Both of these differences can be explained by the increase in media:lumen ratio whereby a

greater mass is displaced inwards on constriction thus reducing the lumen more than in a normotensive vessel irrespective of the vasoactive agent (Folkow & Karlstrom, 1987). An *in situ* model (Qiu *et al* 1995) and the use of resistance vessel rings (Mulvany *et al* 1978; Whall *et al* 1980) have also demonstrated an increased responsiveness to vasoactive agonists.

A shift of the dose-response curve to the left, implying an increase in sensitivity (since the preparation would respond at a lower concentration of the drug than normal and thus have a lowered threshold sensitivity), would indicate that functional changes have also taken place (Kong *et al* 1991; Triggle & Laher, 1985). Leftward shifts in concentration-response curves of a number of agonists have been demonstrated. However, this leftward shift is mainly associated with receptor mediated activation (Haeusler & Haefely, 1970; Bhattacharya *et al* 1977; Collis & Vanhoutte, 1977; Lais & Brody, 1978; Ekas & Lokhandwala, 1981; Morton *et al* 1990). In contrast, Inoue *et al* (1990) demonstrated that in perfused mesenteric preparations from SHRs an increased sensitivity was seen in response to noradrenaline but not to potassium chloride. These findings were observed in preparations from 6 week old SHRs but absent in SHRs 11 and 18 weeks of age (Inoue *et al* 1990). Other investigators have also shown both in ring and perfused mesenteric resistance vessel preparations that sensitivity to noradrenaline is similar in SHR and WKY preparations. However, the addition of cocaine unmasks a selective supersensitivty to noradrenaline in preparations from SHRs indicating that there is a greater neuronal uptake of noradrenaline in these preparations which would normally mask the increased sensitivity to noradrenaline (Mulvany *et al* 1980; Cassis *et al* 1985; Kong *et al* 1991).

In contrast to resistance arteries, conduit vessels (mainly the aorta), from

SHRs have been reported to have similar (Carvalho *et al* 1987; Sada *et al* 1989). decreased (Shibata *et al* 1973; Couture & Regoli, 1980(a); Sun & Hanig, 1983; Laher & Triggle, 1984) or increased (Sudhir & Angus, 1990) responsiveness to vasoactive agents when compared with responses derived from WKY vessels. Discrepancies also exist with regard to sensitivity, whereby both an increase (Hallback *et al* 1971; Field *et al* 1972) and no change (Shibata *et al* 1973; Sudhir & Angus, 1990) in sensitivity have been observed in the aorta from SHRs. It is unclear why such discrepancies exist.

By way of summary it would appear that in the SHR, resistance vessels are hyperresponsive to various agonists and that this hyperresponsiveness may be associated with an enhanced sensitivity.

1.2.8 Contractile proteins

Contraction of smooth muscle involves the contractile proteins, actin and myosin, with each thick myosin filament being surrounded by 15-18 actin filaments (Folkow, 1982). Contractile protein content in smooth muscle has been correlated closely with the amount of contractile activity of the tissue (Seidel & Murphy, 1979; Fatigati & Murphy, 1984). However, evidence as to whether altered vascular responsiveness seen in hypertension correlates with a specific change in contractile protein content is sparse and conflicting.

In the case of the aorta, it has been demonstrated that absolute amounts of actomyosin are greater in the media from SHRs, but when expressed per smooth muscle cell the amount of actomyosin is similar to those observed in smooth muscle cells from WKY rats (Seidel, 1979; Matsumura *et al* 1991). In contrast.

Owens *et al* (1981) demonstrated an increase in actin per aortic smooth muscle cell from SHRs when compared to smooth muscle cells from WKY rats but not when expressed as a fraction of total cell protein, suggesting that there was an increase in the size of the smooth muscle cells and a proportional increase in all proteins. Other studies have shown that α-actin gene expression in the aorta (Le Jemtel *et al* 1993) and superior mesenteric artery (King *et al* 1992) from SHRs is greater than that in vessels from normotensive rats, but whether this is then reflected into an increased level of α-actin protein is not known.

With regard to resistance vessels, Brayden *et al* (1983) established that an increase in both actin and myosin content was associated with a concomitant increase in DNA, this being indicative of an increased smooth muscle cell mass in the SHR mesenteric resistance arteries. When normalised on a DNA basis, the contractile protein content of mesenteric resistance vessels from SHRs did not differ from WKY rats (Brayden *et al* 1983). These results are consistent with the intrinsic cellular stress-generating capabilities of WKY and SHR mesenteric vessels which are also the same (Brayden *et al* 1983). However, in the SHR cerebral vessels, although there was an increase in DNA content, the contractile protein content did not increase. In fact, when normalised on a DNA basis, actin and myosin content decreased (Brayden *et al* 1983). In contrast to the above study, it has been demonstrated that the absolute actin content in mesenteric resistance vessels from SHRs both at 6 and 8 weeks of age was not different by comparison with vessels from WKY rats (Kadirgamanathan, 1995). One reason for the discrepancy between the two studies maybe the age of the animal used, since Kadirgamanathan (1995) used young SHRs, whereas Brayden *et al* (1983) used 25 week old rats.

In addition to directly measuring actin and myosin, 3-methylhistidine, a post-

translationally modified amino acid found uniquely in actin and myosin of muscle, has been used as a biochemical marker for changes in contractile protein content in vascular smooth muscle (Jonsson *et al* 1991). Jonsson et al (1991) documented increases in cellular 3-methylhistidine when expressed per gram of protein in mesenteric resistance vessels and the caudal artery of SHRs when compared with WKY vessels, suggesting a specific increase in contractile proteins. An enhanced 3-methylhistidine content in the mesenteric vascular bed from SHRs has also been demonstrated by Smid *et al* 1993.

Due to the conflicting data regarding both contractile protein and contractility it still remains to be determined whether there is a positive correlation between the two.

1.2.9 Relationship between blood pressure, structure, contractility and contractile proteins

The background information regarding the properties of the cardiovascular system in the SHR has been presented in the foregoing sections of this Chapter and the key findings are summarised in Table 1.2. The key challenge is to integrate the relevant findings to progress toward a unified hypothesis.

Regardless of the large amount of research, it remains difficult to determine whether the structural and functional alterations seen in blood vessels, and in particular, resistance vessels, are causal or adaptive with respect to increased blood pressure. A number of approaches have been used to unravel this puzzle.

Correlating blood pressure with structural changes has been one approach. However, in the SHR there is controversy as to when blood pressure increases,

Table 1.2. Key characteristics of the SHR.

Haemodynamics	Blood pressure increases with age Young SHRs → ↑ in cardiac output Adult SHRs → normal cardiac output → ↑ in total peripheral resistance
Perfused Vascular preparations	↑ in responses to periarterial nerve stimulation ↑ in maximum reactivity to a variety of agonists ↑ in sensitivity
Structure	↑ in media:lumen ratio Resistance vessels - hyperplasia + hypertrophy &/or remodeling Conducting vessels - hypertrophy
Contractile proteins	3-methylhistidine content - ↑ in mesenteric resistance vessels - ←→ in aorta Actin\myosin - ↑ in mesenteric resistance vessels - ←→ in mesenteric resistance vessels - ←→ in aorta

making it difficult to determine whether the structural changes are a cause or consequence of increasing blood pressure. It has been demonstrated by some investigators that blood pressure is already elevated in the SHR at birth (Gray, 1982) although others have shown that blood pressure does not begin to rise untilafter 4 weeks of age (Sen *et al* 1974; Lee, 1985; Grammas *et al* 1991). Furthermore, Mulvany and Korsgaard (1983) using hypertensive/normotensive hybrid rats saw no correlation between blood pressure and either relative heart weight or media/lumen ratio in mesenteric resistance arteries. Since blood pressure was not elevated in these rats, but vascular hypertrophy existed, it suggests that other factors, independent of blood pressure, influence the vascular structure. These observations make it difficult to determine whether the altered structure is a cause or a consequence of blood pressure.

The use of antihypertensive agents has provided further evidence for a dissociation between vascular structure and blood pressure. For example, treatment of four week old SHRs with hydralazine for twenty weeks prevented the rise in blood pressure whilst scarcely affecting the media:lumen ratio of mesenteric resistance vessels (Christensen *et al* 1989). Similar results have been obtained using the calcium antagonist felodopine (Nyborg & Mulvany, 1985) and the ß-blocker metoprolol (Korsgaard *et al* 1991). Due to some studies demonstrating that blood pressure may be elevated in the SHR from in utero and that elevated maternal blood pressure may also have an effect on vascular structure, Smeda and Lee (1991) performed studies whereby male SHRs were treated in utero and continued postnatally on hydralazine up to 28 weeks of age. The results showed that in the mesenteric vasculature, hydralazine was able to produce structural changes in both SHRs and WKY rats. However the medial cross-sectional area of

the mesenteric arteries from SHRs remained greater than that in vessels from WKY rats suggesting structural changes occurred which could not be prevented by maintaining blood pressure at a normotensive level (Smeda & Lee, 1991). In contrast to the mesenteric arteries, media thickness of renal arteries from SHRs was not affected by hydralazine treatment (Smeda *et al* 1988). Similar results have been obtained with the calcium antagonist verapamil when administered in utero and continued postnatally. In that study blood pressure was reduced in the SHR but there was no effect on the media to lumen ratio of mesenteric arteries (Gohlke *et al* 1992). On the other hand, studies where treatment with hydralazine/chlorothiazide and reserpine (Limas *et al* 1983), the ß- blocker propranolol (Weiss, 1975) or with an the ACE inhibitor captopril (Lee *et al* 1991) was used have demonstrated not only the prevention of the rise of blood pressure normally seen in SHRs but also the alterations in blood vessel structure.

A number of studies have extended the above observations by examining the effect of withdrawal of antihypertensive therapy on blood pressure and vascular structure. Freslon and Giudicelli (1983) demonstrated that both the ACE inhibitor captopril and the vasodilator hydralazine were able to reduce blood pressure after 14 weeks of treatment. However, whereas captopril prevented the increase in wall:lumen ratio of mesenteric resistance vessels, hydralazine only had a slight effect on this ratio. Withdrawal of the treatments resulted in captopril alone having a longlasting effect in opposing the rise in blood pressure and the morphological alterations in blood vessels which occur during hypertension development in the genetic model ie. the SHR (Freslon & Giudicelli, 1983). Similarly, Christensen *et al* (1989) (using a series of antihypertensive agents) demonstrated that the treatment which produced the least effect on structure was the one in which blood pressure

rose rapidly after withdrawal of the treatment. Therefore, the available evidence indicates that a regression of vascular structural changes is required to prevent the rise of blood pressure after the discontinuation of antihypertensive therapy.

With respect to contractility, Freslon and Giudicelli (1983) showed that when SHRs were treated with hydralazine the slight reduction in media:lumen ratio was associated with a slight reduction in contractility in mesenteric rings in response to noradrenaline. Similarly it has been demonstrated that a treatment which had no effect on vascular structure also did not alter responsiveness. For example, although felodopine reduced blood pressure it had no effect on structure and also did not alter the enhanced contractile response to noradrenaline seen in mesenteric resistance vessel ring preparations from untreated SHRs (Nyborg & Mulvany, 1985). Christensen *et al* (1989) demonstrated that treatment of SHRs with regimens of metoprolol, isradipine and hydralazine which had a small or no effect on the media:lumen ratio of mesenteric resistance vessels also had no effect on functional parameters of these vessels. On the other hand, perindopril reduced media:lumen ratio and the ability of mesenteric resistance vessels to contract, and this remained when treatment had been withdrawn for 12 weeks. In addition, Warshaw *et al* (1980) demonstrated that treating 25 week old SHRs with a combination of hydralazine, hydrochlorothiazide and reserpine resulted in a reduction in blood pressure and contractility of mesenteric arteries which was also associated with a reduction in smooth muscle cell mass. The above studies indicate that a reduced smooth muscle mass is associated with decreased contractility of arterial vessels. In contrast, Weiss (1975) showed that treatment of adult SHRs with propranolol normalised blood pressure and reduced maximal pressor responses of perfused hindquarters but the "steepness" of the resistance curves, (which reflected

wall/lumen ratio) remained increased. In addition, when propranolol was withdrawn for two weeks blood pressure rose but maximal responses to agonists remained attenuated. In contrast to the above study, Smid (1995) demonstrated that responses to noradrenaline were attenuated in perfused mesenteric preparations from captopril treated SHRs; however, upon withdrawal of captopril treatment, maximum contractility returned to the level of the preparations from untreated SHRs even though blood pressure remained well below the level of untreated SHRs.

The discrepancies seen in the foregoing studies could be attributed to differences in treatment periods, to techniques used for measuring contractility or potencies of the antihypertensives used. Nonetheless, it remains difficult to determine the relationship between structure, blood pressure and contractility.

1.3 Features common to hypertension in the SHR and human

The SHR has proved to be a valuable tool in evaluating the natural history of essential hypertension since it is possible to identify certain similarities between it and primary hypertension in man. Both in adult SHRs and humans (in the established phase of hypertension), the increase in blood pressure is associated with an elevated total peripheral resistance and a normal cardiac output. Neural mechanisms seem to play an important role in both rat and human particularly in the early stages of hypertension. In both forms of hypertension structural alterations of blood vessels have been observed ie. an increase in the media to lumen ratio. Maximum reactivity of resistance vessels from SHRs has been demonstrated to be increased; however, there seems to be controversy as to whether resistance vessels from hypertensive patients are hyperreactive. Furthermore, both SHRs and

humans are responsive to antihypertensive agents.

1.4 Key unresolved aspects in the pathogenesis of hypertension

There are a number of unresolved issues regarding the aetiology of hypertension. These include:

(1) whether alterations in vascular structure are a cause or a consequence of increased arterial pressure.

(2) the precise involvement of both the sympathetic nervous system and renin-angiotensin system.

(3) whether hyperreactivity of resistance vessels is attributable to vascular hypertrophy or a specific increase in contractile proteins.

(4) the precise mechanisms involved in hypertrophy or remodeling of the vascular wall.

1.5 Aim

The principal aim of the present study was:

To elucidate the relationship between contractile proteins, contractility, angiotensin II and systolic blood pressure development in the SHR.

To achieve this aim the following studies were undertaken to:

1) determine whether hyperreactivity of resistance vessels is critical to the development of hypertension.

2) determine whether increased reactivity of the mesenteric vasculature isassociated with a specific increase in contractile proteins in the genetic rat model of hypertension, ie. SHR.

3) determine the relationship between systolic blood pressure, contractile proteins and vascular reactivity in both genetic and experimental rat models of hypertension using antihypertensive agents.

4) determine the involvement of the renin-angiotensin system and sympathetic nervous system in the development of hypertension and their influence on the associated changes in vascular structure and function in both the genetic and experimental rat models of hypertension.

CHAPTER 2
METHODS

2.1 Animals

Male spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were obtained from the Glenthorne Division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO). All experimentation was approved by the CSIRO animal ethics committee. The animals were 5 weeks of age at the commencement of the experiments and were housed in wired-bottomed cages in environmental conditions of 21-24°C, 50% humidity and a 12 hour light/dark cycle. Animals were fed standard rat chow and drinking water ad libitum for the duration of each study the only exception being in the study where the WKYs were treated with deoxycorticosterone (DOCA) when the animals received 0.9% sodium chloride solution as drinking water.

2.2 Drug administration

The antihypertensive agents used were administered orally by way of the rat's drinking water. The drug solutions were freshly prepared every 2-3 days and the concentration of the drugs was adjusted depending on the volume of drug solution per body weight consumed by the rats in each cage.

2.3 Indirect blood pressure measurements

Systolic blood pressure was measured using an indirect tail-cuff procedure based upon a photoelectric tail-cuff detector (IITC, Life Sciences, California, USA).

Rats were placed in ventilated perspex tubes and the tail slid through a

sensing cuff which was mounted on the back wall of the holder. So as to obtain stable readings the animals were placed initially in a large warming cabinet maintained at 29°C for 30 minutes before making the recordings. The sensing cuff was automatically inflated until there was occlusion of the tail artery and hence the disappearance of the pulse. The cuff was automatically deflated at a slow rate and the first onset of the pulse taken as the systolic point. The measurements were repeated until at least three consistent readings were obtained for each animal.

2.4 Surgery - unilateral nephrectomy

For the 12 hours before surgery, rats were fasted and the antibiotic "Clavulox" (80ul of 62.5 mg/ml in 100 ml of H_2O) (12.5 mg potassium clavulanate, 50 mg amoxycillin trihydrate), was administered in their drinking water. On the day of surgery, rats were anaesthetised with a pentobarbitone sodium (60 mg/ml):methohexitone sodium (10 mg/ml) mixture (1:9) (5 ml/Kg body weight). The hair from the surgical site was removed using electric clippers. The animal was placed on a heating pad on its right side and its tail was taped down so as to prevent movement during surgery. The incision site and an area around the incision were wiped over with gauze soaked in antiseptic. A sterile drape was then placed over the animal leaving only the head and the surgical site exposed. A skin incision was made just below the rib cage down the left side and into the abdominal cavity. The left kidney was gently exteriorised by grasping the perirenal fat. A scalpel was used to gently incise the renal capsule and the kidney was then extruded from the capsule. The renal blood vessels and ureter were clamped with haemostats adjacent to the kidney and a single synthetic thread ligature was placed around

these structures, below the haemostats, and tied with two double reef knots followed by two single knots. The kidney was then cut away using a scalpel. The peritoneal membrane and the muscle layer were then closed with interrupted stitches. The skin incision was closed with single interrupted stitches using silk thread. The incision and its surroundings were then wiped with antiseptic and the animals placed on a heating pad and covered with a towel. As the animal began to recover consciousness, a subcutaneous (s.c.) injection of the analgesic buprenorphine hydrochloride ("Temgesic" 0.1 mg/kg) was administered. Animals were housed individually for several days and antibiotic treatment was continued for 7 days.

2.5 Tissue harvest and homogenisation

Animals were sacrificed by decapitation. The required tissues were removed, cleaned of loosely adhering fat and connective tissue, blotted dry, weighed, wrapped in aluminium foil and snap frozen in liquid nitrogen. They were stored at -80°C until required for analysis. The tissues were homogenised, on ice, in cold phosphate buffered saline (pH 7.4) using a glass/glass motorised homogeniser, at approximately 200-400 rpm. Appropriate aliquots of the homogenate for each biochemical assay were taken and either used on the day or stored at -80°C.

2.6 In vitro experiments

2.6.1 Aortic ring preparations

The procedure was adapted from that described by Head *et al* 1987. The thoracic aorta was removed and placed in oxygenated Krebs solution consisting of the following (mM) : NaCl 113, KCl 4.8, KH_2PO4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 5, glucose 11.2 and ascorbic acid 0.6. The aorta was cleaned of surrounding connective and adipose tissue, carefully cut into 3-4 mm rings and mounted on stainless steel stirrups. The preparation was then transferred to a 15 ml water jacketed organ bath which contained Krebs solution continuously aerated with 95% O_2 - 5% CO_2 and maintained at 37°C. Tissues were equilibrated for 45 minutes under a resting tension of 4 g, after which time they were contracted with 20 mM potassium chloride (KCl) to assess tissue viability and ensure stabilisation of the preparation. Once maximum contraction was achieved, the KCl was washed out and the aorta allowed to return to baseline. After stabilisation of the preparation, concentration-response curves to vasoactive agents were obtained by cumulative increases in the total concentration of the agonist. After each concentration-response curve was completed the agonist was washed out and the tissue allowed to re-equilibrate. Responses were measured with an FT03 force displacement transducer, connected to a JRAK amplifier and responses recorded on a Graphtec Linearcorder FW33701. The apparatus was calibrated before each preparation using standard weights where 25 mm represented 1 gram of tension. At the completion of the dose response curves, the aorta was removed from the organ bath, blotted and weighed.

2.6.2 Perfused mesenteric vascular bed preparation

The perfused mesenteric vascular bed preparation was the same as that described by Longhurst *et al* (1986). Upon isolating the abdominal aorta and the superior mesenteric artery a polyethylene catheter (OD 1.0 mm ,ID 0.5 mm) was inserted into the mesenteric artery at its junction with the aorta and tied firmly in place. The aorta was then cut on either side of the mesenteric artery and the entire mesenteric bed plus the intestinal tract were removed from the rat and placed in ice-cold saline. The stomach and caecum were removed, the intestinal contents flushed out with saline and the entire preparation was mounted on a perspex rod embedded with two platinum electrodes for electrical field stimulation. The mesenteric artery was then secured with the ligatures near the proximal electrode. The distal electrode was positioned 2 cm away in the tissue fascia to permit electrical field stimulation. The preparation was then mounted in a 50 ml water jacketed organ bath containing aerated Krebs solution, gassed with 95% O_2-CO_2 and maintained at 37°C. The preparation was perfused through the cannula which was connected to a Gilson Minipuls 2 peristaltic pump delivering Krebs solution (aerated with 95% O_2-CO_2 and maintained at 37°C) at a flow rate of 4 ml/min. Changes in perfusion pressure were monitored using a Statham P23 AC pressure transducer connected to a Graphtec Linearcorder FW33701 via a series of JRAK pressure amplifiers. The apparatus was calibrated for pressure responses before each set of preparations and the flow rate checked every second day.

Noncumulative dose- and frequency-response curves were determined. Agonists were injected intraluminally into the perfusion fluid via a silicon injection port as a bolus dose in a volume 0.1 ml. Frequency response curves were obtained

using a Grass S44 stimulator at a supramaximal voltage (75 mV) with a delay and duration of 1 millisecond and as single repeated pulses. Each stimulation was given for 15 seconds every 3 minutes and the frequency was increased from 2 Hz to a maximum of 64 Hz. Three frequency-response curves were performed for each preparation. Noncumulative dose-response curves to noradrenaline (0.01-20.0 μg), phenylephrine (0.1-10.0 μg), potassium chloride (1-32 mg) and arginine vasopressin (9×10^{-13} - 8.9×10^{-8} M) were performed. In the case of arginine vasopressin , the drug was infused at rate of 0.4 ml/min.

At the end of the protocol period the preparation was transferred into a petri dish containing saline (placed on ice) where the mesenteric bed was dissected from the intestinal tract and the adhering adipose tissue and the vein were carefully cleared away. The mesenteric tree was dissected into the superior mesenteric artery and its resistance vessels. The tissues were blotted, weighed, wrapped in aluminium foil, snap frozen in liquid nitrogen and stored at -80°C until required.

2.7 In situ perfusion fixation and morphometric analysis

Animals were injected intraperitoneally with the anaesthetic pentobarbitone sodium (60 mg/ml):methohexitone sodium (10 mg/ml) mixture (1:9) (5ml/Kg body weight) containing heparin sodium (125 units per 15 ml of blood). The abdominal aorta was isolated via an abdominal incision and clamped well below its junction with the superior mesenteric artery (this was done to prevent perfusion of the hindlimbs with fixative). The abdominal incision was continued to the sternum. The sternum was held with a pair of haemostats, cut on each side, folded back and held in place with the haemostats. The pericardial membrane around the heart was

excised and the diaphragm freed. Holding the heart with haemostats the perfusion needle was pushed through the inferior portion of the left ventricle into the aorta and held in place with the haemostats. Perfusion with phosphate buffered saline (PBS) (pH 7.4) plus 1% sodium nitrite was commenced and the right atrium cut and maintained until all of the blood had been removed. The sodium nitrite was used in order to fully dilate the blood vessels. Following initial perfusion with PBS the animal was perfused with a 4% formaldehyde, 0.5% gluteraldehyde solution for 15 minutes. In each case, the perfusion pressure was maintained at the conscious systolic blood pressure of the animal throughout the perfusion. The thoracic aorta and entire mesenteric vascular bed were removed and stored in the 4% formaldehyde, 0.5% gluteraldehyde solution at 4°C overnight. The tissues were then stored in PBS plus sodium azide (0.01%) until required for histological preparation.

A section of the thoracic aorta and type 2 branch of the mesenteric vasculature were embedded in epoxy resin, cross-sections 0.5 μm thick were cut and stained with 0.5% toluidine blue. Six sections from each sample were taken for evaluation. Measurements of medial thickness were performed using a microscale TM/TC video image analysis system (Digithurst) connected to a light microscope with a video camera (Olympus Vanox). Ten random measurements of the media were made on each section and the mean value used for analysis.

2.8 Biochemical analyses

2.8.1 3-Methylhistidine Assay

3-methylhistidine (3-MH) was assayed by high-pressure liquid chromatography (HPLC) using the method described by Smid et al (1995) with minor modifications.

To each aliquot of tissue homogenate, standard and quality control (QC) samples, 3-ethylhistidine (3-EtH; 15 nmoles) was added as an internal standard (3-EtH was synthesised by Dr D. Ward, Department of Chemistry, University of Adelaide, Australia). Samples were then hydrolysed in hydrochloric acid (HCl, 6 M) at 110°C for 20 hours. After the addition of HCl, the glass vials were flushed with nitrogen and sealed. The hydrolysate was dried under vacuum using a Savant Centrifugal drier and reconstituted in milli-Q water to give a final tissue concentration of 10 mg/ml. Samples were vortexed vigorously, transferred to polypropylene tubes (Sarstedt) and centrifuged at 10,000 g in a microfuge (Beckman, Microfuge E) for 5 minutes. Fifty µl of the supernatant were mixed with 125 µl 0.2 M sodium borate (pH 12.2) and while vortexing 125 µl of acetonitrile containing fluorescamine (1.6 mg/ml) was added. The tubes were allowed to sit at room temperature for 5 minutes after which time 37.5 µl of concentrated perchloric acid (diluted 1 in 2) were added while vortexing the tube. The tubes were capped and the mixture was incubated in a heated water bath at 80°C for 1 hour. The derivative was cooled to room temperature and the acid neutralised with 25 µl of 0.5 M morpholinopropanesulfonic acid (MOPS) in 3 M sodium hydroxide (NaOH).

The fluorine derivative of 3-MH and 3-EtH was assayed by HPLC in conjunction with a fluorescence detector. The HPLC apparatus consisted of a LC

1500 HPLC pump, LC1610 Autosampler, LC 1250 Fluoro Detector, DP 800 Data Interface, Data Management Program (all ICI Instruments, Australia) and 10 μ C_{18} 30cm x 4.6mm Spherisorb S100DS2 packed column (Adelab, Adelaide). 3-MH and 3-EtH were eluted from the column using 26% acetonitrile in 10mM sodium phosphate (pH 7.4) at a flow rate of 1 ml/min and were detected by the fluorimeter at excitation and emission wavelengths of 365 nm and 460 nm respectively. Chromatographic peaks were recorded and peak area analysed by a data acquisition system. Typical illustrative traces are shown in Fig. 2.1 and 2.2.

The standard curve was linear between 0.0276 and 0.5517 nmol/ml (Fig. 2.3). The intra- and interassay coefficients of variation were determined at three concentrations of 3-MH in mesenteric vascular homogenate, representing 15 assays. Intraassay coefficients of variation were as follows: 0.166 nmol/ml 2.55%, 0.259 nmol/ml 3.37% and 0.519 nmol/ml 2.40%. Interassay coefficients of variation were as follows: 0.166 nmol/ml 3.89%, 0.259 nmol/ml 4.20% and 0.519 nmol/ml 3.5%.

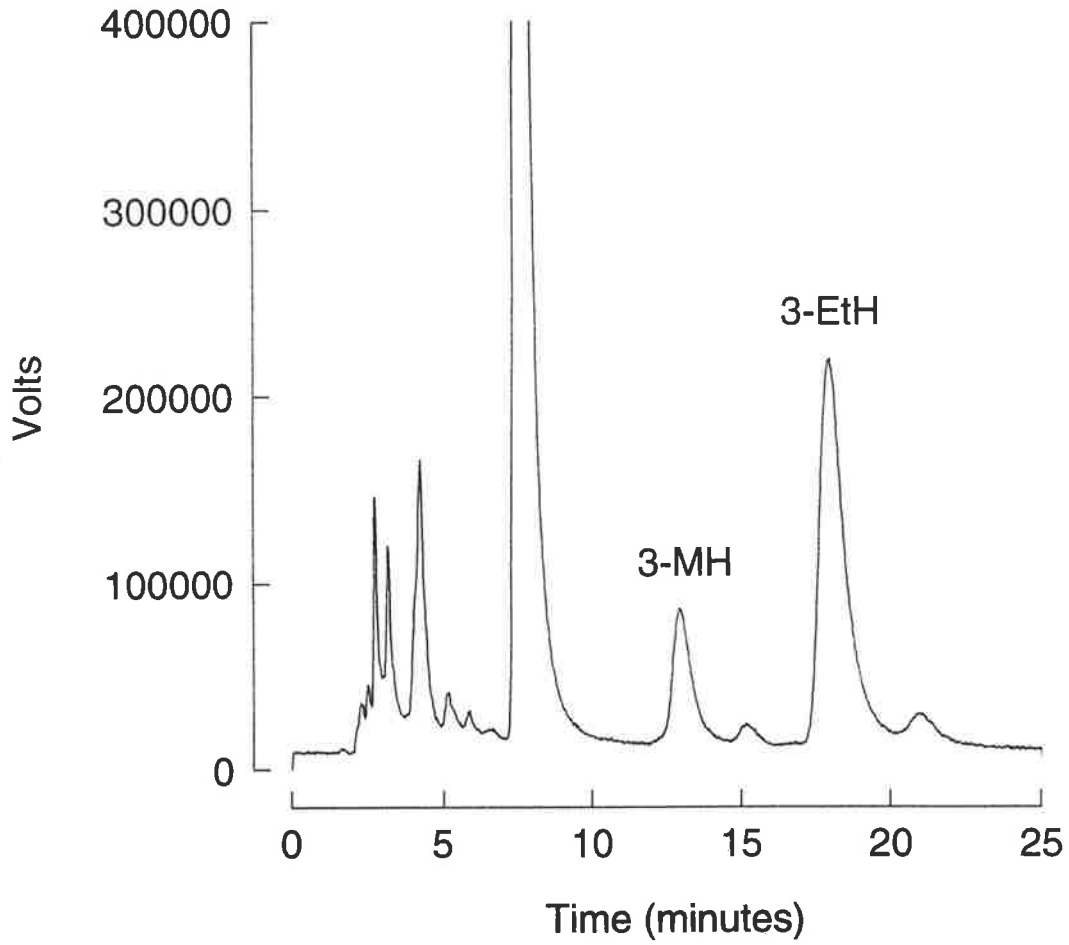


Fig. 2.1. HPLC chromatogram representing tissue samples containing 3-methylhistidine (3-MH) and 3-ethylhistidine (3-EtH).

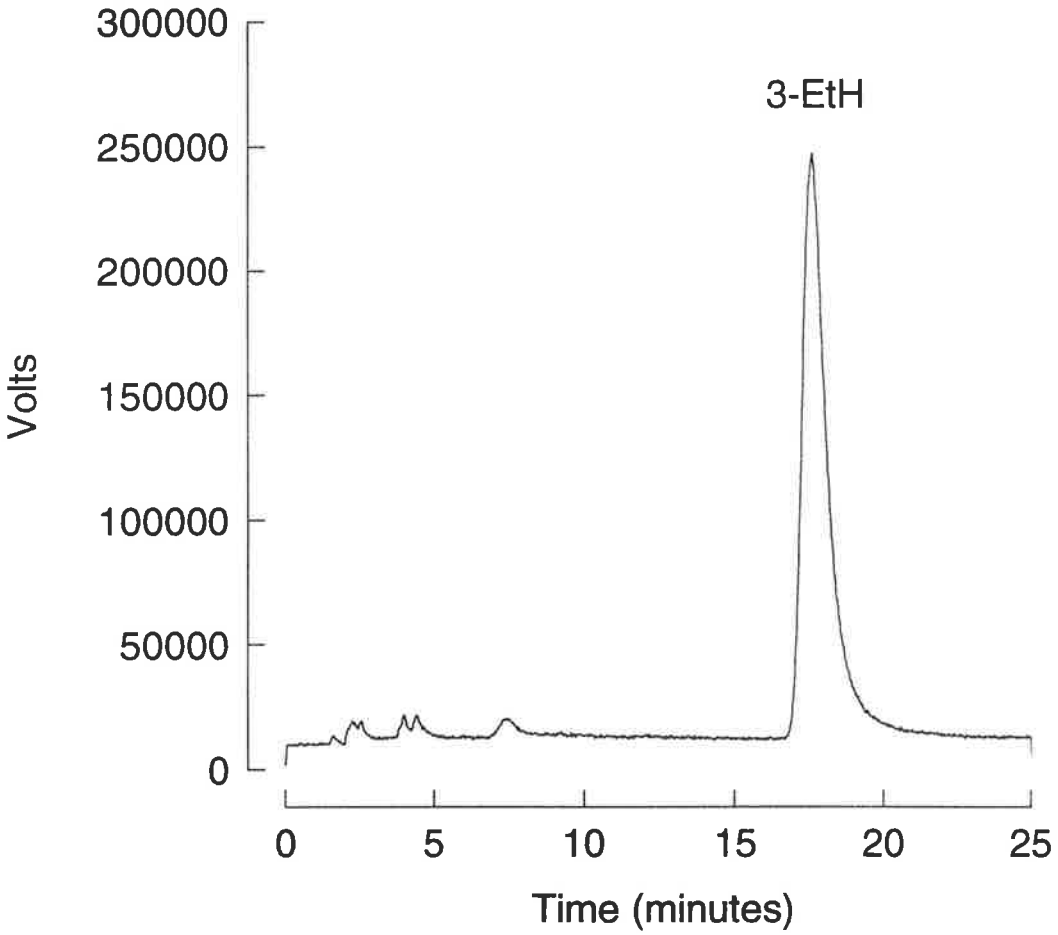


Fig. 2.2. HPLC chromatogram representing the internal standard 3-EtH.

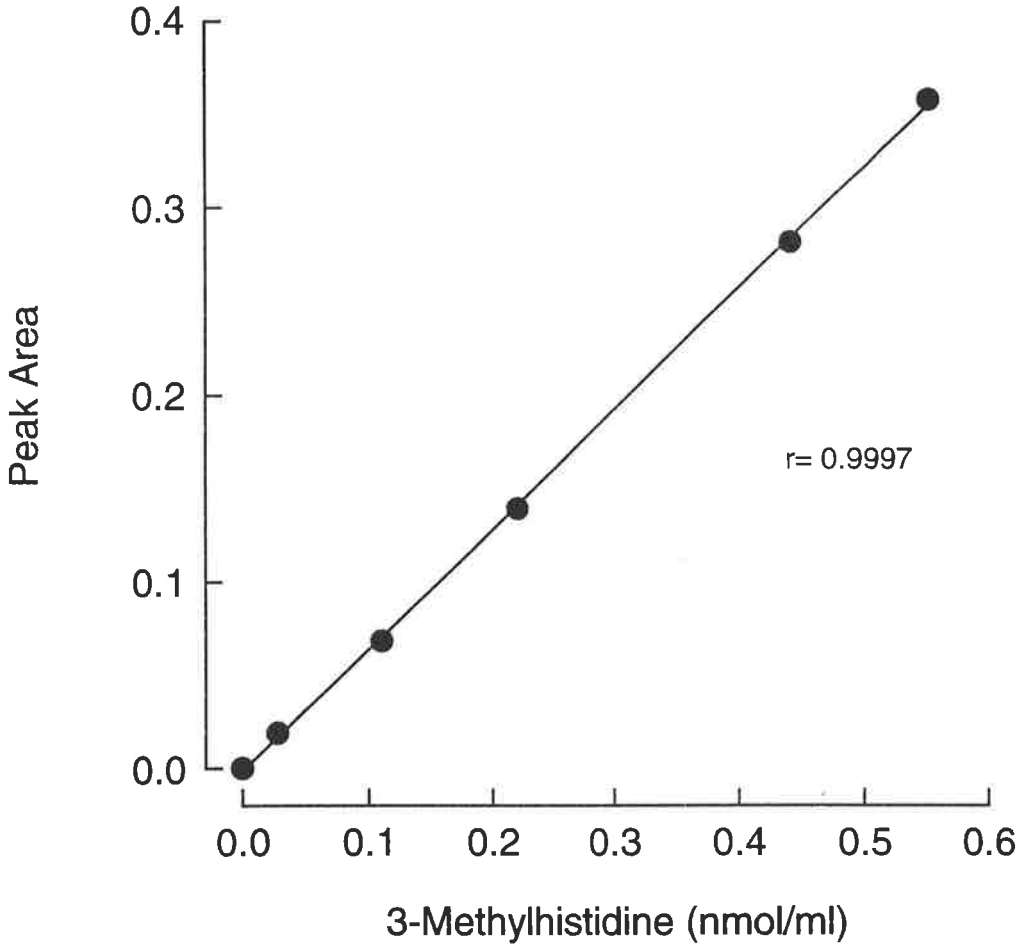


Fig. 2.3. Standard curve of 3-methylhistidine for 5 standards.

2.8.2 DNA Assay

Determinations of DNA were carried out as described by LaBarca & Paigen (1980).

Fifty µl aliquots of tissue homogenate, standard DNA (calf thymus extract, Sigma) and QC sample were mixed with 950 µl phosphate-saline buffer (pH 7.4) containing the fluorochrome H33258 (Calbiochem) at a final concentration of 1 µg/ml in 1.5 ml microfuge tubes. Samples were sonicated for 15 seconds after which they were centrifuged at 10,000 g in a microfuge for 5 minutes. Fluorescence was measured using a Hitachi fluorescence spectrophotometer (Tokyo, Japan). The excitation and emission wavelengths were 356 and 458 nm, respectively.

The standard curve was linear over the range 0.125 and 4.0 µg/ml (Fig. 2.4). The intra- and interassay coefficients of variation for 2.33 µg/ml of DNA in mesenteric vascular homogenate were 1.66% and 4.8%, respectively (representing 15 assays).

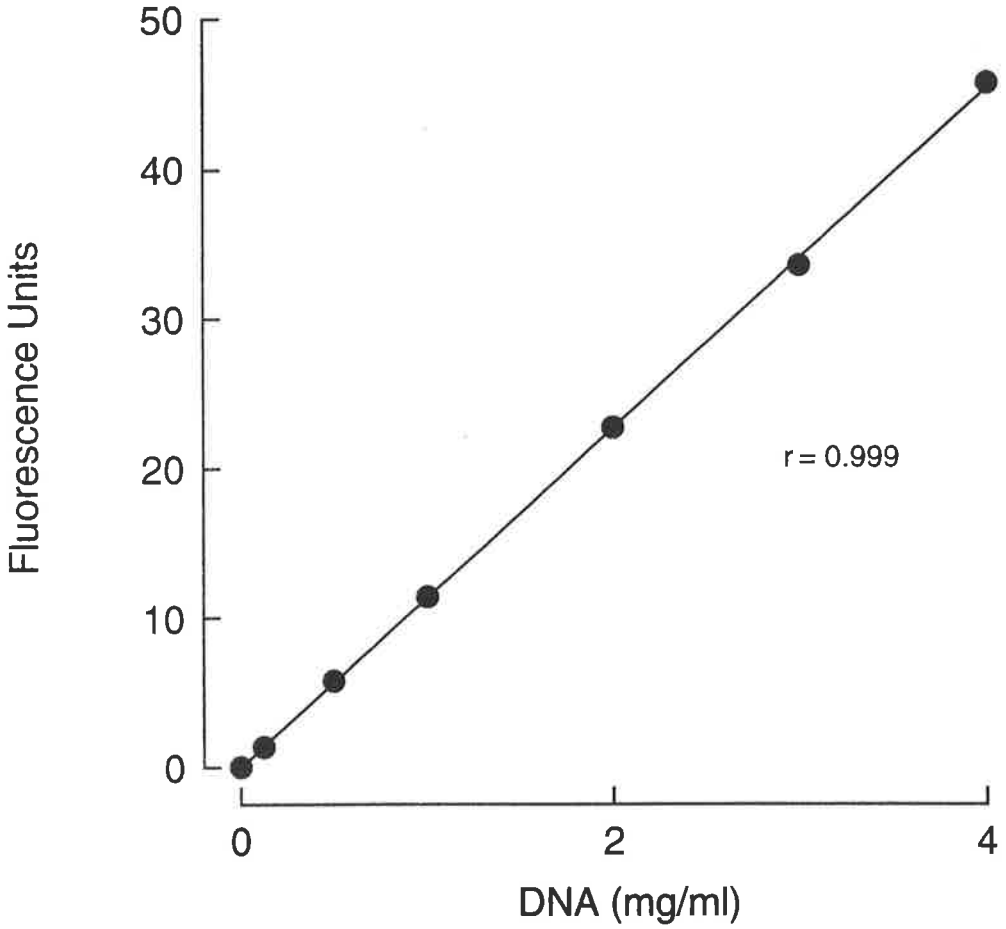


Fig. 2.4. Standard curve of DNA for 6 standards.

2.8.3 Protein Assay

The protein content of the tissue homogenates was measured using the spectrophotometric method of Lowry *et al* 1951.

To 50 µl tissue homogenate, standard (Bovine serum albumin (BSA), Sigma) and QC sample 50 µl 2M NaOH was added and then incubated in a water bath at 60°C for 2 hours. Milli-Q water (400µl) was added and 50 µl of the mixture was taken for the assay. To this aliquot 2.0 mls of the working reagent (alkaline tartrate and copper sulphate reagent) was added while vortexing and the solution allowed to stand for 10 minutes. Two hundred µl of dilute Folin-Ciocalteu's reagent were added while mixing and allowed to stand for 45 minutes after which absorbance was read on a spectrophotometer (Beckman, Du-65) using visible light at 750 nm.

The standard curve was linear over the range 2.2-13.3 µg/ml (Fig. 2.5). The intra and inter-assay coefficients of variation at 7.8 µg/ml of BSA were 2.0% and 4.7% respectively (representing 15 assays).

2.8.4 QC Samples

Mesenteric branches were dissected from 24 rats, pooled and homogenised as described above. Appropriate dilutions were made and sufficient aliquots were prepared so as to incorporate QCs with each individual 3-methylhistidine and DNA assay performed. For the protein assay, BSA was used as the source material for QCs.

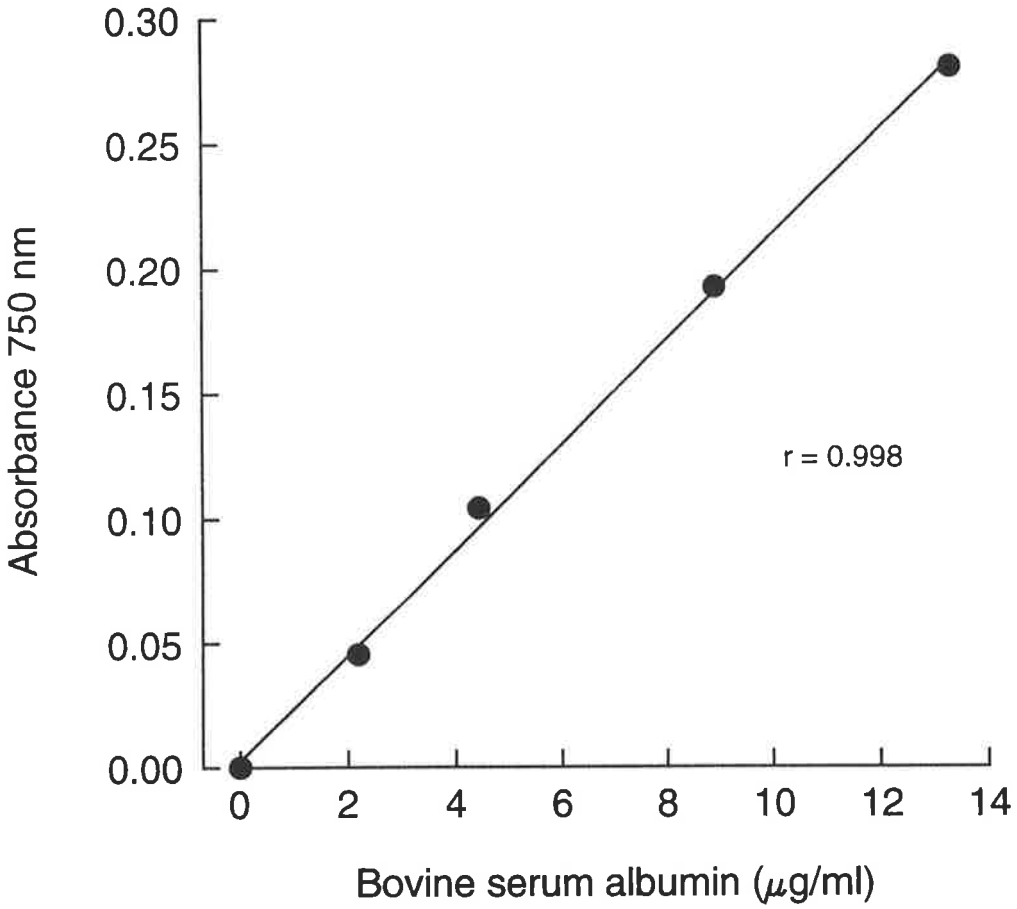


Fig. 2.5. Standard curve of bovine serum albumin for 4 standards.

2.9 Calculations and Statistical analysis

The sensitivity of individual aortic and perfused mesenteric preparations was determined by the dose required to produce 50% of the maximum response (EC_{50}) using the DResponse program (courtesy of G.A.Crabb, University of Adelaide). The responses of perfused mesenteric vascular bed preparations to certain agonists did not reach maximum. In such instances, if the slopes between groups did not differ, the concentration producing a selected increase in perfusion pressure was calculated using GraphPad Prism program (GraphPad Software, California, USA) in order to determine shifts in the concentration-response curves. These values are termed EC_{50mmHg}, $EC_{100mmHg}$ and $EC_{125mmHg}$. Mean logs were determined and statistical analyses were performed on the mean logs. The mean logs were converted to antilogs to provide the geometric mean EC with 95% confidence interval as described by Fleming *et al* 1972. Slopes of the concentration-response curves were determined by linear regression analysis of the linear portion of the log concentration-response curves.

Values were expressed as the means ± standard errors of the mean (s.e.m.) of the number of observations (n) within each control and treatment group. Statistical analysis of the data was performed using the GraphPad Instat program (GraphPad Software, California, USA). Bartlett's test for homogeneity of variances was performed followed by either a parametric or non-parametric test. All parametric data were subjected to a one-way analysis of variance (ANOVA) and where differences were demonstrated the Student-Newman-Keuls post test was performed. Comparisons were also made using the lowest significant differences (lsd) which were conducted by Angela Reid, the biostatistician at CSIRO, Division of

Human Nutrition, Adelaide, Australia. Nonparametric data were subjected to the Kruskal-Wallis test. Correlation analysis was tested for significance using the Pearson correlation test. A probability of $p<0.05$ was taken as an indication of significance.

CHAPTER 3

Vascular Reactivity, Contractile Proteins and Blood Pressure Development in the Hypertensive WKY Rat.

3.1 INTRODUCTION

A key characteristic of hypertension in spontaneously hypertensive rats (SHRs) is an increased peripheral resistance which has been postulated to be due to an altered vascular structure (Webb & Bohr, 1981). In turn, the altered vascular structure is believed to be associated with changes in the responsiveness of blood vessels. Induction of hypertension (which can be achieved using a variety of procedures including chronic mineralocorticoid treatment) has also been demonstrated to alter the structure and function of the vasculature which in turn contribute to an elevated peripheral resistance and thus to the development and/or the maintenance of hypertension (Finch & Haeusler, 1974). However, in the majority of studies, hypertension has been induced in normotensive rat strains such as the Sprague Dawley and Wistar with very few studies using the normotensive control for SHRs, the Wistar-Kyoto (WKY) rat. Thus, it is not known with certainty whether the WKY rat, if made hypertensive, will exhibit similar characteristics as the SHR with regard to vascular structure and contractility.

The present study was designed to : (1) further investigate vascular reactivity in the aorta (conduit vessel) and mesenteric resistance vessels of WKY rats made hypertensive using chronic mineralocorticoid treatment (2) measure contractile protein levels in these vessels in order to determine the relationship which exists between high blood pressure, contractility and contractile protein and (3) determine what influence three different classes of antihypertensive agents would have on blood pressure, contractility and contractile protein levels in the aorta and mesenteric vascular bed. Furthermore, by inducing hypertension in the genetic normotensive control for the SHR it was hoped to trigger and reveal mechanisms

for elevating blood pressure which are linked causally to hypertension in the SHR and would therefore be absent when using normotensive strains with different genetic backgrounds.

3.2 METHODS

3.2.1 Animal and drug treatments

Five week old male WKY rats were maintained as described previously (Chapter 2). Animals were allowed to acclimatise to the holding facility, after which control levels for body weights and systolic blood pressure were established. The rats were randomly divided into two groups. One group of animals underwent left nephrectomy under pentobarbitone sodium (60 mg/ml):methohexitone sodium (10mg/ml) (1:9) anaesthesia (5ml/Kg body weight). The control animals had left flank incisions, but nephrectomy was not performed (sham operated controls) (see Chapter 2 for details). One week after surgery the nephrectomised rats were assigned to either untreated, quinapril HCl (ACE inhibitor) (10 mg/Kg/day), doxazosin mesylate (α_1-adrenoceptor antagonist) (10 mg/Kg/day) or diltiazem HCl (calcium channel blocker) (30 mg/Kg/day) groups. They received 20 mg/Kg DOCA dissolved in sesame oil s.c. twice weekly and had free access to 0.9% saline drinking solution. The antihypertensive agents were dissolved in the 0.9% sodium chloride solution. Control animals were injected s.c. with sesame oil and were maintained on normal drinking water. For simplicity, DOCA-salt groups will be referred to as hypertensive WKY (H-WKY).

All animals were subjected to weekly measurements of weight. Systolic blood

pressures using an indirect tail-cuff method (Chapter 2) were measured weekly up to 4 weeks of treatment and thereafter at 6 weeks of treatment. After 6 weeks of treatment the animals were killed by decapitation . The kidney, heart, thoracic aorta and vas deferens were removed. With respect to the heart, the left and right ventricles were separated, the atria were cut away and the left ventricle weighed separately.

3.2.2 Aortic ring preparations

The thoracic aortic rings were prepared as previously described (Chapter 2) Cumulative concentration-response curves to noradrenaline (0.12 - 8000 nM) and acetylcholine (1 - 10000 nM) were performed.

3.2.3 Perfused mesenteric vascular bed

The perfused mesenteric vascular bed was prepared as previously described (Chapter 2). Frequency-response curves (2-64 Hz) and noncumulative dose-response curves to noradrenaline (0.01-20.0 μg), phenylephrine(0.1-10.0 μg) and arginine vasopressin ($9x10^{-12}$ - $9x10^{-8}$ M) were performed as described previously (Chapter 2).

At the end of the protocol, the preparation was transferred to a petri dish containing saline (which was kept on ice) where the mesenteric bed was dissected from the intestinal tract and the adhering adipose tissue and the vein were carefully cleared away. The mesenteric tree was dissected into the superior mesenteric artery and its resistance vessels. The tissues were blotted, weighed, wrapped in

aluminium foil, snap frozen in liquid nitrogen and stored at -80°C until required.

3.3.4 Biochemical analyses

3-Methylhistidine, DNA and protein assays were performed as described previously in Chapter 2. Data are expressed as normalised per mg of protein and absolute amounts. (Data expressed per mg of wet tissue weight are presented in Appendix II).

3.3 RESULTS

3.3.1 Development of DOCA-salt hypertension

One week after nephrectomy and administration of DOCA-salt, there was a significant increase in systolic blood pressure in the DOCA treated group when compared with the normotensive controls (Fig. 3.1). This rise in systolic blood pressure became progressively greater with time and DOCA-salt treatment. In general, none of the antihypertensive treatments significantly prevented the increase in blood pressure (Fig. 3.1). Right kidney to body weight and left ventricular weight to body weight ratios for H-WKY rats increased when compared to ratios of normotensive WKY rats (Fig. 3.2).

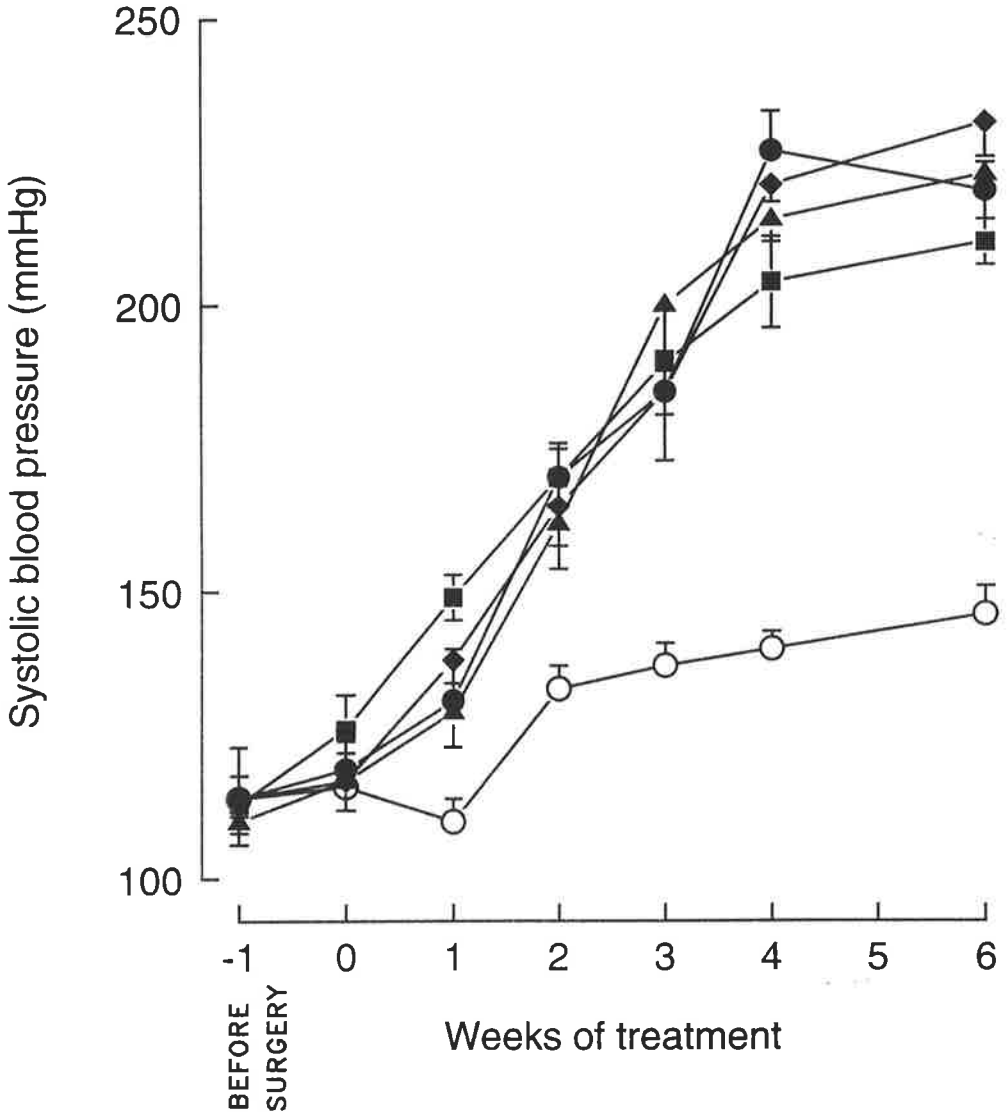


Fig. 3.1. Systolic blood pressure of ○ normotensive WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diltiazem. Each point represents the mean value for 7 to 9 rats ± s.e.m.
Systolic blood pressure of normotensive WKY rats was significantly different from all H-WKY treated with quinapril or diltiazem at week 1 of treatment and from all H-WKY groups at all time points from 2 weeks of DOCA-salt treatment ($p<0.05$).

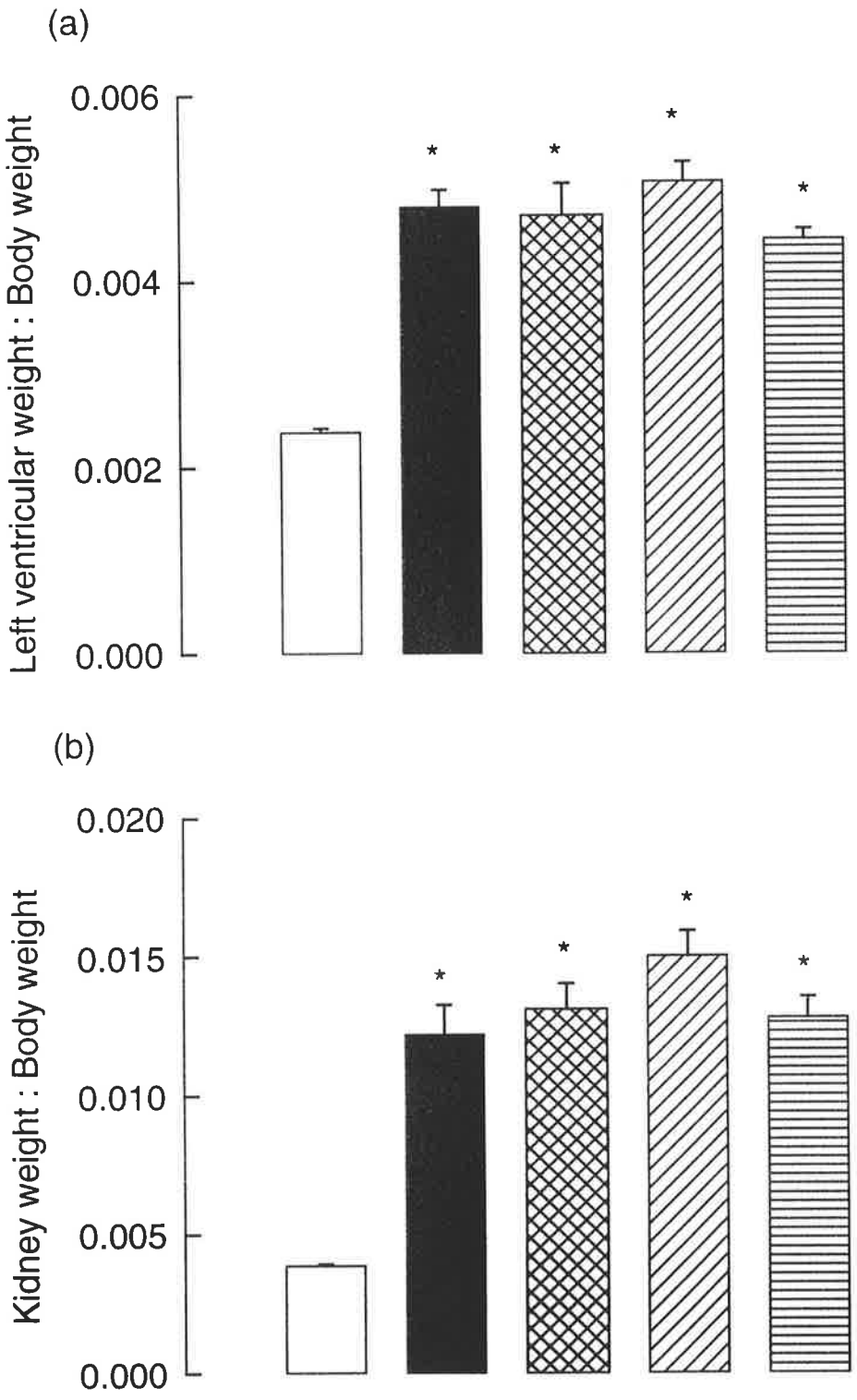


Fig. 3.2. Bar graphs show (a) left ventricular weight: body weight ratio and (b) right kidney weight : body weight ratio for ☐ normotensive WKY rats ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Each bar represents the mean value for 7 to 10 rats ± s.e.m.

* $p<0.001$ when compared with normotensive WKY rats.

3.3.2 Vascular reactivity

3.3.2.1 Aortic ring preparations

Maximal responses elicited by noradrenaline in the thoracic aorta were significantly smaller in aortae from H-WKY rats compared to aortae from normotensive WKY rats (Fig. 3.3). Furthermore, aortae taken from H-WKY rats treated with quinapril, doxazosin or diltiazem also produced maximal responses which were smaller than those of the normotensive control rats but were not significantly different from their respective H-WKY control group (Fig. 3.3). Responses in aortic rings from the four groups of H-WKY rats were significantly more sensitive (lower EC_{50}) to noradrenaline than those from normotensive control rats (Table 3.1).

The vasodilator effect of acetylcholine was reduced in the aortae of all of the H-WKY (ie both untreated and treated) (Fig. 3.4).

3.3.2.2 Perfused mesenteric vascular bed

Responses obtained to periarterial nerve stimulation were significantly lower (at 32 and 64 Hz) in preparations from H-WKY rats compared with preparations from normotensive WKY (Fig. 3.5). Preparations taken from H-WKY rats treated with doxazosin produced responses that were significantly smaller than those seen in any of the other H-WKY groups (Fig. 3.5). Responses to noradrenaline were not significantly different between untreated H-WKY and control rats (Fig. 3.6).

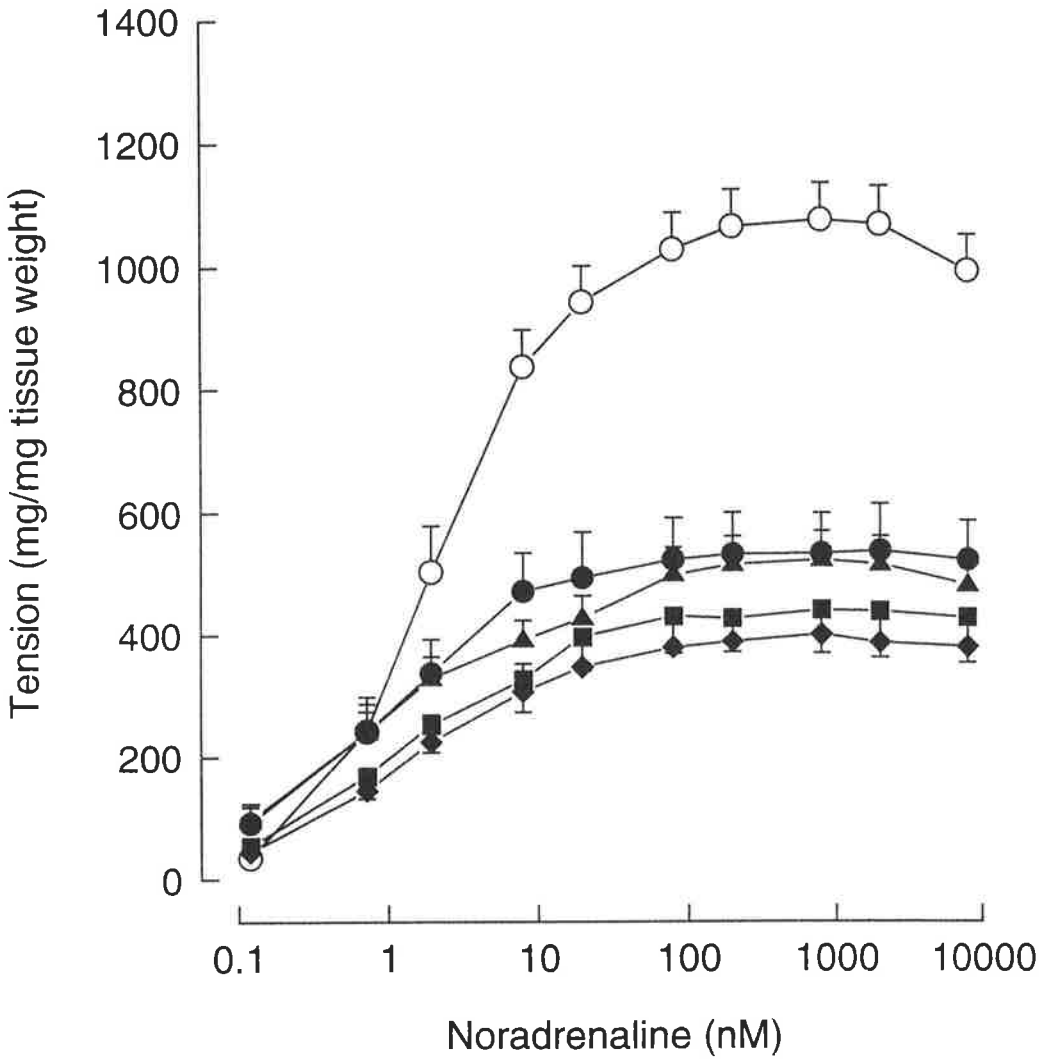


Fig. 3.3. Concentration-response curves for noradrenaline in aortic rings from ○ normotensive WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY treated with diltiazem. Each point represents the mean value for 7 to 10 rats ± s.e.m. Responses from normotensive WKY rats were significantly different from H-WKY rats treated with quinapril or diltiazem at the noradrenaline concentration 1.92 nM ($p<0.05$) and significantly different from all H-WKY groups at noradrenaline concentrations of 7.92 nM and greater ($p<0.001$).

Table 3.1. Sensitivity to noradrenaline of aortic rings from normotensive WKY and untreated and treated H-WKY rats.

	Groups				
	Normotensive WKY (n=10)	H-WKY Control (n=7)	H-WKY quinapril treated (n=7)	H-WKY doxazosin treated (n=7)	H-WKY diltiazem treated (n=6)
EC_{50} (nM ± s.e.m.)	2.52 ± 0.43	1.33 ± 0.37*	1.15 ± 0.22*	1.08 ± 0.18*	1.40 ± 0.28*

* $p<0.05$ compared with normotensive WKY rats.

Table 3.2. Basal perfusion pressure and sensitivity of perfused mesenteric vasculature from normotensive WKY and untreated and treated H-WKY rats.

Groups	Basal perfusion pressure (mmHg ± s.e.m.)	Arginine Vasopressin (nM)	
		$EC_{100\ mmHg}$ (95% CI)	$EC_{50\ mmHg}$ (95% CI)
Normotensive WKY (n=8-9)	51.16 ± 3.77	7.15 (2.91-17.6)	1.51 (0.86-2.64)
H-WKY control (n=5-7)	62.43 ± 10.53	11.1 (3.67-33.3)	3.70 (2.22-6.15)
H-WKY quinapril treated (n=5-7)	57.79 ± 7.87	3.90 (1.82-8.36)	1.25 (0.58-2.68)
H-WKY diltiazem treated (n=4-6)	66.17 ± 11.57	12.6 (1.38-115)	4.67 (1.13-19.2)
H-WKY doxazosin treated (n=7-9)	57.72 ± 5.91	9.73 (3.95-24.0)	2.17 (1.29-3.62)

$EC_{100\ mmHg}$ = dose producing a 100 mmHg increase in perfusion pressure;

$EC_{50\ mmHg}$ = dose producing a 50 mmHg increase in perfusion pressure; CI = confidence interval.

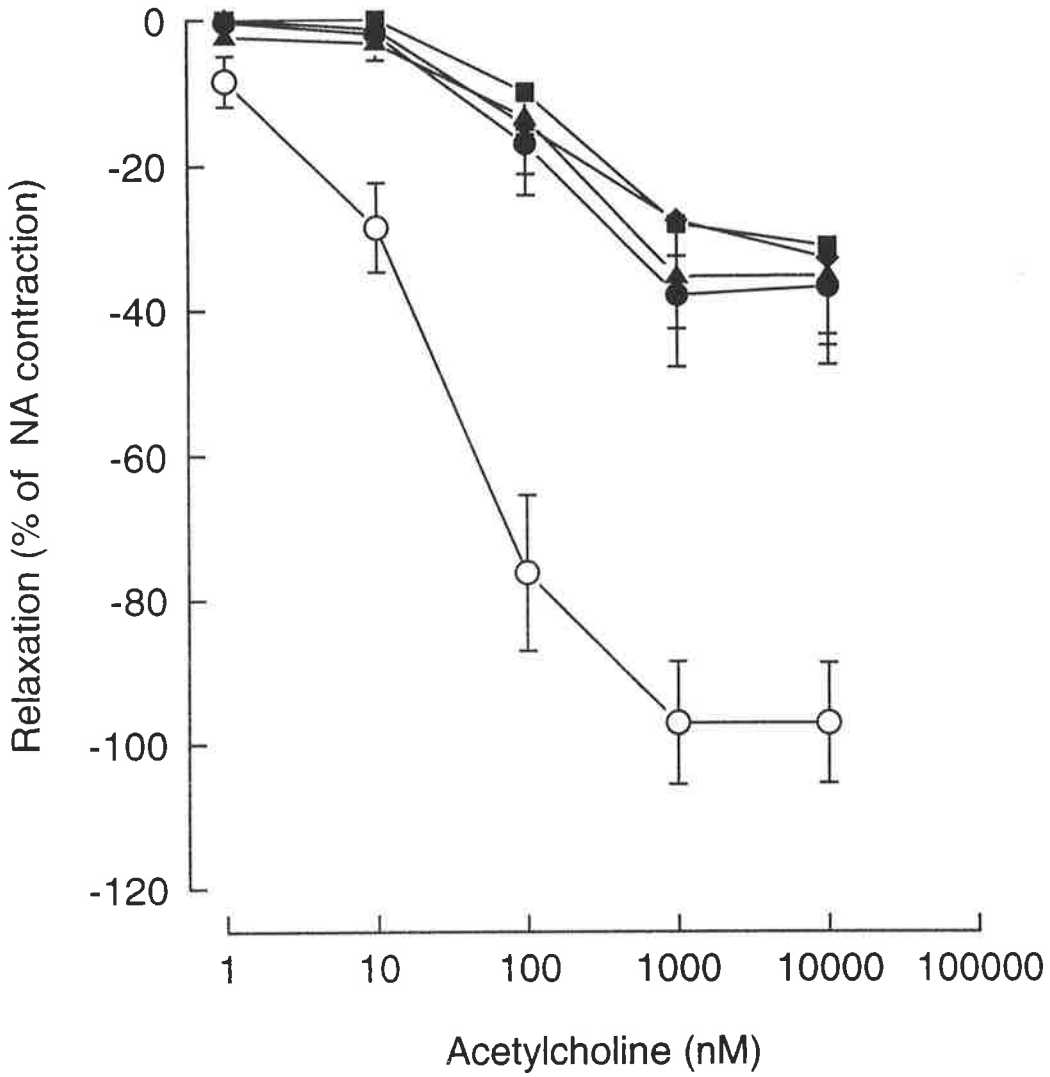


Fig. 3.4. Concentration-response curves to acetylcholine in aortic ring preparations from ○ normotensive WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diltiazem. Each point represents the mean value for 4 to 10 rats ± s.e.m. All H-WKY groups were significantly different from normotensive WKYrats at acetylcholine concentrations 10nM and greater ($p<0.01$).

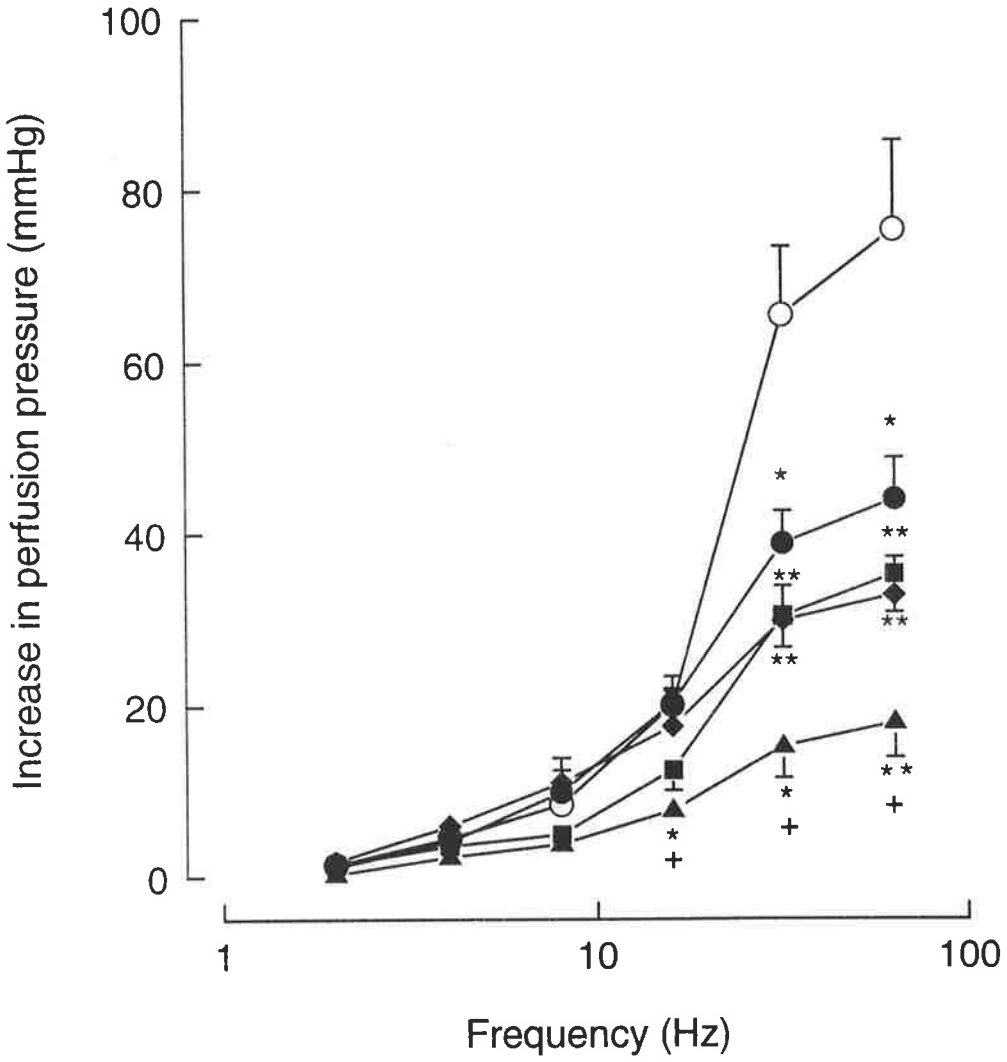


Fig. 3.5. Frequency-response curves in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diltiazem. Each point represents the mean value for 6 to 9 rats ± s.e.m.

* $p<0.01$, ** $p<0.001$ compared with normotensive WKY.

+ $p<0.05$ compared H-WKY control rats.

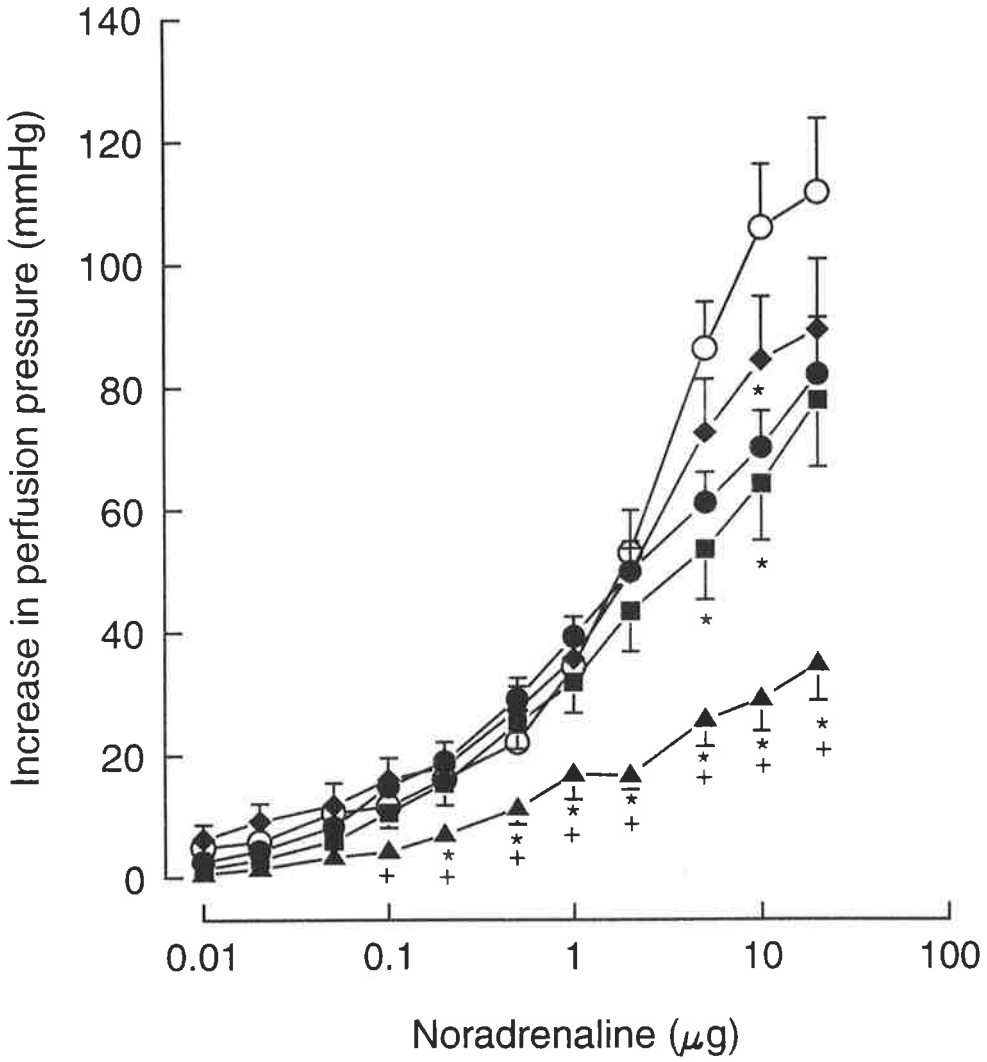


Fig. 3.6. Concentration-response curves to noradrenaline in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diltiazem. Each point represents the mean value for 6 to 9 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p<0.05$ compared with H-WKY control rats.

However, at the higher concentrations of noradrenaline, although not significant, the responses appeared to be attenuated in preparations from untreated H-WKY when compared with responses in preparations from normotensive WKY rats (Fig. 3.6). A similar trend was also seen in preparations from H-WKY rats treated with quinapril and to a lesser degree in preparations from H-WKY rats treated with diltiazem (Fig. 3.6).

Preparations from H-WKY rats treated with doxazosin not only produced responses significantly lower than the normotensive group but also lower than the other H-WKY groups (Fig. 3.6). Responses to phenylephrine in preparations from untreated and quinapril and diltiazem treated H-WKY rats were not significantly different from responses in preparations from normotensive rats (Fig. 3.7). However, preparations from doxazosin treated H-WKY rats displayed lower responses to phenylephrine by comparison with the preparations from the normotensive animals, although significance was only reached at 10 μg (Fig. 3.7). Dose-response curves for arginine vasopressin were not significantly different between any of the treatment groups (Fig. 3.8).

The sensitivity of preparations from H-WKY groups to vasopressin, as indicated by the geometric means of doses producing an increase in perfusion pressure of 50 and 100 mmHg, were not significantly different from preparations from normotensive WKY rats (Table 3.2).

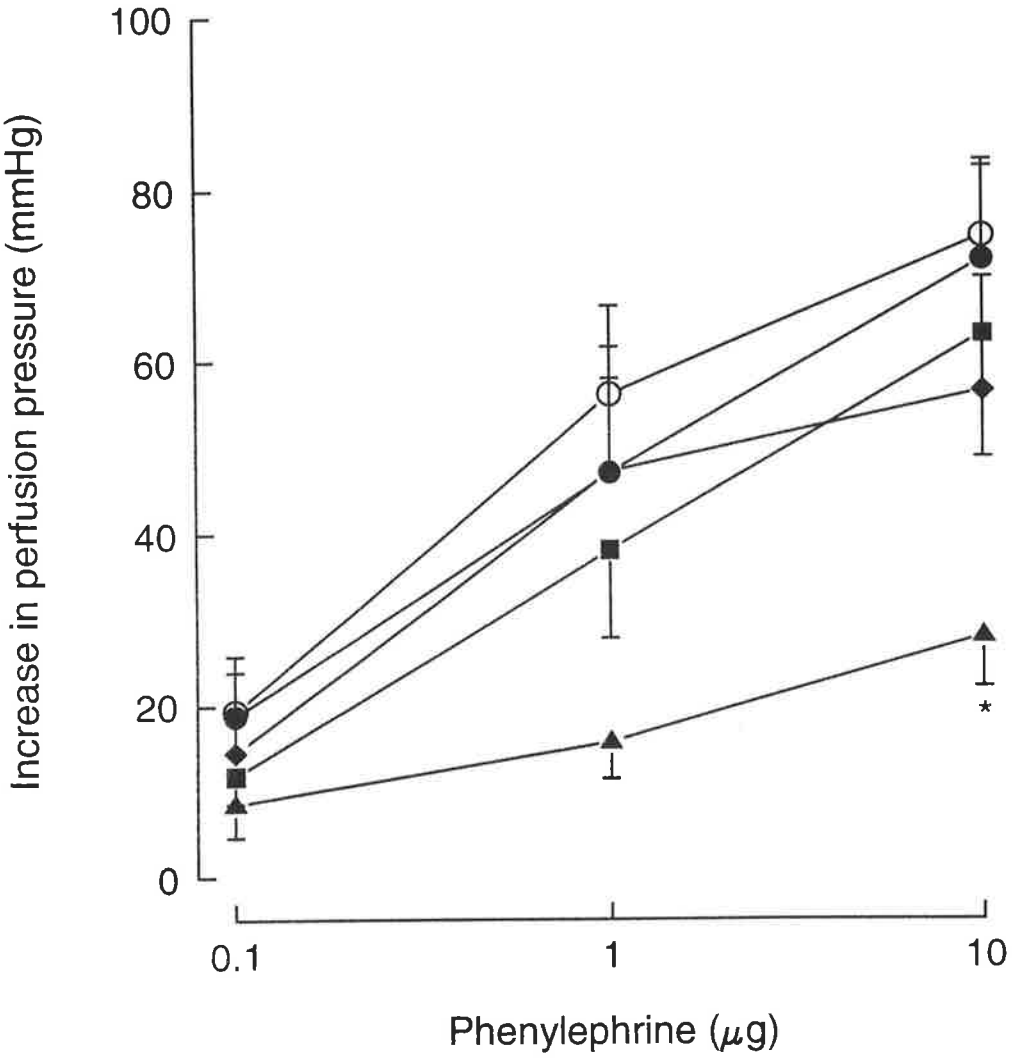


Fig. 3.7. Concentration-response curves to phenylephrine in the isolated perfused mesenteric vasculature from ○ normotensive WKY control rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diiltiazem. Each point represents the mean value for 6 to 9 rats ± s.e.m.
* $p<0.05$ compared with normotensive WKY and H-WKY rats.

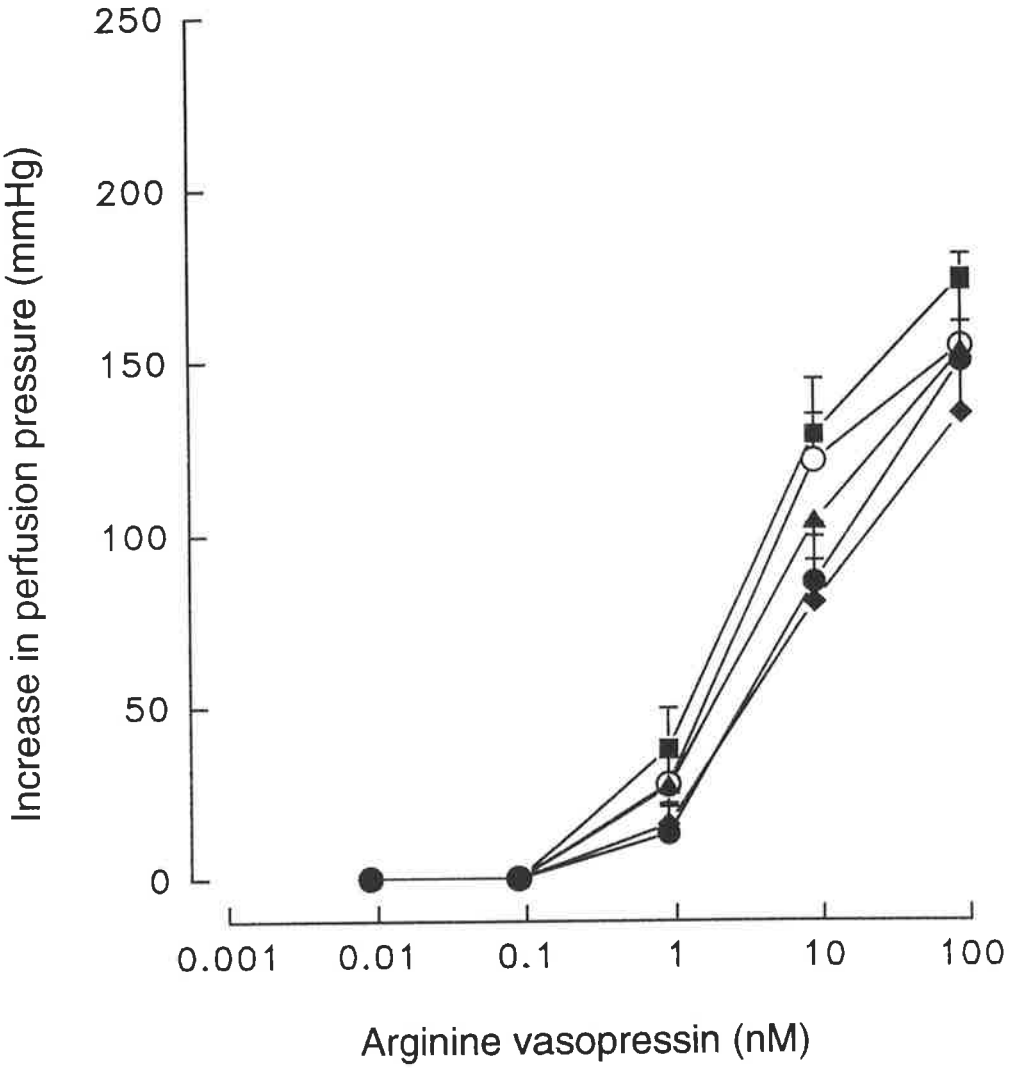


Fig. 3.8. Concentration-response curves to arginine vasopressin in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● H-WKY rats, ● H-WKY control rats, ■ H-WKY rats treated with quinapril, ▲ H-WKY rats treated with doxazosin and ◆ H-WKY rats treated with diltiazem. Each point represents the mean value for 6 to 9 rats ± s.e.m.

3.3.3 Biochemical parameters

3.3.3.1 3-Methylhistidine content

The level of 3-methylhistidine in the aorta from treatment and control groups (expressed per mg of protein) is shown in Fig. 3.9. There was no significant difference in the level of 3-methylhistidine in the aorta from H-WKY untreated rats when compared with normotensive control rats. In general, 3-methylhistidine values in the aorta were not influenced by the antihypertensive drug therapies (Fig. 3.9). Fig. 3.10(a) shows the level of 3-methylhistidine when expressed per mg of protein in the superior mesenteric artery where it can be seen that there were no differences in 3-methylhistidine levels in this tissue between all groups. Absolute values of 3-methylhistidine levels (ie. independent of wet tissue weight and protein content) were significantly increased in tissues from untreated, quinapril and diltiazem treated H-WKY rats when compared with tissues from normotensive WKY rats (Fig. 3.10 (b)). In contrast, chronic treatment of H-WKY rats with doxazosin caused a decrease in the absolute 3-methylhistidine content of the superior mesenteric artery by comparison with levels in tissues from H-WKY controls. Absolute 3-methylhistidine levels were not significantly different between doxazosin treated H-WKY and normotensive control rats (Fig. 3.10(b)).

The levels of 3-methylhistidine per mg of protein in branches of the mesenteric artery are shown in Fig. 3.11(a). A decrease in the levels of 3-methylhistidine was obtained in all H-WKY groups when compared with the content in mesenteric branches from normotensive control rats. In contrast to the decrease obtained when expressing the levels of 3-methylhistidine per mg of protein, the absolute levels of 3-methylhistidine in the mesenteric branches from H-WKY groups

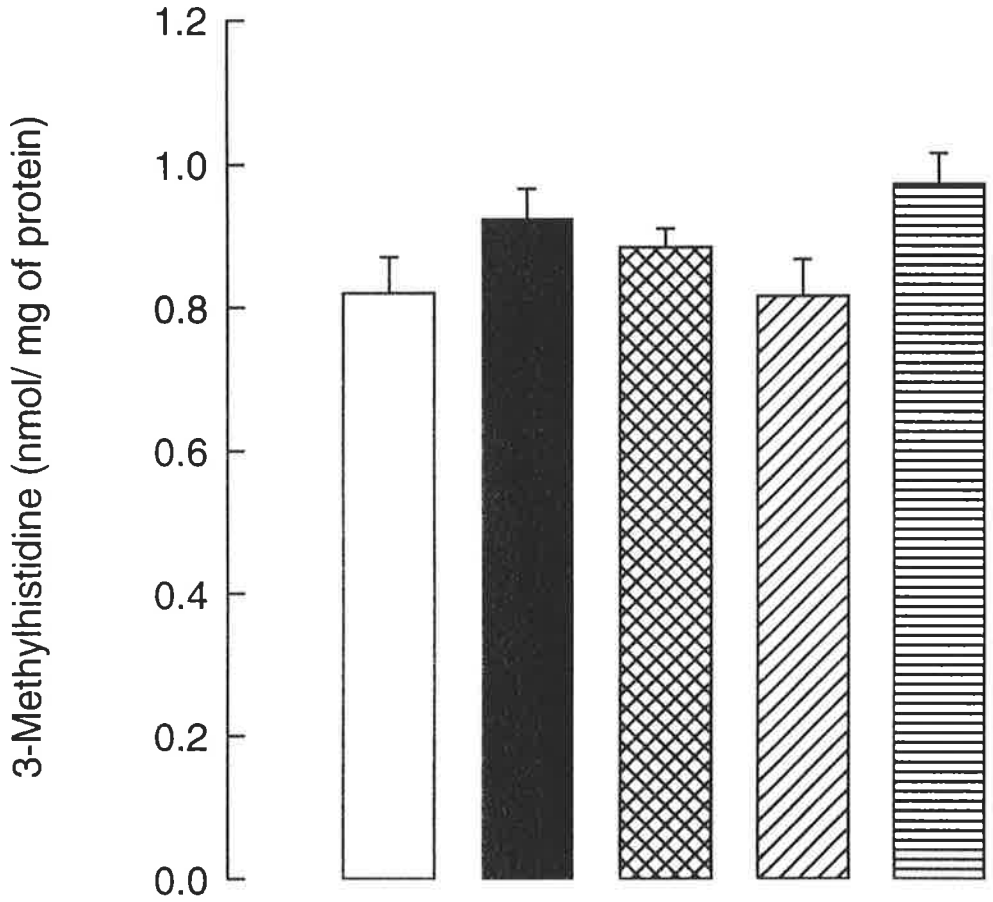


Fig. 3.9. Aortic 3-methylhistidine levels from ▭ normotensive WKY rats, ▬ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Each bar represents the mean value for 6 rats ± s.e.m.

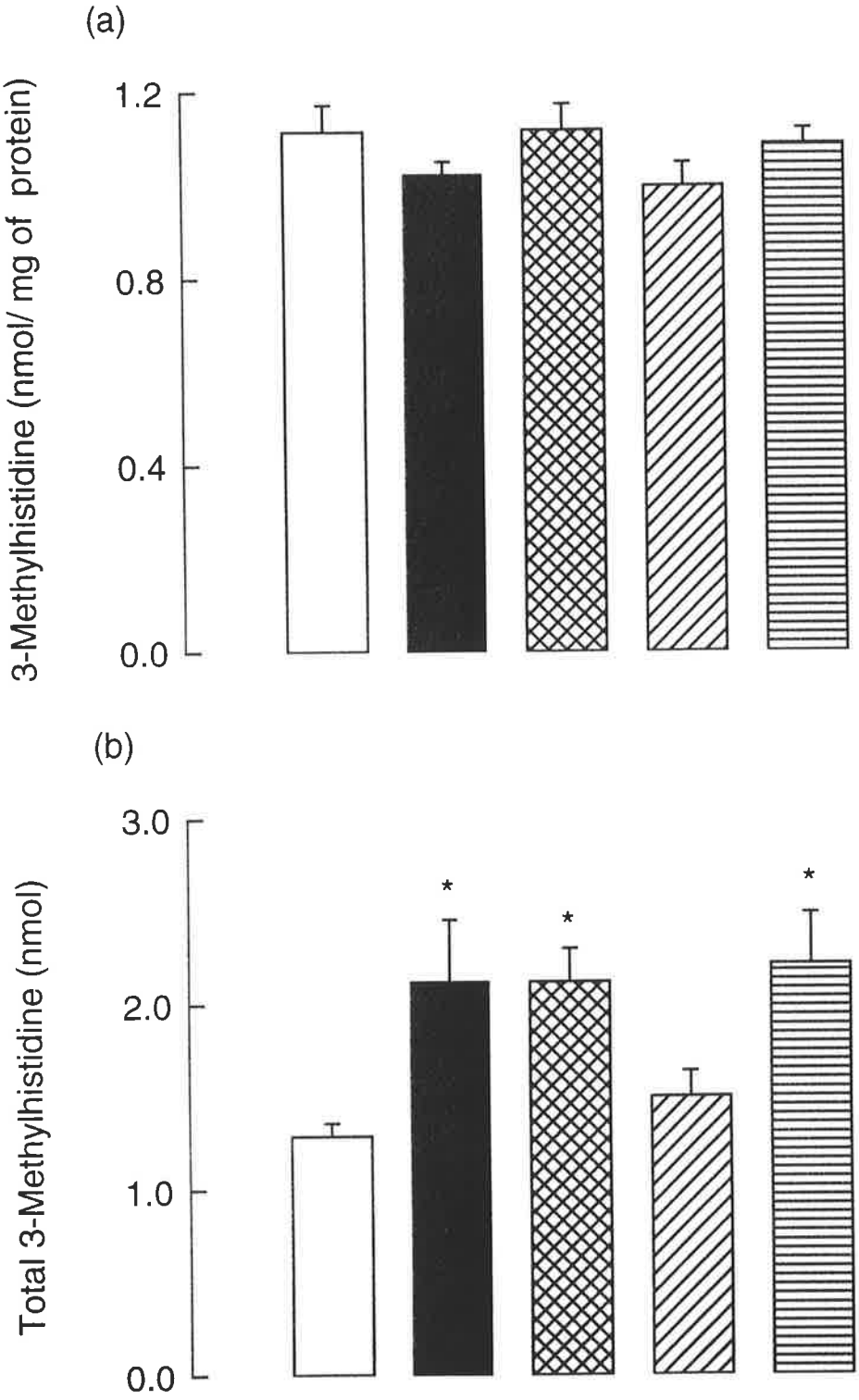


Fig. 3.10. 3-methylhistidine levels found in the superior mesenteric artery from normotensive WKY rats, H-WKY control rats, H-WKY rats treated with quinapril, H-WKY rats treated with doxazosin and H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean for 5 to 6 rats ± s.e.m.

* $p<0.02$ compared with normotensive WKY rats.

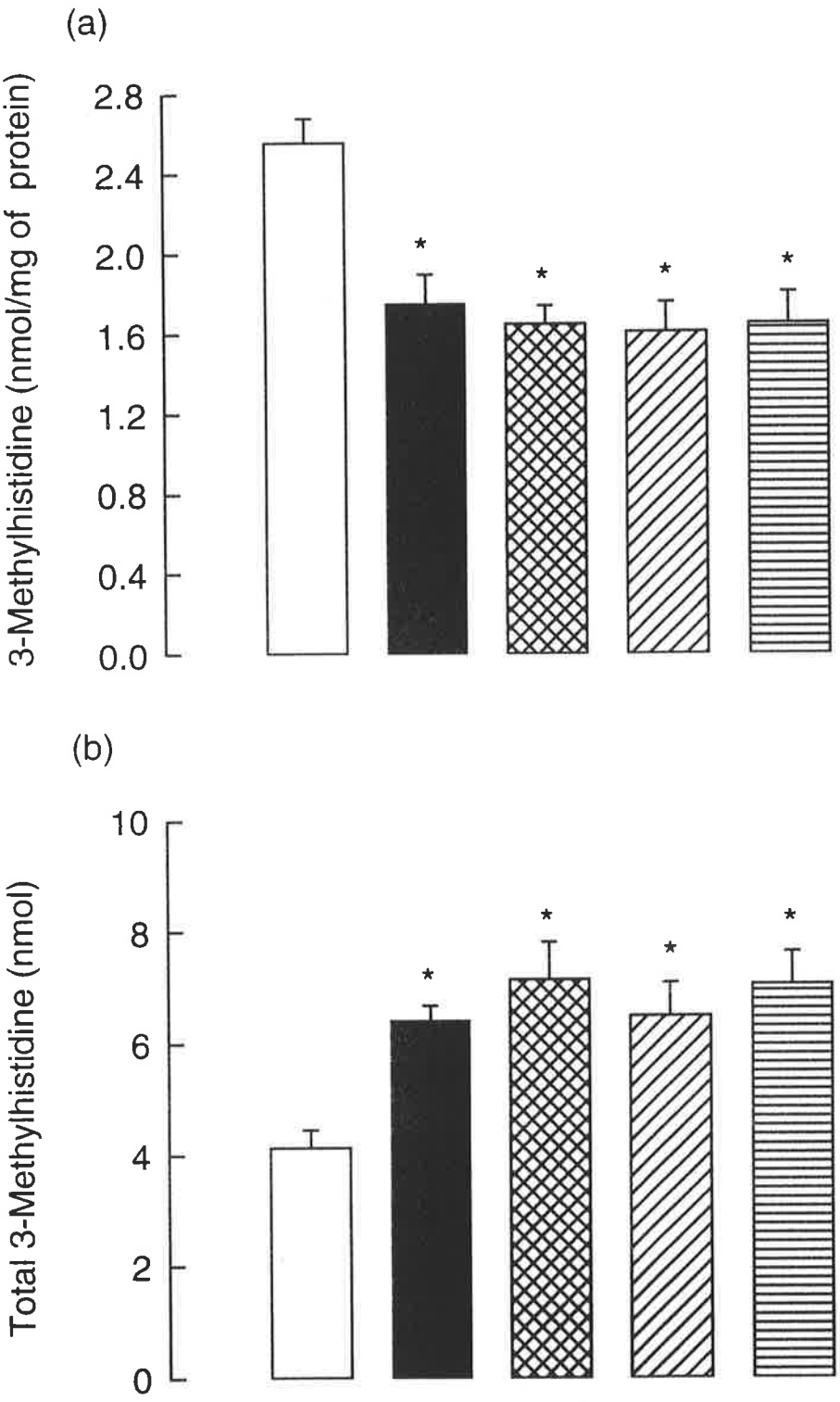


Fig. 3.11. 3-methylhistidine levels found in mesenteric branches from ☐ normotensive WKY rats, ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value for 5 to 6 rats ± s.e.m.

* $p<0.001$ compared with normotensive WKY rats.

were greater than those seen in tissues from normotensive WKY rats (Fig. 3.11(b)).

The vas deferens (a nonvascular tissue) was used to determine whether the effects of hypertension and the antihypertensive agents were specific for vascular tissue.Fig. 3.12(a) shows that there were no significant differences in the levels of 3-methylhistidine (when normalised to protein) in the vas deferens between the H-WKY groups and the normotensive control rats. In contrast, absolute levels of 3-methylhistidine in the vas deferens from H-WKY groups were significantly lower than those seen in the vas deferens from normotensive rats (Fig. 3.12(b)). Although not significantly different the level of 3-methylhistidine in the vas deferens from H-WKY rats treated with doxazosin appeared to be lower than the levels seen in tissues from untreated H-WKY rats (Fig. 3.12(b)).

3.3.3.2 DNA content

Fig. 3.13 shows DNA levels in the aorta. No significant difference was seen between tissues from treatment and control groups.

The DNA content in the superior mesenteric artery was not significantly different between treatment and control groups when expressed per mg of protein (Fig. 3.14(a)). However, absolute levels of DNA in this tissue from H-WKY rats which underwent antihypertensive drug therapy and control H-WKY rats were significantly greater than in normotensive control rats (Fig. 3.14(b)).

In the mesenteric branches, normalised and absolute levels of DNA were increased in all H-WKY groups by comparison with the control group (Fig. 3.15(a) & (b)).

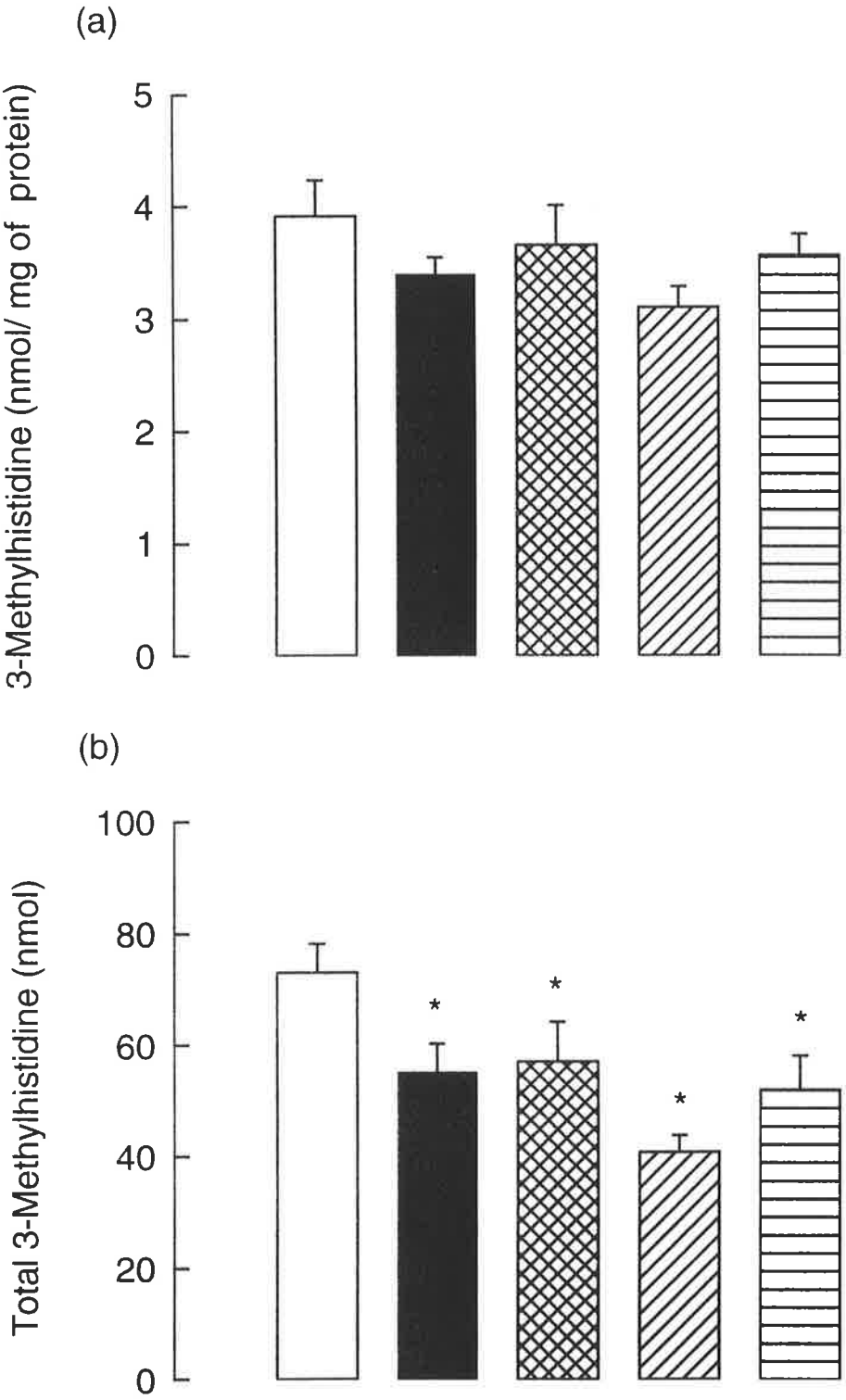


Fig. 3.12. 3-methylhistidine levels found in the vas deferens from normotensive WKY rats, H-WKY control rats, H-WKY rats treated with quinapril, H-WKY rats treated with doxazosin and H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean for 5 to 6 rats ± s.e.m.

* $p<0.01$ compared with normotensive WKY rats.

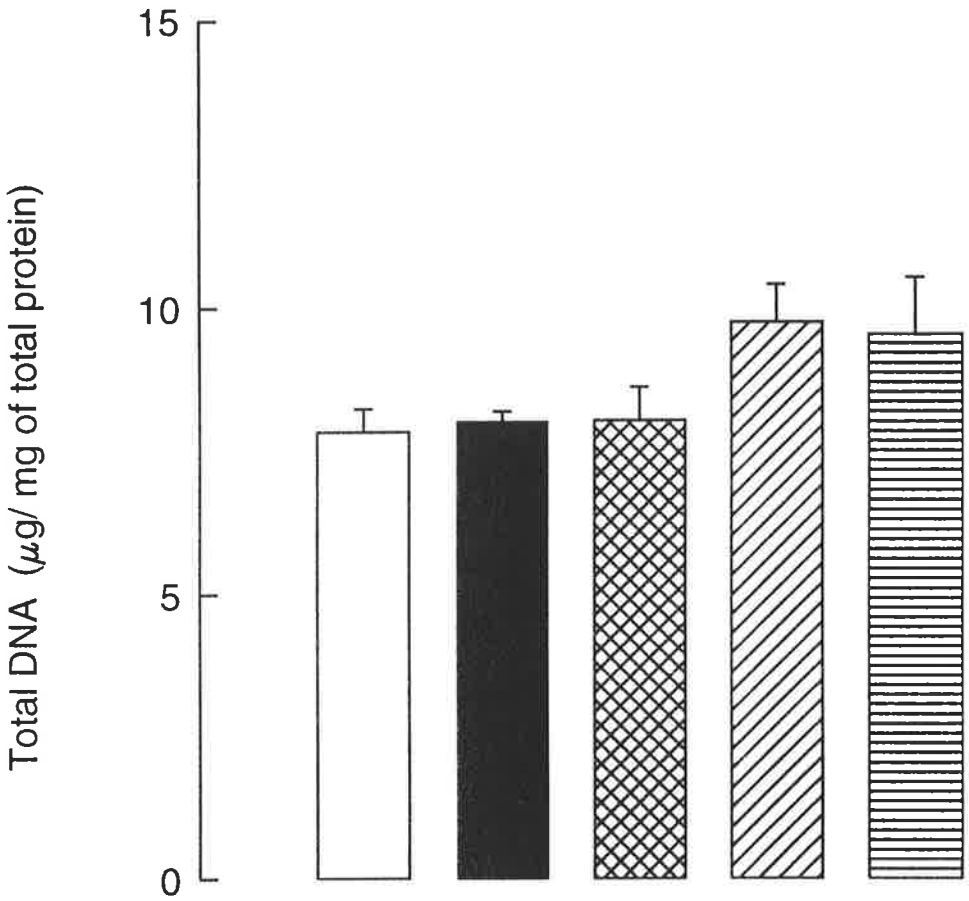


Fig. 3.13. Aortic DNA levels from ☐ normotensive WKY rats, ■ H-WKY control rats, ▨ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Each bar represents the mean value for 5 to 6 rats ± s.e.m.

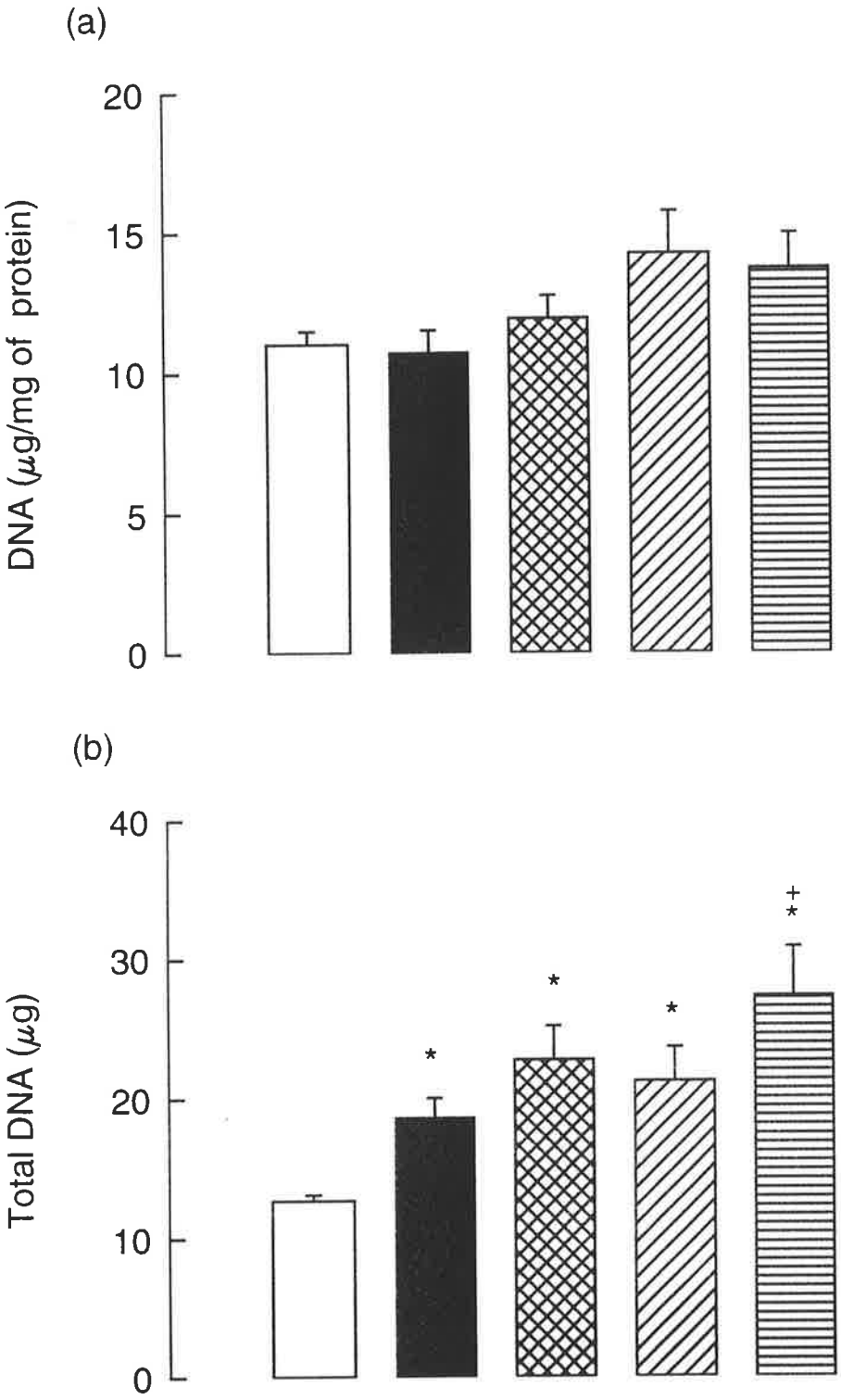


Fig. 3.14. DNA levels found in the superior mesenteric artery from ☐ normotensive WKY rats, ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean 5 to 6 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p< 0.05$ compared with H- WKY control rats.

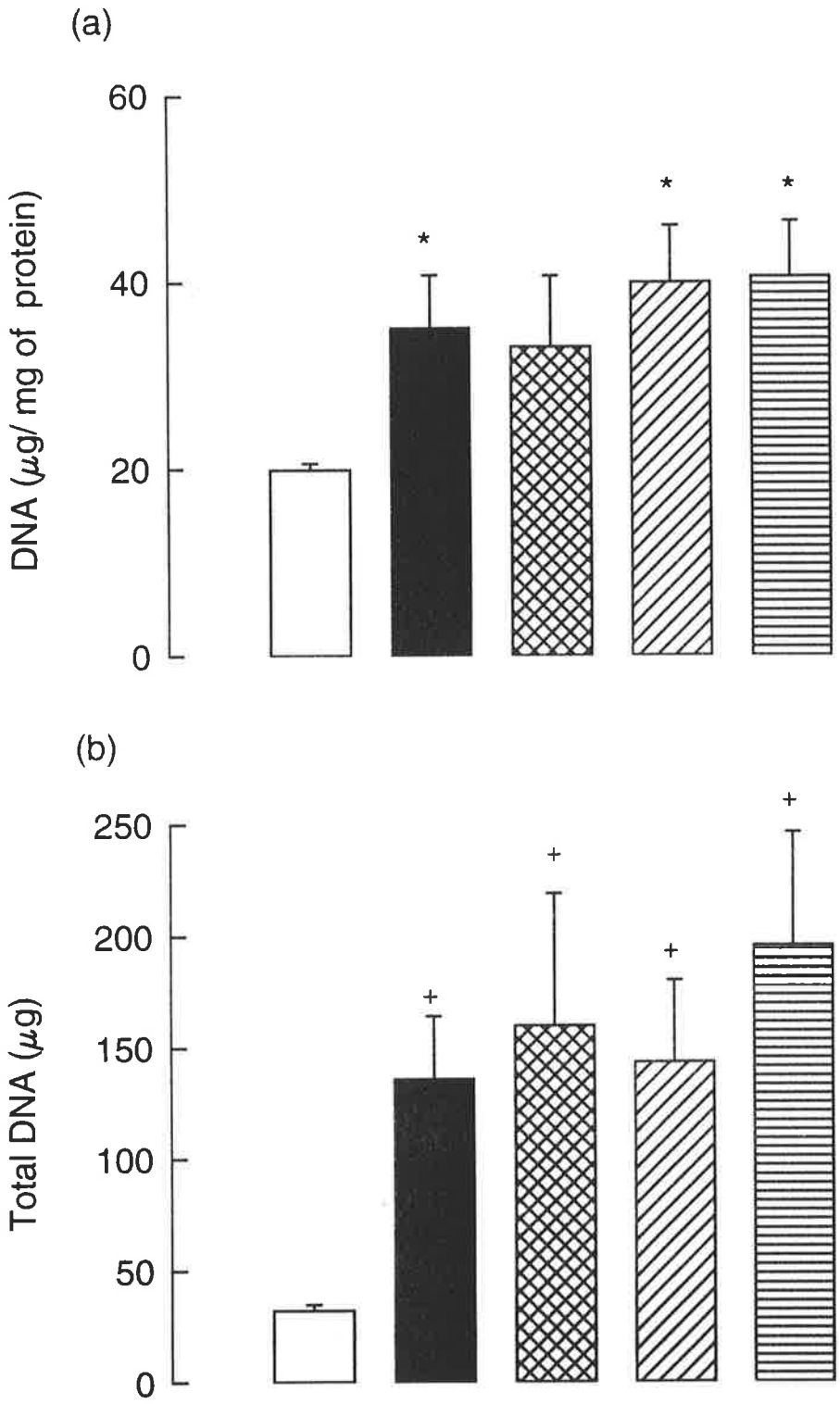


Fig. 3.15. DNA levels found in mesenteric branches from ☐ normotensive WKY rats, ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean 5 to 6 rats ± s.e.m.

* $p<0.001$ compared with normotensive WKY rats.

+ $p< 0.001$ of log values compared with normotensive WKYrats.

Fig. 3.16(a) & (b) shows DNA levels in the vas deferens. There was a significant increase in the DNA level when expressed per mg of protein in the vas deferens from all H-WKY groups when compared to levels found in tissues from normotensive rats (Fig. 3.16(a)). In addition, the DNA levels in the vas deferens from H-WKY rats treated with doxazosin were also significantly greater than those in vas deferens from control H-WKY rats (Fig. 3.16(a)). When data were expressed in absolute terms there was no significant difference in DNA levels among all groups (Fig. 3.16(b)).

3.3.3.3 Protein content

Absolute levels of protein significantly increased in both the superior mesenteric artery (Fig. 3.17(a)) and mesenteric branches (Fig. 3.17(b)) from treated H-WKY and control H-WKY rats when compared with normotensive controls. The vas deferens from untreated and treated H-WKY rats demonstrated a significant reduction in the level of total protein (Fig. 3.18). However, doxazosin treatment further reduced the total level of protein (Fig. 3.18).

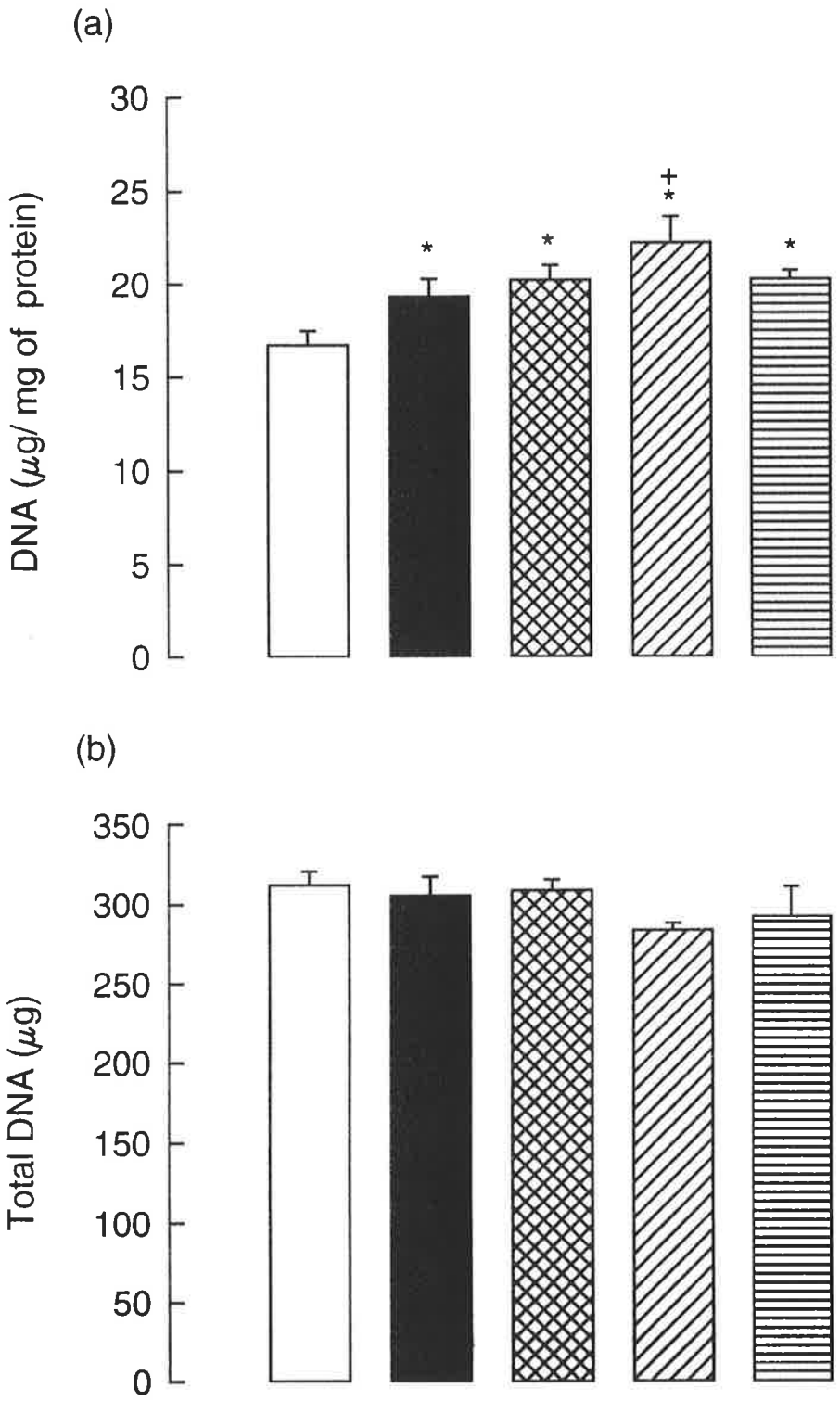


Fig. 3.16. DNA levels found in the vas deferens from ☐ normotensive WKY rats, ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean for 5 to 6 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p< 0.05$ compared with H- WKY control rats.

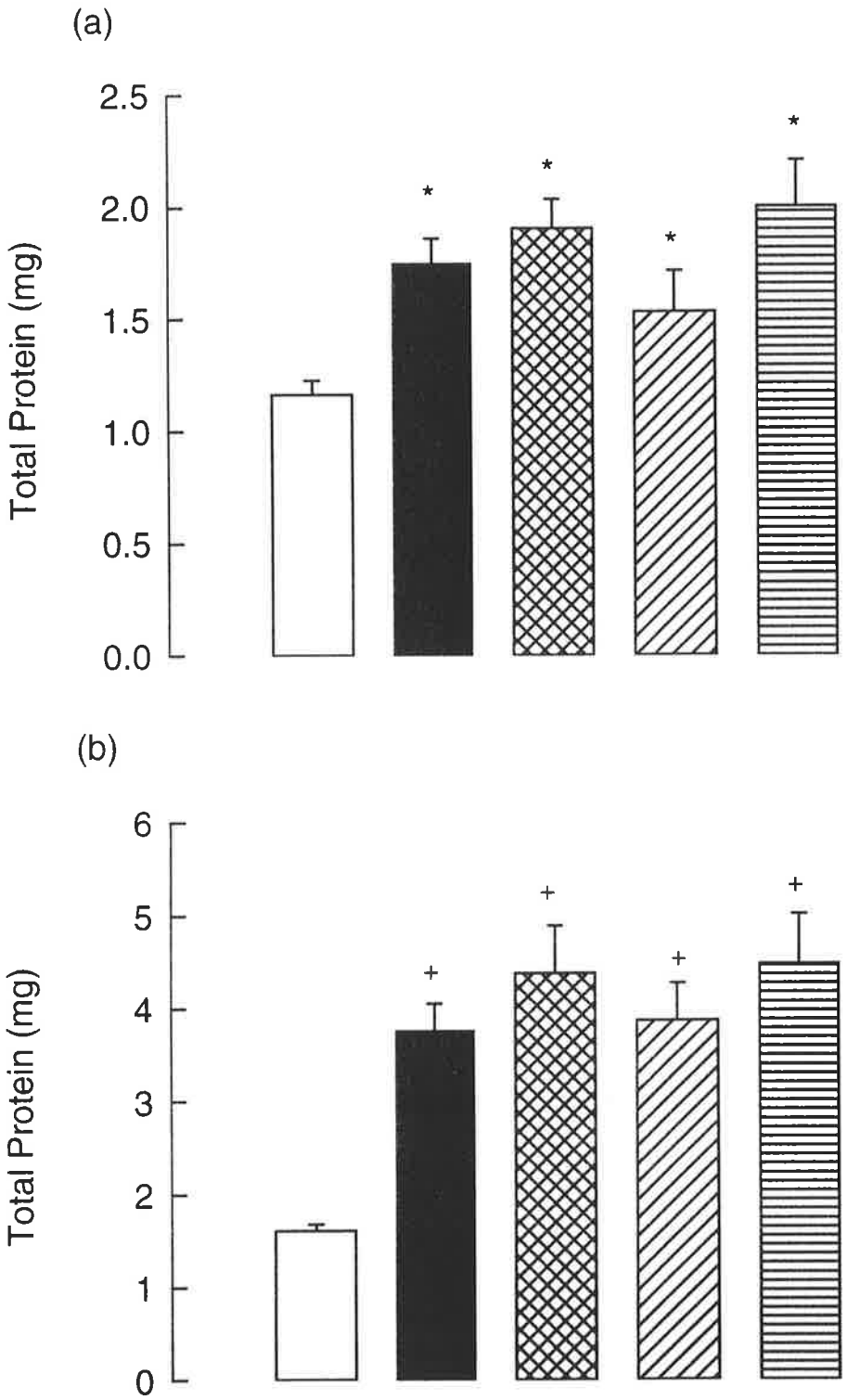


Fig. 3.17. Absolute protein levels found in (a) superior mesenteric artery and (b) mesenteric branches from ☐ normotensive WKY rats, ■ H-WKY control rats, ▨ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Each bar represents the mean value 5 to 6 rats ± s.e.m.

* $p<0.01$ compared with normotensive WKY rats.

+ $p< 0.001$compared with normotensive WKYrats.

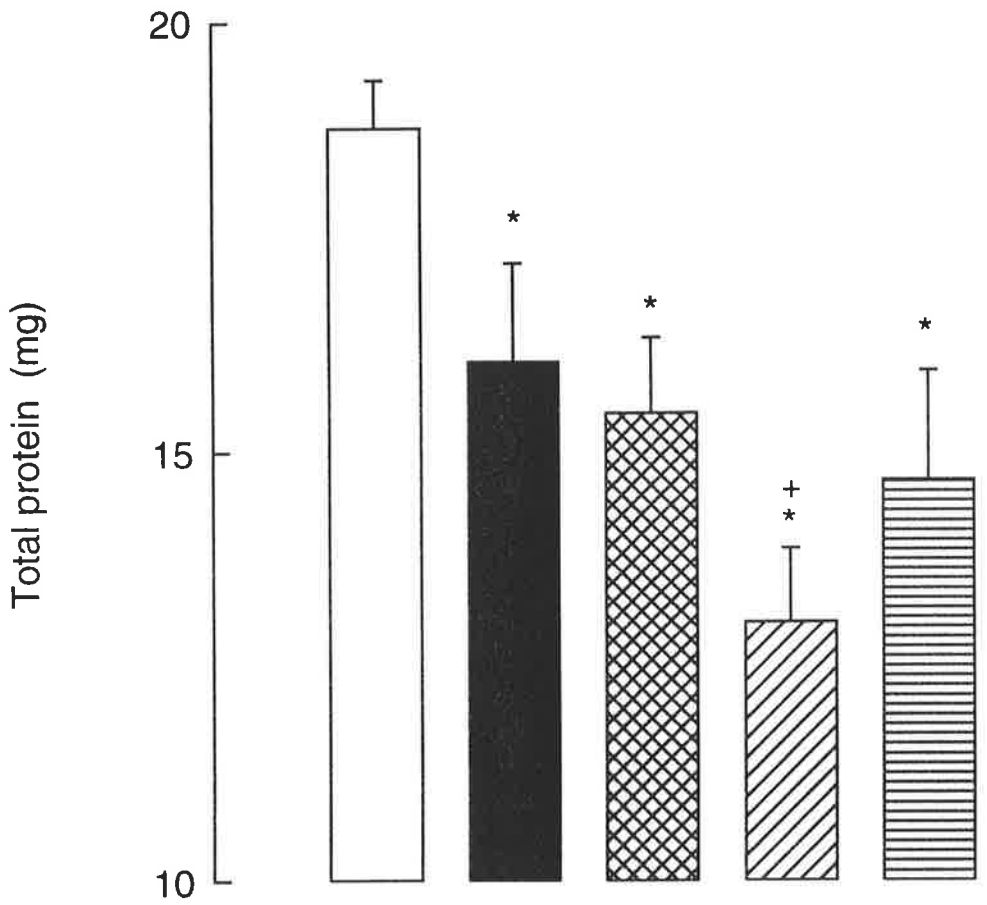


Fig. 3.18. Absolute protein levels found in the vas deferens from ☐ normotensive WKY rats, ■ H-WKY control rats, ▩ H-WKY rats treated with quinapril, ▨ H-WKY rats treated with doxazosin and ▤ H-WKY rats treated with diltiazem. Each bar represents the mean value for 5 to 6 rats ± s.e.m.

* $p<0.01$ compared with normotensive WKY rats.

+ $p<0.05$ compared with H-WKY control rats.

3.4 DISCUSSION

3.4.1 Development of DOCA-salt hypertension

In the present study a significant increase in blood pressure was detected within two weeks of treatment of unilaterally nephrectomised rats with DOCA-salt treatment. The increase in blood pressure that was associated with DOCA-salt hypertension was not prevented by quinapril, doxazosin or diltiazem treatment. These results are consistent with the literature with regard to ACE inhibitors (Douglas *et al* 1979; Porsti *et al* 1986; Tanaka *et al* 1991). However, in contrast to the present data, alpha-adrenoceptor antagonists (Lampa *et al* 1980; Sanchez *et al* 1988) and calcium channel blockers (Lampa *et al* 1980; Hall & Hungerford, 1983; Sanchez *et al* 1988; Li *et al* 1996) have been shown to prevent this rise in blood pressure in mineralocorticoid-salt hypertension. A number of factors may contribute to the discrepancies such as differences in route of administration of the antihypertensive agents i.e. agents have been administered either subcutaneously, intramuscularly, intraperitoneally or by gavage rather than in the animals drinking water. Differences in the amount of and the route of administration of the mineralcorticoid used (e.g. 35 mg/Kg s.c. weekly, 5mg/rat 3 times a week i.m., 30mg s.c. twice weekly, 40mg or 200mg pellet implant) and the use of DOCA-salt treated two kidney rats rather than one kidney rats may also contribute to the discrepancies seen between the present study and those mentioned above.

The increase in blood pressure was associated with cardiac and renal hypertrophy which is consistent with the findings of other investigators (Hall & Hungerford, 1983; Lee *et al* 1989; Li *et al* 1996). However, it has been postulated

that the kidney enlargement is mainly the result of water retention (Lee *et al* 1989). The antihypertensive agents used in the present study were unable to prevent cardiac or renal hypertrophy. It has been demonstrated that antihypertensive agents which prevent the rise in systolic blood pressure during mineralocorticoid treatment are also associated with reduced cardiac hypertrophy (Lampa *et al* 1980; Li *et al* 1996) suggesting that cardiac hypertrophy is secondary to the increase in systolic blood pressure.

3.4.2 Vascular Reactivity

3.4.2.1 Aortic Ring Preparations

The increase in blood pressure was accompanied by a reduction in tension developed by the aortic rings from H-WKY rats in response to noradrenaline which is consistent with other studies (Pang & Sutter, 1980(a)). The attenuated responses to noradrenaline seen in aortae from H-WKY may be attributed to structural alterations such as increased scleroprotein content and relative wall thickness which have been associated with a reduced distensibility (Berry & Greenwald, 1976). Furthermore, aortae from H-WKY rats have demonstrated an enhanced spontaneous tone which may also contribute to the attenuated responses seen in the aortae from hypertensive animals (Rinaldi & Bohr, 1989). In addition, the increased spontaneous tone appears to be endothelium mediated and therefore may be the result of diminished vasodilatation since in the present study responses to acetylcholine were attenuated. In contrast, other investigators have demonstrated no change in the maximal responses of conduit arteries to a range of agonists (Katovich *et al* 1984; Perry & Webb, 1988; Cordellini *et al* 1990; Storm & Webb.

1992; White *et al* 1996). These discrepancies may be due to differences in experimental procedures.

In addition to attenuated responsiveness, aortae from H-WKY rats were more sensitive to noradrenaline, as indicated by the dose of noradrenaline required to cause 50% of maximal contraction, in comparison to aortae from normotensive WKY rats. The present findings are compatible with those of other investigators (Couture & Regoli, 1980(b); Katovich *et al* 1984; Perry & Webb, 1988; Cordellini *et al* 1990; Storm & Webb, 1992). The precise mechanism for the increased sensitivity to α-adrenergic agonists is unclear. It has been shown that the affinity of these agents for adrenoceptors is not altered when compared with normotensive rats (Storm & Webb, 1992). An enhanced release of Ca^{2+} from the intracellular stores (Mecca & Webb, 1984; Storm & Webb, 1992), increased vascular smooth muscle membrane permeability (Bohr & Sitrin, 1970; Jones & Hart, 1975) and an increased number of adrenoceptors (Meggs *et al* 1988) have all been suggested to contribute to an increased sensitivity of conduit vessels. Lack of endothelial nitric oxide has also been demonstrated to increase sensitivity to phenylephrine in carotid arteries from DOCA-salt rats (White *et al* 1996). Consistent with the findings of the above study, it was demonstrated in the present investigation that responses to acetylcholine in aortic rings from H-WKY rats were attenuated. This could also reflect a diminished release and/or production of nitric oxide and therefore contribute to the increased sensitivity to noradrenaline seen in the aorta from H-WKY rats.

Treatment of H-WKY rats with quinapril, doxazosin or diltiazem was unable to prevent the functional changes that were seen in the aorta. However it has been reported that the prevention of elevated blood pressure by administering

hydralazine to rats treated with DOCA-salt was associated with reduced responses to noradrenaline of aortic rings (Pang and Sutter, 1980(b)). It is therefore suggested that the functional changes seen in the aortae of DOCA-salt treated rats may be adaptive changes that occur as a result of high systemic pressure.

3.4.2.2 Perfused mesenteric vascular bed

The responses to periarterial nerve stimulation were significantly decreased in the H-WKY rats. The data obtained in this study is not consistent with the observations of other investigators. For example Ekas and Lokhandwala (1980) and Tsuda *et al* (1986) demonstrated that there was an increase in sensitivity and maximal responses to nerve stimulation in mesenteric preparations from DOCA-salt hypertensive animals. On the other hand Longhurst *et al* (1988) showed little indication of enhanced sensitivity to transmural nerve stimulation and increased responses were confined to the lower portion of the frequency-response curve. It is unclear as to why these discrepancies exist since in the present study the experimental procedure and induction of hypertension were similar to Longhurst *et al* (1988). The only difference between the two studies was the rat strain. The attenuated responses to nerve stimulation observed in this study may have been due to reduced levels of noradrenaline being released from the sympathetic nerve terminals since responses to intraluminal noradrenaline were not attenuated. Depletion of noradrenaline in mesenteric arteries of DOCA-salt hypertensive rats has been reported by others (Crabb *et al* 1980). One explanation for this decrease in noradrenaline levels is the belief that it is due to a decrease in the retention and storage of endogenous noradrenaline in sympathetic nerve terminals (Crabb *et al*

1980). However, if this was the case, then one may expect an increase in basal perfusion pressure in the preparations from the hypertensive animals due to the leakage of noradrenaline from the nerve terminals; however, in this study there was no significant difference in basal perfusion pressure between hypertensive and normotensive preparations. It has also been demonstrated that the noradrenaline turnover rate is increased in a number of tissues from hypertensive rats (de Champlain *et al* 1969) which could also result in reduced endogenous noradrenaline levels.

The responses to noradrenaline, phenylephrine and arginine vasopressin were not significantly different from control animals, although the responses to noradrenaline at the upper part of the dose-response curve tended to be attenuated in preparations from untreated H-WKY rats in comparison to preparations from normotensive WKY rats. This suggests that there may be a down regulation or decreased affinity in α_2-adrenoceptors since it was not seen in response to phenylephrine which is specific for α_1-adrenoceptors.

Furthermore, the results indicate that at least at the lower and middle portions of the concentration-response curves to arginine vasopressin sensitivity in preparations from H-WKY was not significantly different from normotensive WKY rats although there was a tendency for preparations from normotensive WKY rats to be slightly more sensitive to vasopressin. The literature has demonstrated that DOCA-salt treated rats are less sensitive to vasopressin as result of a downregulation of vasopressin receptors (Lariviere *et al* 1988). In contrast to the data presented in this chapter, other investigators have demonstrated hyperresponsiveness to various agonists in the mesenteric vasculature from DOCA-salt hypertensive rats (Ekas & Lokhandwala, 1980; Longhurst *et al* 1988; Stallone,

1995). Similarly, increases in sensitivity and maximal responses to noradrenaline have been observed in perfused hindquarter and kidney preparations (Finch & Haeusler, 1974; Bereck *et al* 1980).

In contrast to the reactivity studies, the biochemical studies which were undertaken indicated an increase in contractile protein content (as measured by 3-methylhistidine) and growth of blood vessels (as measured by DNA and protein) in the mesenteric vasculature which could be interpreted to be associated with an enhanced reactivity. Evidence exists to suggest that endothelial cell function may compensate for increased vascular responsiveness in the mesenteric vasculature in mineralocorticoid hypertension since disruption of endothelial function potentiated constrictor responses in both normotensive and hypertensive groups but the magnitude of potentiation in hypertensive rats was greater (King & Webb, 1988). It could then be speculated that in the present study the endothelium may be masking enhanced contractility and sensitivity of the mesenteric vascular bed which is in contrast to what is seen with the thoracic aorta where endothelium dependent responses were reduced in the hypertensive rats.

On the other hand, the absolute increases in 3-methylhistidine content and therefore contractile protein maybe misleading in the light of reports which have demonstrated changes in smooth muscle cell phenotypes and switches in contractile protein isoforms. Although the relationship between growth and differentiation of vascular smooth muscle cells is not fully understood, it has been demonstrated that in the proliferative state smooth muscle cells exhibit a reduced expression of smooth muscle contractile proteins (such as α-actin, myosin heavy chains) with an increased expression of nonmuscle variants of these proteins (Morano, 1992; Owens, 1995). In the contractile state the opposite exists. Similarly,

during development, there is a shift from primarily nonmuscle to smooth muscle variants of contractile proteins (Owens & Thompson, 1986; Morano, 1992). In the renovascular model of hypertension it has been demonstrated that there is an increase in aortic smooth muscle cells containing the non-muscle myosin isoform compared with controls and these cells are referred to as "immature" (Pauletto *et al* 1994). In light of the above evidence it is then hypothesised that in the H-WKY rats the smooth muscle cells were in a state in which the expression of non-smooth muscle variants of contractile proteins was increased which would in turn influence contractility of the mesenteric vasculature regardless of an increase in 3-methylhistidine content and cell number.

Another hypothesis that can be put forward regarding the unaltered contractility but increases in vascular 3-methylhistidine, DNA and protein content is that together with an increase in vessel wall there was a proportional increase in the lumen diameter in which case the media to lumen ratio would not increase and thereby contractility would not be enhanced. Morphometric studies have demonstrated that at least in the large mesenteric arteries from DOCA-salt treated animals there is an increase in both lumen and medial cross-sectional area, when compared with vessels from normotensive controls (Lee *et al* 1989).

Responses to nerve stimulation, noradrenaline, phenylephrine and vasopressin in preparations from H-WKY rats treated with quinapril or diltiazem were not significantly different from the those of the H-WKY untreated rats. In the case of doxazosin treated H-WKY rats, the responses to nerve stimulation, noradrenaline and phenylephrine were significantly lower than in the other three H-WKY groups. However, responses to vasopressin were similar between all groups therefore suggesting that the decreased responses to the α-agonists and nerve

stimulation is a specific effect of doxazosin on the adrenoceptors either by still occupying receptors in the mesenteric preparation after its removal from the animal or by affecting the number and/or affinity of postsynaptic α_1-receptors.

Collectively the data from the present study suggest that there are no major differences in responsiveness of the perfused mesenteric vascular preparation from H-WKY rats in comparison with preparations from normotensive WKY rats.

3.4.3 Biochemical parameters

3-methylhistidine has previously been used as a marker for contractile proteins in the mesenteric vasculature from SHRs and is hence indicative of hypertrophy of the vessel wall (Jonsson *et al* 1991; Smid *et al* 1993). In this study, the 3-methylhistidine content, (in absolute terms), in the mesenteric branches from H-WKY rats was enhanced. However, this increase in contractile protein was not specific, ie there was an increase in proteins in general. Furthermore, the increase in the other proteins appeared to be to a greater degree than that obtained with 3-methylhistidine since the expression of 3-methylhistidine on a protein basis resulted in the level of 3-methylhistidine being lower in mesenteric branches from hypertensive animals as compared with levels in tissues from normotensive animals. In addition to the increase in proteins it was also demonstrated that the DNA content was enhanced, both when normalised and as absolute values, in the mesenteric branches from the hypertensive animals. It can be suggested that the increase in the biochemical parameters measured reflect growth of the resistance vessel wall and that the number of smooth muscle cells increased, based on the assumption that the cells are diploid, which would suggest an increase in medial

area. The results are consistent with morphometric studies that have shown an increase in medial cross-sectional area not only in mesenteric resistance vessels but also renal vessels, from DOCA-salt hypertensive animals, and that the increase in the medial cross-sectional area has been attributed to an increase in the number of smooth muscle cells (hyperplasia) (Berry & Henrichs, 1982; Lee *et al* 1989; Li *et al* 1996). Morphological studies have also demonstrated increases in collagen in resistance vessels (Ooshima *et al* 1975; Iwatsuki *et al* 1977) which would also contribute to the increase in protein seen in this study.

In the case of the superior mesenteric artery, total 3-methylhistidine, proteins in general and DNA content were also enhanced in this tissue from H-WKY animals when compared with tissues from normotensive WKY rats. Morphometrically it has been shown that the medial area increases in the superior mesenteric artery from DOCA-salt hypertensive rats and the increase attributed to hyperlpasia of smooth muscle cells (Lee *et al* 1989). In addition, increases in adventitial area of the superior mesenteric artery have also been observed suggesting increases in proteins such as collagen, elastin or other extracellular proteins (Lee *et al* 1989).

The 3-methylhistidine and DNA levels in the aorta were similar in DOCA-salt hypertensive and normotensive animals. Unfortunately, a defined anatomical length of the aorta was not taken. Therefore it was not possible to determine the absolute levels of 3-methylhistidine, DNA and proteins, making it difficult to speculate as to what changes had taken place. If proportional increases occurred in all biochemical parameters measured then normalisation of the data would mask any increase that occurred. Previous studies have shown both increased (Berry & Greenwald, 1976; Soltis *et al* 1991; Ashen & Hamlyn, 1994) and no change (Berry & Henrichs, 1982)

in the medial area in aortae from DOCA-salt hypertensive animals. In other experimental models of hypertension such as aortic coarctation the aorta has been associated with hyperplasia of smooth muscle cells (Owens & Reidy, 1985). Furthermore, in the abdominal aorta (another conducting vessel) from DOCA-salt hypertensive rats it has been demonstrated that hyperplasia of smooth muscle cells contributed to medial enlargement (Berry & Henrichs, 1982). This is consistent with the change seen in the superior mesenteric artery (a conduit vessel) in the present study . In contrast, it has been shown that isolated aortic smooth muscle cells from DOCA-salt treated rats exhibit tetraploid nuclei and cellular hypertrophy (Lichtenstein *et al* 1986; Chobanian *et al* 1987). In most studies, collagen and elastin levels are increased in conduit vessels from DOCA-salt hypertensive animals (Wolinsky, 1970; Ooshima *et al* 1975; Berry & Henrichs, 1982; Lee *et al* 1989) although Berry & Greenwald (1976) demonstrated increases in elastin but not collagen.

The reduced 3-methylhistidine and protein content found in the vas deferens from H-WKY rats suggests that the DOCA treatment exerts specific effects on this non vascular tissue without affecting cell number. Whether similar trends are seen with other nonvascular tissues is not known.

The mechanisms involved in vascular growth in hypertension are unclear , although it has been suggested that both the sympathetic nervous and renin-angiotensin systems may be involved. Although plasma renin levels are low in DOCA-salt animals (Porsti *et al* 1986; Li *et al* 1996) little information is available concerning the local vascular renin-angiotensin system. Michel *et al* (1994) demonstrated that changes in plasma renin activity did not account for the regulation of tissue angiotensin converting enzyme activity and that this enzyme

was subject to independent regulation in each organ (Michel *et al* 1994). DOCA-salt treatment induced hypertrophy in both heart and kidney which was associated with an increase in angiotensin converting enzyme activity suggesting the possible involvement of angiotensin II (Michel *et al* 1994). Therefore an ACE inhibitor (quinapril) and α-adrenoceptor antagonist (doxazosin) were used to determine whether these antagonists could inhibit any trophic and/or mitogenic effects that angiotensin II and noradrenaline respectively may have on blood vessels regardless of whether the rise in blood pressure was prevented or not. In addition a calcium channel blocker (diltiazem) was also used since alterations in calcium have been demonstrated in hypertension which may influence contractility and trophic process (Kwan, 1985; Lau & Eby, 1985; Chobanian, 1990; Michel *et al* 1990). The drug therapies used in this study were unable to prevent the increases seen in 3-methylhistidine, proteins and DNA in the above tissues with the exception that the absolute levels of 3-methylhistidine in the superior mesenteric artery from doxazosin treated DOCA-salt rats were lower than those in the superior mesenteric artery from H-WKY untreated rats.

The explanation for such a decrease is unclear, but it may reflect the difference in sympathetic innervation per unit of crosssectional wall area which is larger in mesenteric resistance vessels than in the superior mesenteric artery (Smeda & Lee, 1991). Therefore, noradrenaline may have a specific effect on contractile proteins and in the superior mesenteric artery (where innervation is less) doxazosin can inhibit the effects of noradrenaline but not in the mesenteric branches.

Consistent with this study Li *et al* (1996) also demonstrated that an angiotensin converting enzyme inhibitor (cilazapril) was unable to prevent structural

CHAPTER 4

Vascular Reactivity, Contractile Proteins and Blood Pressure Development in the Spontaneously Hypertensive rat.

4.1 INTRODUCTION

Evidence has accumulated over the years to demonstrate that structural changes in peripheral blood vessels can develop early in the genetic rat model of hypertension the SHR (Nordborg & Johansson, 1979; Nordborg *et al* 1983). More importantly, it has been shown that vascular hypertrophy may not depend completely on the level of arterial pressure, since antihypertensive agents are able to prevent the rise of systolic blood pressure without effect on the vascular structure, but may also depend on neuroendocrine systems such as the sympathetic nervous system and the renin-angiotensin system.

Studies undertaken in vivo and in vitro have implicated both systems in having trophic influences on vascular smooth muscle cells. Lee *et al* (1987) reported that neonatal sympathectomy of SHRs prevented hyperplastic changes in the mesenteric vasculature. Bevan (1975) has also observed the important trophic influence of the sympathetic nervous system on the structure of the rabbit ear artery. In addition, it has been demonstrated that α_1-adrenergic receptor activation with methoxamine induced the activation of mesenteric vasculature and aortic ornithine decarboxylase concomitantly with mean arterial pressure increases in normotensive rats (orthinine decarboxylase is the rate-limiting enzyme in the formation of polyamines, a key regulatory enzyme in growth processes)(Thompson *et al* 1992). Studies using cultured vascular smooth muscle cells have in addition demonstrated that activation of adrenergic receptors can increase the rate of cell proliferation (Blaes & Boissel, 1983) and stimulate polyploidy (Yamori *et al* 1987). In contrast to the above studies four day phenylephrine infusion in young WKY rats did not result in small artery hypertrophy even though blood pressure increased (Boonen *et al* 1993).

The trophic effects of the renin-angiotensin system have been demonstrated by the chronic infusion of angiotensin II into normotensive rats whereby vascular hypertrophy in the mesenteric circulation was produced in the absence of elevated blood pressure as the latter was controlled with hydralazine (Griffin *et al* 1991). Studies with cultured smooth muscle cells have also demonstrated the ability of angiotensin II to induce cellular growth ie an increase in protein synthesis and polyploidy (Turla *et al* 1991; Berk & Rao, 1993).

Associated with vascular hypertrophy have been alterations in the contractility of blood vessels. Although the structural changes may be the major determinant of the degree of contractility the vessel is able to achieve, there is some evidence to suggest that vasoconstrictor agents such as angiotensin II and vasopressin, which have a widespread influence on the level of proteins in smooth muscle cells, can also have a further selective influence on the level of the contractile protein α-actin (Turla *et al* 1991). However, the evidence involving contractile protein levels and contractility is very sparse.

The objective of the present study was to examine the effect of ACE inhibition, (thereby preventing the formation of angiotensin II) and α_1-adrenoceptor blockade (since α_1-adrenoceptors are the key subtype involved in responses of the vasculature to sympathetic nerve stimulation (Van Zwieten, 1988)) on the development of hypertension and cardiovascular responses in the SHR. In addition, a calcium channel blocker was used since altered calcium handling of vascular smooth muscle cells has been associated with the development of hypertension and calcium is involved in both contractility and growth (Chobanian, 1990; Michel *et al* 1990). Therefore, the aim of this study was to determine whether a relationship exists between blood pressure, contractility, contractile protein levels and growth

using three different classes of antihypertensive agent.

4.2 METHODS

4.2.1 Animal and drug treatments

Male SHR and WKY rats were maintained as described previously (Chapter 2). At 5 weeks of age, SHRs were randomly assigned to either untreated (control), quinapril, doxazosin or diltiazem treated groups. The antihypertensive agents were administered as previously described (Chapter 2). The WKY rats were maintained on water. The treatment period was from 5 to 18 weeks of age. All animals were subjected to weekly measurements of body weight. Systolic blood pressure was measured at four weekly intervals. After the 13 weeks of treatment the animals were killed by decapitation and appropriate tissues were removed (see Chapter 3) and stored as described in Chapter 2.

4.2.2 Aortic ring preparations

The thoracic aortic rings were prepared as previously described (Chapter 2). Cumulative concentration-response curves to noradrenaline (0.12 - 8000 nM) were performed.

4.2.3 Perfused mesenteric vascular bed

The perfused mesenteric vascular bed was prepared as previously described (Chapter 2). Frequency-response curves (2-64 Hz) and noncumulative dose-response curves to noradrenaline (0.01-20.0 μg), phenylephrine (0.1-10.0 μg), arginine vasopressin (9×10^{-13} - 9×10^{-8} M) and potassium chloride (1-32 mg) were performed. At the end of the protocol the preparation was transferred to a petri dish (on ice) containing saline where the mesenteric bed was dissected as described in Chapter 3.

4.2.4 Biochemical analyses

3-Methylhistidine, DNA and protein assays were performed as described previously (Chapter 2). Data are expressed as normalised per mg of protein and absolute amounts. (Data expressed per mg of wet tissue weight are presented in Appendix II).

4.3 RESULTS

4.3.1 Blood Pressure

In the untreated SHR, systolic blood pressure rose with age and was significantly greater than that for the WKY group (Fig. 4.1). Chronic quinapril treatment prevented the development of hypertension in the SHR and also maintained a systolic pressure lower than that of the WKYs although this was not significantly different at 12 weeks of treatment (Fig. 4.1). In contrast to quinapril treatment, both doxazosin and diltiazem therapies were not able to completely prevent the increase in systolic blood pressure in the SHR although at both 8 and 12 weeks of treatment the systolic blood pressure of these animals was significantly lower than that of untreated SHRs (Fig. 4.1).

Relative left ventricular mass (left ventricular weight:body weight ratio) of untreated SHRs was significantly increased in comparison to relative left ventricular mass of WKY rats (Fig. 4.2(a)), indicative of cardiac hypertrophy in the former strain. Cardiac hypertrophy was completely prevented by quinapril treatment and to a lesser degree by diltiazem (Fig. 4.2(a)). Doxazosin treatment resulted in a small degree of regression of cardiac hypertrophy, but this did not reach significance (Fig. 4.2(a)). Systolic blood pressure level significantly correlated with the degree of cardiac hypertrophy (Fig. 4.3). Relative renal mass did not differ significantly between any of the groups (Fig. 4.2(b)).

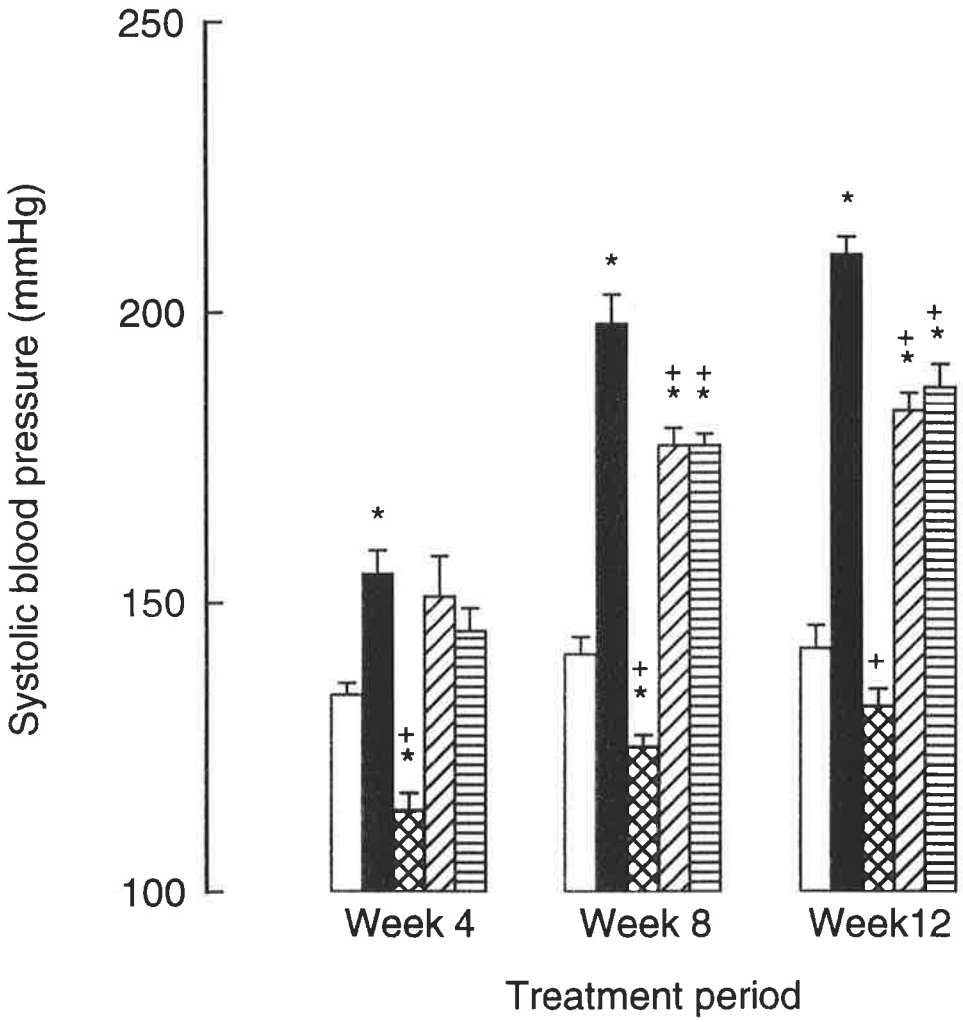


Fig. 4.1. Systolic blood pressure profile of ☐ normotensive WKY rats, ■ SHR controls, ▩ SHRs treated with quinapril, ▨ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Each bar represents the mean value for 10 rats ± s.e.m.

* $p < 0.05$ compared with normotensive WKY rats.

+ $p < 0.001$ compared with SHR controls.

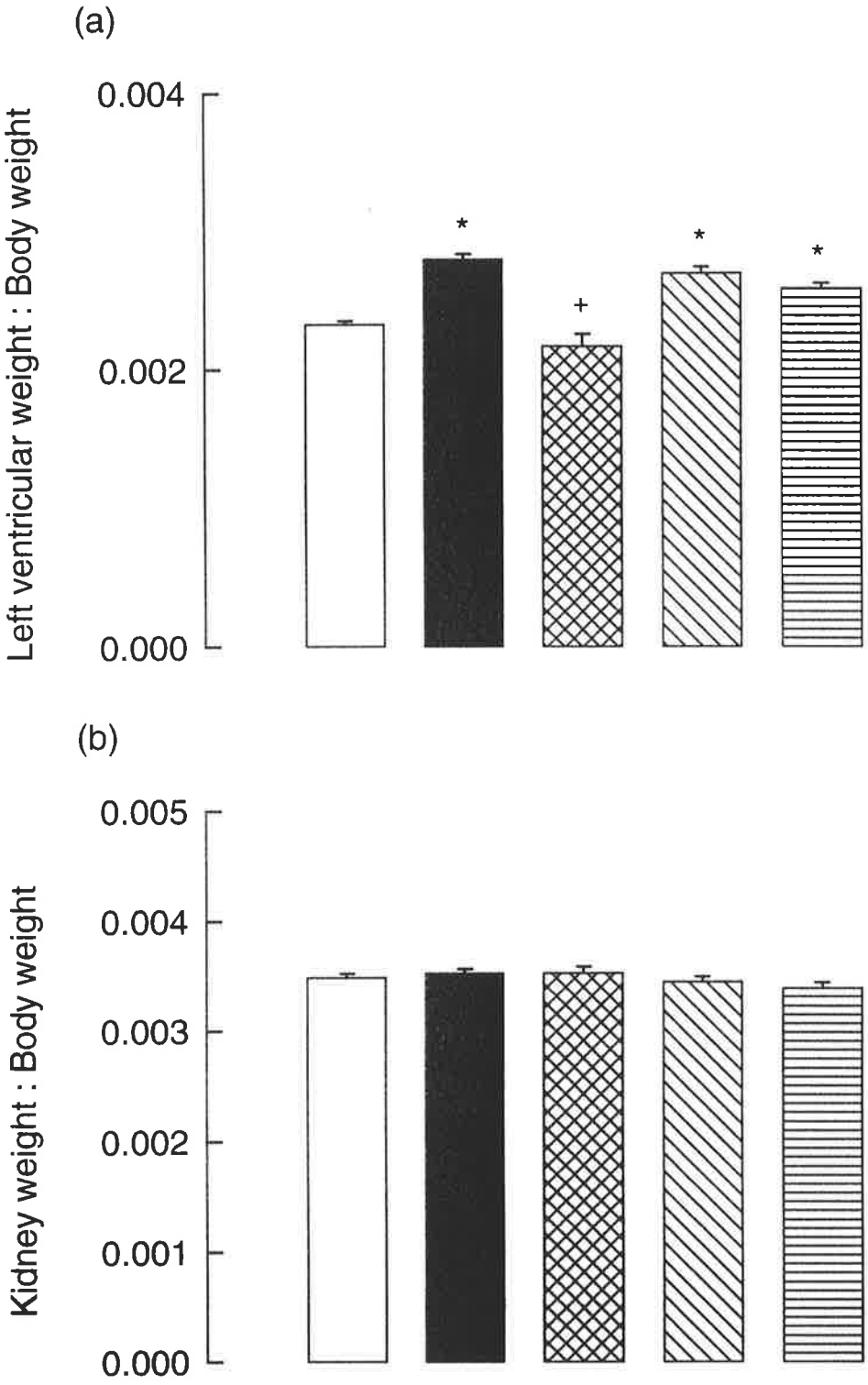


Fig. 4.2. Bar graphs showing (a) left ventricular weight: body weight ratio and (b) kidney weight : body weight ratio for ☐ normotensive WKY rats, ■ SHR controls , ▨ SHRs treated with quinapril, ▧ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Each bar represents the mean value of 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p<0.001$ compared with SHR controls.

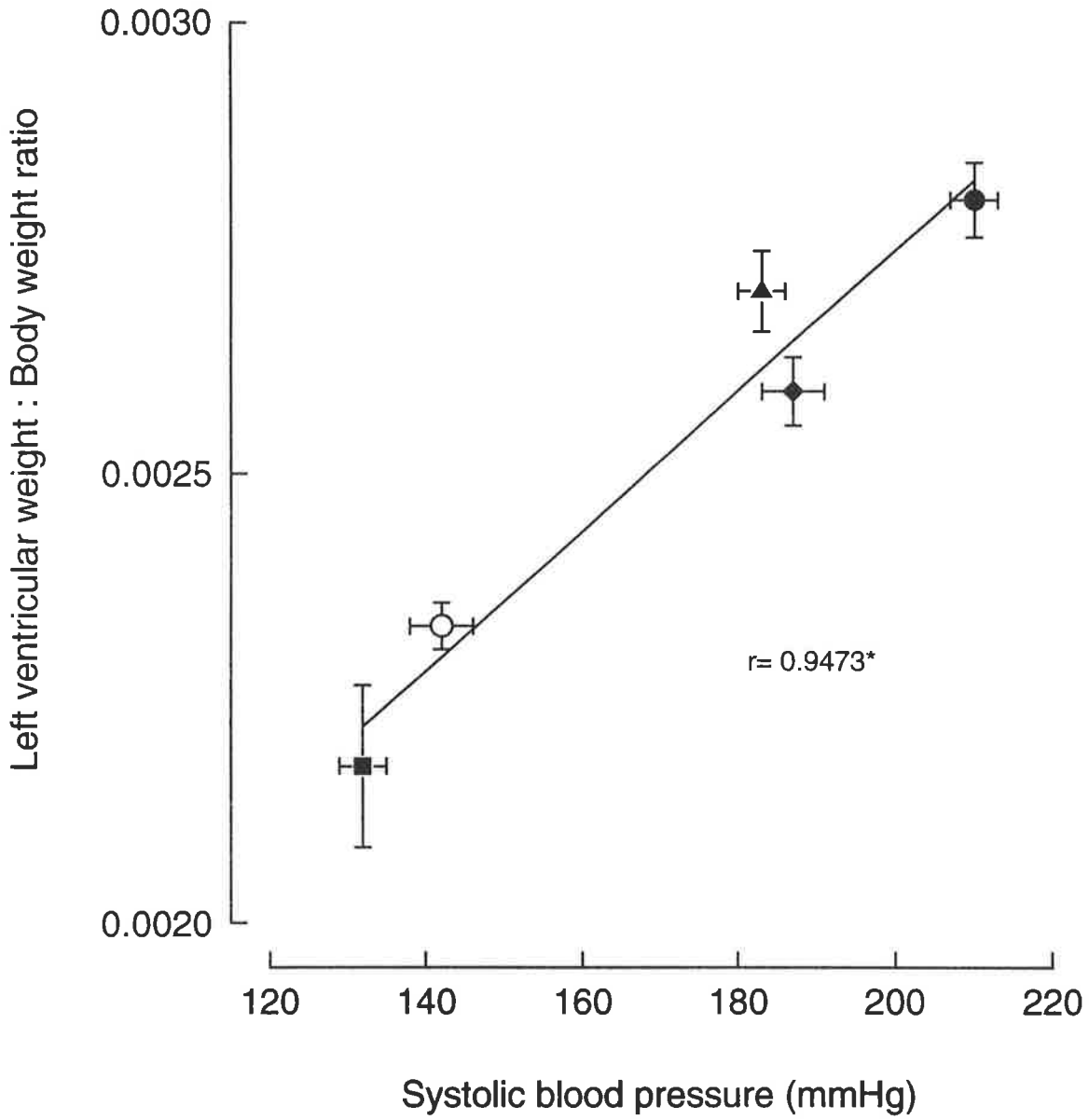


Fig. 4.3. Relation between mean systolic blood pressure and left ventricular weight of ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem.
* significant correlation of all groups, p=0.005.

4.3.2 Vascular reactivity

4.3.2.1 Aortic ring preparations

Maximal responses elicited by noradrenaline in the thoracic aorta were significantly lower in aortae from untreated SHRs when compared to aortae from WKY control rats (Fig. 4.4(a)). Furthermore, aortae taken from SHRs treated with doxazosin or diltiazem also produced maximal responses lower than the WKY control rats and these responses were not significantly different from the untreated SHR group (Fig. 4.4(b)). In contrast to doxazosin and diltiazem treatments, quinapril therapy resulted in significantly augmented responses to noradrenaline in aortae from these animals when compared to aortae from the untreated SHRs (Fig. 4.4(a)).

However, the EC_{50} values for noradrenaline were not significantly different between any of the groups (Table 4.1).

4.3.2.2 Perfused mesenteric vascular bed

Responses obtained by periarterial nerve stimulation were markedly lower at 16, 32 and 64 Hz in preparations from normotensive WKY rats when compared with preparations from untreated and diltiazem treated SHRs (Fig. 4.5). Chronic quinapril and doxazosin treatment resulted in the attenuation of vascular reactivity to nerve stimulation; however, at 32 and 64 Hz contractility in preparations from the quinapril treated SHRs was still greater than that seen in preparations from WKY rats (Fig. 4.5).

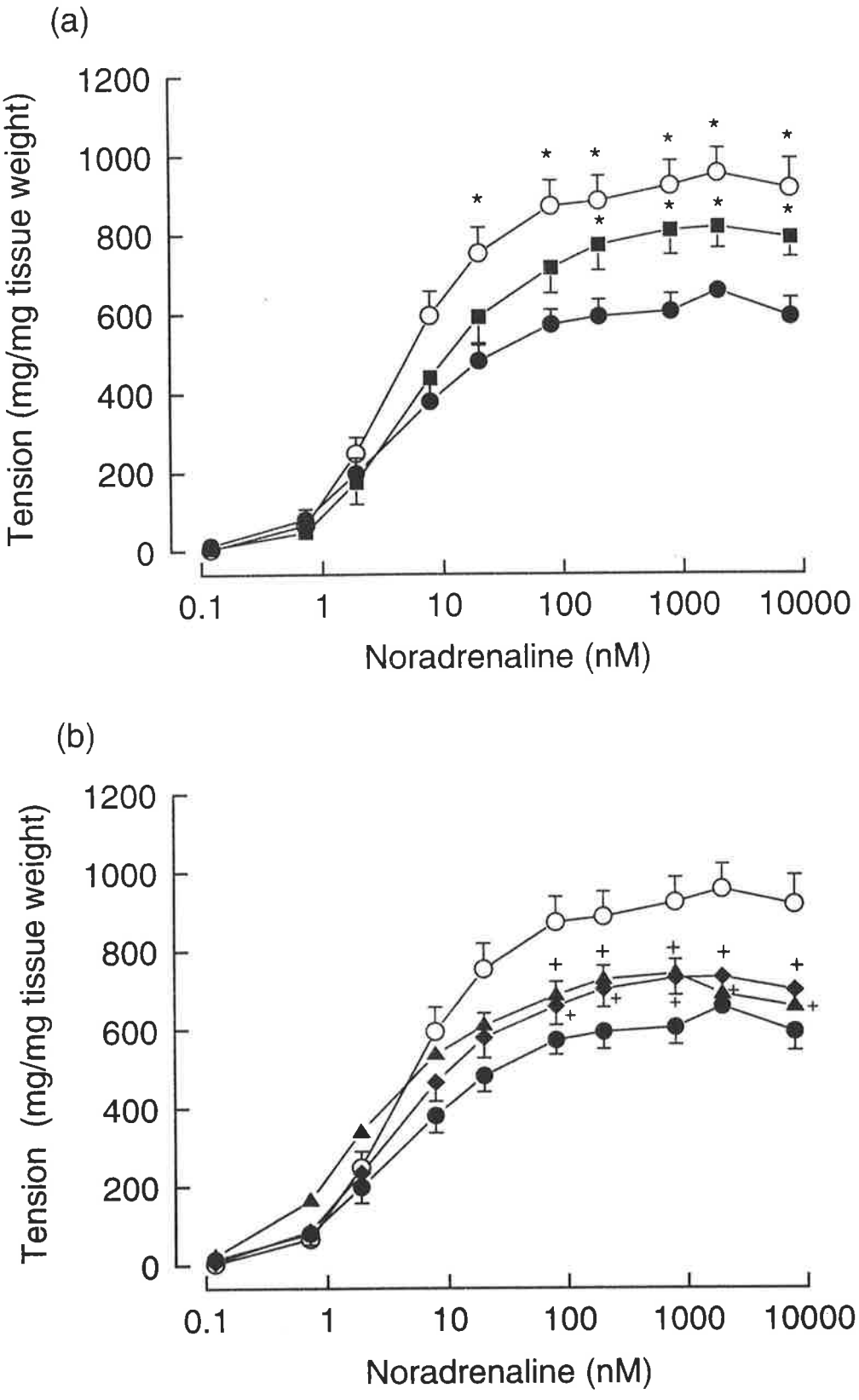


Fig. 4.4. Concentration-response curves to noradrenaline of aortic rings from (a) ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril and (b) ○ normotensive WKY rats, ● SHR controls, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 6 to 8 rats ± s.e.m.

* $p<0.05$ compared with SHR controls.

\+ $p<0.05$ compared with normotensive WKY rats.

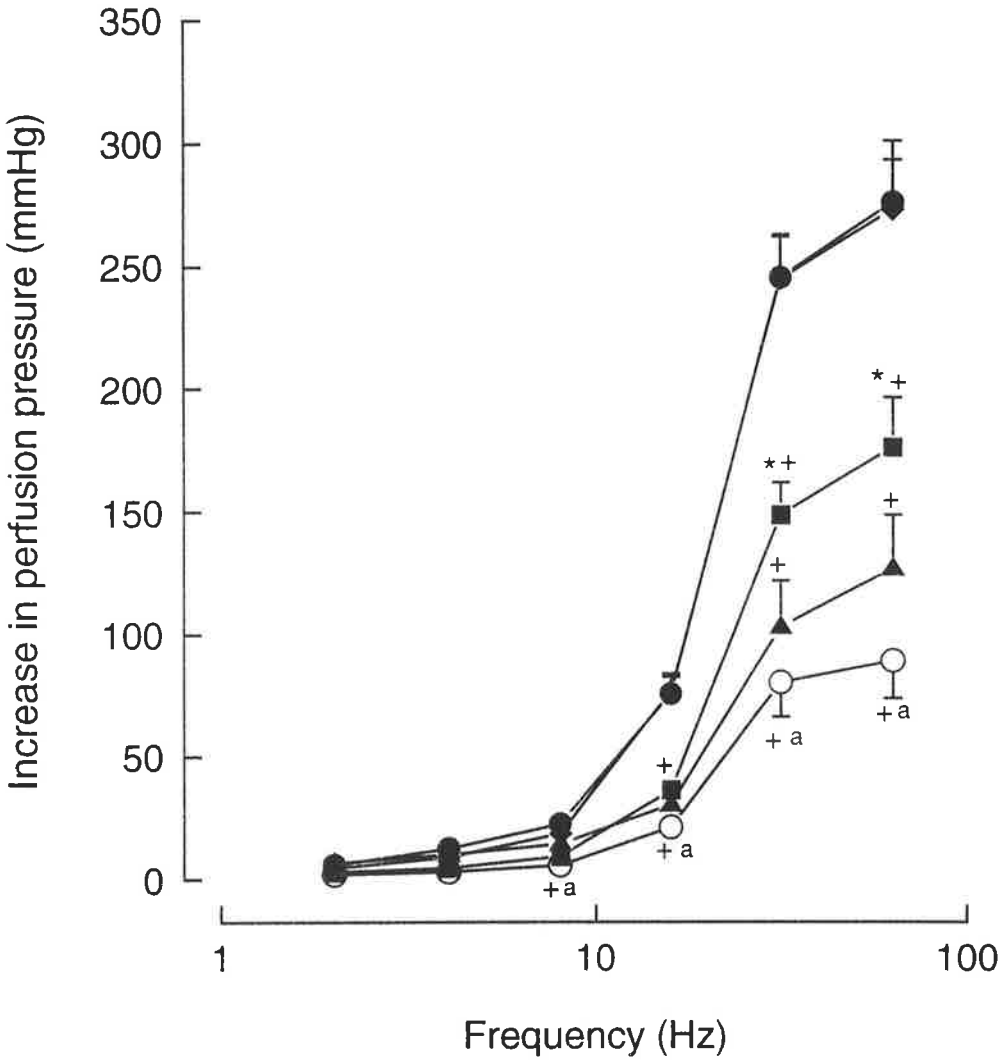


Fig. 4.5. Frequency-response curves in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 5 to 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p<0.05$ compared with SHR controls.

a $p<0.005$ compared with diltiazem.

A similar trend to that seen with nerve stimulation was seen with responses to intraluminal noradrenaline and phenylephrine (Fig. 4.6 & 4.7).

Responses to arginine vasopressin and potassium chloride were augmented in preparations from control, doxazosin and diltiazem treated SHRs when compared with preparations from WKY rats (Fig. 4.8 & 4.9). On the other hand, chronic quinapril therapy of SHRs attenuated responses to both agonists and in the case of potassium chloride the responses of preparations from this treatment group and the WKY group were superimposable (Fig 4.8 & 4.9). Sensitivity (EC_{50}) of mesenteric preparations to potassium chloride was similar amongst all groups (Table 4.2). Furthermore, slopes for potassium chloride response curves were greater in preparations from untreated, diltiazem and doxazosin treated SHRs in comparison to slopes from WKY and quinapril treated SHRs (Table 4.2). In contrast, dose-response curves to arginine vasopressin of preparations from untreated and treated SHRs were shifted to the left when compared to dose-response curves of preparations from WKYs this being indicative of an increased sensitivity of the SHR vasculature to arginine vasopressin which was not influenced by any of the antihypertensive agents (Table 4.2).

4.3.3 Biochemical parameters

4.3.3.1 3-Methylhistidine content

The content of 3-methylhistidine in the aorta from treated and control groups (expressed per mg of protein) is displayed in Fig. 4.10. There was no significant difference in the content of 3-methylhistidine between groups (Fig. 4.10).

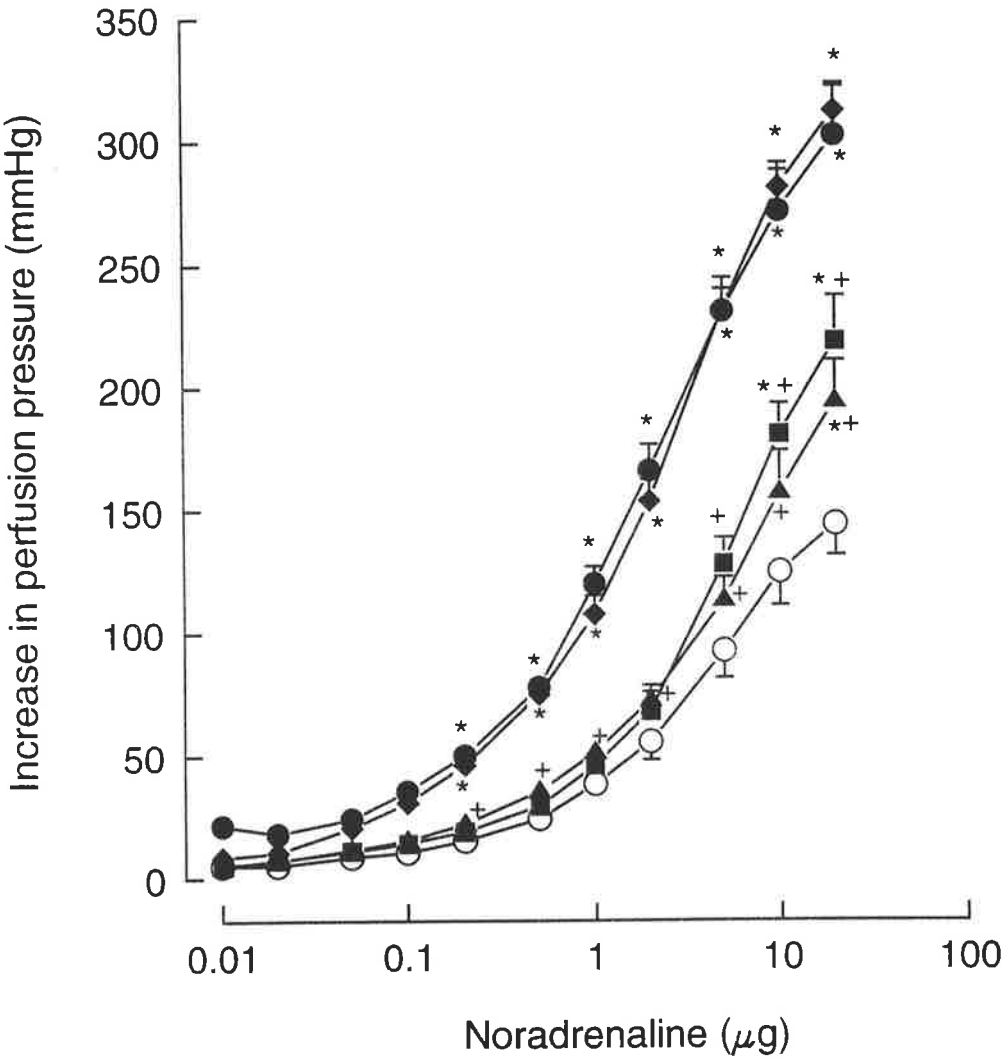


Fig. 4.6. Concentration-response curves to noradrenaline in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 6 to 8 rats ± s.e.m.
* $p<0.05$ compared with normotensive WKY rats.
+ $p<0.01$ compared with SHR controls.

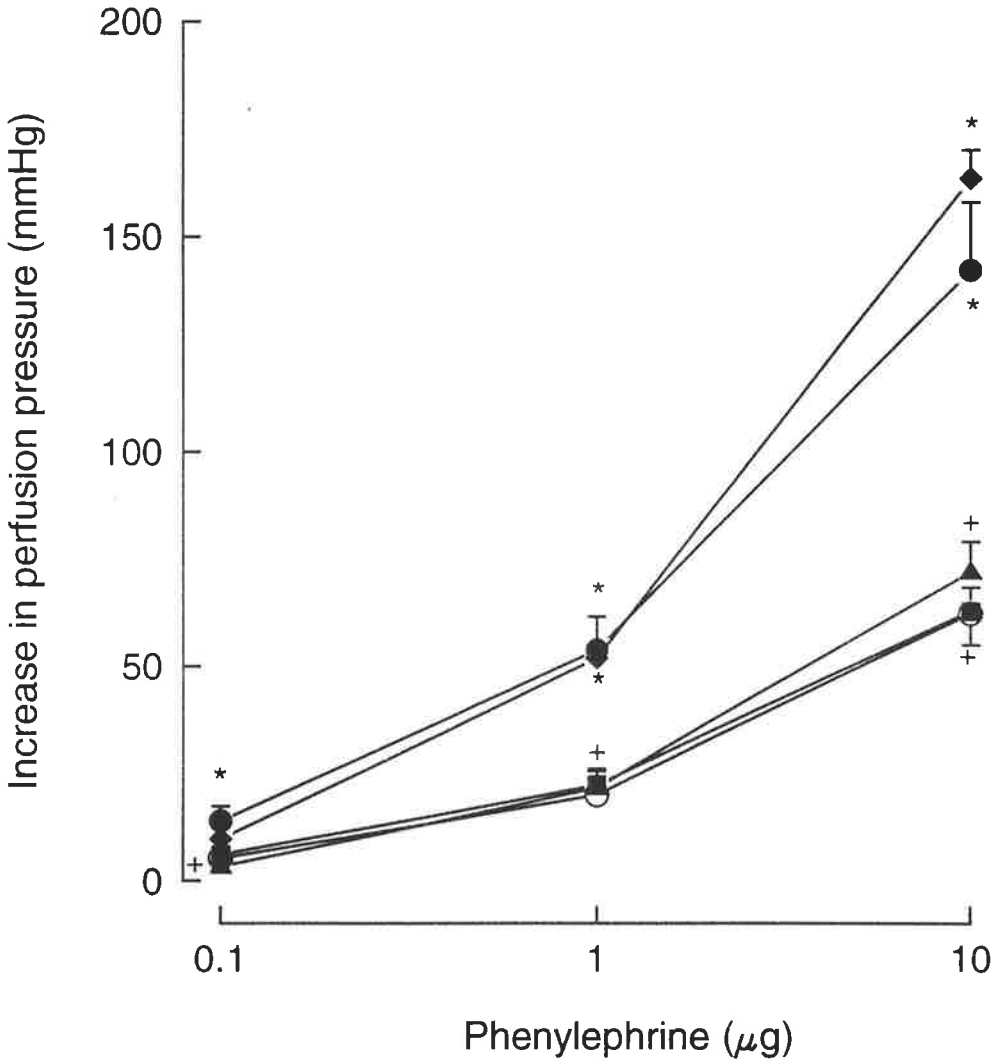


Fig. 4.7. Concentration-response curves to phenylephrine in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 6 to 8 rats ± s.e.m.
* $p<0.05$ compared with normotensive WKY rats.
+ $p<0.05$ compared with SHR controls.

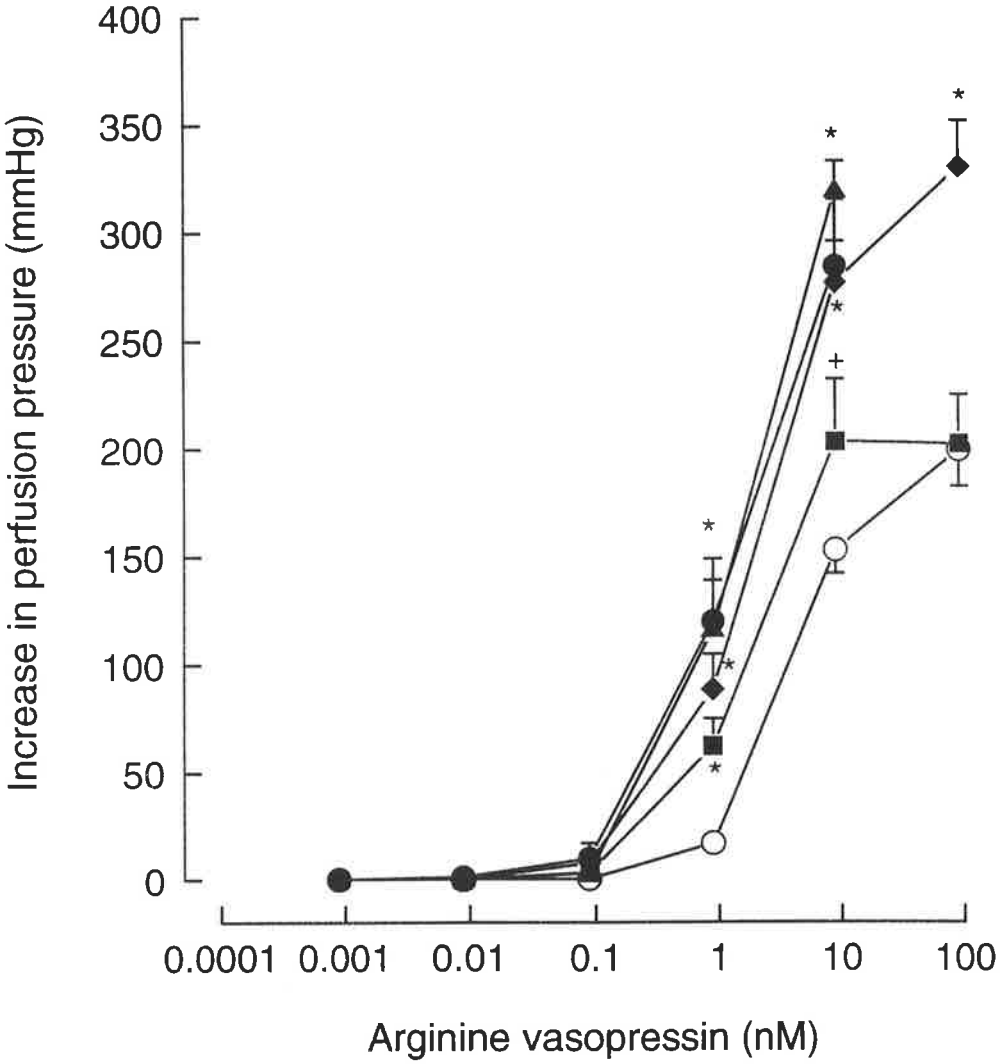


Fig. 4.8. Concentration-response curves to vasopressin in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 5 to 8 rats ± s.e.m.
* $p<0.01$ compared with normotensive WKY rats.
+ $p<0.05$ compared with SHR controls.

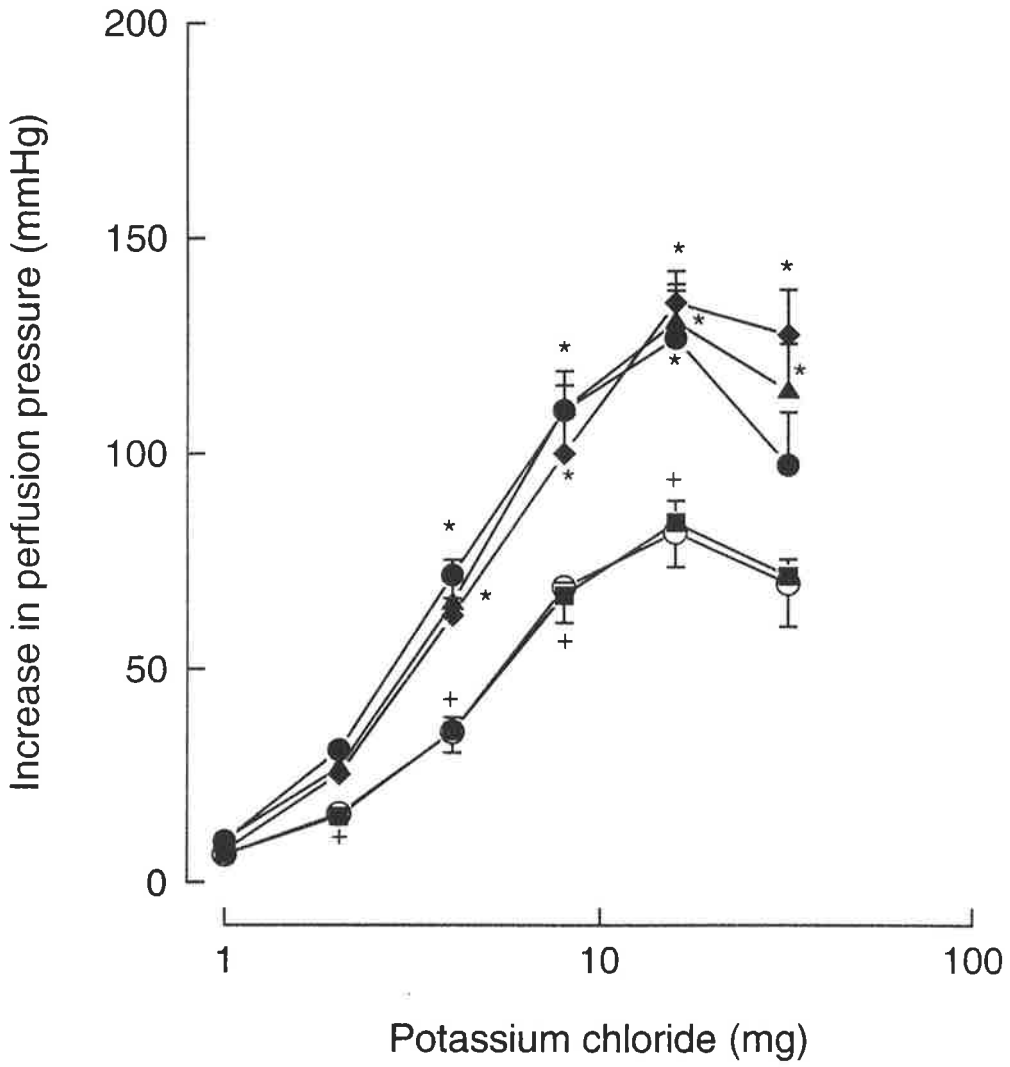


Fig. 4.9. Concentration-response curves to potassium chloride in the isolated perfused mesenteric vasculature from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value for 6 to 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p<0.01$ compared with SHR controls.

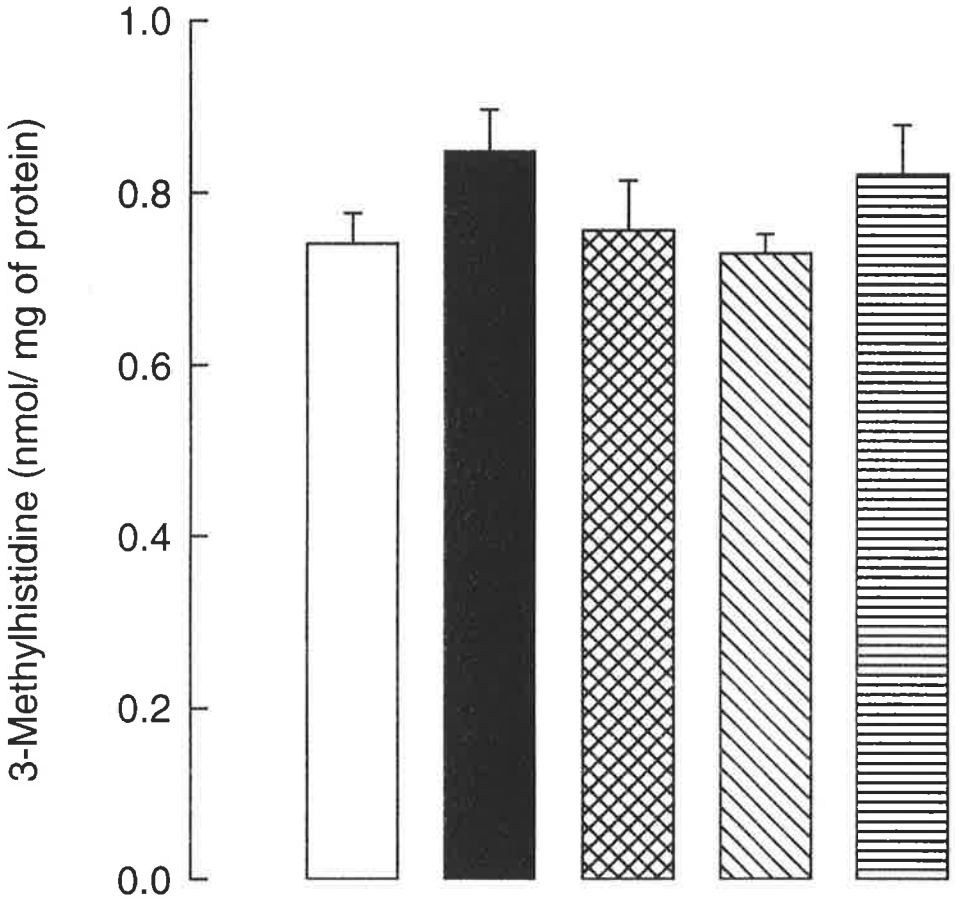


Fig. 4.10. Aortic 3-methylhistidine levels from ☐ normotensive WKY rats, ■ SHR controls, ▩ SHRs treated with quinapril, ▨ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem.
Each bar represents the mean value for 8 rats ± s.e.m.

Fig 4.11(a) shows the level of 3-methylhistidine relative to protein content in the superior mesenteric artery. There was no difference in 3-methylhistidine levels in this tissue between groups (Fg. 4.11 (a)). Absolute 3-methylhistidine levels were significantly increased in tissues from untreated SHRs and doxazosin and diltiazem treated SHRs when compared to tissues from WKY rats (Fig. 4.11(b)). However, chronic treatment of SHRs with quinapril resulted in lower absolute 3-methylhistidine levels in the superior mesenteric artery in comparison to levels in tissues from untreated SHRs but levels were similar to those seen in tissues from WKY rats (Fig. 4.11(b)).

The concentrations of 3-methylhistidine per mg of protein in the mesenteric branches are shown in Fig 4.12(a). A difference in 3-methylhistidine level between strains and after drug treatments was not seen. However, when the results were expressed in absolute terms quinapril treatment alone caused a marked and significant reduction in mesenteric branch 3-methylhistidine levels (Fig. 4.12(b)). Between strains and after doaxzosin and diltiazem treatment, the absolute levels were not significantly different (Fig. 4.12(b)).

3-methylhistidine levels were also measured in the vas deferens, in order to determine whether the differences seen in blood vessels are specific for vascular smooth muscle. No significant changes were observed in the 3-methylhistidine levels between SHR, WKY and treated SHR when expressed per mg of protein (Fig 4.13(a)). However, a strain difference did exist when the data were expressed in absolute terms but the values were not affected by treatment (Fig. 4.13(b)).

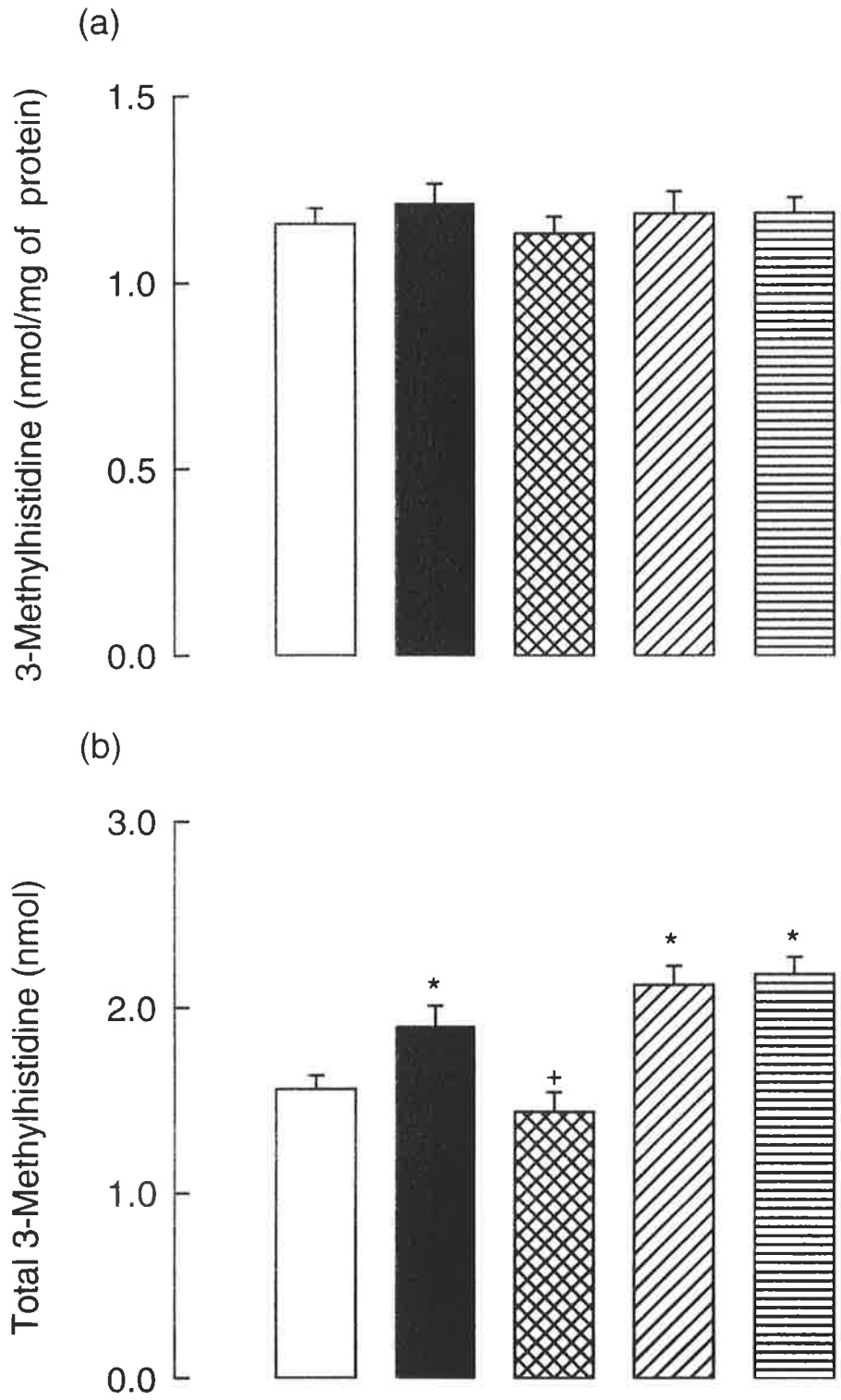


Fig. 4.11. 3-methylhistidine levels found in the superior mesenteric artery from normotensive WKY rats, SHR controls, SHRs treated with quinapril, SHRs treated with doxazosin and SHRs treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean for 8 rats ± s.e.m.

* $p<0.001$ compared with normotensive WKY rats.

+ $p<0.001$ compared with SHR controls.

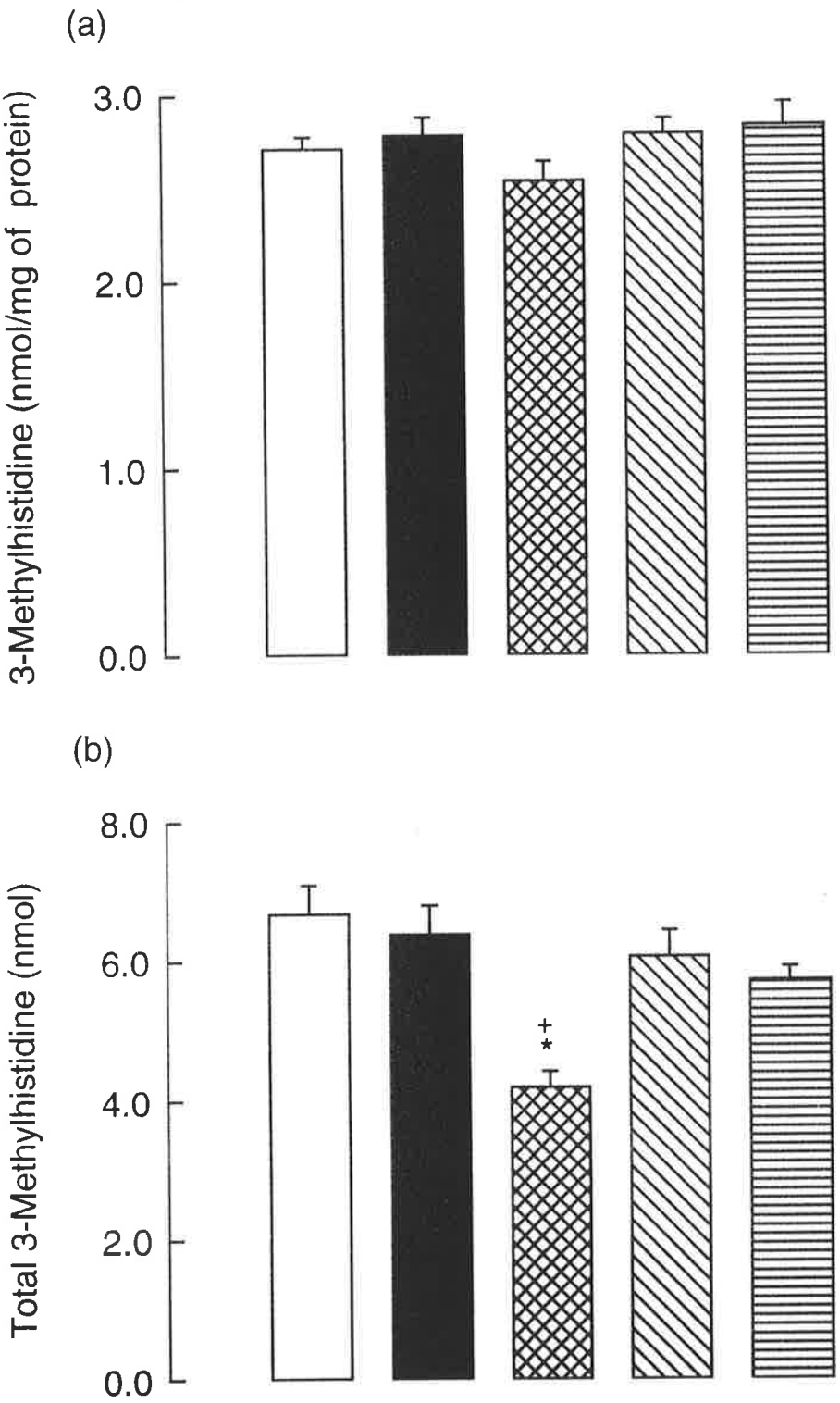


Fig. 4.12. 3-methylhistidine levels found in mesenteric branches from normotensive WKY rats, SHR controls, SHRs treated with quinapril, SHRs treated with doxazosin and SHRs treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean for 8 rats ± s.e.m.

* $p<0.001$ compared with normotensive WKY rats.

+ $p<0.001$ compared with SHR controls.

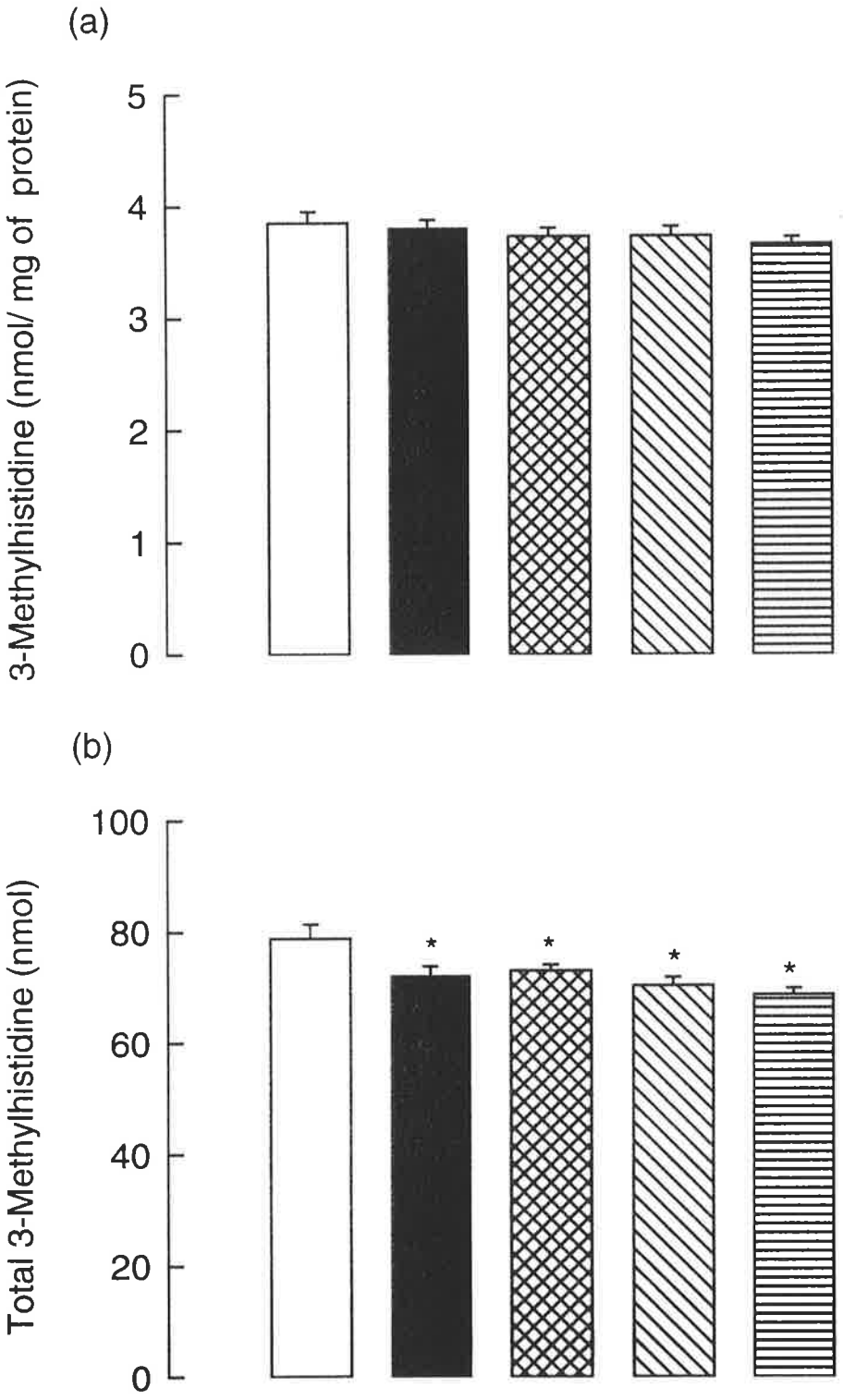


Fig. 4.13. 3-methylhistidine levels found in the vas deferens from ☐ normotensive WKY rats, ■ SHR controls, ▩ SHRs treated with quinapril, ▨ SHRs treated with doxazosin and ▤ SHR treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value for 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

4.3.3.2 DNA content

Fig. 4.14. shows the DNA levels per mg of protein in the aorta. There was no significant difference in the values identified in the tissues from the SHR and WKY rats. With respect to the treatment groups, chronic quinapril therapy enhanced the DNA levels in the aorta (Fig. 4.14).

In the case of the superior mesenteric artery, DNA levels were significantly elevated in the untreated, quinapril and doxazosin treated SHRs in comparison to levels in tissues from normotensive WKY rats when expressed per mg of protein (Fig. 4.15(a)). However, the absolute levels of DNA in this tissue from doxazosin and diltiazem treated SHRs and untreated SHRs were significantly greater than in tissues from normotensive control rats (Fig. 4.15(b)). Although significance was not reached, quinapril treatment prevented the increase in absolute levels of DNA in the superior mesenteric artery when compared with levels in tissues from untreated SHRs.

In the mesenteric branches, the levels of DNA expressed per mg of protein were greater in untreated and treated SHRs when compared to tissues from WKY rats (Fig. 4.16(a)). In addition, mesenteric vessels taken from quinapril treated SHRs displayed a significantly higher level of DNA than tissues from untreated SHRs (Fig. 4.16(a)). In absolute terms, there was no significant difference between strains and treatment groups although tissues from the quinapril treated group did display a lower level of DNA (Fig. 4.16(b)).

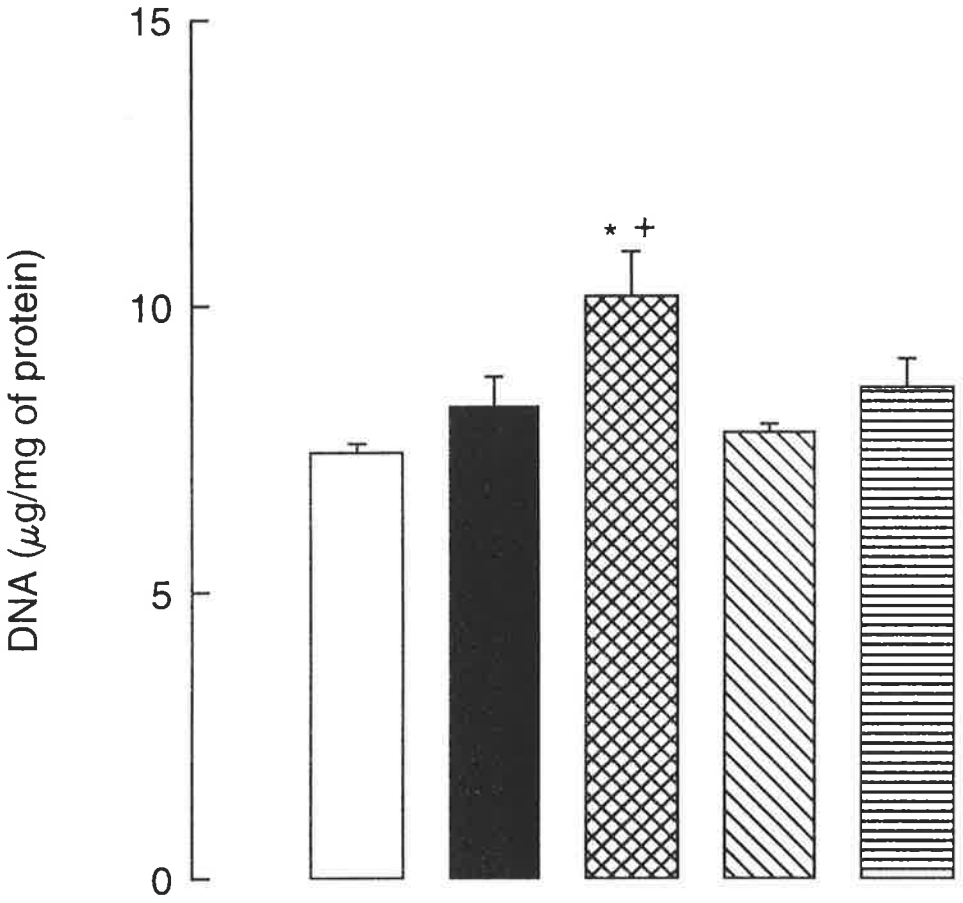


Fig. 4.14. Aortic DNA levels from ☐ normotensive WKY rats, ■ SHR controls, ▨ SHRs treated with quinapril, ▨ SHRs treated with doxzosin and ▤ SHRs treated with diltiazem. Each bar represents the mean value for 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p<0.05$ compared with SHR controls.

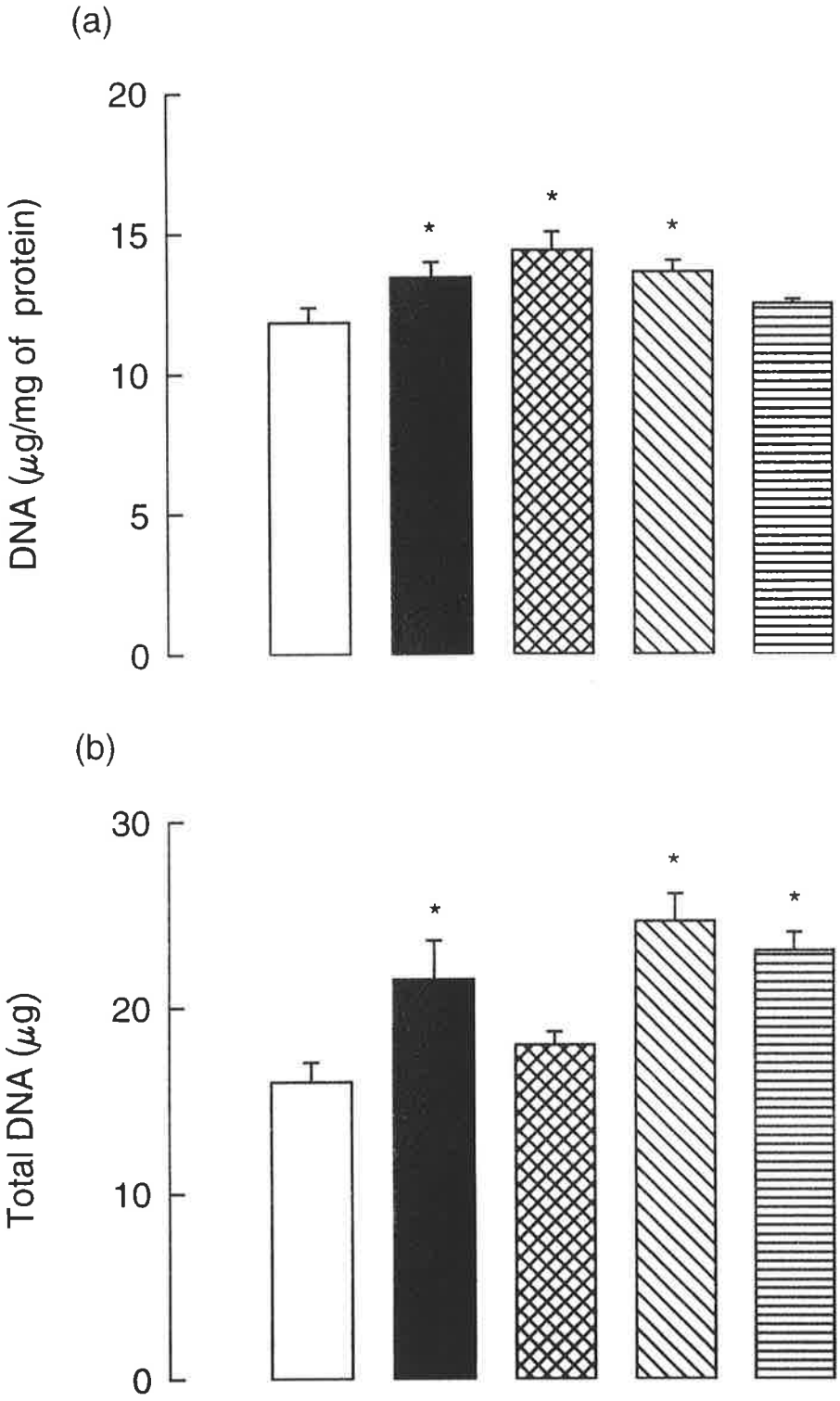


Fig. 4.15. DNA levels found in superior mesenteric artery from ☐ normotensive WKY rats, ■ SHR controls, ▨ SHRs treated with quinapril, ▧ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

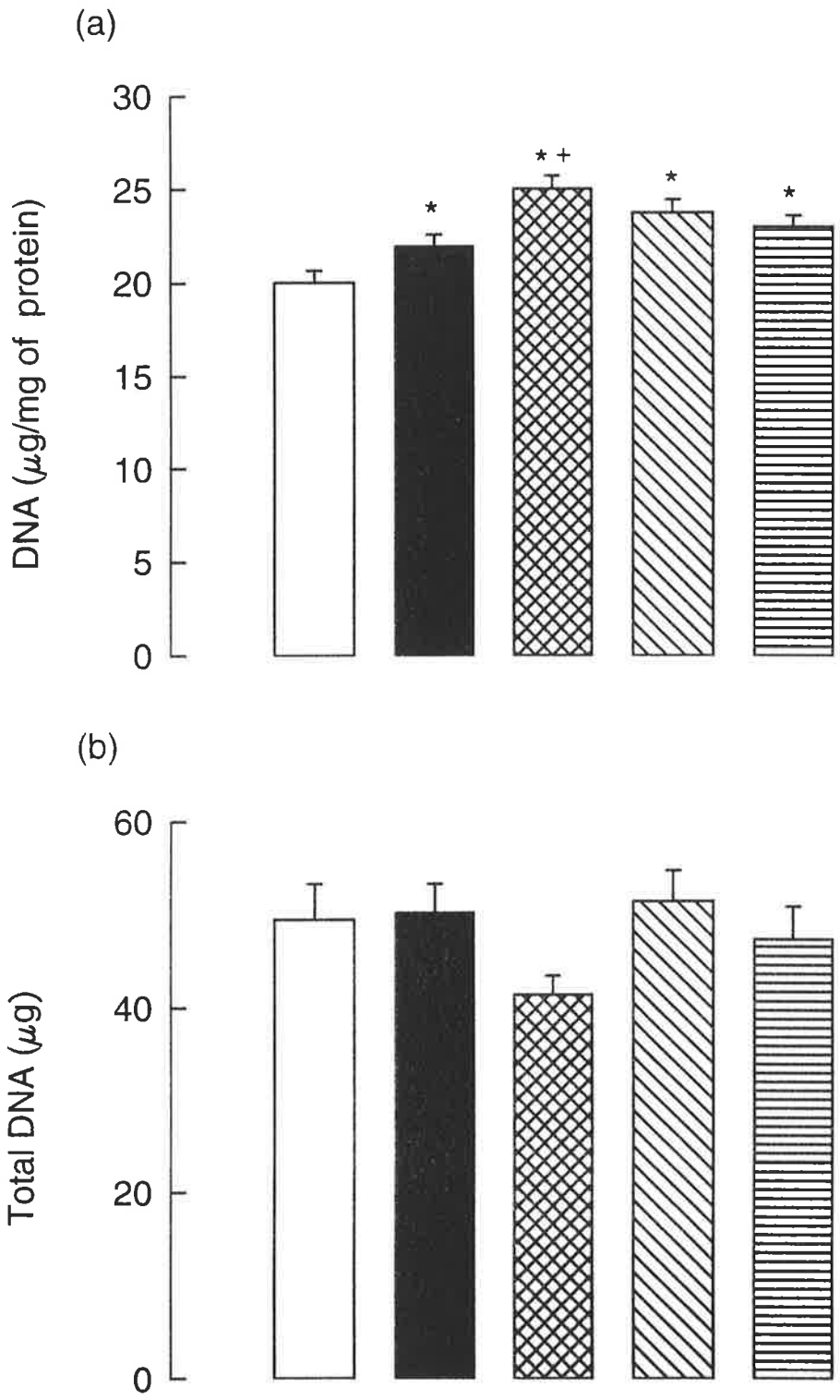


Fig.4.16. DNA levels found in mesenteric branches from ☐ normotensive WKY rats, ■ SHR controls, ▨ SHRs treated with quinapril, ▨ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p< 0.05$ compared with SHR controls.

The vas deferens taken from untreated SHRs demonstrated a small but significant increase in DNA levels relative to protein content when compared to levels in the vas deferens from WKYs but this increase was not affected by any of the treatments (Fig. 4.17(a)). There was no significant difference among the groups when the data was expressed in absolute terms (Fig. 4.17(b)).

4.3.3.3 Protein content

The absolute levels of proteins were significantly decreased in both the superior mesenteric artery (Fig. 4.18(a)) and mesenteric branches (Fig. 4.18(b)) from SHRs treated with quinapril when compared with tissues from untreated SHRs. Mesenteric branches from quinapril treated SHRs also showed significantly reduced levels of proteins when compared to levels in tissues from WKY rats (Fig. 4.18(b)). Chronic doxazosin and diltiazem treatment resulted in an increase in protein levels only in the mesenteric artery when compared to tissues from WKY rats (Fig. 4.18(a)).

Protein levels in the vas deferens were similar amongst all groups (Fig. 4.19).

4.3.4 Correlation analysis

Total 3-methylhistidine, DNA and protein content in both the superior mesenteric artery and its branches did not correlate with the prevailing systolic blood pressure in WKY rats and untreated and treated SHRs (Fig. 4.20, 4.21 and 4.22).

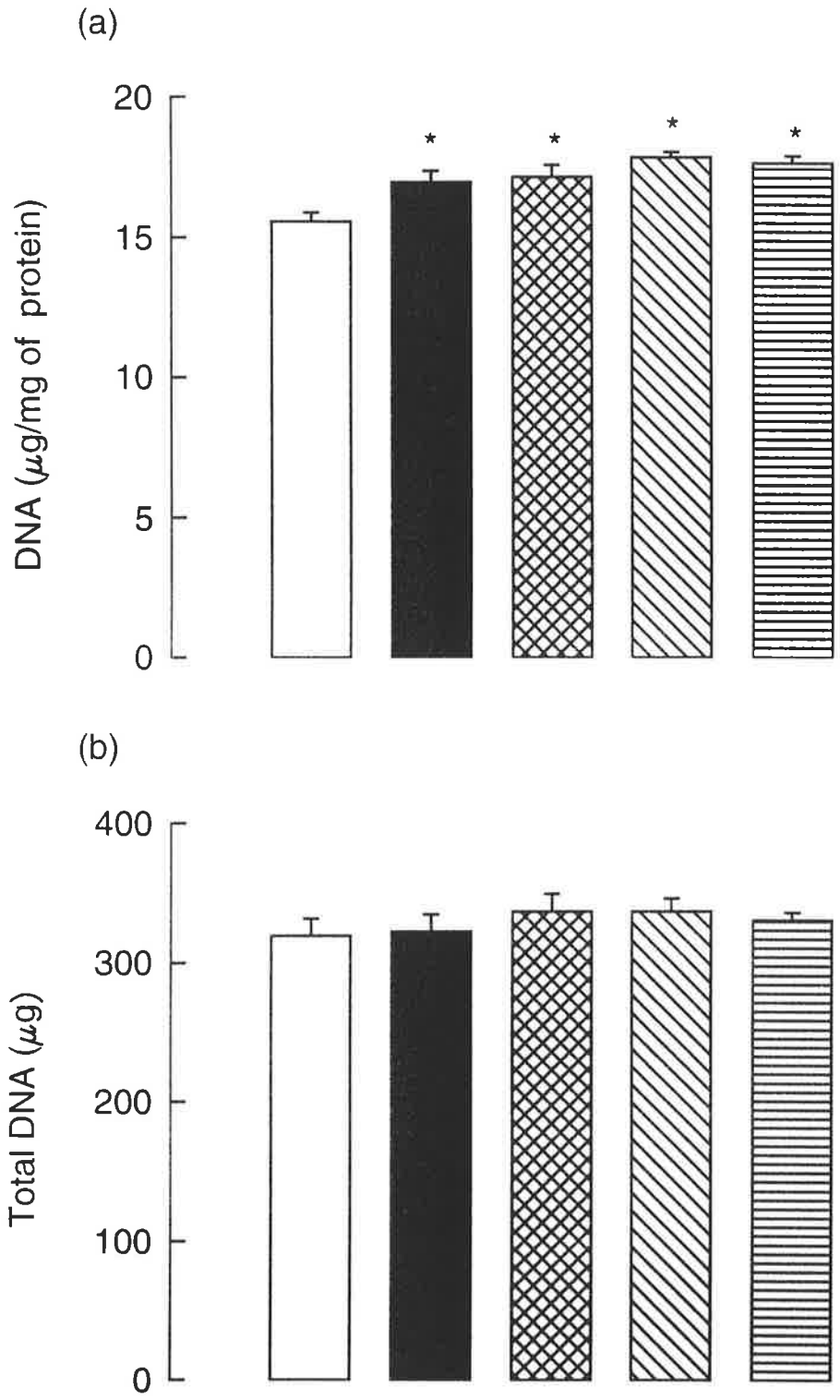


Fig. 4.17. DNA levels found in the vas deferens from ☐ normotensive WKY rats, ■ SHR controls, ▨ SHRs treated with quinapril, ▧ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 8 rats ± s.em.

* $p<0.05$ compared with normotensive WKY rats.

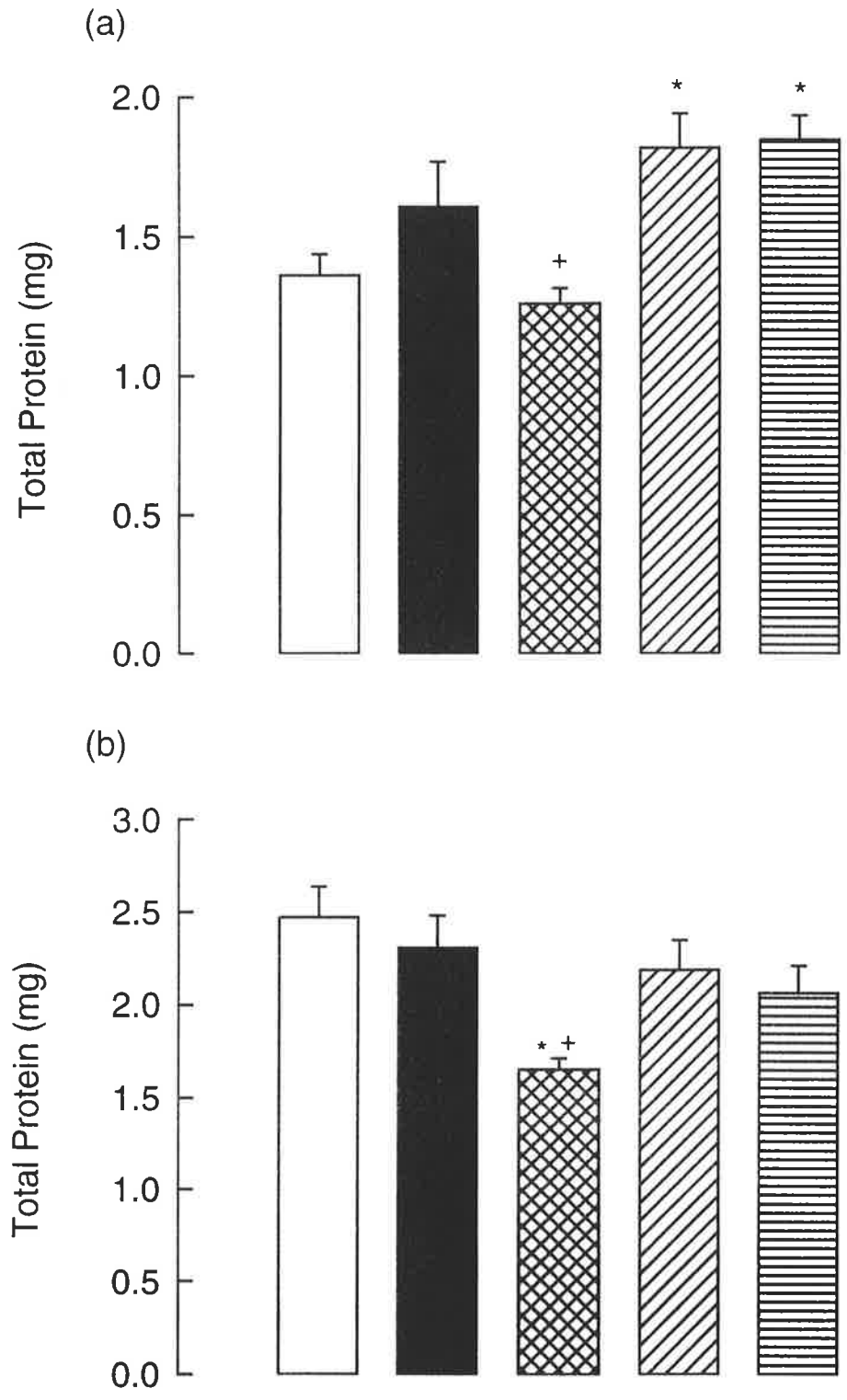


Fig. 4.18. Absolute protein levels found in (a) superior mesenteric artery and (b) mesenteric branches from ☐ normotensive WKY rats, ■ SHR controls, ▨ SHRs treated with quinapril, ▨ SHRs treated with doxazosin and ▤ SHRs treated with diltiazem. Each bar represents the mean value 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

+ $p< 0.05$ compared with SHR controls.

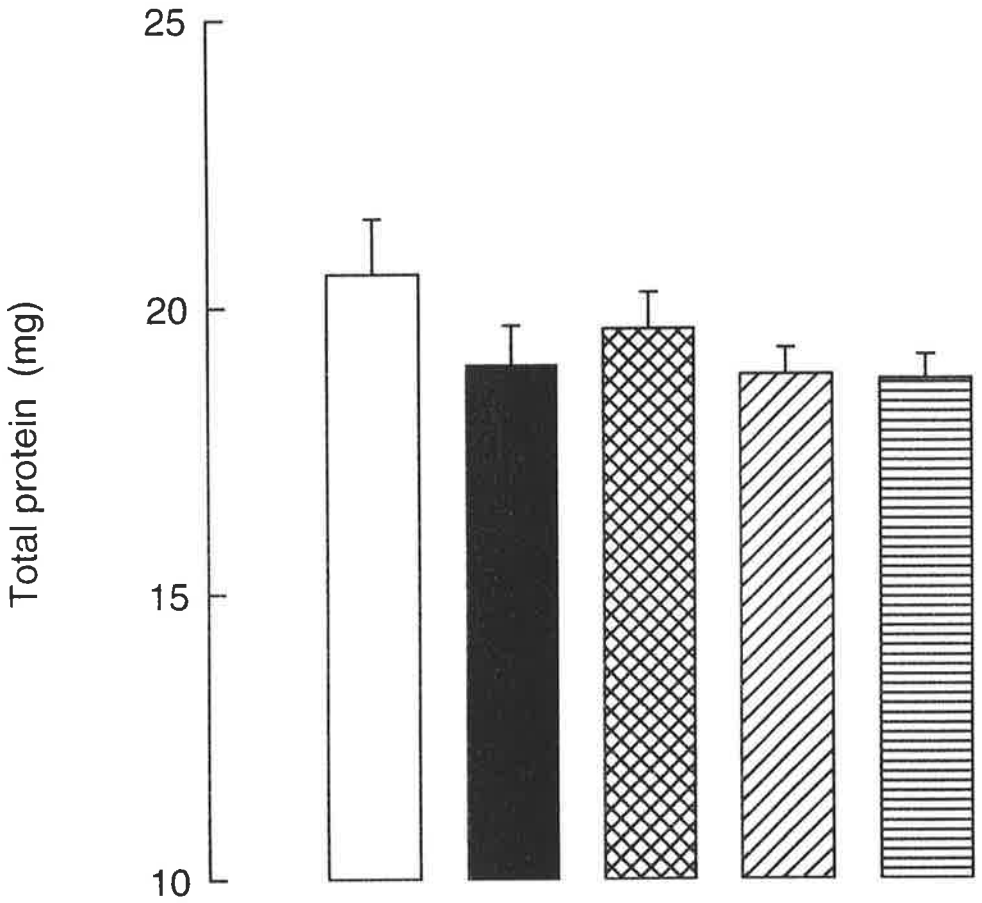


Fig. 4.19. Absolute protein levels found in the vas deferens from normotensive WKY rats, SHR controls, SHRs treated with quinapril, SHRs treated with doxazosin and SHRs treated with diltiazem. Each bar represents the mean value for 8 rats ± s.e.m.

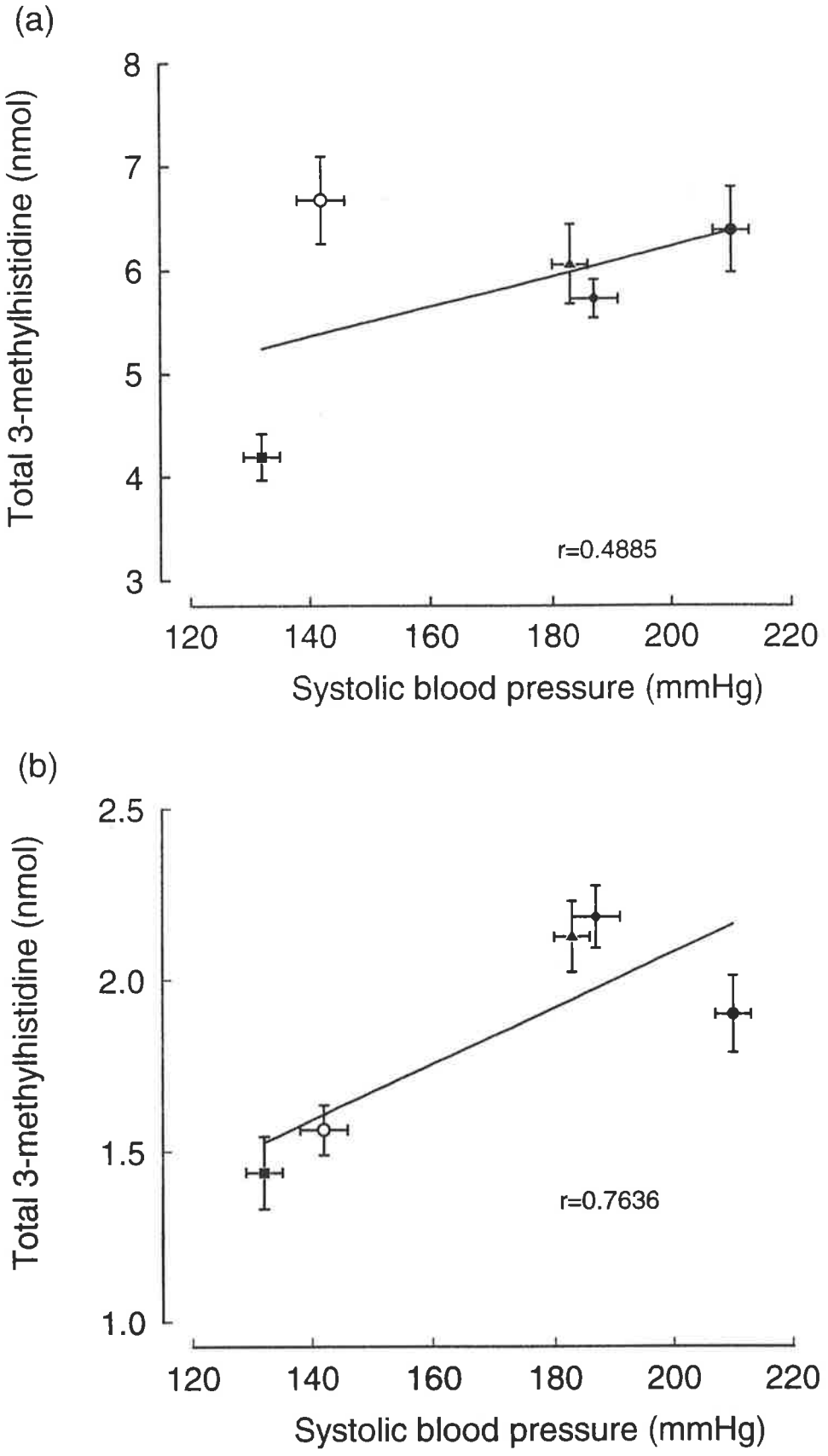


Fig. 4.20. Relationship between mean systolic blood pressure and 3-methylhistidine levels in (a) mesenteric branches and (b) the superior mesenteric artery from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 8 to 10 rats ± s.e.m.

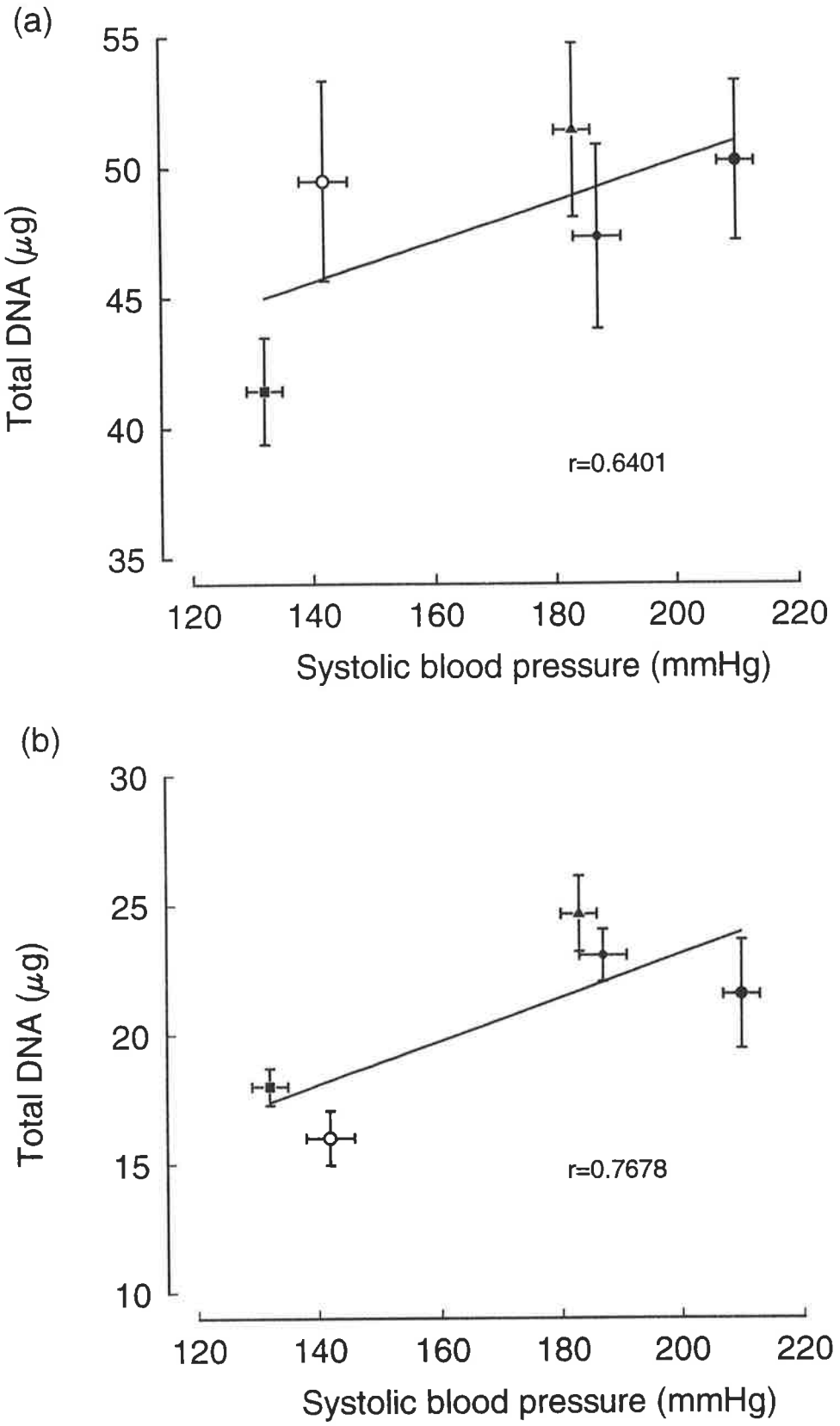


Fig. 4.21. Relationship between mean systolic blood pressure and absolute DNA levels in (a) mesenteric branches and (b) the superior mesenteric artery from o normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 8 to 10 rats ± s.e.m.

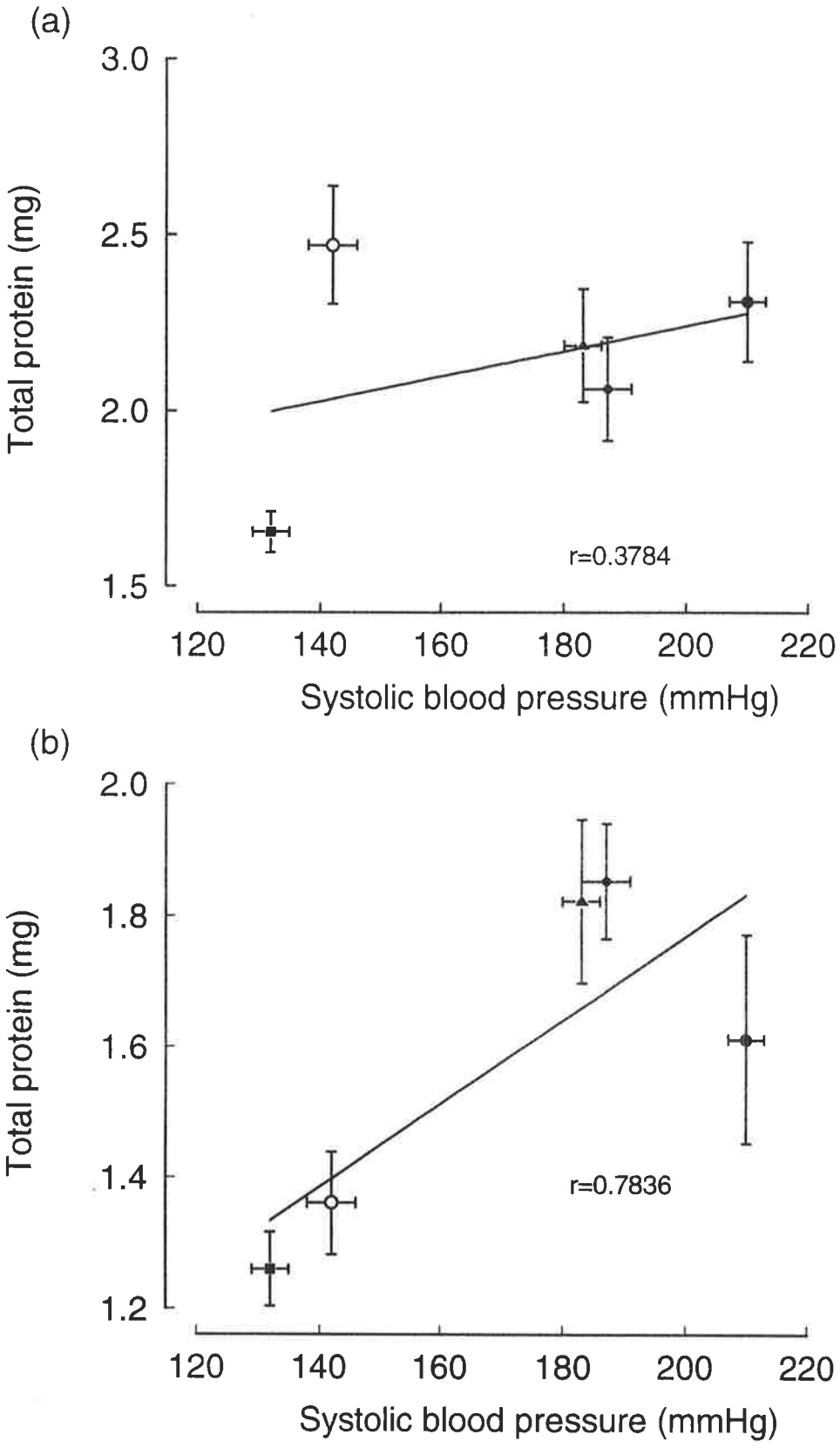


Fig. 4.22. Relationship between mean systolic blood pressure and absolute protein levels in (a) mesenteric branches and (b) the superior mesenteric artery from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 8 to 10 rats ± s.e.m.

Vascular reactivity to potassium chloride in the perfused mesenteric vasculature correlated well with systolic blood pressure in WKY rats and untreated and treated SHRs (Fig. 4.23). However, a simple relationship did not existed between the reactivity of the mesenteric vasculature and 3-methylhistidine content of the mesenteric branches (Fig. 4.24). In contrast vascular contractility to noradrenaline of the thoracic aorta did not correlate with systolic blood pressure in WKY rats and untreated and treated SHRs (Fig.4.25).

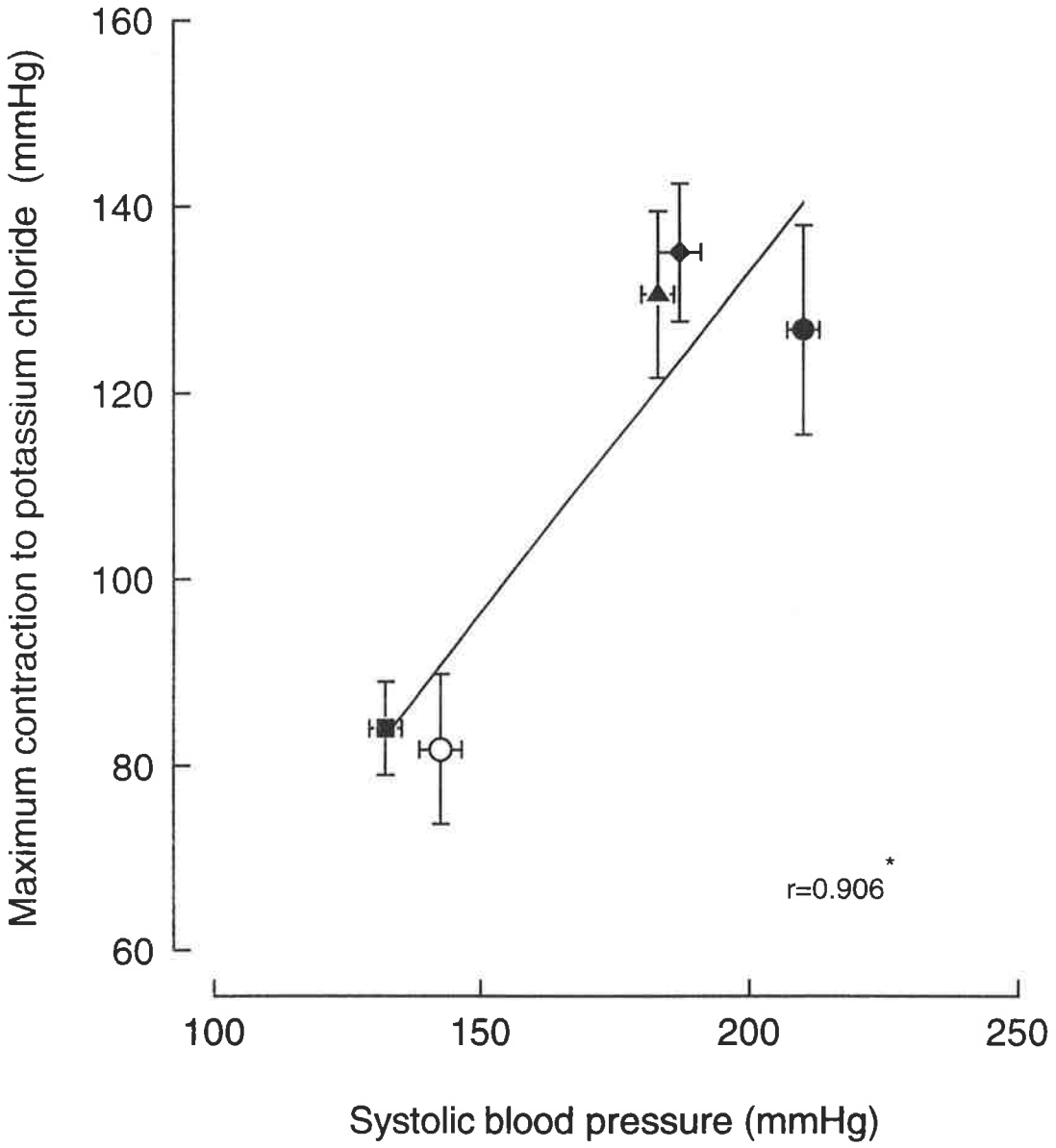


Fig. 4.23. Relationship between mean maximum responses to potassium chloride in perfused mesenteric preparations and systolic blood pressure from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 6 to 10 rats ± s.e.m.

* significant correlation of all groups, p=0.0337.

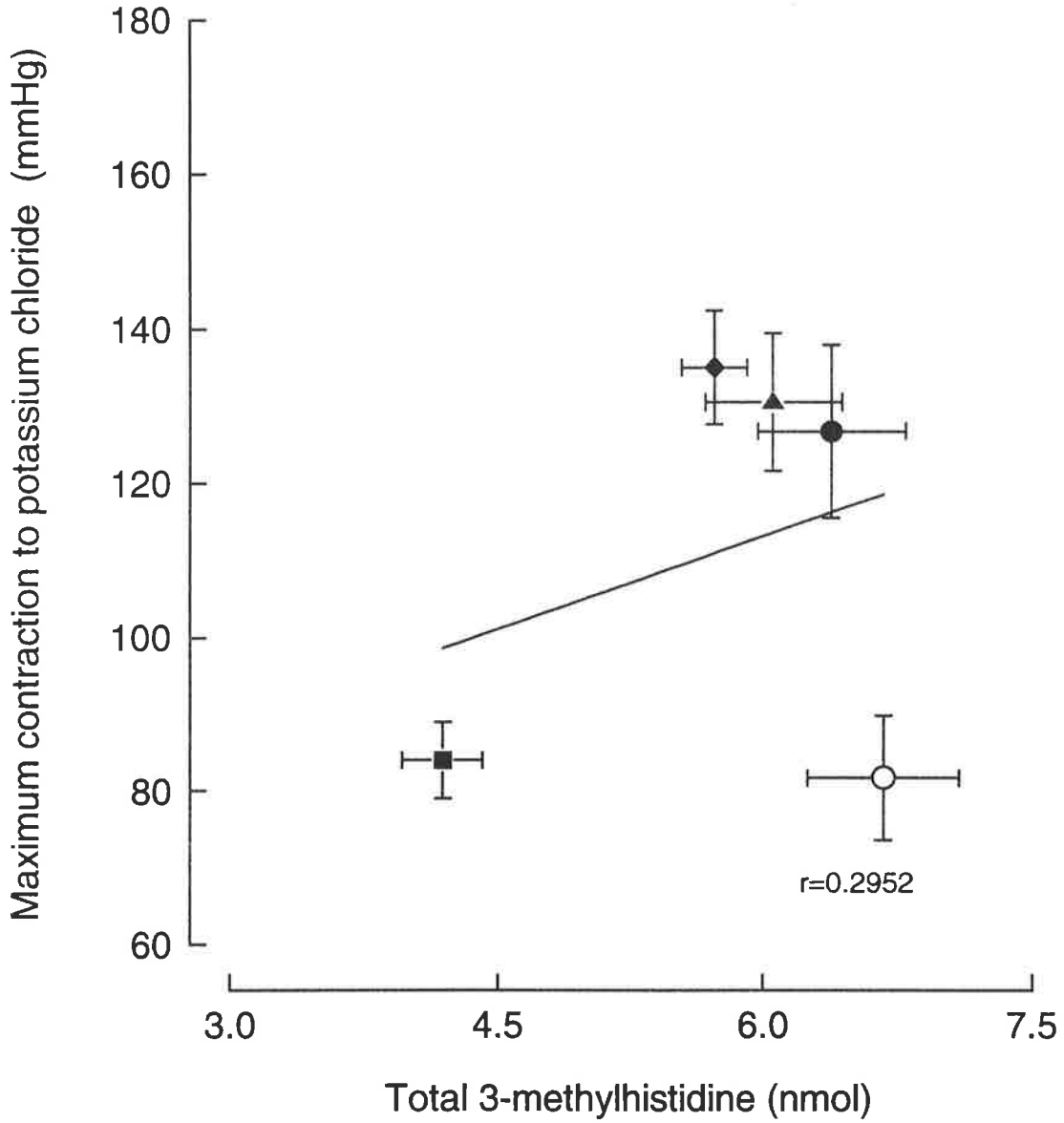


Fig. 4.24. Relationship between mean maximum responses to potassium chloride in perfused mesenteric preparations and 3-methylhistidine levels in mesenteric branches from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 6 to 8 rats ± s.e.m.

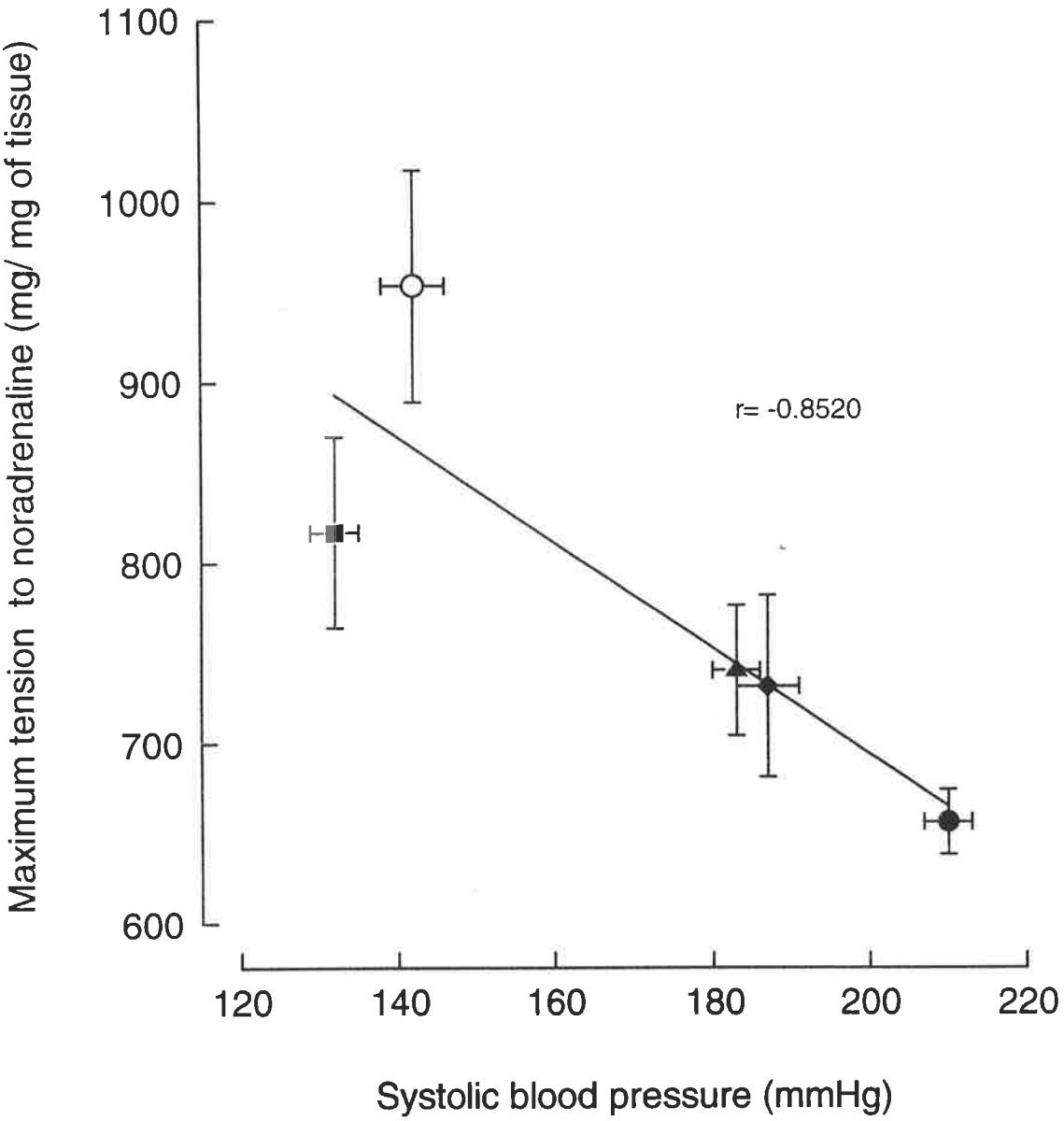


Fig. 4.25. Relationship between mean systolic blood pressure and maximum tension to noradrenaline of thoracic aortae from ○ normotensive WKY rats, ● SHR controls, ■ SHRs treated with quinapril, ▲ SHRs treated with doxazosin and ◆ SHRs treated with diltiazem. Each point represents the mean value of 6 to 8 rats ± s.e.m.

4.4 DISCUSSION

4.4.1 Blood pressure

The present study confirms results of previous studies by demonstrating that early treatment of genetically hypertensive animals with an ACE inhibitor prevents the full expression of hypertension in adulthood (Sada *et al* 1989; Sada *et al* 1990; Richer *et al* 1991). In contrast, the blood pressure of SHRs treated with either diltiazem or doxazosin remained significantly higher than that of WKY rats albeit lower than the untreated SHR. Similar results have been obtained with diltiazem by other investigators (Narita *et al* 1983) but in the case of doxazosin either moderate (Chichester & Rodgers, 1987) or no effects (Young *et al* 1993) on systolic blood pressure have been observed. The differences observed with respect to doxazosin may be due to there being differences in the dose of the antagonist employed and treatment period.

The prevention of full expression of hypertension in the SHR treated with quinapril was also associated with the prevention of cardiac hypertrophy. The part prevention in the rise in blood pressure seen in the SHRs treated with either doxazosin or diltiazem was associated with a small reduction in left ventricular hypertrophy. In the present study, there was a correlation with left ventricular weight and blood pressure suggesting that the incomplete regression of left ventricular hypertrophy may be related to the incomplete control of blood pressure alone. Nonetheless, it should be noted that the literature also presents evidence which suggests that left ventricular hypertrophy may not be completely attributed to the increased pressure load (Sen *et al* 1974; Tubau *et al* 1987). For example, the

ACE inhibitor ramipril when administered at nonhypotensive doses is able to induce complete regression of cardiac hypertrophy provoked by aortic banding in rats (Linz *et al* 1991).

4.4.2 Vascular reactivity

4.4.2.1 Aortic ring preparations

The present study demonstrated that thoracic aortic ring preparations from SHRs have decreased contractility to noradrenaline when compared with those from normotensive rats. These results are consistent with those of a number of other investigators (Shibata *et al* 1973; Sun & Hanig, 1983; Silva *et al* 1994). Attenuated responses in aortic rings from SHRs have not been seen only with noradrenaline but also with potassium chloride, barium and angiotensin II when compared with aortae from normotensive controls (Shibata *et al* 1973). Therefore, the low contractility of untreated SHR aortae is not attributable to alterations of the vascular adrenergic receptors alone. The decreased contractile response may be due to a number of factors such as changes in the wall of the aorta caused by increased formation of collagen (Iwatsuki *et al* 1977; Olivetti *et al* 1982) and therefore reduced distensibility, increased intracellular calcium which is associated with increased myogenic tone (Grammas *et al* 1991) and increased activity of the sodium/potassium pump and overactivity of calcium-dependent potassium channels (Silva *et al* 1994). However, the sensitvity of the SHR aortae (EC_{50}) to noradrenaline was similar to WKY aortae, which is consistent with the findings of other investigators (Hallback *et al* 1971; Shibata *et al* 1973) indicating no change in the affinity and /or number of adrenoceptors.

Of the antihypertensive treatments examined, only quinapril was able to augment contractility to noradrenaline in the aorta without any effect on sensitivity. ACE inhibitors have been shown to reduce collagen and elastin levels (Richer *et al* 1991; Albaladejo *et al* 1994; Benetos *et al* 1994), prevent hypertrophy of the media (Owens, 1987; Richer *et al* 1991; Freslon *et al* 1992), reduce myogenic tone by blocking calcium influx (Sada *et al* 1990) and prevent increased passive permeability of the vascular membrane (Ito *et al* 1981) of the aorta. All of these processes may contribute to the augmented responses seen in aortae from quinapril treated SHRs.

Both diltiazem and doxazosin were unable to prevent the attenuated responses seen in the aorta from untreated SHRs. Production and accumulation of elastin and collagen in the aorta peaks about 4-6 weeks after birth (Keeley *et al* 1991). It has also been reported that the response of increased elastin and collagen production is rapid and begins at relatively low thresholds of increasing blood pressure (Keeley *et al* 1991). Therefore, in the case of both doxazosin and diltiazem therapy, where blood pressure appeared not to be affected after four weeks of treatment, (at this time point the animals were 9 weeks of age), most of the connective tissue could have been laid down thereby reducing distensibility which may contribute to the attenuated responses to noradrenaline seen in the aortae from these SHRs.

In addition, the present study also demonstrated that contractility of the thoracic aorta and systolic blood pressure did not correlate well . However, the power to deny this relationship was not great and therefore caution is required when interpreting these results. It appears that the relationship in the aorta is inverse and may be a result of the structural alterations seen in the aorta when

systolic blood pressure is elevated. Pressure independent mechanisms may also influence contractility. Evidence exists to suggest that at least medial hypertrophy of the aorta is simply not a response to increased systolic blood pressure (Lee & Smeda, 1985; Owens, 1987). Therefore diltiazem and doxazosin may not be able to prevent aortic medial hypertrophy (irrespective of the systolic blood pressure) which could also contribute to the attenuated responses to noradrenaline.

4.4.2.2 Perfused mesenteric vascular bed

In the present study it was evident that responses to nerve stimulation at the higher frequencies were much greater in perfused mesenteric preparations from untreated SHRs when compared with the normotensive WKY controls. The results are in agreement with Ekas & Lokhandwala (1981), Longhurst *et al* (1986), Hano and Rho (1989) and Inoue *et al* (1990) and may in part reflect the greater density of noradrenergic nerve fibres seen in the SHR vasculature (Head, 1989). Vascular reactivity not only to nerve stimulation but also to noradrenaline, phenylephrine, vasopressin and potassium chloride was increased in the preparations from SHRs. The concomitant increase in maximum contractility to potassium chloride indicates that a nonspecific increase in reactivity is present. Taken in conjunction with the increased maximum and the slope of the concentration-response curve the foregoing results are consistent with structural changes ie. an increase in the media:lumen ratio (Folkow & Karlstrom, 1987).

An increased sensitvity of the arteries to agonists may in part contribute to increased contractile activity of the mesenteric vasculature from SHRs. In this study there was a significant leftward shift in the arginine vasopressin-response curves

but not with potassium chloride-response curves in the perfused mesenteric bed of the SHR when compared to controls. The indication of an increase in sensitivity of the mesenteric vasculature to vasopressin but not to potassium chloride suggests that the functional state of the individual smooth muscle cells of the SHR was not altered (Kong *et al* 1991) but there is possibly a change in the cellular events that are triggered upon receptor activation.

An increase in sensitivity to noradrenaline has been demonstrated by Haeusler and Haefely (1970) in the mesenteric vasculature and by Lais and Brody (1978) in the hindquarter vasculature of adult SHRs. In contrast, other investigators have demonstrated an increased sensitivity to noradrenaline only in the presence of cocaine which inhibits the enhanced neuronal uptake and masks the increased sensitivity to noradrenaline in the mesenteric vasculature from SHRs (Mulvany *et al* 1980; Longhurst *et al* 1986). These functional changes may be attributed to a number of factors. Mulvany and Nyborg (1980) have demonstrated that SHR mesenteric resistance vessels are more sensitive to calcium when calcium influx pathways are activated by noradrenaline suggesting that these channels are more permeable to calcium or there is a larger number of them. However, when depolarised by potassium there was no difference in calcium sensitivity suggesting that the potential channels in SHR and WKY vessels were similar (Mulvany & Nyborg, 1980). These results are consistent with the results of the present study whereby sensitivity to potassium chloride in preparations from SHRs was not different to that from preparations from WKYs. Furthermore, since in this study, the dose-response curves to vasopressin were shifted to the left it could therefore be suggested that the increase in calcium sensitivity after activation of calcium influx by noradrenaline (that is seen by other investigators in mesenteric resistance vessels)

may be nonspecifically related to receptor-operated calcium channels.

Furthermore alterations in the Na^+-H^+ exchange of vascular smooth muscle cells (Berk *et al* 1989), enhanced vascular phospholipase C (Uehara *et al* 1988) and altered calcium handling (Kwan, 1985; Lau & Eby, 1985) in the SHR vasculature may also influence contractility. In addition, it has been demonstrated that SHR arteries display significantly faster velocities of narrowing than do the normotensive WKY arteries which can also contribute to the increased ability of SHR arteries to constrict (Packer & Stephens, 1984).

Long term treatment of SHRs with quinapril, but not with diltiazem or doxazosin, was associated with a non specific attenuation of responses in the mesenteric vasculature. The responses obtained to nerve stimulation although attenuated with quinapril treatment still remained significantly greater than responses obtained in preparations from WKY rats. These results could reflect the greater density of sympathetic nerves in the SHR vasculature which exists as early as twenty days of age (Donohue *et al* 1988). Furthermore, it has been demonstrated that the sympathetic nervous system is not affected by ACE inhibition in terms of catecholamine levels since long term therapy with an ACE inhibitor does not alter catecholamine levels in the mesenteric arteries and plasma of the SHR (Lee *et al* 1991). In addition, biosynthesis and storage of catecholamines in the terminal reticulum of the heart and adrenal medulla from SHRs are not influenced by ACE inhibition (Dominiak *et al* 1987). Therefore, in the light of the above studies it could be suggested that the attenuated responses to nerve stimulation seen after chronic quinapril treatment do not reflect its effects on catecholamine synthesis or storage. Nevertheless, ACE inhibition has been demonstrated both (*in vivo* and *in vitro*) to have sympathoinhibitory effects (Antonaccio & Kerwin, 1981; Collis &

Keddie, 1981) since angiotensin II has been shown to enhance noradrenaline release (Nilsson & Folkow, 1982; Jonsson *et al* 1993), inhibit uptake of noradrenaline (Jackson & Campbell, 1979) and because the sensitivity of presynaptic angiotensin II receptor is enhanced in the SHR (Cline, 1985).

Quinapril treatment also lowered the maximal responses to potassium chloride of the perfused mesenteric bed and the slope of the concentration-response curve to potassium chloride which is in agreement with morphometric studies demonstrating that ACE inhibitors reduce media:lumen ratios in mesenteric resistance vessels from SHRs (Lee *et al* 1991; Harrap *et al* 1993; Shaw *et al* 1995). In addition, ACE inhibition has been shown to prevent the increased velocities of shortening seen in the SHR arteries and thus also reduce the degree of narrowing seen in the SHR (Packer & Stephens, 1984).

Consistent with the data from the previous study where DOCA-salt treated WKYs were used (Chapter 3), preparations from doxazosin treated SHRs also demonstrated attenuated responses to nerve stimulation, noradrenaline and phenylephrine but not to arginine vasopressin and potassium chloride suggesting a specific influence of doxazosin on adrenoceptors. Similar responses have been seen *in vivo*, whereby chronic treatment of SHRs with doxazosin resulted in attenuation of blood pressure responses to noradrenaline and phenylephrine and an associated reduction in sensitivity to both agonists (Young *et al* 1993).

Diltiazem treatment was also unable to prevent the augmented responses to any of the agonists or nerve stimulation in perfused mesenteric preparations so characteristic of the untreated SHR. Therefore, the data suggest that both diltiazem and doxazosin therapies were unable to prevent the structural changes of the mesenteric vasculature seen in the untreated SHR.

4.4.3 Biochemical parameters

In this study, the total content of 3-methylhistidine, DNA and proteins in general, regardless of how they were expressed, were not significantly different in the mesenteric branches of SHRs when compared with those from WKY rats suggesting that growth of the vessel wall was not enhanced nor was there a selective increase in the contractile proteins in this model of genetic hypertension. These results are in contrast with the evidence found in the literature in which increases in DNA content (Brayden *et al* 1983) and 3-methylhistidine levels (Jonsson *et al* 1991) have been demonstrated in the mesenteric vasculature of the SHR when compared with the vasculature of WKY rats. However, it should be noted that total 3-methylhistidine, DNA and protein content of the superior mesenteric artery was significantly elevated in the SHR, indicating the significance of regional differences in the mesenteric vascular bed.

Morphometric studies at various ages have also shown predominantly hyperplasia of the smooth muscle cells and increased adventitial area in the mesenteric branches from SHRs (Lee, 1985; Mulvany *et al* 1985; Owens *et al* 1988). However, in the case of contractile proteins the results of the present study are in agreement with results of previous studies from our laboratory in which total actin was measured and no difference in the level of actin in mesenteric branches was found between SHRs and WKY rats (Kadirgamanathan, 1995). It is also not inconceivable that the techniques used in this study were unable to detect small but yet physiologically significant increases in 3-methylhistidine, DNA and protein in the resistance vessels since it has been stated that a 5% increase in growth would be able to cause a substantial increase in peripheral resistance (Korner *et al* 1993).

Unlike diltiazem and doxazosin, quinapril treatment resulted in a decrease in the absolute levels of 3-methylhistidine, DNA and total protein in the mesenteric branches when compared with both untreated SHR and WKY control rats which would be suggestive of an inhibition of vessel growth by quinapril in the SHR.

In addition to the existence of a local, independent renin-angiotensin system in the blood vessel wall, angiotensin II has also been implicated in influencing vascular wall structure (Dzau, 1988); however, the exact mechanism is unclear. Angiotensin II has been demonstrated to induce hypertrophy of cultured rat aortic smooth muscle cells which is associated with widespread but also selective increases in protein synthesis (Turla *et al* 1991). Re et al (1984) have suggested that angiotensin may bind to receptors on nuclear chromatin and initiate nuclear events that result in protein synthesis and cell proliferation (Re *et al* 1984). Increased hydrolysis of phosphoinositides by angiotensin II via receptor occupation could increase DNA synthesis through the Na^+/H^+ antiport (Wang & Prewitt, 1991). Alternatively, potentiation of sympathetic activity by angiotensin II is another possibility, since it has been demonstrated that the sympathetic nervous system has a role in vascular smooth muscle growth (Bevan, 1975; Lee *et al* 1987). Chronic treatment with ACE inhibitors has been shown to interfere with the growth of cardiovascular tissues in normotensive rats suggesting that angiotensin II may also play a role in normal vascular growth (Owens, 1987; Wang & Prewitt, 1991; Keeley *et al* 1992). Furthermore, since it has been demonstrated that quinapril can block the production of angiotensin II in the rat mesenteric vasculature (Weishaar *et al* 1991), it can be assumed that the decrease in growth of the vasculature seen in the present study during quinapril treatment was due to the prevention of angiotensin II formation. Further support for the involvement angiotensin II comes from studies

which have treated SHRs with an angiotensin II receptor antagonist and vascular hypertrophy has been prevented (Shaw *et al* 1995).

Studies involving the aorta indicated that there was no difference in any of the biochemical parameters between SHRs and WKY rats when these were normalised to the protein level. Other studies have demonstrated that in the aorta the increase in aortic smooth muscle cell mass in the untreated SHR is due to an increase in cell size with no increase in cell number and that cell hypertrophy is accompanied by a proportional increase in ploidy (Owens & Schwartz, 1982). It has also been demonstrated that the contractile protein, actin, content of the SHR aorta was greater than that of WKYs but when expressed as a fraction of total cell protein there was no difference between SHR and WKY rats (Owens *et al* 1981). In contrast Jonsson *et al* showed that 3-methylhistidine content of the aorta from SHRs was similar to that seen in aortae from WKY rats (Jonsson *et al* 1991). Collagen levels have also been shown to be increased in the aorta of SHRs when compared to normotensive controls (Iwatsuki *et al* 1977).

Similar results were obtained with the three different therapies, except in the one case where quinapril treatment resulted in increased DNA levels in the thoracic aorta. Albaladejo *et al* (1994) and Richer *et al* (1991) have demonstrated that quinapril treatment was associated with an increase in the density of aortic wall nuclei with the increase in the density of nuclei being consistent with a decrease in the smooth muscle cell size. Consistent with the above study other investigators have shown aortic smooth muscle cell number to be similar in ACE inhibitor treated and untreated SHRs but aortic smooth muscle cell content and weight is reduced (Owens, 1987). It follows that in the present study the increase in DNA level in aortae from quinapril treated SHRs is due to inhibition of cell growth. Long term

treatment of young SHRs with ACE inhibitors has also been shown to reduce the incidence of smooth muscle cell polyploidism and medial hypertrophy (Collis & Vanhoutte, 1977; Owens, 1987; Freslon *et al* 1992).

In the superior mesenteric artery (also a conducting vessel) the levels of 3-methylhistidine and DNA when expressed per mg of protein were not significantly different in the SHR when compared with levels in tissues from WKY rats. However, absolute levels of 3-methylhistidine, DNA and protein were increased in the superior mesenteric artery from SHRs when compared to those from WKY rats. The increase in DNA levels seen in the superior mesenteric artery from untreated SHRs has also been associated with polyploidy rather than an increase in smooth muscle cell number but this is not as pronounced as in the aorta (Black *et al* 1988). It could be speculated that the increase in protein (although not quite significant) and 3-methylhistidine levels seen in the present study would be associated with hypertrophy of the smooth muscle cells, since hyperplasia has not been seen in the superior mesenteric artery (Owens *et al* 1981; Owens & Schwartz, 1982; Black *et al* 1988) and the increase in DNA was due to polyploidy. Doxazosin and diltiazem therapies were unable to prevent the increase in absolute levels of 3-methylhistidine, proteins and DNA in the superior mesenteric artery. It has been demonstrated that although the ß-antagonist propranolol prevented the rise in blood pressure in SHRs, (the systolic blood pressure being 26 mmHg lower than its untreated counterparts), it had no effect on aortic smooth muscle mass (Owens, 1987).

In the case of quinapril, the increase in total 3-methylhistidine, proteins and DNA content of the superior mesenteric artery was prevented, again suggesting the prevention of vascular growth. The results of the present study being consistent

with the outcome of morphological studies in which the ACE inhibitor captopril prevented hypertrophy of the vessel wall of the superior mesenteric artery in the SHR and treatment had a minimal effect on the number of cells in the smooth muscle layer (Lee *et al* 1991).

The effects on the above parameters seen with quinapril were not present in the vas deferens - a nonvascular smooth muscle tissue. Therefore, these changes seen with quinapril treatment were specific for vascular smooth muscle.

4.5 SUMMARY

In summary the present study demonstrated that a relationship existed between the reactivity of the mesenteric vasculature and systolic blood pressure. However, vascular 3-methylhistidine, DNA and protein, particularly in the mesenteric branches, were not related to systolic blood pressure in a straightforward manner. Furthermore, vascular contractile proteins in the mesenteric branches did not simply follow changes in vascular reactivity. It is therefore suggested that vessel remodeling (ie. the rearrangement of the same amount of vessel material around a smaller lumen) may be involved, which would result in enhanced vascular reactivity.

With respect to the antihypertensive agents used, only chronic quinapril therapy prevented the development of hypertension and attenuated reactivity to various agonists and nerve stimulation in the perfused mesenteric vascular preparation in the doses used. Associated with the hypotensive state and attenuated reactivity were reduced levels of vascular 3-methylhistidine, DNA and proteins in general. The data indicate a strong involvement of angiotensin II in vessel growth and possibly in the remodeling process.

CHAPTER 5

Vascular Reactivity, Contractile Proteins and Blood Pressure After the Withdrawal of ACE inhibitor therapy in the SHR. Are the α_1-adrenoceptors Involved ?

5.1 INTRODUCTION

Antihypertensive therapies in general have been shown to prevent the rise in blood pressure that is seen in the SHR, although discontinuation of most treatments results in blood pressure returning to hypertensive levels (Freslon & Giudicelli, 1983; Christensen *et al* 1989). The ACE inhibitors and angiotensin II receptor antagonists have been shown to be the most effective since after withdrawal of these agents blood pressure remains at a level below that of control SHRs (Morton *et al* 1992). [It must be noted that drug withdrawal is associated with an immediate but small rise in blood pressure (Morton *et al* 1992; Harrap *et al* 1993; Chen *et al* 1995(b); Korner & Bobik, 1995)]. The actual mechanism for this persistent effect on blood pressure is unclear but seems to involve angiotensin II levels and the structure of resistance vessels. In contrast to the persistent effect of ACE inhibitors on blood pressure, contractility and contractile protein levels of mesenteric resistance vessels from SHRs have been demonstrated to increase upon withdrawal of ACE inhibitor treatment (Smid, 1995).

The present study set out to determine whether the initial rise in blood pressure after ACE inhibitor withdrawal involved α_1-adrenoceptors suggesting the involvement of the sympathetic nervous system. This was achieved by either combining the ACE inhibitor treatment with an α_1-adrenoceptor antagonist for 13 weeks and then withdrawing the ACE inhibitor but continuing treatment with the α_1-adrenoceptor antagonist for a further 4 weeks or treating the SHRs with the α_1-adrenoceptor antagonist for only 4 weeks once ACE inhibitor treatment was discontinued. In addition, the relationship between blood pressure, contractility,

contractile protein levels and growth of both conduit and resistance vessels would be examined under the above conditions.

5.2 METHODS

5.2.1 Animal and drug treatments

Male SHR and WKY rats were maintained as described previously (Chapter 2). At 5 weeks of age, SHRs were randomly assigned to either untreated (control), quinapril or quinapril plus doxazosin treated groups. The antihypertensive agents were administered as previously described (Chapter 2). After 13 weeks of treatment quinapril treated animals were further divided into 3 groups: continued quinapril treatment, discontinuation from quinapril therapy and maintained on water or discontinuation from quinapril therapy but maintained on doxazosin for a further 4 weeks. The group given the combination quinapril and doxazosin was withdrawn from quinapril but doxazosin treatment was continued for 4 weeks. All animals were subjected to weekly measurements of body weight. Systolic blood pressures were measured at four weekly intervals during the first 13 weeks of treatment and then every 2 weeks during the remaining four weeks of therapy. After a total of 17 weeks of treatment the animals were killed by decapitation and appropriate tissues were removed (see Chapter 3) and stored as described in Chapter 2.

5.2.2 Aortic ring preparations

The thoracic aortic rings were prepared as previously described (Chapter 2) Cumulative concentration-response curves to noradrenaline (0.12 - 8000 nM) were performed.

5.2.3 Perfused mesenteric vascular bed

The perfused mesenteric vascular bed was prepared as previously described (Chapter 2). Frequency response curves (2-64 Hz) and noncumulative dose-response curves to noradrenaline (0.01-20.0 μg), phenylephrine (0.1-10.0 μg) and potassium chloride (1-32 mg) were performed.

At the end of the protocol the mesenteric preparation was transferred to a petri dish (on ice) containing saline where the mesenteric bed was dissected as described in Chapter 3.

5.2.4 Biochemical analyses

3-Methylhistidine , DNA and protein assays were performed as described previously (Chapter 2). Data are expressed as normalised per mg of protein and absolute amounts. (Data expressed per mg of wet tissue weight are presented in Appendix II).

5.3 RESULTS

5.3.1 Blood Pressure

In the untreated SHR systolic blood pressure rose with time and seemed to plateau at 15 weeks of treatment (Fig. 5.1). Chronic quinapril treatment for 17 weeks prevented the development of hypertension in the SHR (Fig. 5.1). After drug withdrawal, blood pressure rose but was still significantly lower than untreated SHRs (Fig. 5.1). The rise in blood pressure seen after drug withdrawal was not prevented by doxazosin treatment whether doxazosin was given for 17 weeks or for only 4 weeks (Fig. 5.1).

Relative left ventricular mass (left ventricular weight: body weight ratio) of untreated SHRs was significantly increased by comparison with relative left ventricular mass of quinapril treated SHRs (Fig. 5.2(a)). Discontinuation of quinapril therapy resulted in cardiac hypertrophy the extent of which was significantly less than that obtained with untreated SHRs (Fig. 5.2(a)). Consistent with changes in blood pressure, the cardiac hypertrophy seen after withdrawal of quinapril therapy was not prevented by doxazosin (Fig. 5.2(a)). The systolic blood pressure level significantly correlated with the degree of cardiac hypertrophy (Fig. 5.3). Relative renal mass did not differ significantly between any of the groups (Fig. 5.2(b)).

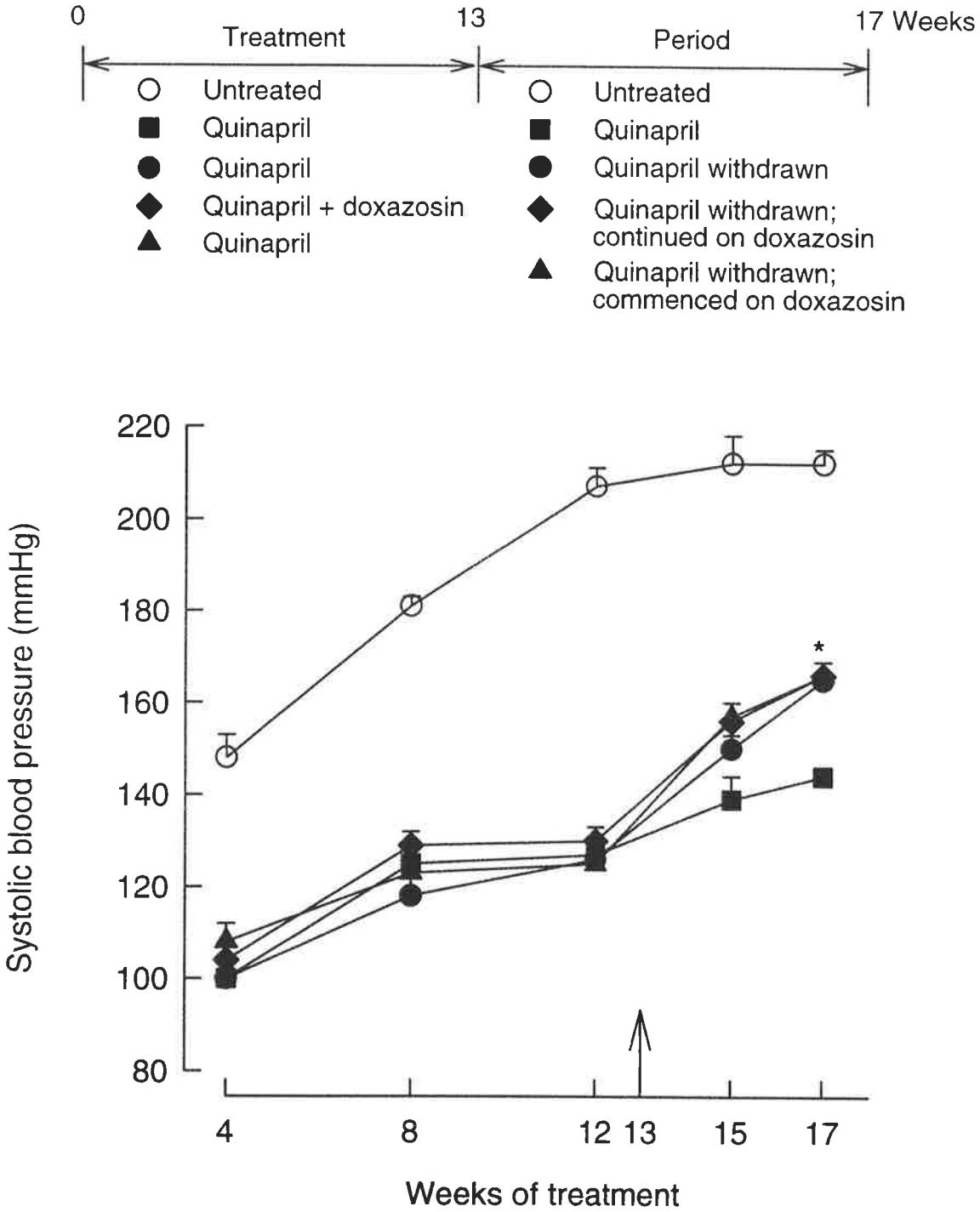


Fig. 5.1. Systolic blood pressure profile of untreated and treated SHRs during a 17 week treatment period. Each point represents the mean value for 5 to 8 rats ± s.e.m. At each time point treated SHR groups were significantly diiferent from untreated SHRs with $p<0.001$.

* $p<0.001$ compared with SHRs treated with quinapril.

↑ = Time point when SHRs were released from quinapril.

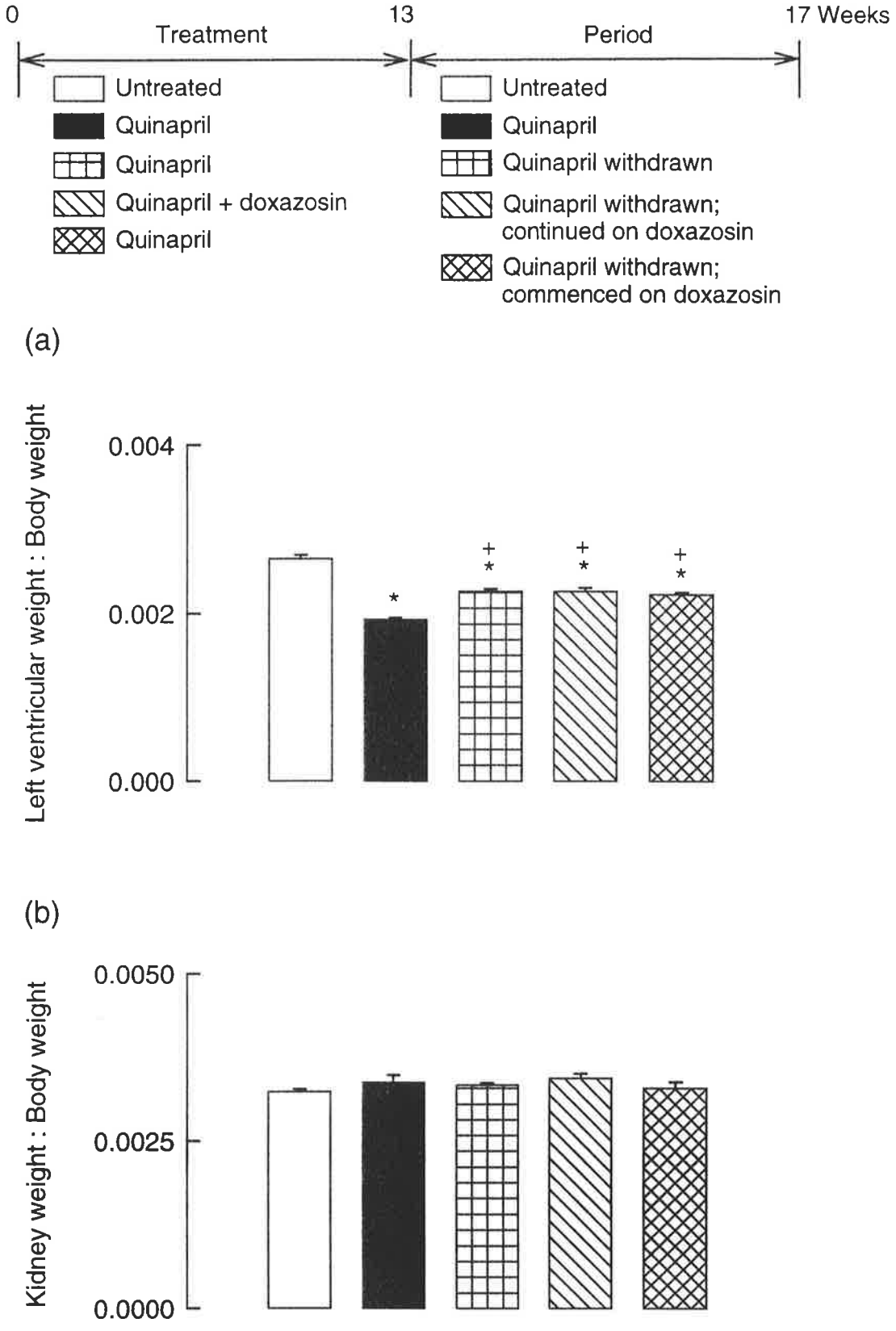


Fig. 5.2. Bar graphs show (a) left ventricular weight: body weight ratio and (b) kidney weight : body weight ratio for untreated and treated SHRs after a 17 week treatment period. Each bar represents the mean value for 8 rats ± s.e.m.

* $p<0.001$ compared with untreated SHRs.

+ $p<0.001$ compared with SHR treated with quinapril.

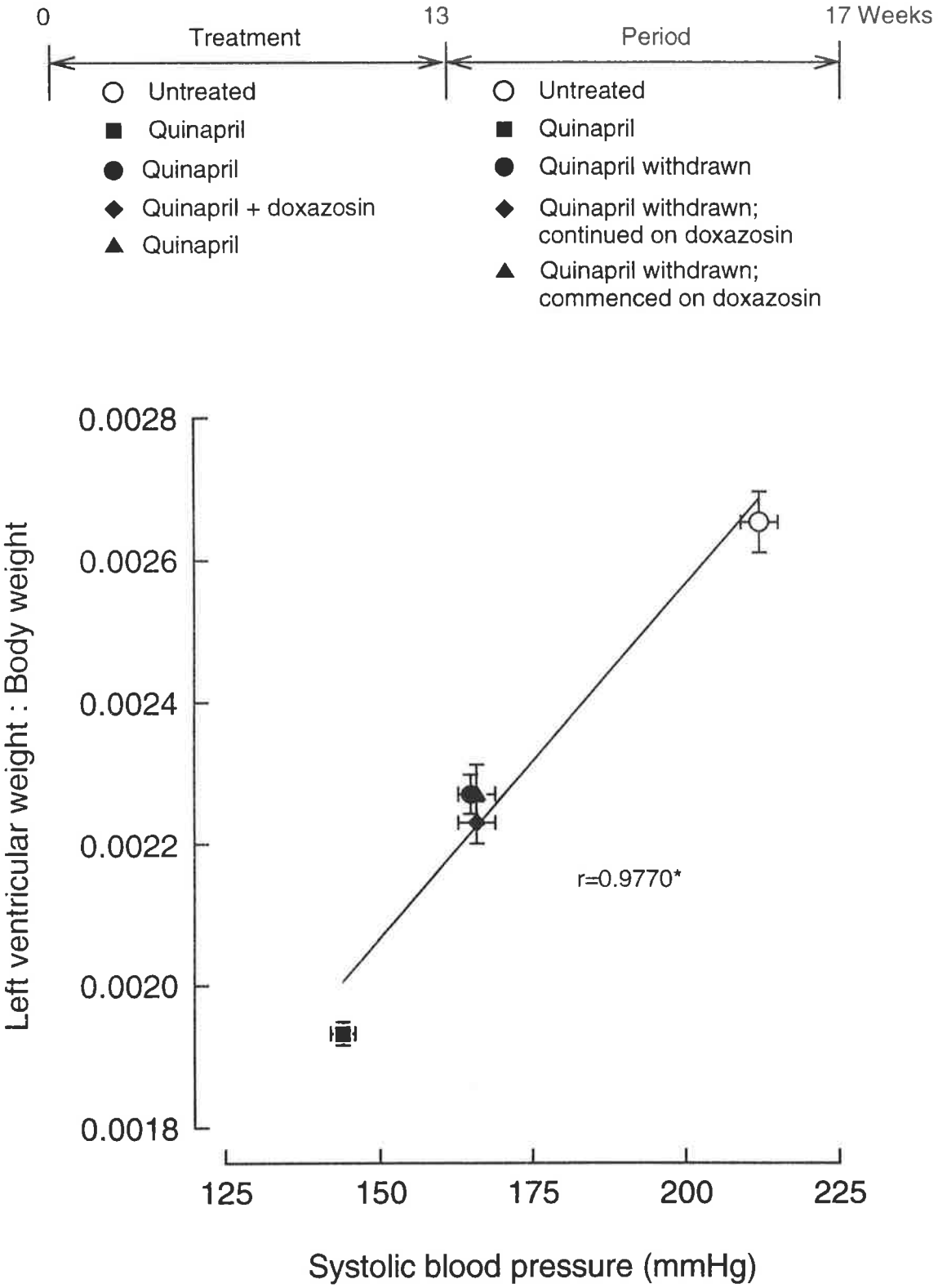


Fig. 5.3. Relationship between mean systolic blood pressure and left ventricular weight to body weight ratio of untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

* significant correlation of all groups, $p<0.05$.

5.3.2 Vascular reactivity

5.3.2.1 Aortic Rings

Maximal responses elicited by noradrenaline in the thoracic aorta were significantly lower in aortae from untreated SHRs compared to aortae from quinapril treated SHRs (Fig. 5.4). Furthermore, aortae taken from SHRs withdrawn from quinapril therapy and maintained on water for 4 weeks also produced maximal responses lower than the aortae from quinapril treated SHRs however significance was not reached (Fig. 5.4). In addition, the responses of aortae taken from SHRs released from quinapril treatment were also not significantly different from those of the untreated group (Fig. 5.4). In contrast, doxazosin treatment during and after quinapril therapy resulted in significantly augmented responses to noradrenaline in aortae from these animals when compared to aortae from the untreated SHRs (Fig. 5.4). The sensitivity of the aortic rings to noradrenaline was not significantly different between any of the groups (Table 5.1).

5.3.2.2 Perfused mesenteric vascular bed

The perfused mesenteric preparations from quinapril treated SHRs showed no significant attenuation in responses to nerve stimulation when compared with the control SHR preparation (Fig. 5.5(a)). Interestingly, after quinapril withdrawal, vascular contractility appeared greater than that displayed in the untreated SHR group, although this was not significantly different (Fig. 5.5(a)). Doxazosin treatment whether for 17 weeks or 4 weeks was able to cause some attenuation of the responses to nerve stimulation after withdrawal of quinapril when compared with

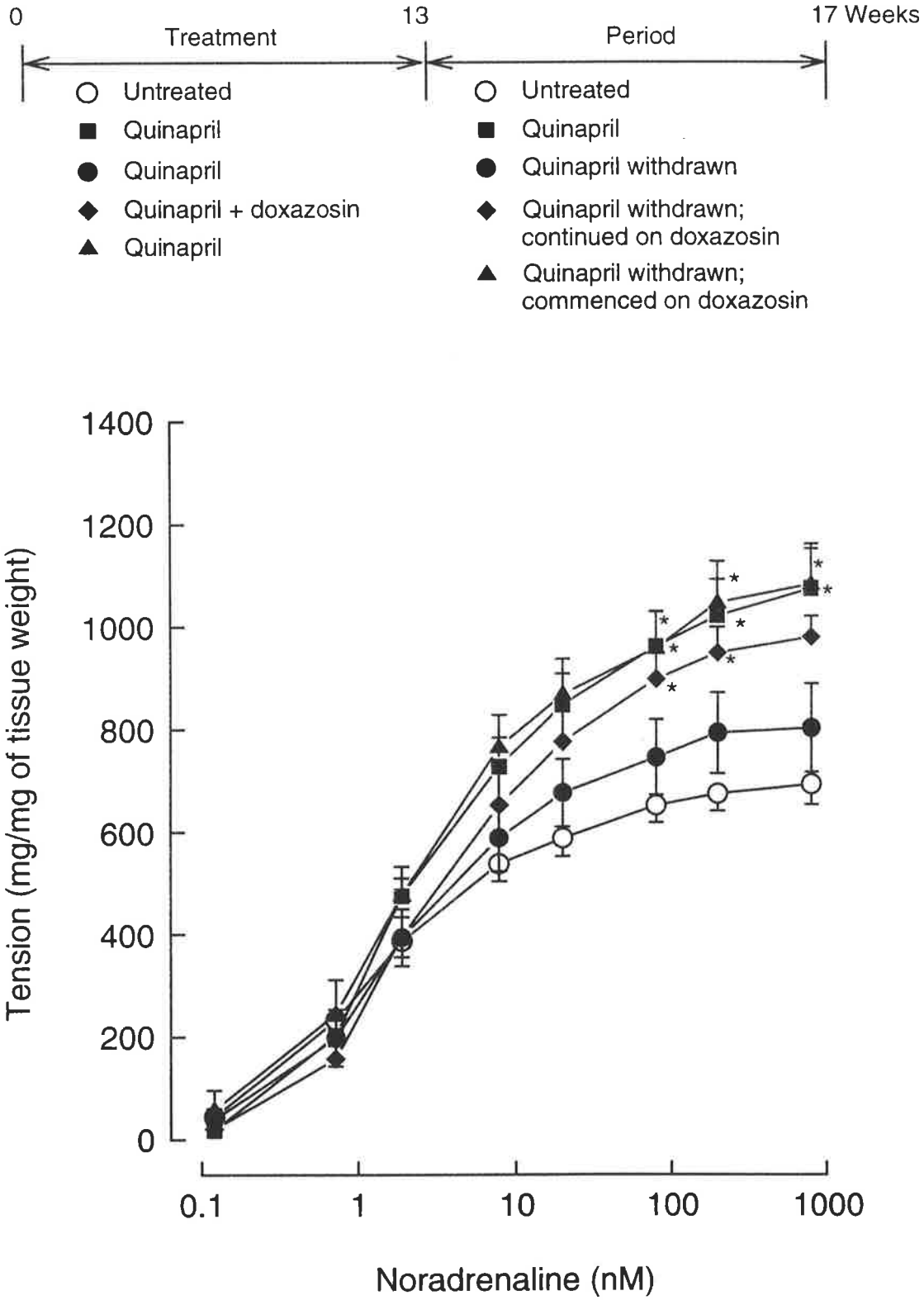


Fig. 5.4. Concentration-response curves to noradrenaline of aortic rings from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 6 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

Table 5.1. Sensitivity to nordrenaline of aortic rings from untreated and treated SHRs.

	Groups				
	SHR Control (n=7)	Quinapril (n=7)	Quinapril released (n=8)	Quin + Dox/ Dox (n=5)	Quin/Dox (n=8)
EC_{50} (nM ± s.e.m.)	1.52 ± 0.23	3.13 ± 0.78	2.69 ± 0.65	3.48 ± 0.55	2.39 ± 0.44

Table 5.2. Sensitivity of perfused mesenteric vasculature from untreated and treated SHRs.

Groups	Potassium Chloride (mg)	
	EC_{50} (95% CI)	Slope ± s.e.m.
SHR control (n=7)	2.99 (2.56-3.49)	113.1 ± 9.44
Quinapril treated (n=7)	2.81 (2.20-3.58)	73.0 ± 7.54 *
Quinapril withdrawn (n=7)	3.72 (2.74-5.09)	92.16 ± 8.45
Quin + Dox / Dox (n=7)	3.44 (2.42-4.87)	86.53 ± 8.68
Quin / Dox (n=7)	3.53 (2.87-4.36)	89.9 ± 5.21

EC_{50} = dose producing a 50 % of the maximum increase in perfusion pressure; CI = confidence interval.
* $p<0.05$ compared with SHR controls.
Quin + Dox/ Dox = SHRs treated with quinapril and doxazosin for 13 weeks then withdrawn from quinapril but continued on doxazosin for a further 4 weeks.
Quin/Dox = SHRs treated with quinapril for 13 weeks then withdrawn from quinapril and commenced on doxazosin treatment for 4 weeks.

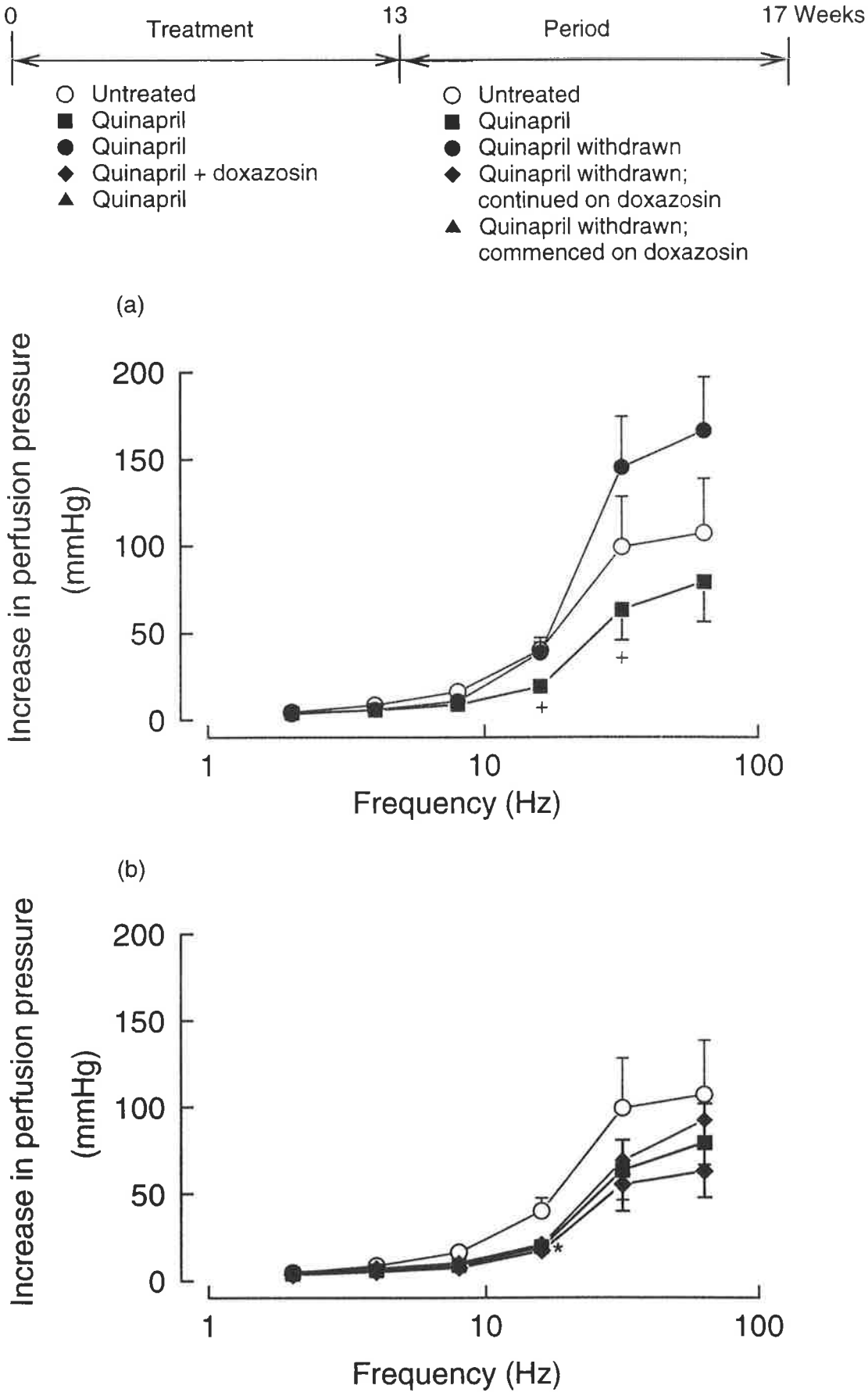


Fig. 5.5. Frequency-response curves in the perfused mesenteric vasculature from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 6 to 8 rats ± s.e.m.

* $p<0.05$ compared with SHR controls.

+ $p<0.05$ compared with SHRs withdrawn from quinapril.

responses from the "released" group although the values were not significantly different (Fig. 5.5(b)).

Responses to intraluminal noradrenaline and phenylephrine were significantly attenuated in preparations from quinapril treated animals (Fig. 5.6 & 5.7). Responses of perfused mesenteric preparations from "released" animals to noradrenaline and phenylephrine were intermediate to those from quinapril and untreated SHR groups (Fig. 5.6 & 5.7). As with nerve stimulation, doxazosin treatment (regardless of its period of administration) prevented this rise in vascular reactivity upon withdrawal of quinapril treatment (Fig. 5.6 & 5.7).

Responses to potassium chloride were also attenuated in preparations from quinapril treated SHRs when compared to preparations from control SHRs (Fig. 5.8). On the other hand, discontinuation of quinapril therapy in SHRs augmented responses to potassium chloride although these responses were still lower than those seen in preparations from control SHRs (Fig. 5.8). The enhanced contractility seen after quinapril withdrawal was also seen in preparations from doxazosin treated animals. Sensitivity of mesenteric preparations to potassium chloride was similar amongst all groups (Table 5.2). Furthermore, slopes for potassium chloride response curves were greater in preparations from untreated, "released" and doxazosin treated SHRs in comparison to slopes from quinapril treated SHRs but slopes only for preparations from untreated SHRs reached significance (Table 5.2).

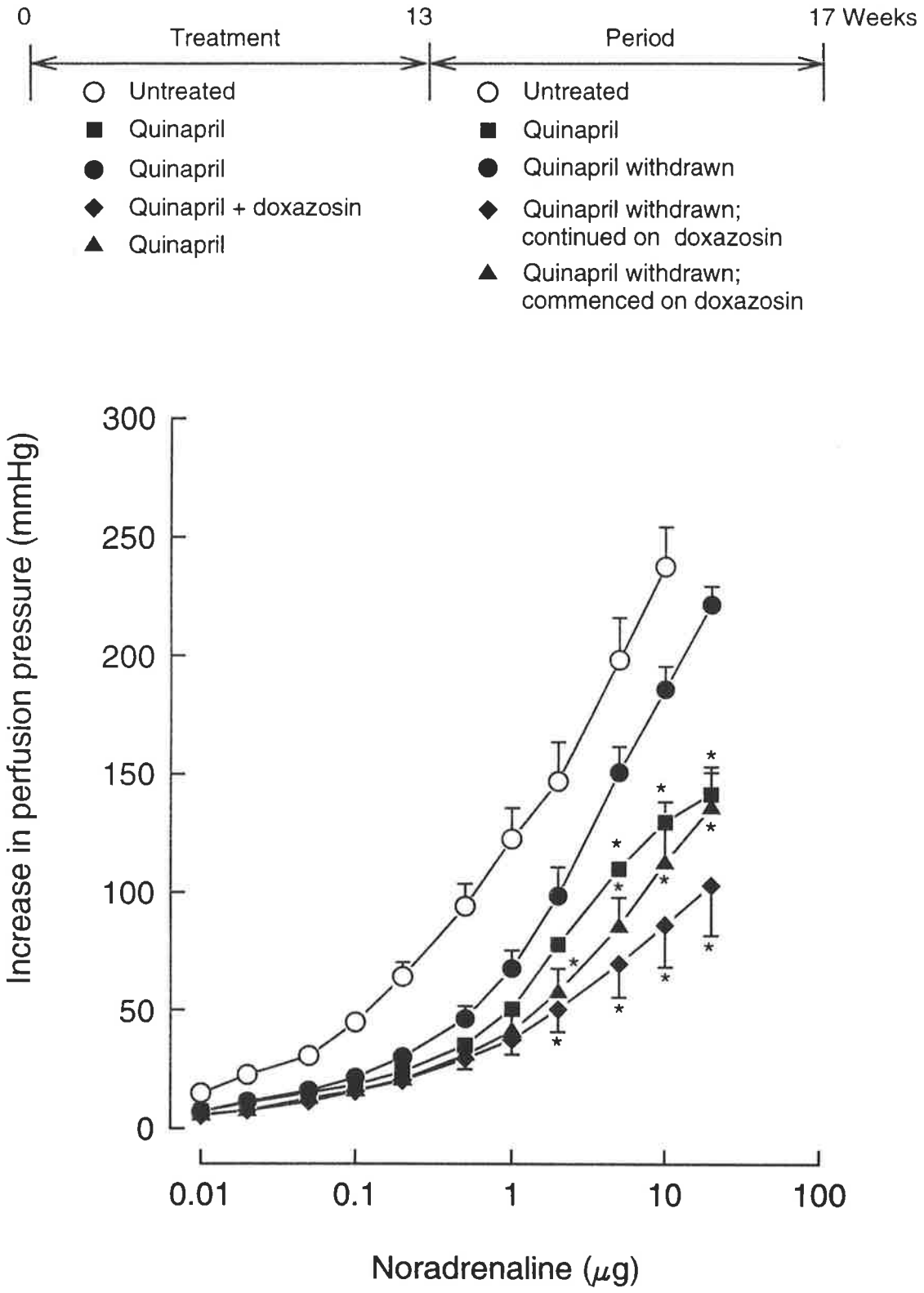


Fig. 5.6. Concentration-response curves to noradrenaline in the perfused mesenteric vasculature from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

Responses to all concentrations of noradrenaline from treated groups were significantly different from those of the untreated group, $p<0.01$.

* $p<0.05$ compared with SHRs withdrawn from quinapril.

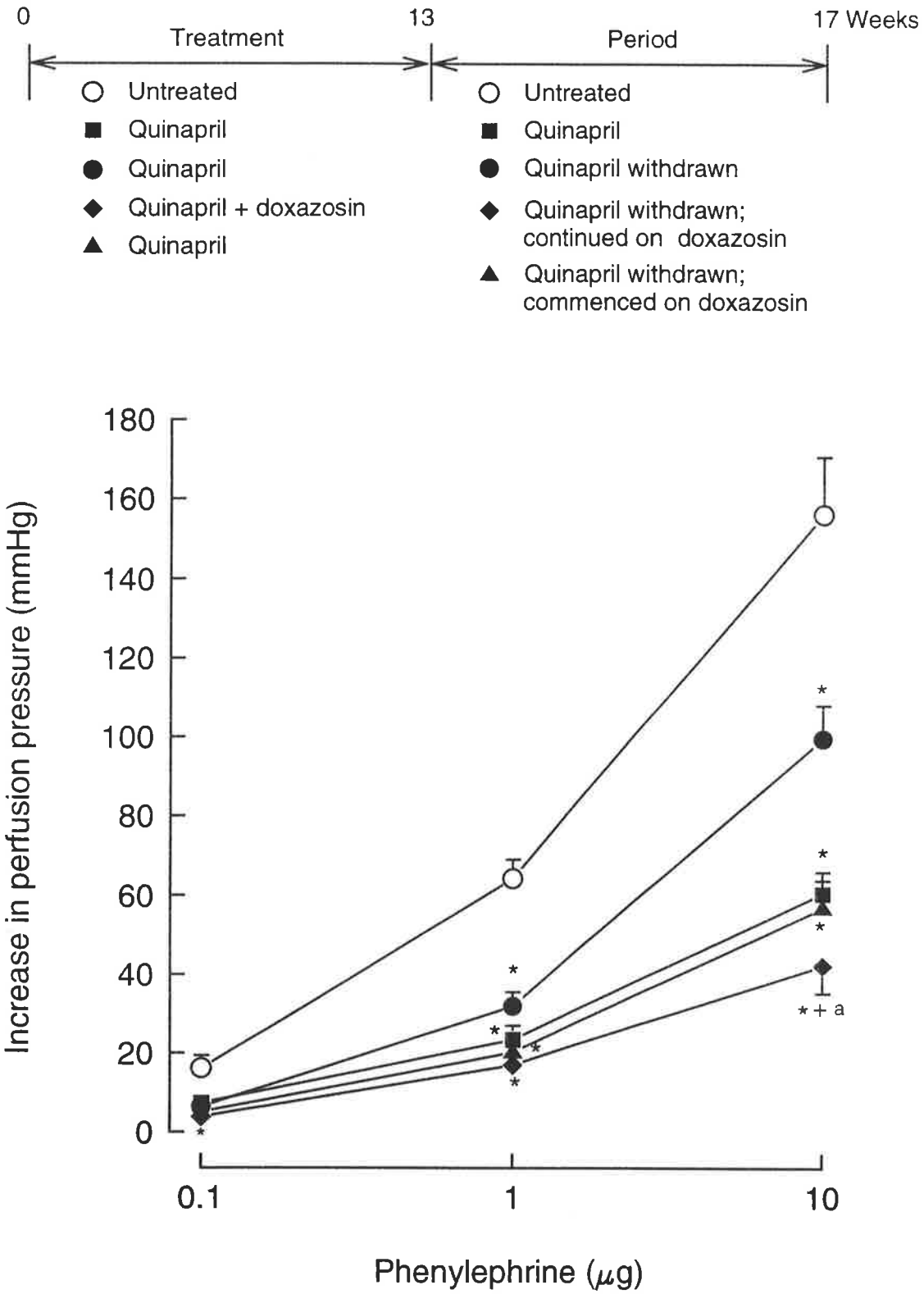


Fig. 5.7. Concentration-response curves to phenylephrine in the perfused mesenteric vasculature from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHRs withdrawn from quinapril.

a $p<0.05$ compared with SHRs treated with quinapril.

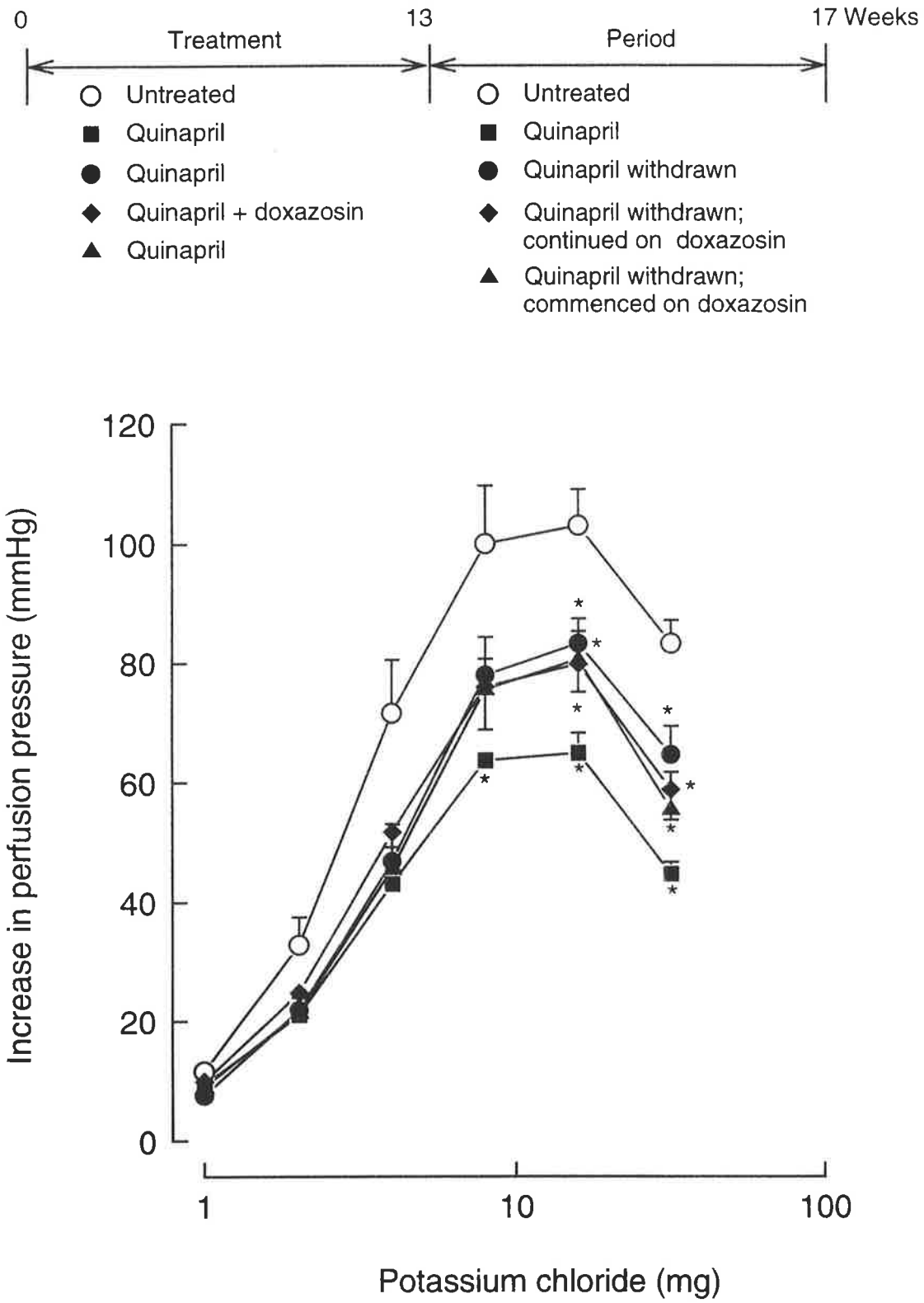


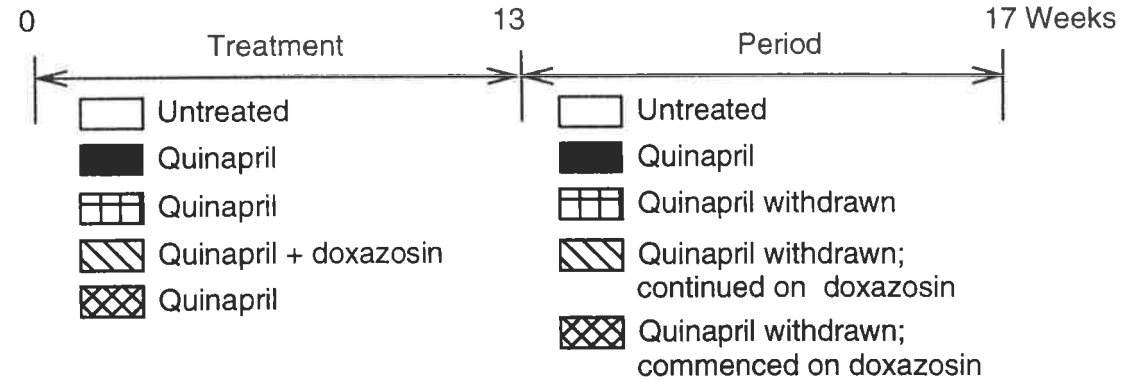
Fig. 5.8. Concentration-response curves to potassium chloride in the perfused mesenteric vasculature from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.
* $p<0.05$ compared with untreated SHRs.

5.3.3 Biochemical parameters

5.3.3.1 3-Methylhistidine content

The content of 3-methylhistidine in the aorta from treated and control groups, (expressed per mg of protein), is shown in Fig. 5.9. Quinapril treatment significantly reduced the 3-methylhistidine level in the aorta and after withdrawal of quinapril the levels of 3-methylhistidine reverted back to control levels. Furthermore, doxazosin treatment for 17 weeks and 4 weeks was able to prevent the increase in 3-methylhistidine levels seen after withdrawal of quinapril (Fig. 5.9).

Fig. 5.10(a) shows the level of 3-methylhistidine in relation to protein in the superior mesenteric artery. There was no difference in 3-methylhistidine levels in this tissue between all groups (Fig. 5.10(a)). Absolute values for 3-methylhistidine were significantly decreased in tissues from quinapril treated SHRs when compared to tissues from untreated SHRs (Fig. 5.10(b)). Discontinuation of quinapril treatment resulted in elevated absolute 3-methylhistidine levels in the superior mesenteric artery in comparison to levels in tissues from quinapril treated SHRs (Fig. 5.10(b)). Although there was a tendency for doxazosin to prevent the rise in absolute 3-methylhistidine levels after quinapril release, significance was obtained only when it was doxazosin was administered for 17 weeks (Fig. 5.10(b)).



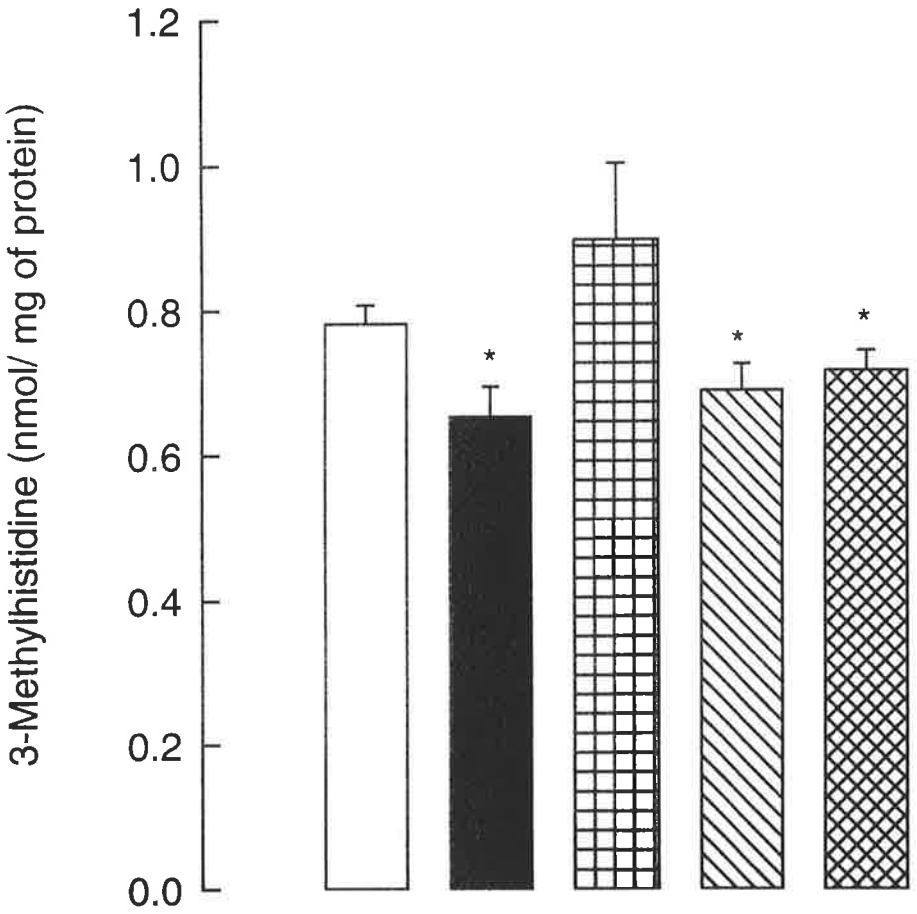
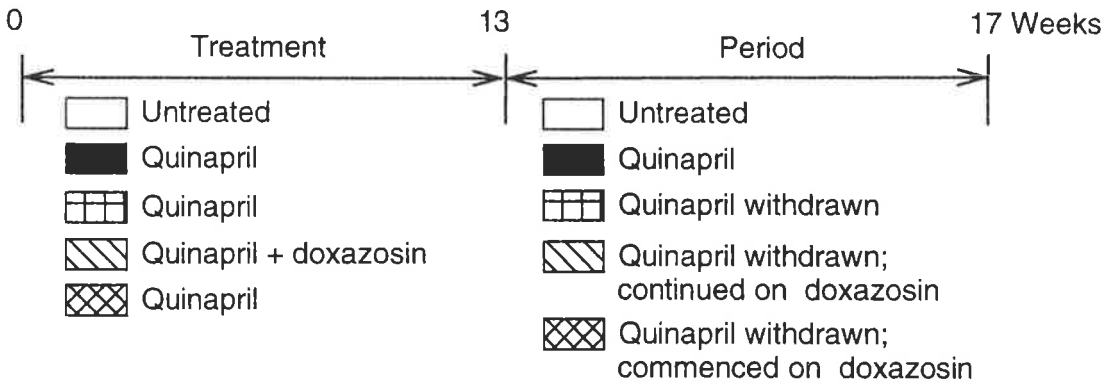


Fig. 5.9. The bar graphs show levels of 3-methylhistidine in the thoracic aorta from untreated and treated SHRs after a 17 week treatment period. Each bar represents the mean value for 8 rats ± s.e.m.
* $p<0.05$ compared with SHRs withdrawn from quinapril.



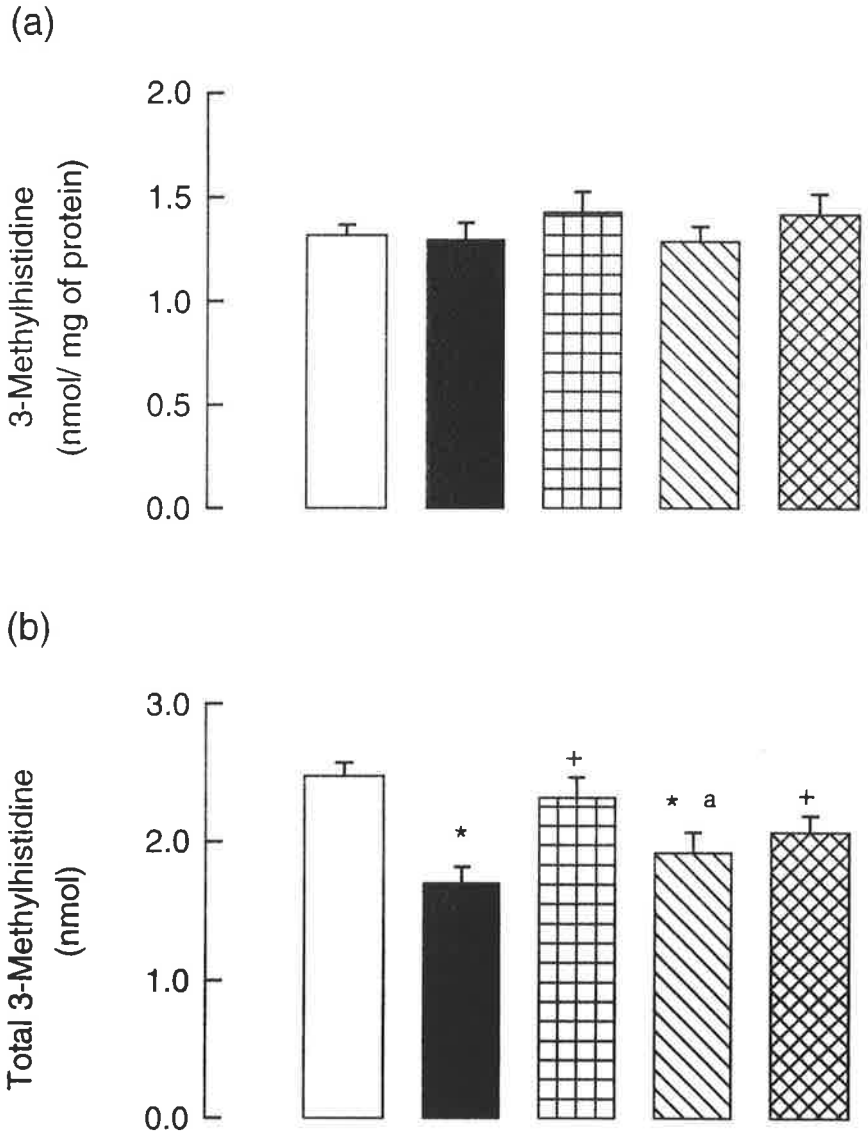


Fig. 5.10. The bar graphs show levels of 3-methylhistidine in the superior mesenteric artery from untreated and treated SHRs. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.01$ compared with SHRs treated with quinapril.

a $p<0.05$ compared with SHRs withdrawn from quinapril.

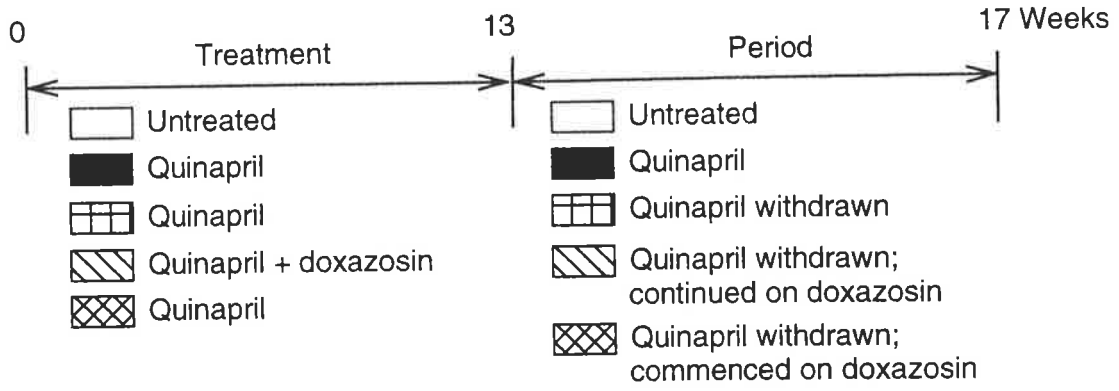
The concentrations of 3-methylhistidine per mg of protein in mesenteric branches are shown in Fig. 5.11(a). Differences in the 3-methylhistidine level between untreated and treated groups were not seen. However, when results were expressed in absolute terms, quinapril treatment alone caused a marked and significant reduction in mesenteric branch 3-methylhistidine levels (Fig. 5.11(b)). The absolute amount of 3-methylhistidine in mesenteric branches after withdrawal of quinapril were similar to levels seen in untreated SHRs and this increase was prevented only when doxazosin was administered for 17 weeks (Fig. 5.11(b)).

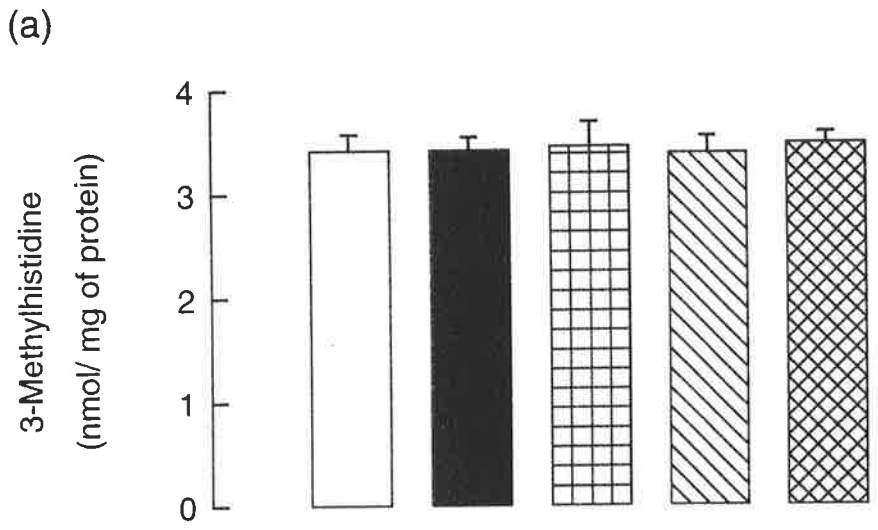
In the case of the nonvascular tissue ie. the vas deferens no significant changes were observed in 3-methylhistidine levels between control SHRs and treated SHRs when expressed per mg of protein or in absolute terms (Fig. 5.12 (a) & (b)).

5.3.3.2 DNA content

Fig. 5.13. displays DNA levels in the aorta. A significant difference was obtained between tissues from control and all treated groups. Between treatments there was a significant difference between the group withdrawn from quinapril and the group which received doxazosin for 4 weeks after the withdrawal of quinapril (Fig. 5.13).

In the case of the superior mesenteric artery, DNA levels were similar in untreated and treated SHRs when expressed per mg of protein (Fig. 5.14(a)). However, absolute levels of DNA in this tissue from SHRs which underwent quinapril therapy were significantly lower than the levels seen in the control group (Fig. 5.14(b)).





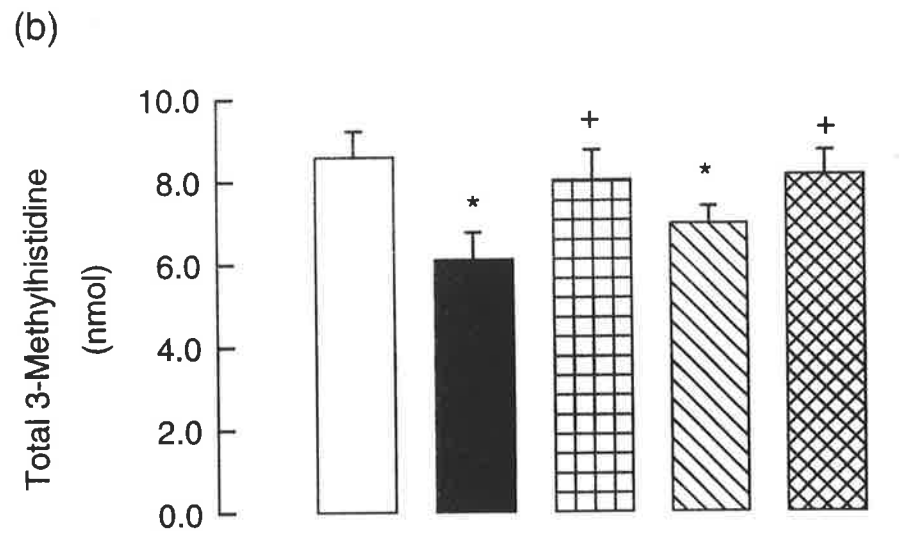
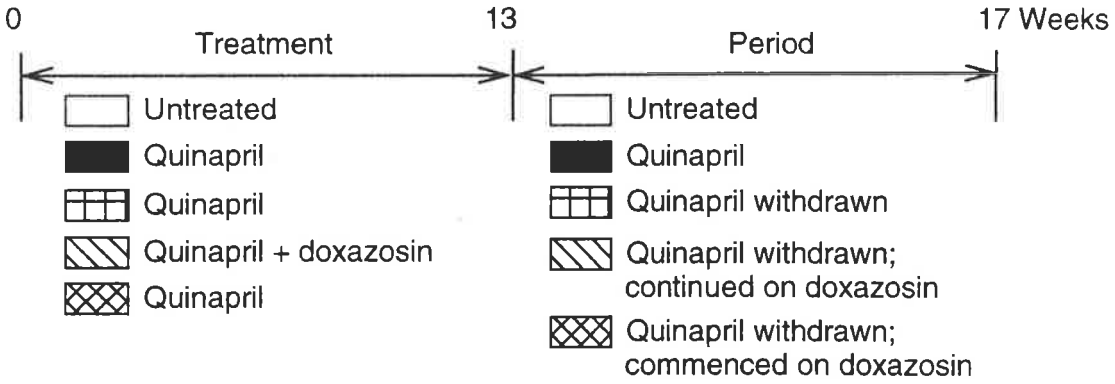
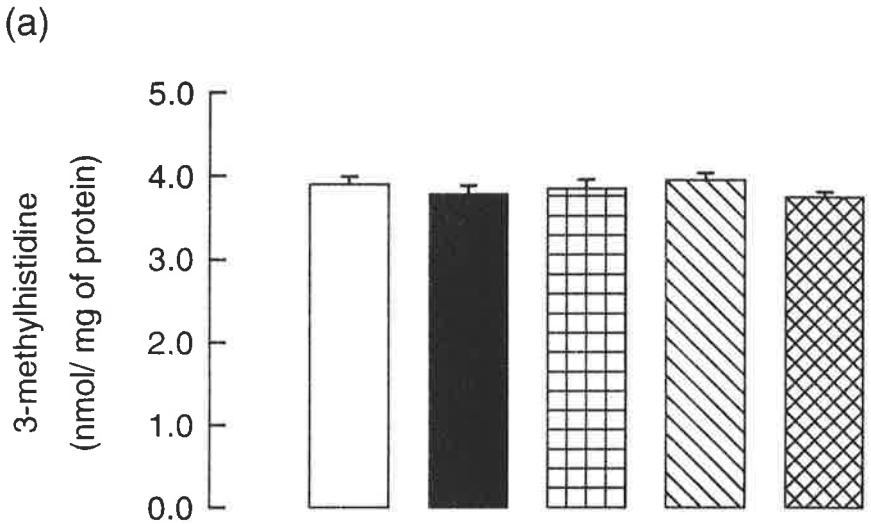


Fig. 5.11. The bar graphs show levels of 3-methylhistidine in mesenteric branches from untreated and treated SHRs after a 17 week treatment period. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHRs treated with quinapril.





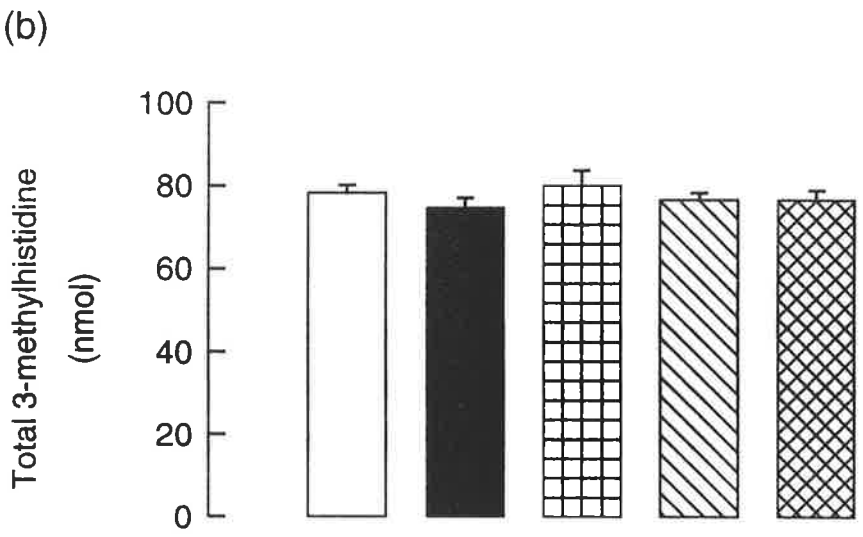
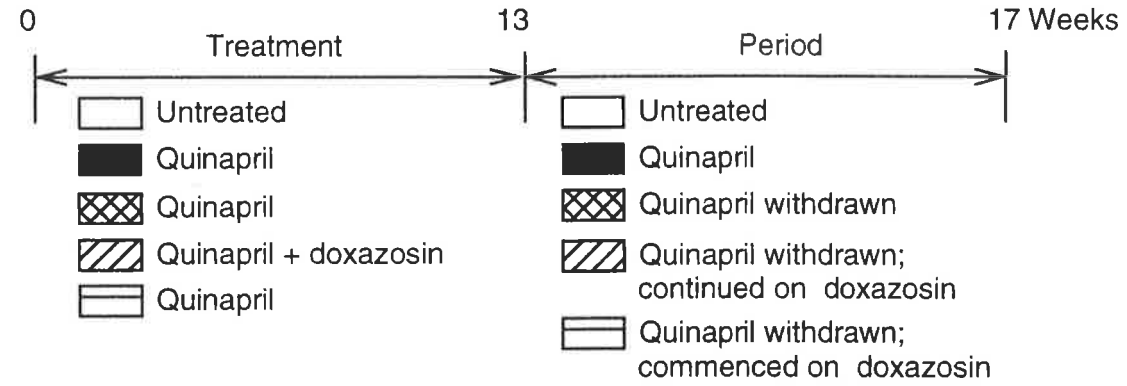


Fig. 5.12. The bar graphs show levels of 3-methylhistidine in the vas deferens from untreated and treated SHRs after a 17 week treatment period. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 7 to 8 rats ± s.e.m.



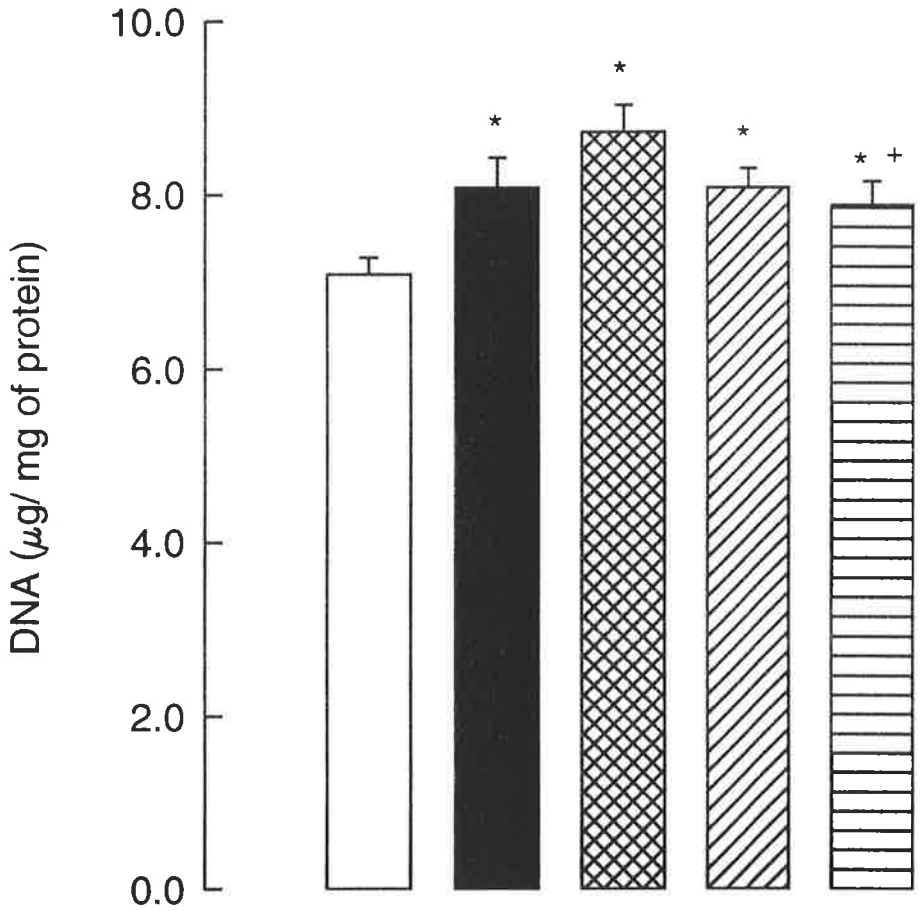
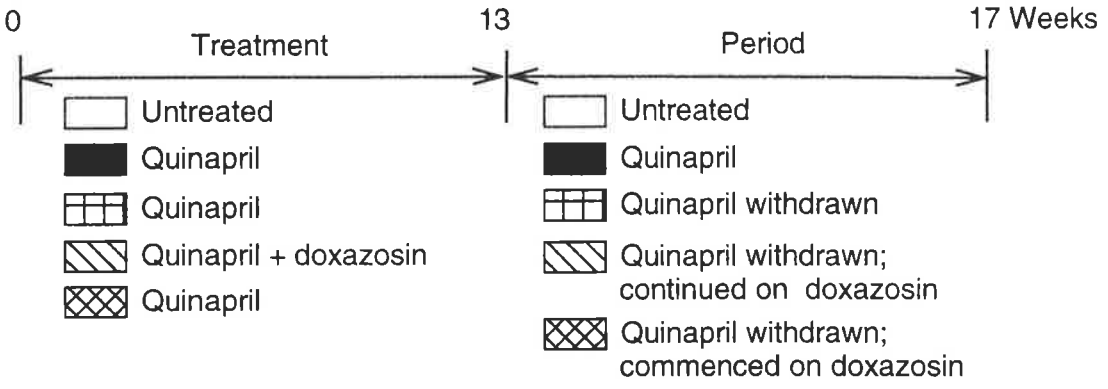
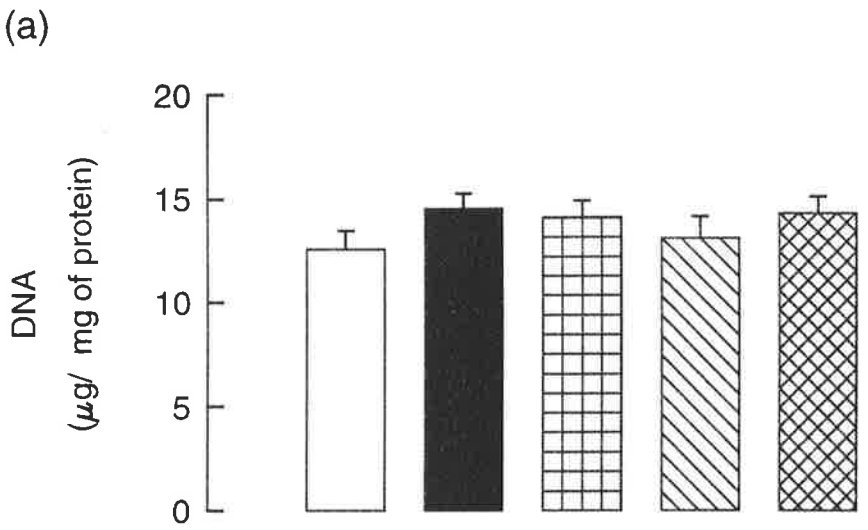


Fig. 5.13. The bar graphs show levels of DNA in the thoracic aorta from untreated and treated SHRs after a 17 week treatment period. Each bar represents the mean value for 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHRs withdrawn from quinapril.





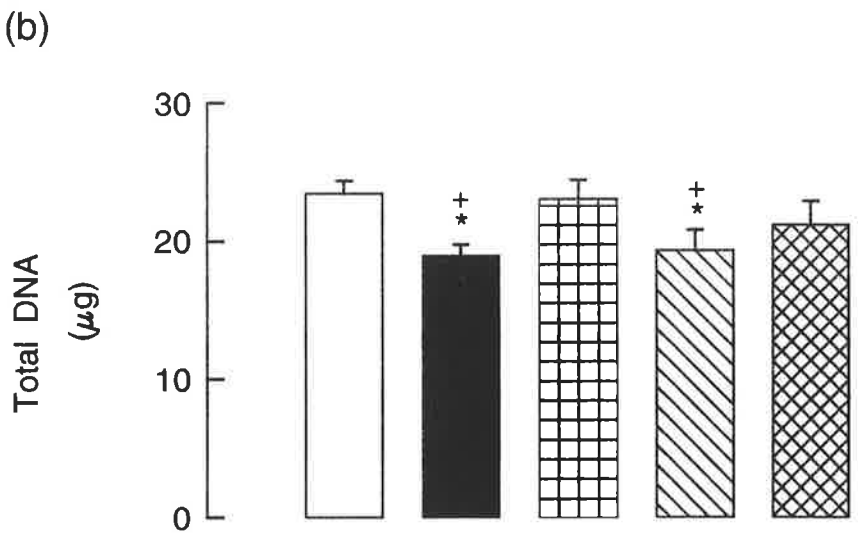


Fig. 5.14. The bar graphs show levels of DNA in the superior mesenteric artery from untreated and treated SHRs after a 17 week treatment period. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts.

Each bar represents the mean value of 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHR withdrawn from quinapril.

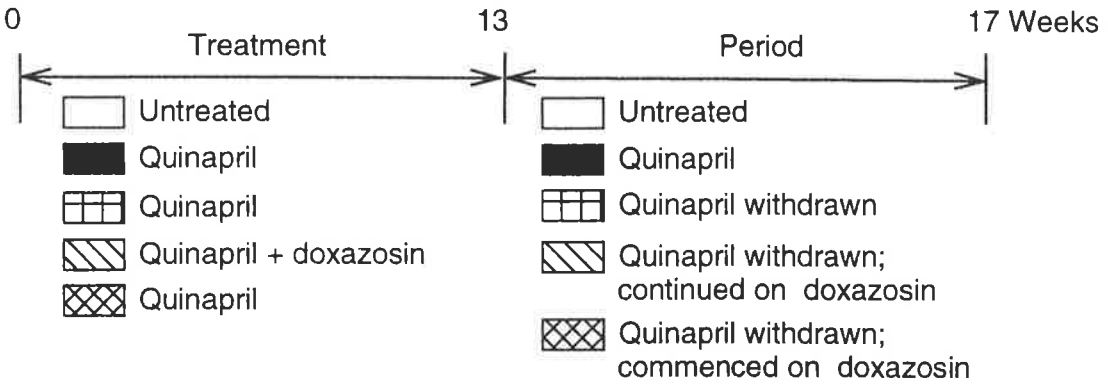
Discontinuation of quinapril therapy resulted in the DNA levels returning to values similar to those of the untreated group. Only 17 weeks of doxazosin treatment was able to significantly prevent the rise in absolute DNA levels after release from quinapril treatment when compared with levels of the tissues from the "release" group (Fig. 5.14(b)).

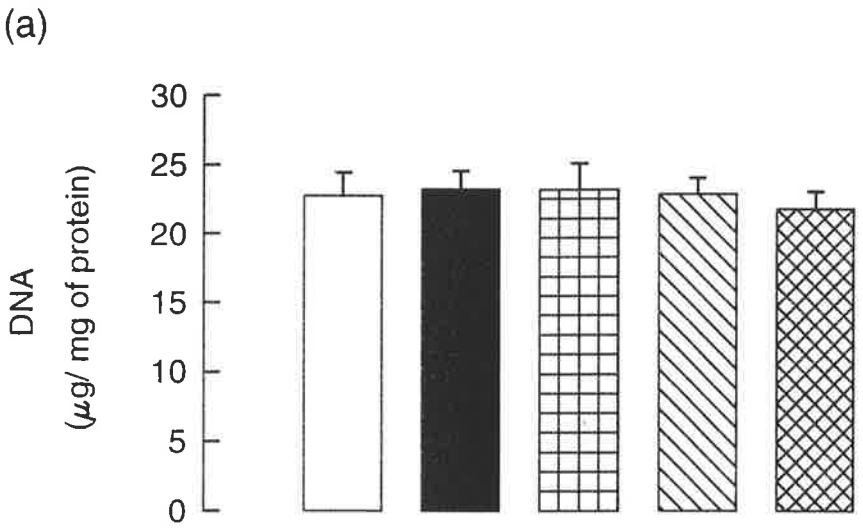
In the mesenteric branches the levels of DNA expressed per mg of protein were similar in untreated and treated SHRs (Fig. 5.15(a)). In absolute terms the reduced levels of DNA seen with chronic quinapril treatment were not maintained after discontinuation of quinapril (Fig. 5.15(b)). Although significance was not reached there was a tendency for doxazosin administered for 17 weeks to slow this rise (Fig. 5.15(b)).

The vas deferens taken from untreated SHRs demonstrated similar DNA levels to those from the treatment groups (Fig. 5.16(a) & (b)).

5.3.3.3 Protein content

The absolute levels of total protein were significantly decreased in both the superior mesenteric artery (Fig. 5.17(a)) and mesenteric branches (Fig. 5.17(b)) from SHRs treated with quinapril when compared with controls. In the case of the superior mesenteric artery when quinapril treatment was ceased there was an increase in protein but to a level which was lower than levels in the tissue from untreated SHR although significance was not quite reached ($p=0.054$) (Fig. 5.17(a)). The increase in protein levels after withdrawal of quinapril was not prevented by doxazosin treatment; again, significance was not achieved when compared with levels from quinapril treated group (Fig. 5.17(a)).





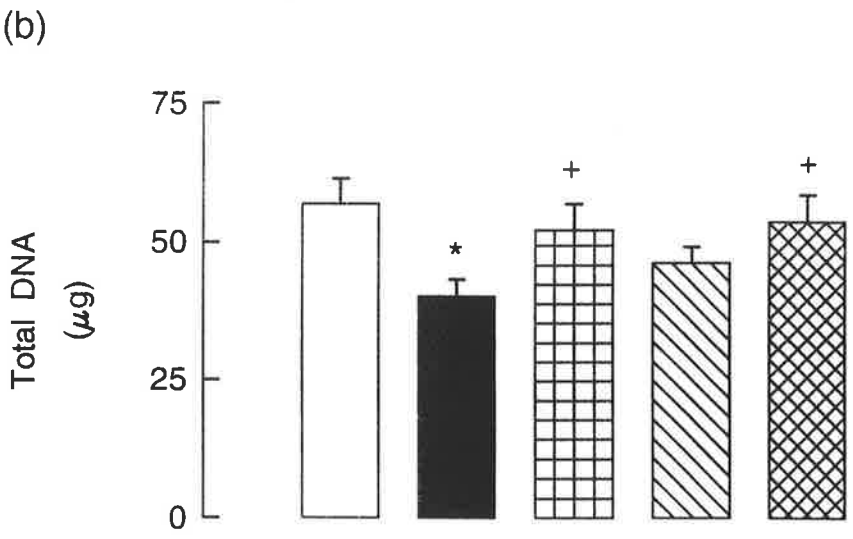
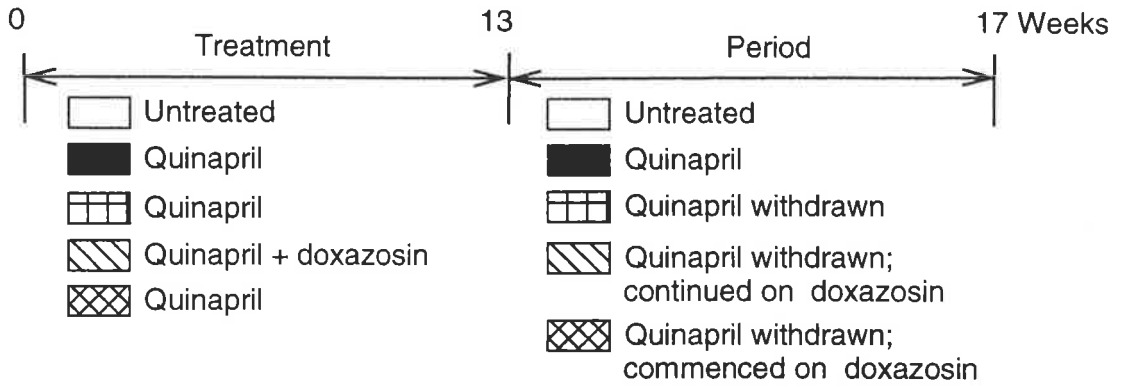
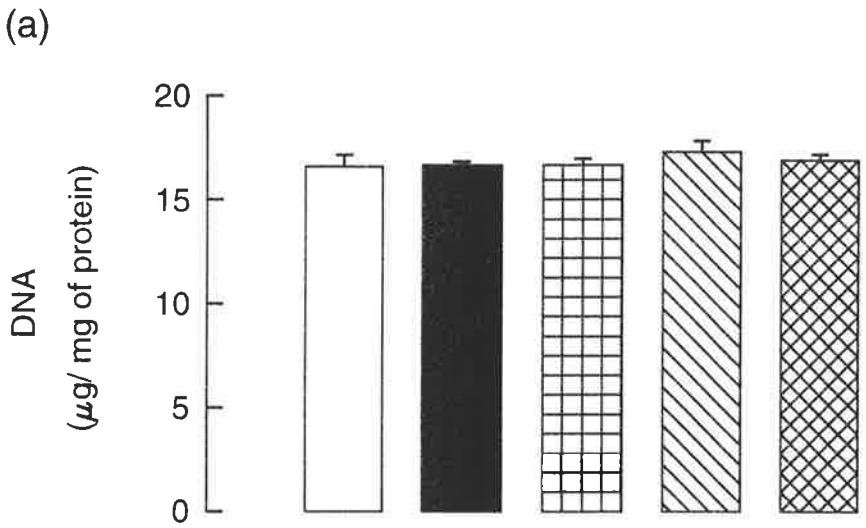


Fig. 5.15. The bar graphs show levels of DNA in mesenteric branches from untreated and treated SHRs after a 17 week treatment period. Data are expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 7 to 8 rats ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHRs treated with quinapril.





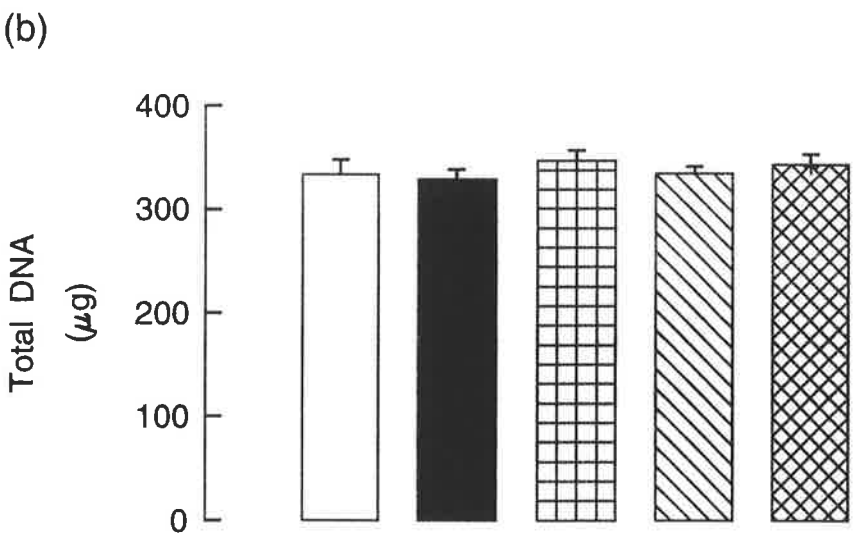
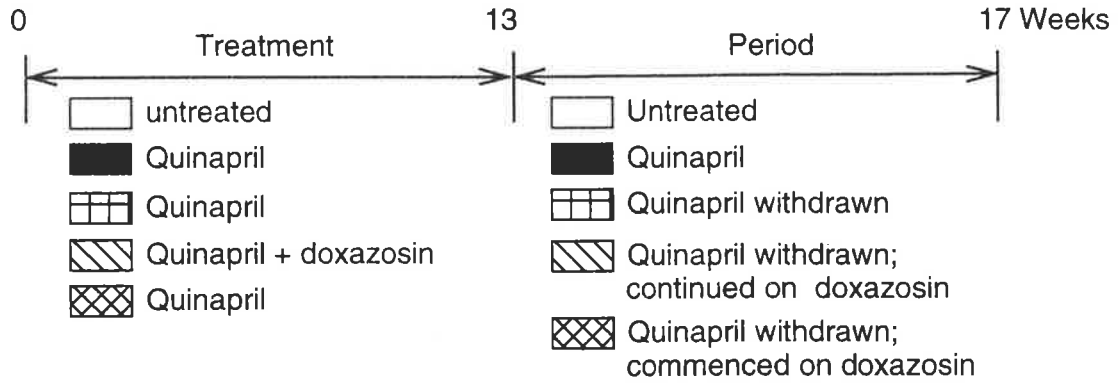
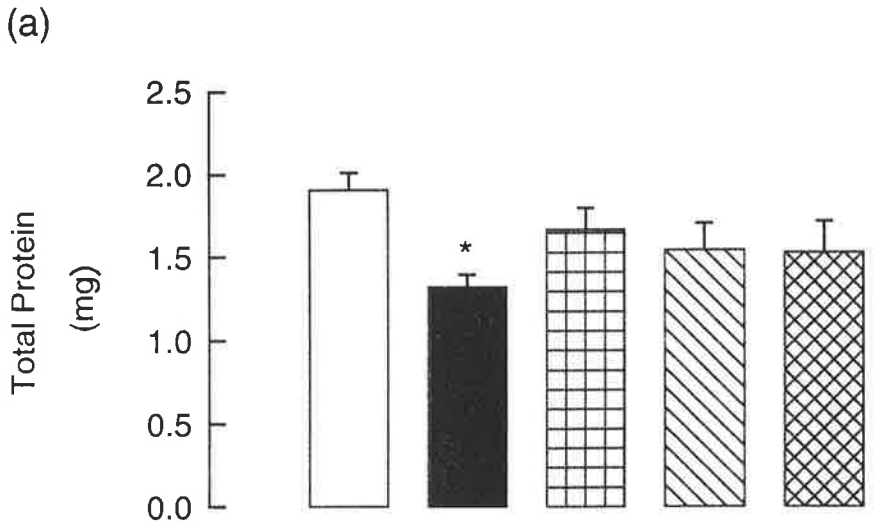


Fig. 5.16. The bar graphs show levels of DNA in the vas deferens from untreated and treated SHRs after a 17 week treatment period. Data is expressed as (a) normalised per mg of protein and (b) absolute amounts. Each bar represents the mean value of 7 to 8 rats ± s.e.m.





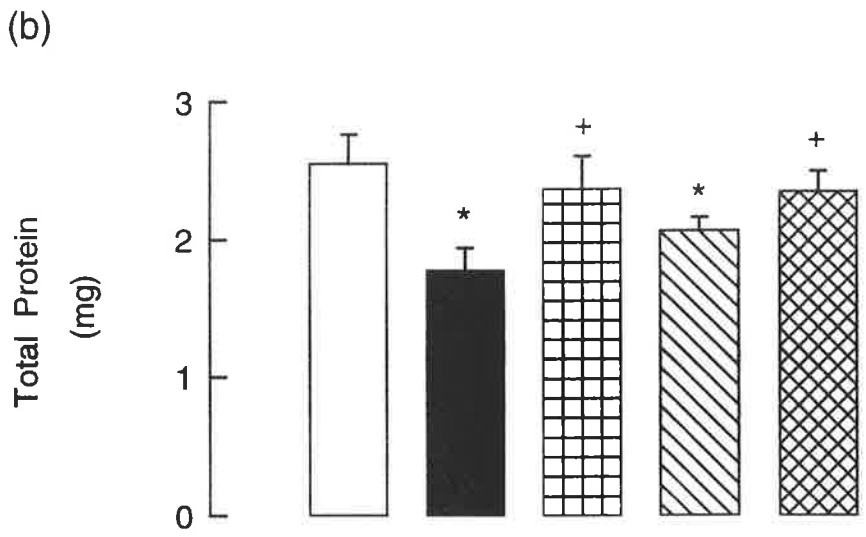


Fig. 5.17. The bar graphs show absolute levels of protein in (a) superior mesenteric artery and (b) mesenteric branches from untreated and treated SHRs after a 17 week treatment period. Each bar represents the mean value of 7 to 8 ± s.e.m.

* $p<0.05$ compared with untreated SHRs.

+ $p<0.05$ compared with SHRs treated with quinapril.

Protein levels in the mesenteric branches increased after quinapril treatment was ceased and doxazosin treatment for 17 weeks appeared to reduce this rise seen upon withdrawal of quinapril (Fig. 5.17(b)).

Fig. 18. shows the total protein content found in the vas deferens. There was no significant difference in the protein content of the treated and untreated tissues.

5.3.4 Correlation analysis

Total 3-methylhistidine, DNA and protein content of mesenteric branches did not correlate with the prevailing systolic blood pressure in untreated and treated SHRs (Fig. 5.19(a), 5.20(a) & 5.21(a)). A similar trend was seen in the superior mesenteric artery except in the case of total protein which correlated with the systolic blood pressure (Fig. 5.19(b), 5.20(b) & 5.21(b)).

Vascular responses to potassium chloride in the perfused mesenteric vasculature correlated well with systolic blood pressure in untreated and treated SHRs (Fig. 5.22). However vascular 3-methylhistidine content did not follow vascular reactivity (Fig. 5.23). In contrast, vascular contractility to noradrenaline of the thoracic aorta appeared to very weakly correlate with systolic blood pressure in untreated and treated SHRs with $p=0.0501$ (Fig. 5.24).

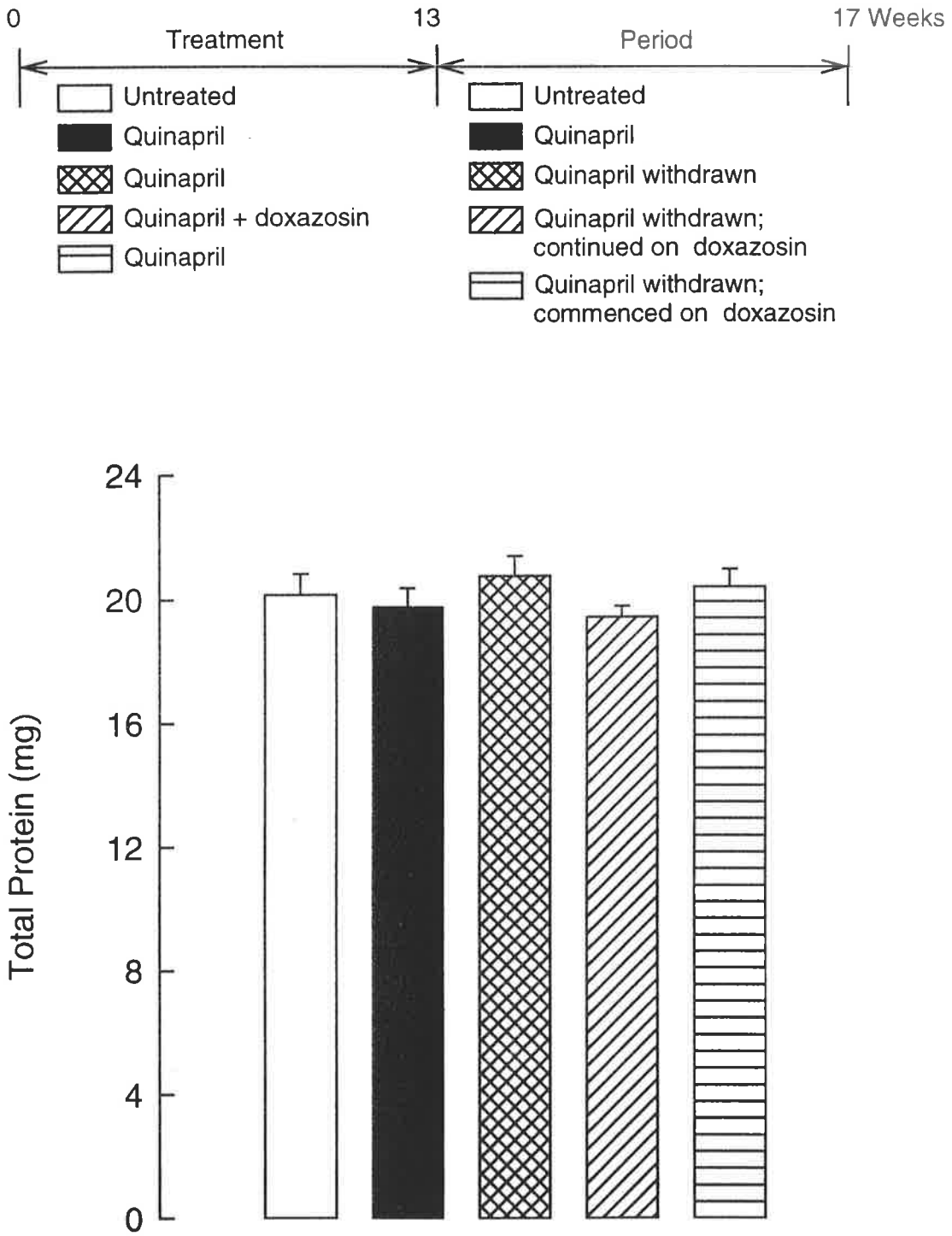


Fig. 5.18. The bar graphs show absolute levels of protein in the vas deferens from untreated and treated SHRs after a 17 weeks treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

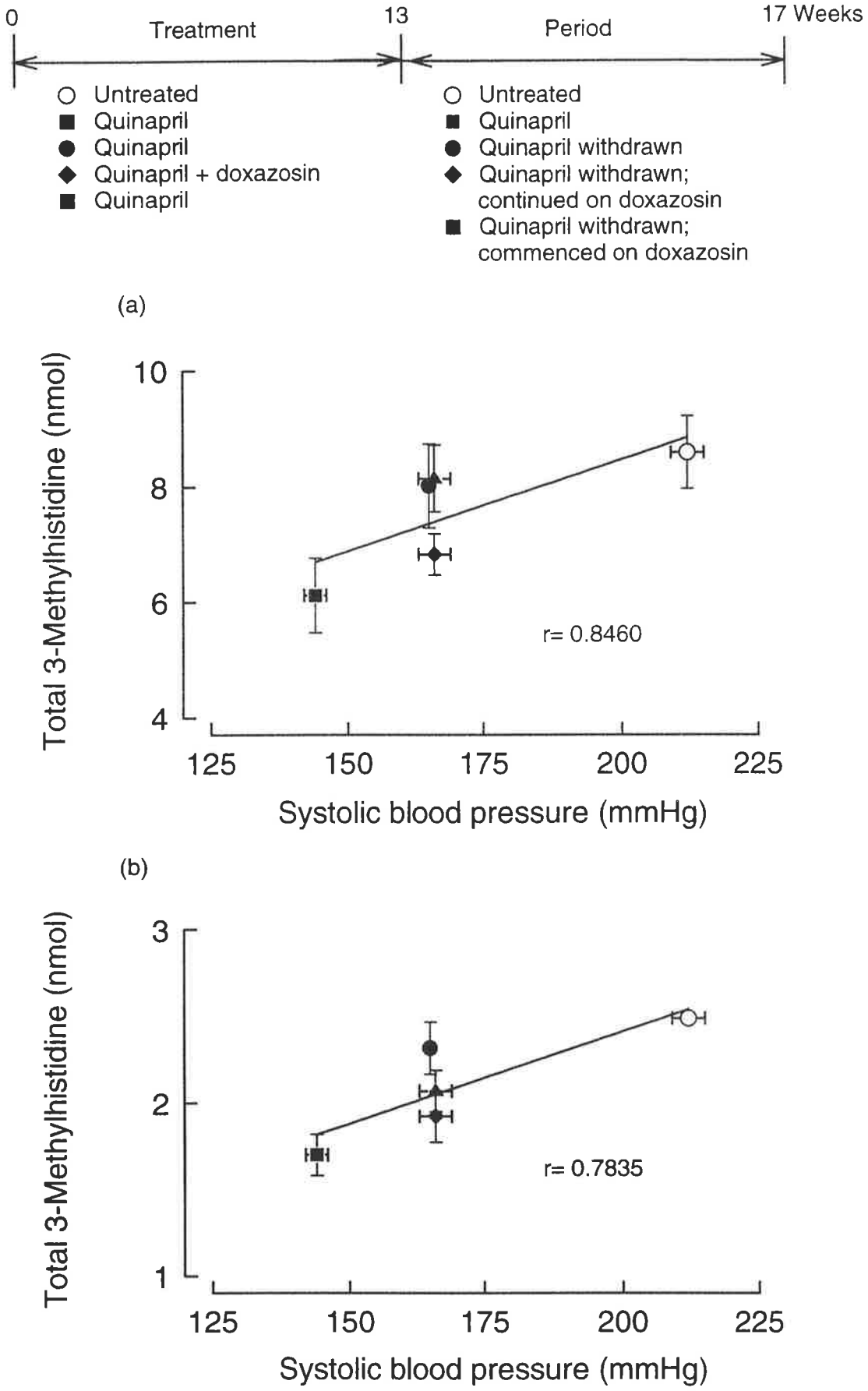


Fig. 5.19. Relationship between mean systolic blood pressure and 3-methylhistidine levels in (a) mesenteric branches and (b) superior mesenteric artery from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

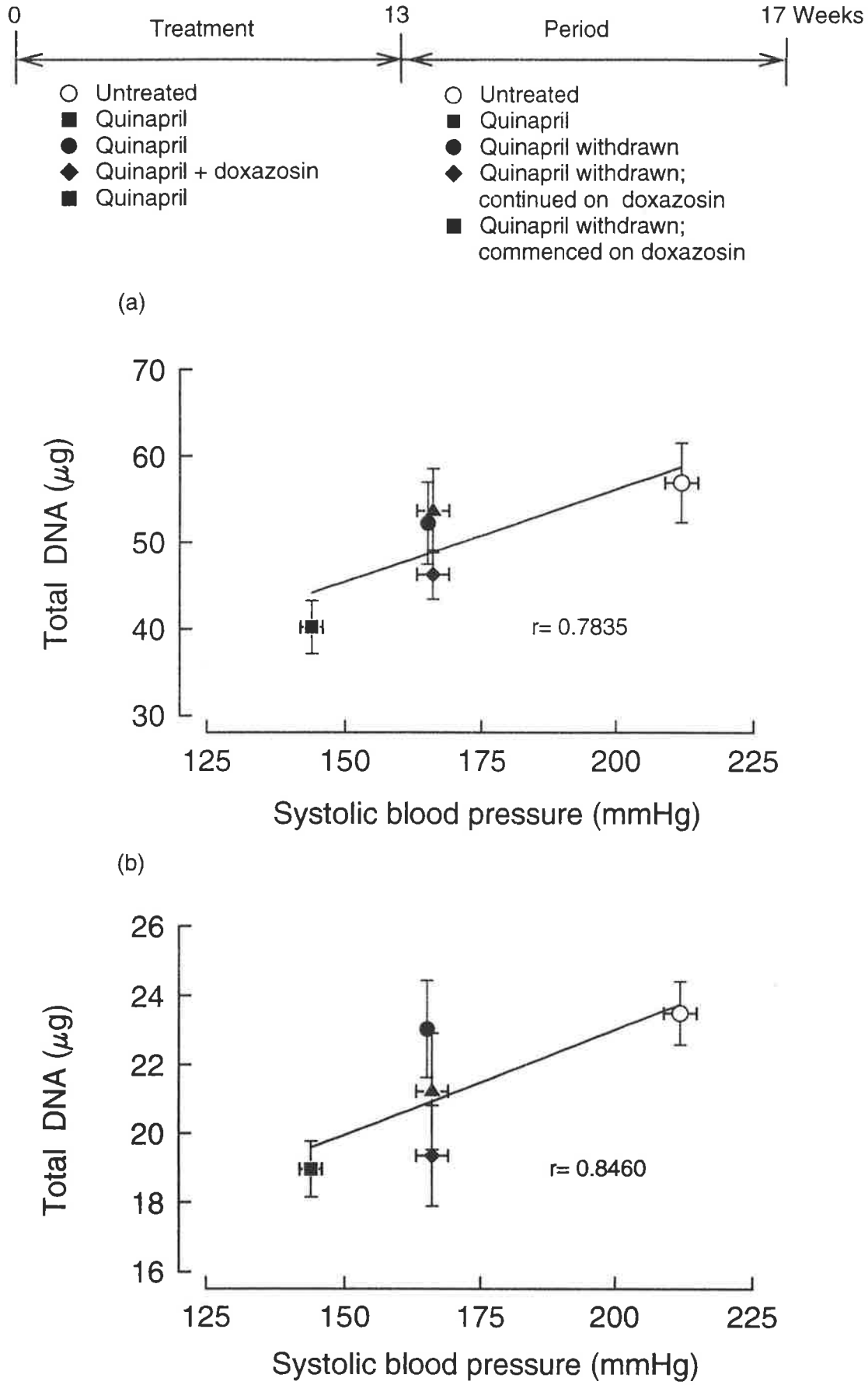
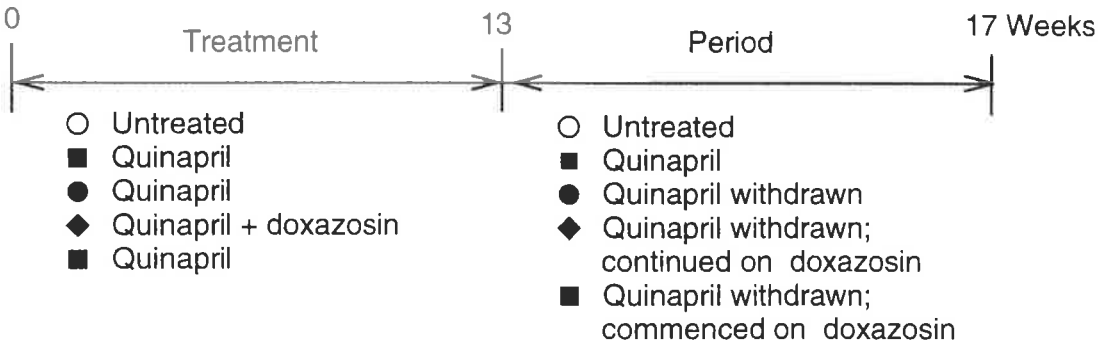
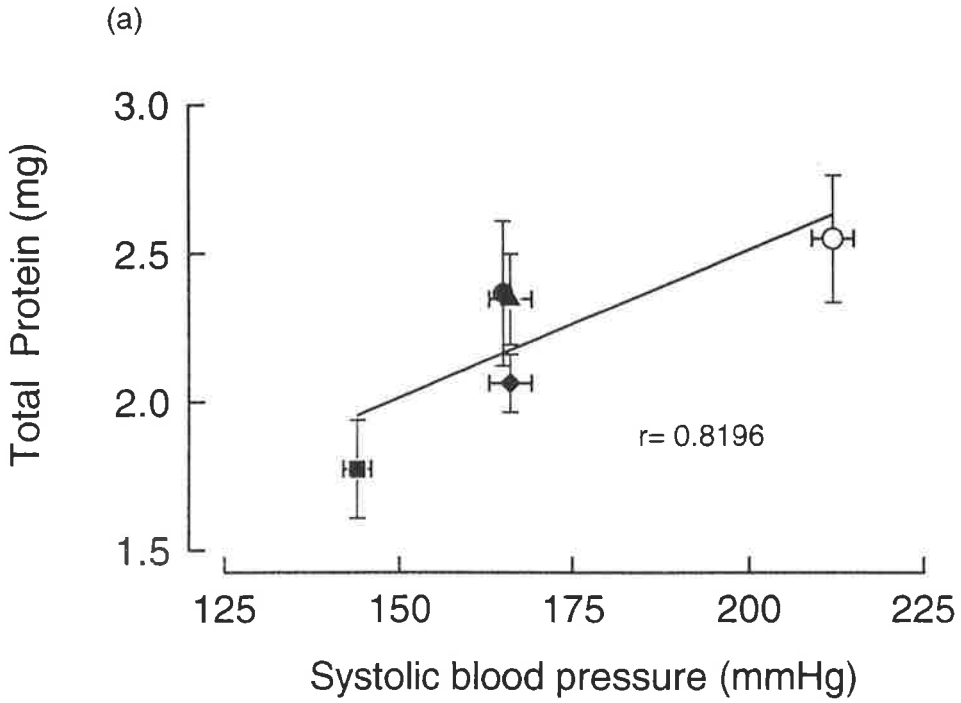


Fig. 5.20. Relationship between mean systolic blood pressure and DNA levels in (a) mesenteric branches and (b) superior mesenteric artery from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.





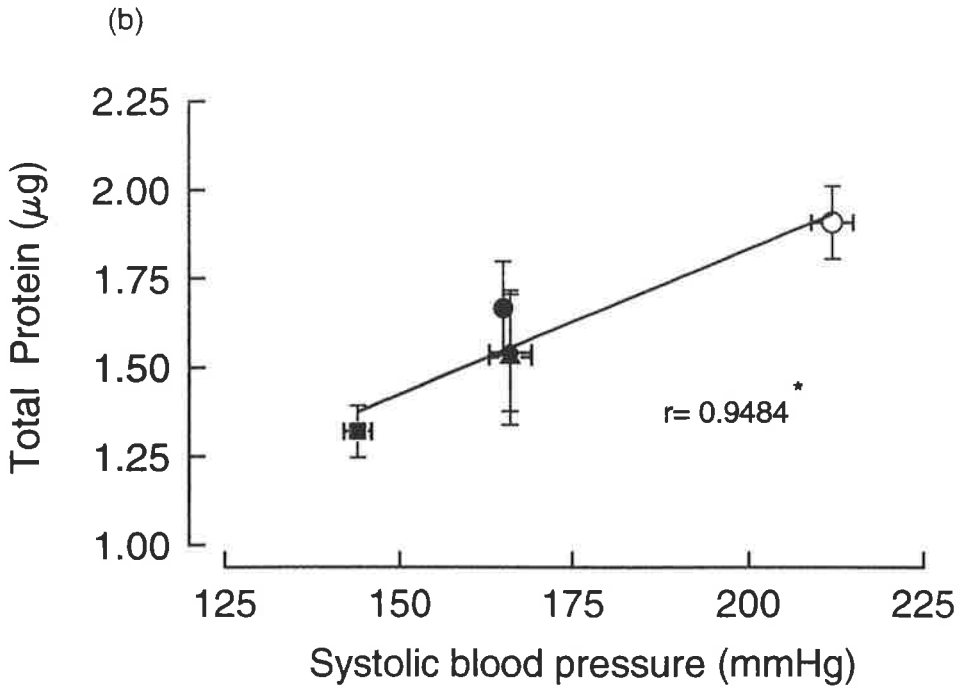


Fig. 5.21. Relationship between mean systolic blood pressure and protein levels in (a) mesenteric branches and (b) superior mesenteric artery from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.
* significant correlation of all groups, p= 0.0140.

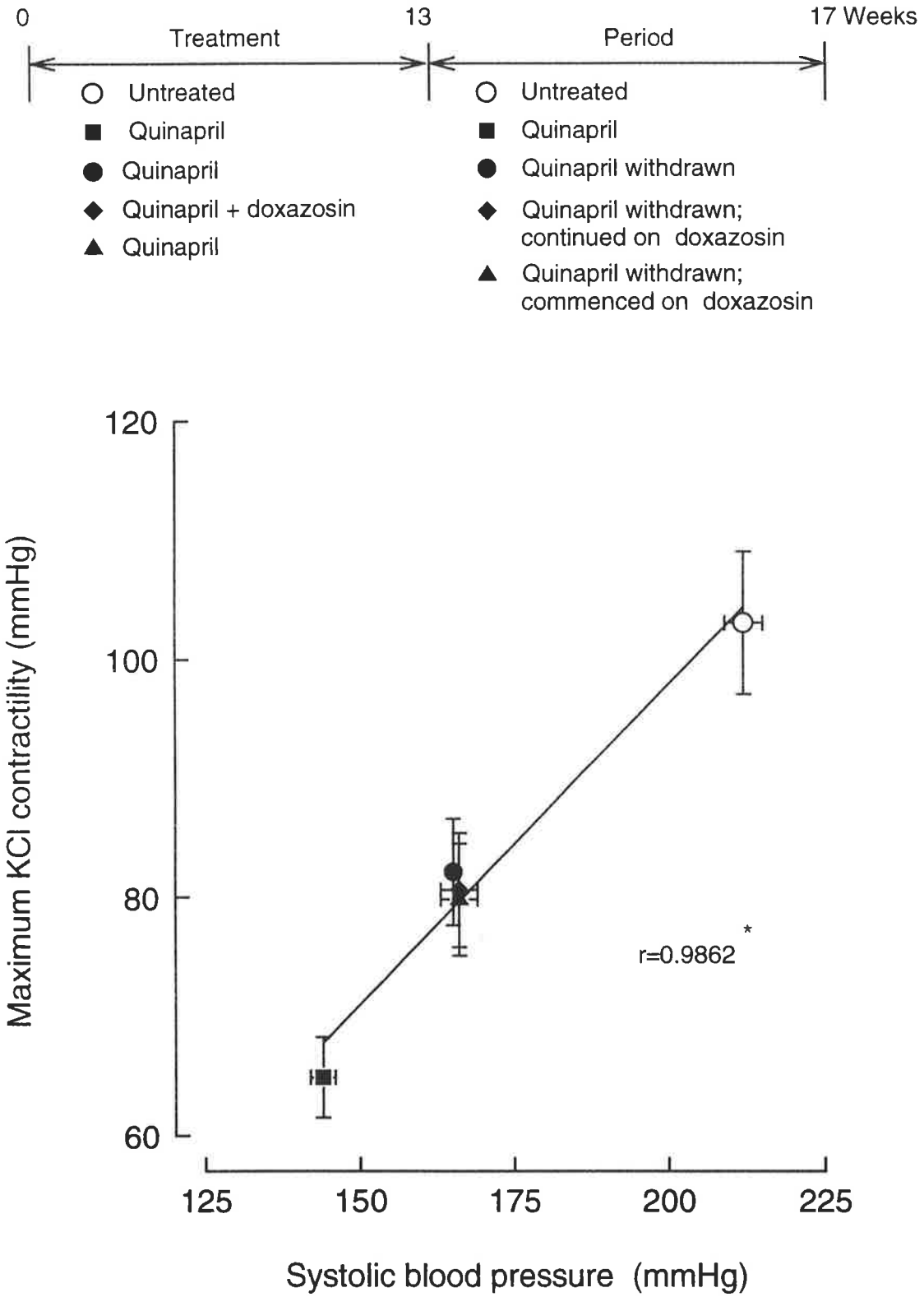


Fig. 5.22. Relation between mean systolic blood pressure and maximum KCl contractility of perfused mesenteric vasculature from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

* significant correlation of all groups, p=0.0019.

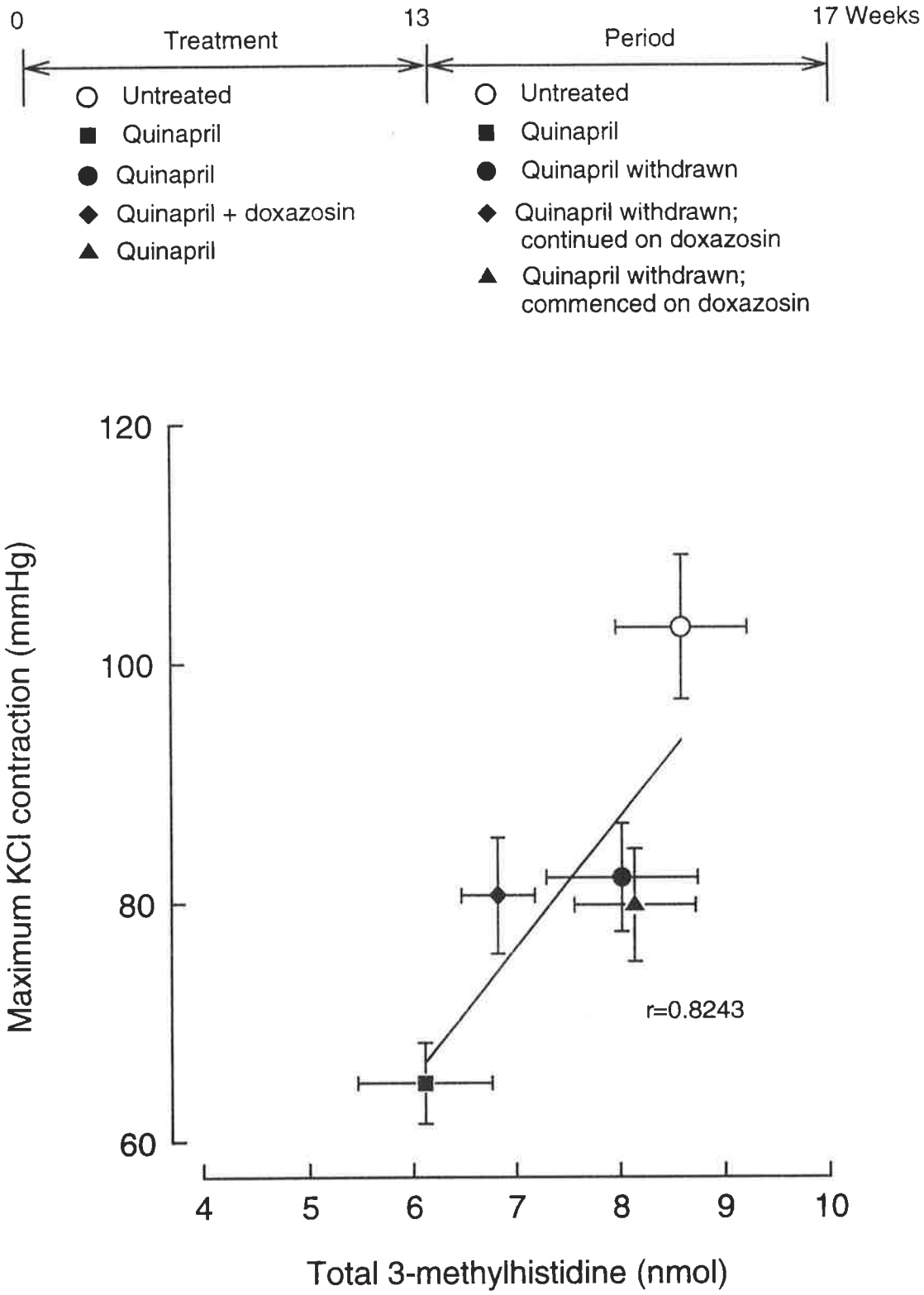


Fig. 5.23. Relation between mean maximum KCl contractility and absolute levels of 3-methylhistidine of mesenteric branches from untreated and treated SHRs after a 17 weeek treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

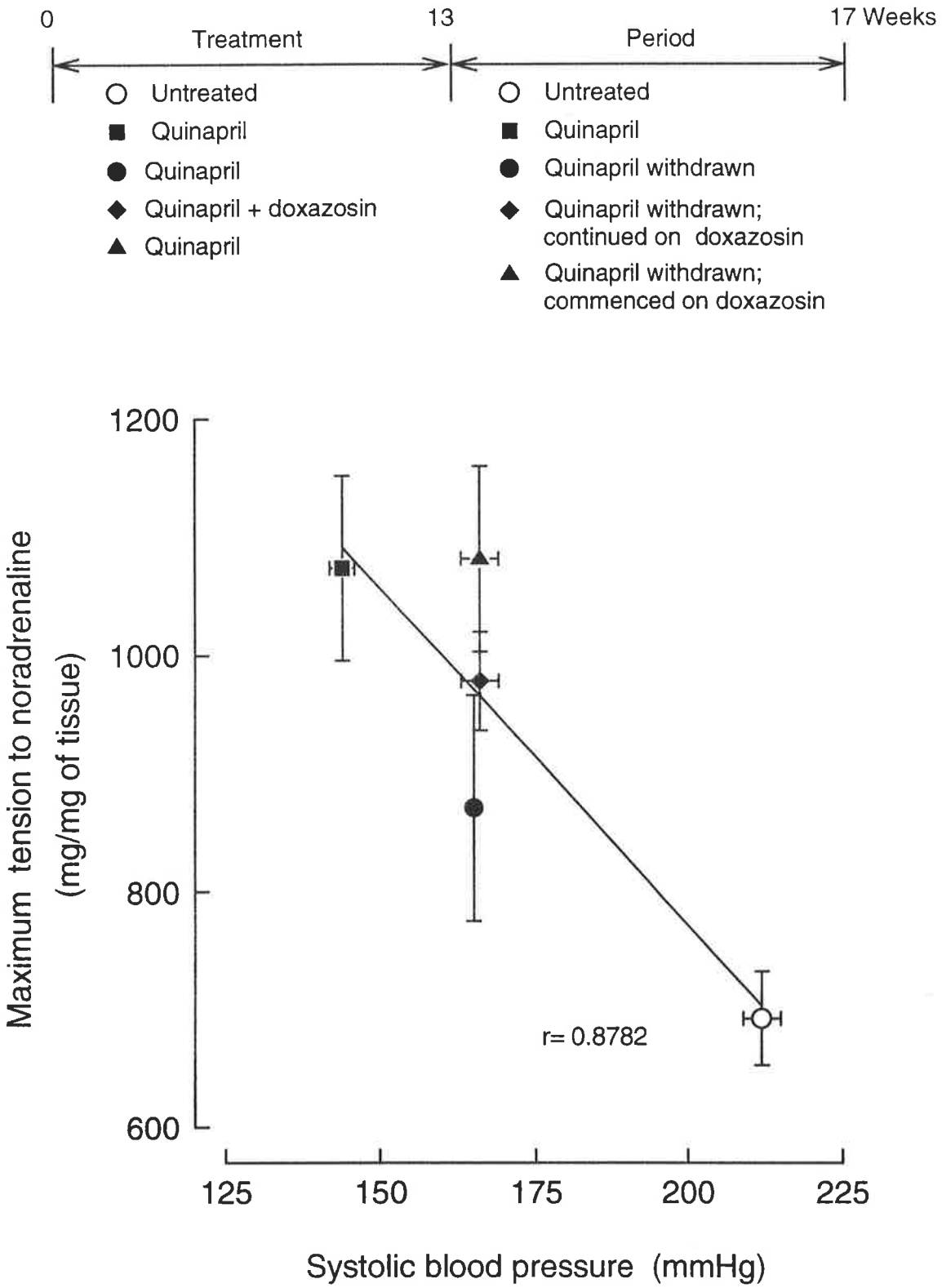


Fig. 5.24. Relationship between mean systolic blood pressure and maximum tension to noradrenaline of the thoracic aorta from untreated and treated SHRs after a 17 week treatment period. Each point represents the mean value for 7 to 8 rats ± s.e.m.

5.4 DISCUSSION

5.4.1 Blood pressure

In the present study there was an increase in systolic blood pressure in the untreated SHR with time which seemed to plateau at 15 weeks of treatment. Treatment of SHRs with quinapril prevented this rise in systolic blood pressure which was consistent with the findings of the previous chapter. However, within 2 weeks of withdrawing quinapril treatment systolic blood pressure had risen by 20 mmHg but continued to be lower than the systolic blood pressure of untreated SHRs. The rise in blood pressure that was seen is in keeping with the observations of other investigators who have demonstrated an immediate rise of blood pressure upon withdrawal of an ACE inhibitor, which is then followed by a persistent reduction of systolic pressure with release periods ranging from 7 to 22 weeks (Giudicelli *et al* 1980; Cadilhac & Giudicelli, 1986; Black *et al* 1989; Christensen *et al* 1988; Harrap *et al* 1990; Kost *et al* 1995). Furthermore, this persistent reduction in blood pressure is generally specific for ACE inhibitors and nonpeptide angiotensin II receptor antagonists, although evidence to the contrary does exist (Weiss, 1975; Limas *et al* 1983; Morton *et al* 1992).

The precise mechanism which is involved in maintaining the reduction of blood pressure upon withdrawal of the antihypertensive therapy is unclear; however, it has been demonstrated that total peripheral resistance is reduced with ACE inhibitor treatment of SHRs (Giudicelli *et al* 1980; Harrap *et al* 1990) and persists after withdrawal of the inhibitor. Therefore, it has been suggested that it is the reduced total peripheral resistance which maintains the persistent reduction in blood

pressure (Harrap *et al* 1990). Another possibility that has been suggested involves the expression of hypertensive genes and that ACE inhibitor treatment in young SHRs may interfere with the genetic process that determines blood pressure and vascular mass (Harrap & Lever, 1989).

It is not clear as to which mechanism is involved in the rise in blood pressure that occurs after withdrawing ACE inhibitor treatment and therefore the possible involvement of the sympathetic nervous system was investigated by blockade of the α_1-adrenoceptors with doxazosin. Doxazosin was not able to prevent the rise in blood pressure upon withdrawal of quinapril regardless of whether it was administered during and after quinapril treatment (17 weeks) or for 4 weeks after quinapril treatment. If the sympathetic nervous system is not involved (as suggested by the results of the present study) the rise in blood pressure that was seen upon quinapril withdrawal may simply be due to a direct physiological effect of angiotensin II.

In agreement with the previous study, quinapril was able to prevent cardiac hypertrophy. The actions of quinapril on left ventricular hypertrophy after cessation of treatment, regardless of whether doxazosin was administered, appeared to largely parallel blood pressure changes these results being consistent with the literature (Giudicelli *et al* 1980; Adams *et al* 1990). It has also been suggested that an intrinsic enhancement of myocardial performance due to left ventricular hypertrophy could also contribute to the redevelopment of hypertension (Adams *et al* 1990).

5.4.2 Vascular reactivity

5.4.2.1 Perfused mesenteric vascular bed

Contrary to the previous study, at the age of 22 weeks there was not a significant difference between preparations from untreated SHRs and quinapril treated SHRs to nerve stimulation. These results suggest that the amount of noradrenaline released upon nerve stimulation was less in the untreated SHR at the age of 22 weeks of age since responses to exogenous noradrenaline were augmented when compared with preparations from quinapril treated SHRs. These results are consistent with Collis and Vanhoutte (1977) who observed that responses evoked by renal nerve stimulation in kidneys from older SHRs were similar to those in kidneys from control rats but responses to exogenous noradrenaline were augmented (Collis & Vanhoutte, 1977). Lais *et al* (1974) demonstrated that adult SHRs responded similarly to WKYs upon stimulating the hindquarters suggesting that once the increased systolic blood pressure has been established the involvement of the sympathetic nervous system is toned down (Lais *et al* 1974).

An interesting finding, was that the cessation of quinapril treatment resulted in the augmentation of responses to nerve stimulation (although only statistically different at 16 and 32 Hz) suggesting the involvement of presynaptic receptors. The enhanced responsiveness may involve increased sensitivity of angiotensin type 1 (AT_1) receptors and $ß_2$-receptors since both facilitate the release of noradrenaline presynaptically (de Champlain, 1990; Jonsson *et al* 1993). In addition, responses to exogenous noradrenaline were significantly augmented at the upper end of the concentration curve in preparations from SHRs withdrawn from quinapril treatment

when compared with responses in preparations from quinapril treated SHRs. It can therefore also be suggested that postsynaptically altered processes (ie. beyond receptor occupancy) may be influencing the responses to stimulation and exogenous noradrenaline after the cessation of quinapril.

Responses to noradrenaline, phenylephrine and potassium chloride, in preparations from "released" rats, were intermediate by comparison with preparations from quinapril treated and untreated SHRs. Responses to potassium chloride in preparations from SHRs withdrawn from quinapril tended to be greater than those in preparations from quinapril treated SHRs although the values did not reach statistical significance. Furthermore, sensitivity was not altered in preparations from any of the groups to potassium chloride. The increased maximal response with no change in sensitivity to potassium chloride in preparations from SHRs withdrawn from quinapril treatment suggests that structural changes may have occurred in the mesenteric resistance vessels. Differences seen between the quinapril treated and quinapril released animals were greatest at maximal response, with no difference at minimal response, a pattern suggesting that the structural change is an increase in vessel wall thickness with small reductions of the lumen diameter (Griffin *et al* 1991). The slopes of the potassium chloride-response curves from the quinapril released group were also slightly greater when compared with slopes of the quinapril treated group, further suggesting that a small degree of structural change may have occurred. These results are consistent with morphological studies in which Morton *et al* (1992) demonstrated (after animals were withdrawn from a 10 week treatment period with losartan or captopril for 15 weeks),that the media/lumen ratio of mesenteric resistance arteries was still lower than that in control animals but had increased from the end of treatment in both cases. Similarly, other investigators

have also demonstrated that withdrawal of an ACE inhibitor results in media:lumen increases from the end of the treatment period; however, they remain lower than control animals (Freslon & Giudicelli, 1983; Harrap *et al* 1993).

With regard to contractility, there seems to be inconsistency in the literature whereby a number of studies have demonstrated that reduced contractility in resistance arteries is similar to that seen before withdrawal of the ACE inhibitor both in perfused and ring preparations (Adams *et al* 1990; Freslon & Giudicelli, 1983). On the other hand, some investigators have demonstrated that contractility reverts to levels seen in untreated SHRs after ACE inhibitor withdrawal (Cadilhac & Giudicelli, 1986; Smid, 1995). Why these discrepancies exist is unclear, particularly since the treatment periods were similar.

The presence of doxazosin in the animal, following withdrawal of quinapril, could not prevent the increase in responses to potassium chloride seen when quinapril was withdrawn even though responses to nerve stimulation, noradrenaline and phenylephrine remained attenuated therefore suggesting (as in the previous chapters) that the reduced contractility seen in response to nerve stimulation, noradrenaline and phenylephrine was due to a specific effect of doxazosin on adrenoceptors.

5.4.2.2 Aortic ring preparations

Quinapril treatment was able to augment contractility in aortic rings when compared with aortae from untreated SHRs which was also consistent with the previous study (Chapter 4). As discussed in the previous chapter, ACE inhibitors have been shown to influence media:lumen ratio (Owens, 1987; Richer *et al* 1991;

Freslon *et al* 1992), collagen and elastin levels (Richer *et al* 1991; Albaladejo *et al* 1994; Benetos *et al* 1994), calcium handling (Sada *et al* 1990),and membrane permeability (Ito *et al* 1981) which could all result in the enhanced contractility that is seen with ACE inhibition. Discontinuation of quinapril treatment resulted in the responses to noradrenaline in the aorta being attenuated. These results are consistent with morphological studies whereby aortic collagen and elastin densities and media thickness increase upon withdrawal of an ACE inhibitor (Richer *et al* 1991) which could result in the attenuation of contractility.

The doxazosin treatment prevented the attenuated responses seen in the aorta after discontinuing quinapril therapy. Studies have demonstrated that both doxazosin and prazosin can inhibit angiotensin II induced increases in medial smooth muscle cell DNA synthesis of the carotid artery and aorta suggesting that these increases in smooth muscle cell DNA synthesis are mediated via stimulation of α_1-adrenoreceptors (van Kleef *et al* 1992; van Kleef *et al* 1996). Angiotensin II has also been demonstrated to potentiate responses of α_1-adrenoceptor agonists (Qiu *et al* 1994). In addition, doxazosin has also been shown to reduce aortic collagen synthesis (Chichester & Rodgers, 1987). It is therefore suggested that the enhanced contractility seen in aortae of animals treated with doxazosin during and after withdrawal of quinapril therapy may be a result of doxazosin inhibiting trophic effects imposed on both the extracellular matrix and smooth muscle cells of the aorta. The aortic 3-methylhistidine data of the present study also suggest that doxazosin may be preventing smooth muscle cell growth after the withdrawal of quinapril since 3-methylhistidine levels (indicative of hypertrophy) appeared to be reduced in the aortae of animals treated with doxazosin when compared with levels in aortae from untreated and "released" SHRs.

Doxazosin treatment for 4 weeks after the withdrawal of quinapril also prevented the attenuated responses seen in aortae after discontinuing quinapril therapy. Although the administration of doxazosin alone (as shown in the previous chapter) could not augment responses in the aorta it may be possible that once normotensive levels are achieved with quinapril treatment then doxazosin can maintain these levels. Since 3-methylhistidine levels (indicative of hypertrophy) were also reduced in the aortae of these animals it is suggested that 4 weeks of doxazosin therapy after withdrawal of quinapril continues to inhibit the trophic effects imposed on the aorta and thereby preventing the attenuation of contractile responses.

Nevertheless, this effect of doxazosin is independent of blood pressure since the rise in blood pressure seen upon withdrawal of quinapril could not be prevented by doxazosin. The results of the present study are consistent with other investigators who have demonstrated that α_1-adrenoceptor antagonists can prevent angiotensin II induced increases in medial DNA without effect on blood pressure (van Kleef *et al* 1992). Therefore it can be suggested that a pressure independent mechanism is also involved in influencing contractility of the aorta.

5.4.3 Biochemical Parameters

DNA levels in aortae from all treatment groups were significantly increased in comparison to levels in aortae from untreated SHRs. With regard to the levels of contractile proteins it was demonstrated that aortic 3-methylhistidine content after discontinuing quinapril treatment for 4 weeks reverted back to levels seen in aortae of the untreated SHR. However, when doxazosin was administered either during

and after quinapril withdrawal or for 4 weeks (after quinapril cessation) the levels of 3-methylhistidine remained similar to levels seen in aortae from quinapril treated animals.

Collectively the data suggests that quinapril and doxazosin treatments prevented hypertrophy of the aortic smooth muscle cells as indicated by the reduced levels of 3-methylhistidine and the increase in DNA levels reflects the decrease in smooth muscle size. However, the aortae from animals which had been withdrawn from quinapril treatment showed increased levels of DNA and 3-methylhistidine. The increased aortic 3-methylhistidine content indicating a selective increase in contractile proteins and suggesting that hypertrophy of smooth muscle cells had occurred. These results are consistent with morphological studies whereby aortic media thickness increases upon withdrawal of an ACE inhibitor (Richer *et al* 1991). Therefore it is suggested that the increase in aortic DNA levels seen upon withdrawal of quinapril treatment in the present study may indicate polyploidy which is associated with hypertrophy.

The decrease in DNA, 3-methylhistidine and protein level (in absolute terms) in the superior mesenteric artery and mesenteric branches after quinapril treatment was consistent with the results of the previous study suggesting that quinapril had inhibited smooth muscle cell growth. Four weeks of release from quinapril treatment was associated with the reversal of the reduced levels of 3-methylhistidine, DNA and proteins in both the superior mesenteric artery and mesenteric branches to levels similar to that seen in untreated SHRs. However, the increase in total proteins in the superior mesenteric artery was not as prominent after release from quinapril. The administration of doxazosin during and after quinapril treatment prevented the 3-methylhistidine and DNA levels increasing after the cessation of

quinapril both in the superior mesenteric artery and mesenteric branches. However, this treatment regimen appeared to be more effective in the superior mesenteric artery. In addition, increases in total protein were also prevented in the mesenteric branches with doxazosin treatment. The administration of doxazosin for 4 weeks after the withdrawal of quinapril appeared less effective in preventing the rise in the biochemical markers in the superior mesenteric artery and its branches after quinapril treatment was discontinued.

Collectively, the data suggest that the increase in contractile protein and DNA content in the mesenteric vasculature after a 4 week withdrawal period from quinapril treatment was due to trophic effects mediated via stimulation of α_1-adrenoceptors. Furthermore, the reactivity studies of the present study demonstrated that responses to nerve stimulation in perfused mesenteric preparations from animals released from quinapril treatment were enhanced. It could also be suggested that it is the sympathetic nervous system which is having trophic influences on the structure of the mesenteric vasculature and these influences are mediated by α_1-adrenoceptors activation. This influence in turn could be enhanced by angiotensin II since angiotensin II production would occur after cessation of quinapril and angiotensin II has been shown to facilitate presynaptic noradrenaline release (Nilsson & Folkow, 1982; Jonsson *et al* 1993) and inhibit noradrenaline uptake (Jackson & Campbell, 1979).

The differences seen between the superior mesenteric artery and mesenteric branches with regard to doxazosin's ability to prevent the increases in the biochemical parameters upon withdrawal of quinapril may reflect the degree of sympathetic innervation, since sympathetic innervation per unit of cross-sectional wall area is smaller in the superior mesenteric artery when compared with

mesenteric branches (Smeda & Lee, 1991). In addition, the difference in the type of smooth muscle cell growth which occurs in the two types of vessel, ie. hypertrophy versus hyperplasia, may also influence the degree to which doxazosin can prevent the increases in the biochemical parameters measured.

The inability of the 4 week treatment period with doxazosin after withdrawal of quinapril to be as effective in preventing the increases in 3-methylhistidine and DNA content of the superior mesenteric artery and its branches may suggest that when doxazosin is co-administered with quinapril, the ACE inhibitor potentiates doxazosin's α_1-adrenoceptor antagonist action (Tierney *et al* 1989) and possibly inhibits growth of the vessel wall to a greater degree than quinapril alone thereby masking any vessel growth that may result upon withdrawal of quinapril treatment.

5.5 SUMMARY

In summary, cessation of quinapril therapy was associated with an increase in systolic blood pressure and contractility of the mesenteric vasculature but remained lower than untreated SHRs. In contrast, the 3-methylhistidine, protein and DNA content of the mesenteric vasculature reverted to levels seen in tissues of untreated SHRs. Furthermore, it was shown that the α_1-adrenoceptors appeared to be involved in increasing 3-methylhistidine, protein and DNA levels of the mesenteric vasculature but not involved in increasing systolic blood pressure.

The present study also demonstrated that reactivity of the mesenteric vascular bed related well to systolic blood pressure. However vascular reactivity did not significantly correlate with the vascular contractile protein content in a simple manner. It is suggested that a process such as remodeling may be involved in

influencing vascular reactivity rather than growth of the vessel wall upon withdrawal of ACE inhibitor therapy.

With regard to the thoracic aorta, it is suggested that a pressure independent mechanism mediated via α_1-adrenoceptor activation may also be involved in altering contractility of the aorta. In addition, stimulation of α_1-adrenoceptors may also influence growth of the vessel wall.

CHAPTER 6

The Effect of Quinapril Treatment and its Withdrawal on Medial Thickness in the Aorta and Mesenteric Circulation of the SHR

6.1 INTRODUCTION

The previous chapter, using both biochemical and functional markers. identified that quinapril treatment of SHRs prevented alterations in the vascular structure of both conduit and resistance vessels that are seen in untreated SHRs . In addition, the effect of discontinuing quinapril treatment was also investigated. To further understand the changes seen in the previous study a preliminary evaluation was conducted to investigate the morphology of both conducting and resistance vessels.

The aim of this preliminary study was to examine: (1) whether chronic quinapril treatment of SHRs affects the medial thickness of blood vessels and (2) whether any effect on this layer, which is seen with quinapril treatment persists upon withdrawal of the quinapril.

6.2 METHODS

6.2.1 Animal and drug treatments

Male SHR and WKY rats were maintained as described previously (Chapter 2). At 5 weeks of age, SHRs were randomly assigned to either untreated (control) or quinapril treated groups. The antihypertensive agent was administered as previously described (Chapter 2). After 13 weeks of treatment quinapril treated animals were further divided into 2 groups: ie. those that were continued on quinapril treatment or those that were discontinued from quinapril therapy and maintained on water for a further 4 weeks. All animals were subjected to weekly

measurements of weight. Systolic blood pressures using an indirect tail-cuff method (described in Chapter 2) were measured at four weekly intervals during the first 13 weeks of treatment and then every 2 weeks during the remaining four weeks of therapy. After a total of 17 weeks of treatment the blood vessels were fixed using the perfusion fixation procedure described in Chapter 2.

6.2.2 Morphometry

The thoracic aorta and type 2 mesenteric resistance branches (Fig. 6.1) were dissected after the blood vessels were fixed. Tissues were then embedded, sectioned and stained as described in chapter 2. The histological sections were prepared at the Centre For Electron Microscopy and Microstructure Analysis (CEMMSA), University of Adelaide, South Australia. The thickness of the media in these vessels was measured using an image analysis program (see Chapter 2 for details).

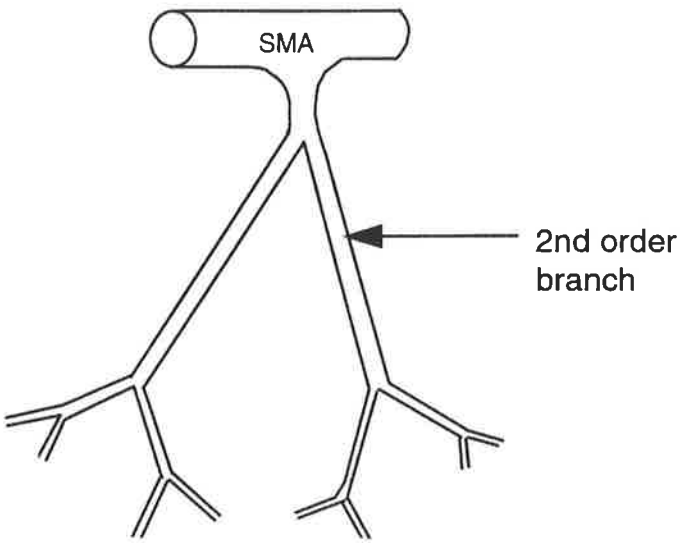


Fig. 6.1. Schematic representation of the mesenteric vascular bed which shows the segment of the mesenteric circulation which was used in the morphometric assessment of medial hypertrophy. SMA = superior mesenteric artery.

6.3 RESULTS

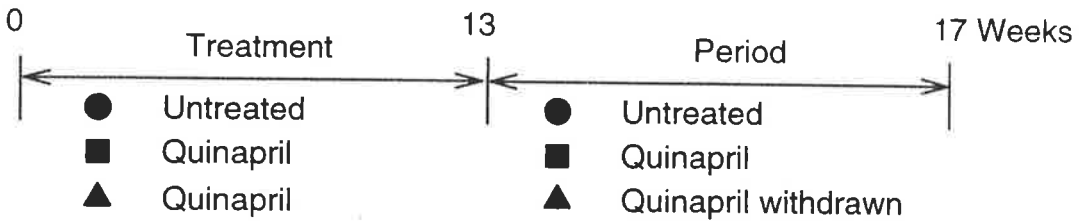
6.3.1 Blood Pressure

In the untreated SHR the systolic blood pressure rose with age and seemed to plateau at 22 weeks of age (Fig. 6.1). Chronic quinapril treatment for 22 weeks prevented the development of hypertension in the SHR (Fig. 6.1). After drug withdrawal, blood pressure rose but was still significantly lower than that in untreated SHRs (Fig. 6.1).

6.3.2 Morphometry

Aorta : The medial thickness of aortae from quinapril treated SHRs was significantly less than the medial thickness of aortae from both untreated SHRs (Fig. 6.2). Discontinuing quinapril treatment resulted in an increase in the medial thickness of aortae from these latter animals when compared with aortae from quinapril treated SHRs. However, the values were remained significantly less than the medial thickness of vessels from untreated SHRs (Fig. 6.2).

Type 2 mesenteric resistance vessel : In this group of vessels although significance was not reached there was a tendency for vessels from both the quinapril treated and quinapril treatment discontinued groups to have medial thicknesses which were less than the medial thickness of vessels from untreated SHRs (Fig. 6.3). In addition, it appeared that the medial thickness of mesenteric arteries from SHRs withdrawn from quinapril treatment were slightly greater than the media in vessels from quinapril treated rats (Fig. 6.3).



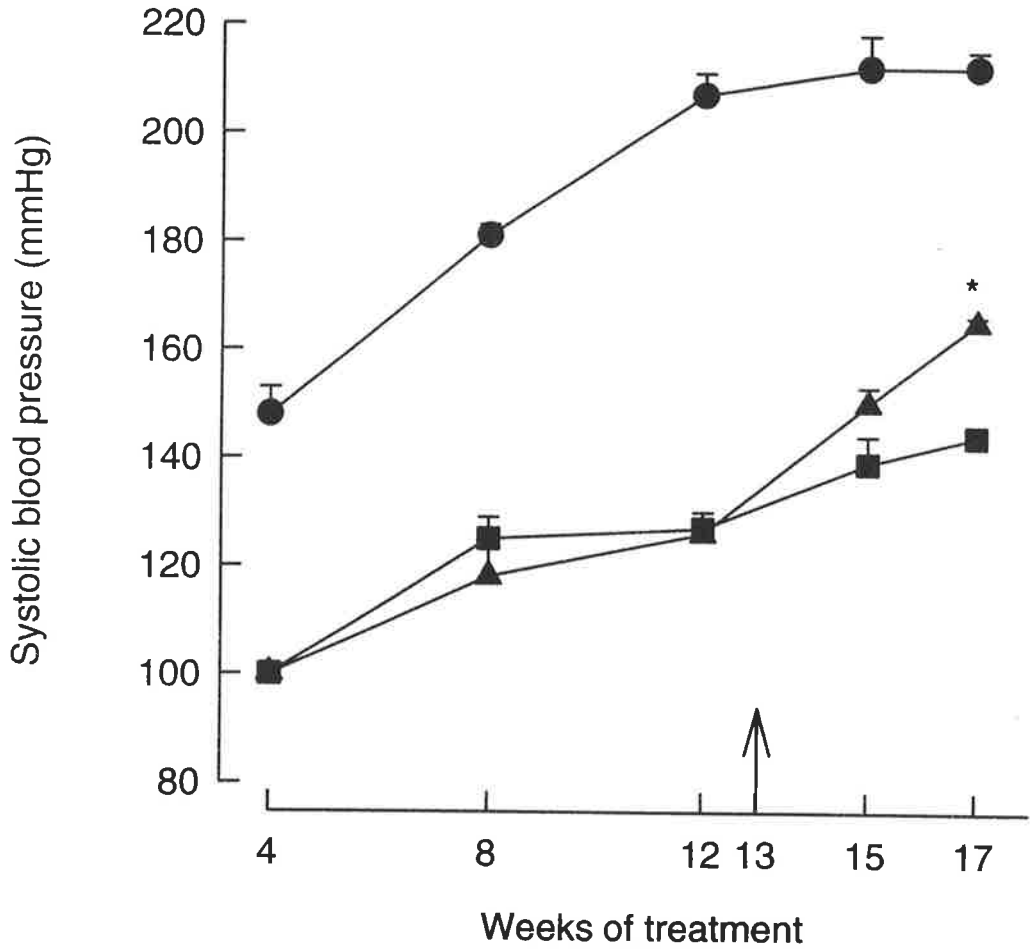


Fig. 6.1. Systolic blood pressure profile of untreated and treated SHRs during a 17 week treatment period. Each point represents the mean value for 5 to 8 rats ± s.e.m. At each time point treated SHR groups were significantly diiferent from untreated SHRs ($p<0.001$).

* $p<0.001$ compared with SHRs treated with quinapril.

↑ = Time point when SHRs were released from quinapril.

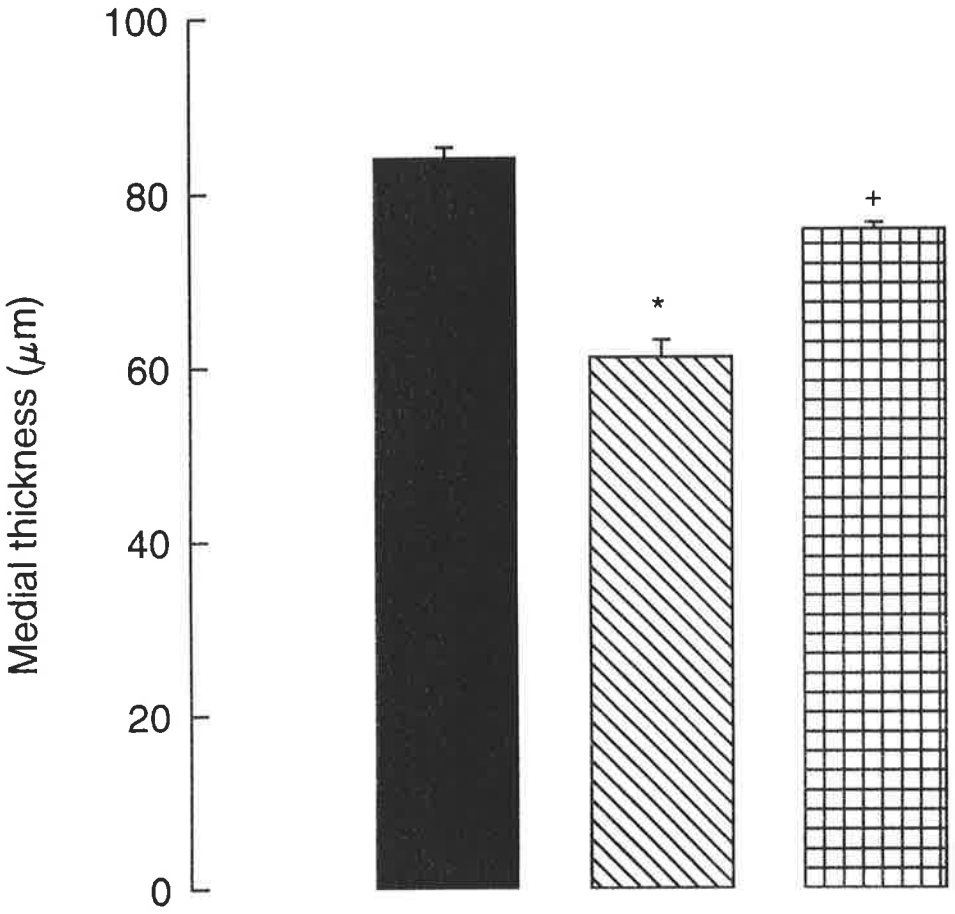


Fig. 6.2. Medial thickness of thoracic aorta from ■ untreated SHRs, ▨ SHRs treated with quinapril and ⊞ SHRs withdrawn from quinapril. Each bar represents the mean of 4 rats ± s.e.m.

* $p<0.001$ compared with untreated SHRs and those withdrawn from quinapril.

+ $p<0.01$ compared with untreated SHRs.

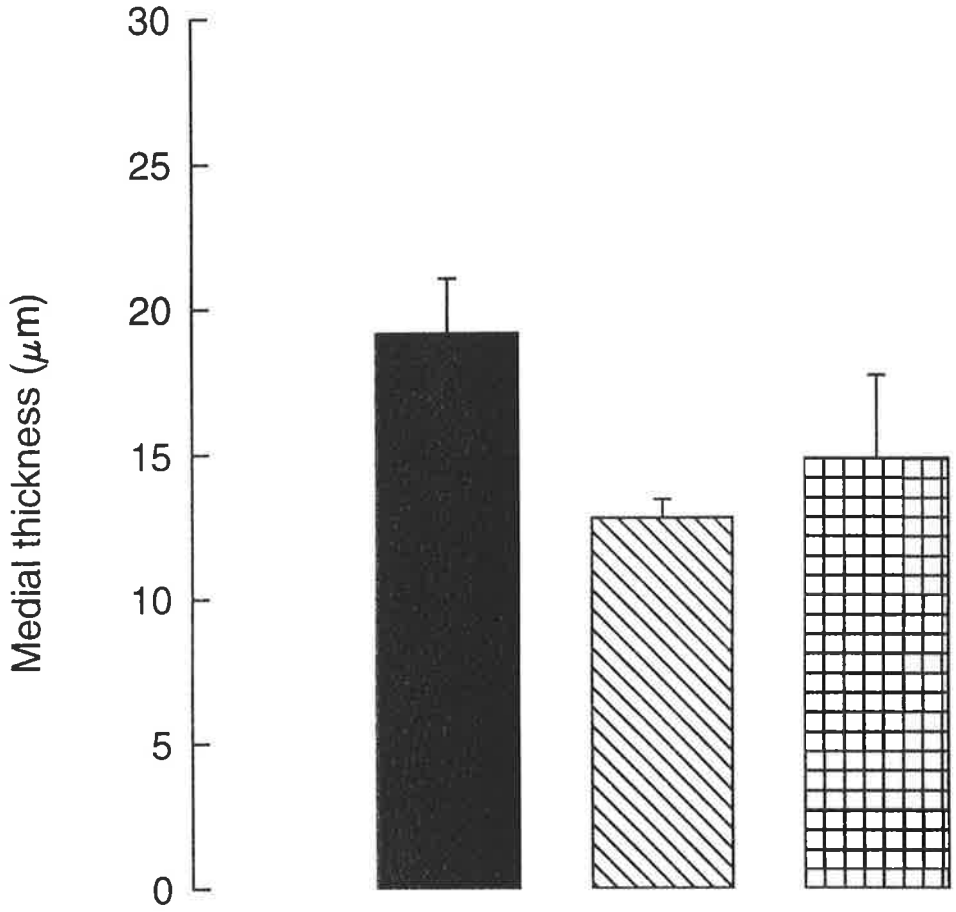


Fig. 6.3. Medial thickness of type 2 mesenteric branches from ■ untreated SHRs, ▨ SHRs treated with quinapril and ▦ SHRs withdrawn from quinapril. Each bar represents the mean of 4 rats ± s.e.m.

6.4 DISCUSSION

In the present morphological study quinapril treatment prevented hypertrophy of the media of the thoracic aorta in the SHR. These results are consistent with other investigators using ACE inhibitors and those obtained in the superior mesenteric artery of SHR, another conducting vessel (Freslon & Giudicelli, 1983; Lee *et al* 1991). Chronic treatment of young SHRs with ACE inhibitors has been shown to prevent medial hypertrophy and smooth muscle cell polyploidy (Owens, 1987) and to reduce the wall:lumen ratio of the aorta (Freslon & Giudicelli, 1983). Similarly, in the superior mesenteric artery , long term captopril treatment of SHRs resulted in a reduction of the medial area (Lee *et al* 1991).

Cessation of quinapril resulted in an increase in medial thickness of the aorta when compared to aortae from quinapril treated SHRs but this did not increase to the level seen in aortae from untreated SHRs. In the present study a relationship between systolic blood pressure and medial hypertrophy of the aorta has been shown to exist. However, in light of the data obtained in the previous chapter a pressure-independent mechanism may also be involved. It is therefore suggested that the increase in medial width which is seen after withdrawal of quinapril is the result of the availability of angiotensin II which either exerts direct trophic effects or acts through potentiating the trophic effects of noradrenaline via α_1-adrenoceptors.

In the mesenteric resistance vessels of the SHR, although significance was not achieved (presumably due to the small number of vessels examined), there was a strong indication that quinapril treatment reduced the medial thickness in these vessels. The morphometric studies were in agreement with the biochemical analysis discussed in the previous chapter suggesting an inhibition of smooth muscle cell

growth. In addition, the results from the perfused mesenteric vasculature preparations which related to reactivity showed that quinapril treatment lowered maximal responses of the mesenteric vascular bed to potassium chloride, which is in agreement with the reduced medial mass due to treatment. These comparisons are made with caution, as the morphometry was conducted on one mesenteric vessel size but the biochemistry and functional studies were performed on the entire bed. Due to methological limitations it was not possible to use the vessels tested in the reactivity study for morphometry. Nevertheless, these results are in agreement with other morphometric studies which have demonstrated that ACE inhibitor therapy reduces medial mass in both large and small mesenteric resistance vessels (Lee *et al* 1991; Harrap *et al* 1993). In addition it has also been demonstrated, using the myograph, that reduced medial thickness in mesenteric resistance vessels from ACE inhibitor treated SHRs was associated with reduced contractility when compared with controls (Christensen *et al* 1989).

Withdrawal of quinapril treatment appeared to result in a small increase in medial thickness in the mesenteric vessels from SHRs. Support for an increase in medial thickness in mesenteric resistance vessels comes from other studies which have also demonstrated that the medial area in mesenteric vessels from SHRs increases upon withdrawal of chronic ACE inhibitor treatment but that the medial thickness remains reduced when compared to that seen in vessels from untreated animals (Freslon & Giudicelli, 1983; Morton *et al* 1992; Harrap *et al* 1993). Such results would also be consistent with the results from the reactivity studies in the previous chapter in which the maxima and slope of the potassium chloride-response curve of mesenteric preparations from SHRs which were withdrawn from quinapril indicated that the media:lumen ratio may have increased.

In the present study the data indicate that vascular structure influences (or is influenced) by blood pressure. After withdrawal of treatment there appeared to be a good relationship between blood pressure and medial thickness of the thoracic aorta and mesenteric branches but this does not exclude pressure independent mechanisms being involved as well.

CHAPTER 7

GENERAL DISCUSSION

The aetiology of essential hypertension has been under intense investigation since the early 1800's. Despite these intensive research efforts a unified cause-effect relationship or hypothesis for the disorder has not as yet been established. However, over the years, the availability of animal models of hypertension and a range of pharmacological therapies have become important tools in understanding the relationship between hypertension and alterations in cardiovascular structure and function.

It has been well established that in the genetic rat model of hypertension, the SHR, the elevated systolic blood pressure is associated with an increase in total peripheral vascular resistance (Trippodo & Frohlich, 1981; Nordlander, 1988). This increase in total peripheral resistance is thought to be the consequence of morphological alterations in the resistance vessels (Webb & Bohr, 1981). It is agreed that the structural changes are due to an increase in vessel thickness and smooth muscle mass resulting in an increase in media to lumen ratio. The structural alterations seen in hypertension are believed to be responsible for the hyperresponsiveness of the resistance vessels to various agents. From a functional standpoint there are two enhanced (amplified) responses in hypertension, one neurogenic and the other non-neuronal. Studies in the SHR suggest an increased sympathetic innervation (hypernoradrenergic innervation) which supports neurogenic enhanced responses (Head, 1989). Non-neurogenic enhanced or amplified responses are characterised by dramatically enhanced contraction of blood vessels to a variety of (non-specific) vasoactive agents. Functional changes such as

alterations in membrane permeability and intracellular calcium handling may contribute to the increased reactivity.

Evidence has also accumulated over the years to implicate both the sympathetic nervous system (SNS) and the renin-angiotensin system (RAS) in influencing systolic blood pressure and altering vascular structure . Sympathectomy or ACE inhibition have both been demonstrated to prevent the development of hypertension, vascular hypertrophy and hyperreactivity of resistance vessels in the SHR (Lee *et al* 1987; Lee *et al* 1991; Korner *et al* 1993).

The main aim of this thesis was to examine the basis of amplified responses in blood vessels in hypertension. This involved investigating the relationship between contractile proteins, contractility, angiotensin II, and systolic blood pressure development in the SHR.

In chapter 3 of the thesis it was demonstrated that mineralocorticoid therapy produced an increase and sustained elevation in systolic blood pressure in the normotensive WKY rat. The increased systolic blood pressure was associated with marked increases in total 3-methylhistidine, DNA and protein content of the mesenteric vasculature suggesting hypertrophy of the blood vessels. However, contractile responses of the perfused mesenteric vascular bed preparation from hypertensive WKY rats were similar to responses seen in preparations from normotensive WKY rats. In addition it was also suggested that an increase in a marker for contractile proteins (3-methylhistidine) is not a prerequisite for enhanced contractility. Moreover, the elevated systolic blood pressure and increases in vascular total 3-methylhistidine, DNA and protein content did not appear to be influenced by ACE inhibition, α_1-adrenoceptor antagonism and calcium channel blockade.

With regard to the SHR, it was shown that maximum contractility of the mesenteric vascular bed from the SHR was greater than in the corresponding vascular bed of the normotensive WKY rat. In contrast to the hypertensive WKY, in the SHR the total 3-methylhistidine, DNA and protein content was increased only in the superior mesenteric artery but not in the mesenteric branches. Moreover, chronic treatment of SHRs with an ACE inhibitor (but not with an α_1-adrenoceptor antagonist and calcium channel blocker) prevented the development of hypertension and appeared to normalise the contractile responses. Interestingly, ACE inhibition consistently decreased the levels of 3-methylhistidine, DNA and protein in the superior mesenteric artery and its branches.

The study discussed immediately above was extended to determine the effect of withdrawal of the ACE inhibitor on blood pressure, contractility and the levels of 3-methylhistidine, DNA and protein. Upon cessation of the ACE inhibitor systolic blood pressure and vascular contractility remained significantly lower than that seen in untreated SHRs. By contrast, total 3-methylhistidine, DNA and protein content of the mesenteric vasculature, which were all depressed with ACE inhibition, reverted to levels seen in vessels from untreated SHRs. However, the coadministration of an α_1-adrenoceptor antagonist with the ACE inhibitor and its continued administration after the withdrawal of the ACE inhibitor, resulted in maintenance of the reduced level of total 3-methylhistidine, DNA and protein in the superior mesenteric artery and branches. This treatment was unable to prevent the increase in systolic blood pressure and contractile responses.

In the SHR the analysis of type 2 mesenteric branches by histological methods indicated that ACE inhibition reduced the medial thickness of the mesenteric branches. Furthermore, withdrawal of the ACE inhibitor appeared to

result in a small increase in media thickness.

A number of key features are evident from these studies and provided information into the basis of the enhanced contractile responses to vasoactive agents seen in the mesenteric vascular bed of the SHR.

Firstly it was demonstrated that making the genetic normotensive control for the SHR, the WKY, hypertensive failed to reveal a pattern of cardiovascular functional changes similar to that which is characteristic of the SHR. In the hypertensive WKY rats it was shown that enhanced contractility of the resistance vessels was not critical to the development and/or maintenance of hypertension. It was also demonstrated that ACE inhibition and α_1-adrenoceptor blockade did not influence the levels of total 3-methylhistidine, DNA and protein in the mesenteric vasculature suggesting systems other than the RAS and SNS are involved in the growth of blood vessels in this model. Although a number of hypotheses were addressed in chapter 3 it is also suggested that enhanced levels of total 3-methylhistidine, DNA and protein (hypertrophy) may be an adaptive response to the increasing systolic blood pressure consequent on volume expansion and not necessarily involved in the maintenance of the hypertension in this model. In addition, fluid retention and other characteristics of DOCA-salt may not be appropriate surrogates for genetic mechanisms of the SHR. It is also possible that WKY rats may carry genes that prevent pressure rises in response to hypertensive stimuli and thereby not "reveal" components of the hypertensive process.

Secondly, (and in contrast to the hypertensive WKY), the studies in which the SHR was used demonstrated that in this model of hypertension a relationship existed between systolic blood pressure and contractility of resistance vessels. It was demonstrated that enhanced contraction was associated with hypertension. For

example the prevention of hypertension with chronic ACE inhibitor therapy was associated with attenuated contractile responses in the perfused mesenteric preparation. Moreover, withdrawal of the ACE inhibitor resulted in the systolic blood pressure rising which was also associated with the maximum contractility of the perfused mesenteric preparation increasing in magnitude. Blood pressure and contractility were well correlated, suggesting that enhanced contraction of the mesenteric vasculature should not be ignored as a potential cause of the elevated blood pressure.

Thirdly it was observed that there was not a straightforward relationship between contractility and contractile protein content (as measured by 3-methylhistidine). In both models of hypertension (H-WKY and SHR) the content of total 3-methylhistidine in the mesenteric vasculature did not necessarily reflect the degree of contractility produced by this vascular bed ie. an increased level of 3-methylhistidine was not necessarily associated with enhanced contractility. In the SHR, (but not in the hypertensive WKY rat), ACE inhibition resulted in both contractility of the perfused mesenteric preparation and total 3-methylhistidine content of the mesenteric vasculature being lowered. It could therefore be argued that under these precise conditions a relationship may exist between maximum contractility and 3-methylhistidine content. It is possible that this relationship is simply one of growth ie. treatment with an ACE inhibitor at an early age in the SHR causes vascular growth to be reduced and this is reflected in a decreased 3-methylhistidine content and decreased maximum contraction. However, the administration of an α_1-adrenoceptor antagonist during and after ACE inhibitor therapy maintained a reduced level of total 3-methylhistidine in the mesenteric vasculature with increased contractility. Therefore, under conditions of ACE inhibitor

withdrawal and the presence of α_1-adrenoceptor antagonist, the influence the ACE inhibitor has on the 3-methylhistidine content is independent of its influence on contractility. It is worth noting that the total 3-methylhistidine content of the mesenteric bed closely follows the levels of total DNA and protein and thereby (as mentioned above) vessel growth.

Fourthly, it was also demonstrated that the enhanced contractility of the mesenteric vasculature was not influenced by vascular smooth muscle mass (as indicated by levels of total DNA and protein) in a simple fashion. In the literature there is evidence which suggests that an increase in vessel thickness and smooth muscle mass results in an increase in media to lumen ratio which in turn is responsible for the hyperresponsiveness of resistance vessels seen in the SHR (ref). As mentioned earlier, the SHR characteristically displays enhanced contractility in the perfused mesenteric vascular bed which was not associated with increased levels of total DNA and protein in the mesenteric branches although there was evidence for increased 3-methylhistidine, DNA and protein content in the superior mesenteric artery. The latter suggests that the degree of vessel wall growth and smooth muscle mass is similar in the mesenteric branches in both the SHR and WKY.

Although the biochemical analyses suggested that the mesenteric resistance vessels from the SHR were not associated with increased vascular growth the reactivity data of the present thesis suggested that structural alterations occurred resulting in the enhanced contractility of the perfused mesenteric preparation. Both the maxima and slope of the concentration-response curves for preparations from SHRs had increased, indicating an increase in the media to lumen ratio in the mesenteric resistance vessels. The rearrangement of the same amount of vessel

wall material around a smaller lumen would result in the media to lumen ratio being increased. It is therefore suggested that eutrophic inward remodeling (Mulvany *et al* 1996) occurs in the mesenteric resistance vessels of SHRs resulting in an increased media to lumen ratio which in turn contributes to the increased maximum contraction of the mesenteric vasculature in this model.

Finally, the evidence obtained in the present thesis shows a strong involvement of the renin-angiotensin system in the development of hypertension in the SHR. Systolic blood pressure, vascular reactivity, medial thickness of the mesenteric branches and vascular growth were sensitive to ACE inhibition and therefore the inhibition of angiotensin II production. It also appears that the mesenteric levels of total 3-methylhistidine, DNA and protein under appropriate conditions were sensitive to α_1-adrenoreceptor blockade, thereby suggesting the involvement of the sympathetic nervous system. However, the involvement of adrenoreceptors in influencing the levels of vascular 3-methylhistidine, DNA and protein were only seen when the α_1-adrenoreceptor antagonist was administrated during and after ACE inhibitor therapy. Withdrawal of the ACE inhibitor was associated with the total 3-methylhistidine, DNA and protein content of the mesenteric vasculature returning to levels seen in tissues from untreated SHRs. However when the α_1-adrenoreceptor antagonist was administrated during and after ACE inhibitor therapy total 3-methylhistidine, DNA and protein content of the mesenteric vasculature was maintained at reduced levels. The administration of the α_1-adrenoreceptor antagonist was without effect on systolic blood pressure which was seen to increase upon cessation of ACE inhibitor therapy. It is then postulated that angiotensin II has two roles: 1) an indirect role which also involves the α_1-adrenoreceptors and influences vascular total 3-methylhistidine, DNA and protein

content and thereby growth of the vessel wall. 2) a direct role which may result in remodeling which affects contractility of the resistance vessels and systolic blood pressure.

The evidence presented in this thesis supports the significant role of the renin-angiotensin system in the development of hypertension in the SHR. However, while the precise details involved in the enhancement of contractility in the mesenteric vasculature cannot be elucidated, the mechanisms involved can be speculated upon in at least two distinct model systems.

Model 1. The mesenteric resistance vessels remodel during the developmental phase of hypertension in the SHR. The remodeling results in an increase in the media to lumen ratio in the mesenteric branches without altering the vessel mass (therefore DNA and protein content) and accordingly enhances maximum contractility. In this model, the administration of an ACE inhibitor during high blood pressure development prevents the remodeling and growth (as indicated by decreased levels of 3-methylhistidine, DNA and protein) both in the resistance vessels and superior mesenteric artery in the SHRs and results in a blood vessel with a diminished capacity for maximum contraction. Upon withdrawal of the ACE inhibitor, growth is reinstated by angiotensin II with 3-methylhistidine, DNA and protein levels returning to untreated levels. Moreover, contractility increases but fails to achieve the levels seen in untreated animals suggesting that the degree of remodeling of the mesenteric resistance vessels is to a lesser degree than that seen in vessels of untreated SHRs. The increase in growth seen upon withdrawal of the ACE inhibitor may be a measure of the capacity of the older rats to "makeup"

for the earlier suppression of vascular development by the ACE inhibitor (Korner & Bobik, 1995).

Model 2. In a similar fashion to Model 1, the mesenteric resistance vessels have increased media to lumen ratios in the SHR during development. A consequence of this diminished lumen there is an increased resistance to flow in the branches and an increased distal pressurisation of the intimal surface of the superior mesenteric artery. The superior mesenteric which is normally a conducting vessel is now under increased tone and is able to significantly contribute to contraction. The large vascular smooth muscle mass of the superior mesenteric artery is responsible for the enhanced maximal contraction seen in the perfused mesenteric bed of the SHR. In a similar fashion to Model 1, ACE inhibition prevents remodeling and normal growth in the branches and thereby eliminates the distal influence on the superior mesenteric artery and hence its role in contributing to contraction. This model also provides an explanation for the influence of α_1-adrenoceptor blockade after ACE inhibitor withdrawal. As a consequence of distal pressurisation of the superior mesenteric artery is hypertrophy. It was demonstrated in the DOCA-salt treated WKY that the hypertrophic response was indicated by increases in 3-methylhistidine, DNA and protein levels. In the SHR, the superior mesenteric artery and not the branches displayed an increase in 3-methylhistidine, DNA and protein levels ie. hypertrophy. It can be assumed that hypertrophy of the superior mesenteric artery is an event similar to that seen in most conducting vessels and is related to pressure and α_1-adrenoceptor stimulation. α_1-adrenoceptor blockade alone will not alter the angiotensin II dependent remodeling and growth in the mesenteric branches. However, after the release from the ACE inhibitor, remodeling

and growth reoccurrs in the mesenteric branches and its distal influence induces hypertrophy in the superior mesenteric artery. Under these conditions in the presence of an α_1-adrenoceptor antagonist the hypertrophy is inhibited despite the pressure influences consequent on remodeling in the branches. This model suggests that while the superior mesenteric artery may contribute to enhanced contractility in the SHR it need not be hypertrophied for its contribution to blood pressure elevation since the α_1-adrenoceptor antagonist prevented hypertrophy without an influence on blood pressure.

The principal objective of this thesis was to explore the mechanisms underlying the enhanced contractility seen in the mesenteric vascular preparations in the SHR. The results of this study have further characterised the properties of this process and have highlighted the critical role of angiotensin II in the development of the exaggerated responses. In addition, the results have drawn attention to the complex interaction between structure, function and growth. Finally, the results have suggested at least two model systems which may be explored to further define this process.

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Appendix I

Table 3.1. Body weights (grams) of animals at the end of the treatment period.

Normotensive WKY	H-WKY control	H-WKY quinapril treated	H-WKY doxazosin treated	H-WKY diltiazem treated
354.91 ± 6.21	259.5 ± 11.94	263.82 ± 12.57	257.55 ± 8.95	259.09 ± 9.87

Data represents the mean value for 7 to 10 rats ± s.e.m.

APPENDIX II

Table 3.1. 3-Methylhistidine levels expressed as nmol per mg wet tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	0.166 ± 0.00746	0.192 ± 0.0090	0.331 ± 0.0119	0.474 ± 0.0356
H-WKY control	0.193 ± 0.00456	0.180 ± 0.0143	0.178 ± 0.0270*	0.402 ± 0.0290
H-WKY quinapril-treated	0.179 ± 0.00634	0.198 ± 0.0060	0.166 ± 0.0234*	0.442 ± 0.0532
H-WKY doxazosin-treated	0.170 ± 0.00595	0.148 ± 0.0051	0.151 ± 0.0220*	0.340 ± 0.0471
H-WKY diltiazem treated	0.184 ± 0.00971	0.1956 ± 0.0228	0.150 ± 0.0211*	0.439 ± 0.0286

Table 3.2. DNA levels expressed as µg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	1.582 ± 0.0246	1.906 ± 0.1142	2.574 ± 0.0419	2.026 ± 0.0812
H-WKY control	1.686 ± 0.0785	1.955 ± 0.1515	3.313 ± 0.3228	2.259 ± 0.0831
H-WKY quinapril-treated	1.615 ± 0.0863	2.114 ± 0.0900	2.993 ± 0.3418	2.407 ± 0.0892
H-WKY doxazosin-treated	2.032 ± 0.0975*	2.065 ± 0.1155	3.475 ± 0.3734	2.373 ± 0.2899
H-WKY diltiazem-treated	1.790 ± 0.1729	2.361 ± 0.1671	3.539 ± 0.5509	2.476 ± 0.0697

Data represents the mean value for 5 to 6 rats ± s.e.m.
* $p<0.05$ compared with normotensive WKY rats.

Table 3.3. Protein levels expressed as mg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	0.205 ± 0.0105	0.175 ± 0.0133	0.130 ± 0.00563	0.121 ± 0.0017
H-WKY control	0.212 ± 0.0124	0.175 ± 0.0123	0.099 ± 0.0071*	0.118 ± 0.0062
H-WKY quinapril-treated	0.202 ± 0.0046	0.179 ± 0.0084	0.099 ± 0.0110*	0.120 ± 0.0046
H-WKY doxazosin-treated	0.211 ± 0.0105	0.149 ± 0.0096	0.092 ± 0.0764*	0.104 ± 0.0136
H-WKY diltiazem-treated	0.190 ± 0.0087	0.178 ± 0.0189	0.089 ± 0.0069*	0.122 ± 0.0022

Data represent the mean value for 5 to 6 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

Table 4.1. 3-Methylhistidine levels expressed as nmol per mg wet tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	0.156 ± 0.0057[a]	0.176 ± 0.0067	0.356 ± 0.0134	0.492 ± 0.0149
SHR control	0.174 ± 0.0031[a]	0.189 ± 0.0064	0.364 ± 0.0010	0.444 ± 0.0093*
SHR quinapril-treated	0.134 ± 0.0048	0.181 ± 0.0081	0.337 ± 0.0141	0.437 ± 0.0058*
SHR doxazosin-treated	0.159 ± 0.0047[a]	0.204 ± 0.0087	0.375 ± 0.0145	0.446 ± 0.0065*
SHR diltiazem-treated	0.165 ± 0.0047[a]	0.219 ± 0.0089*§	0.388 ± 0.0132	0.429 ± 0.0138*

Table 4.2. DNA levels expressed as µg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	1.577 ± 0.0271	1.804 ± 0.0971	2.630 ± 0.1114	1.991 ± 0.0537
SHR control	1.692 ± 0.0403*	2.123 ± 0.1140*	2.876 ± 0.0760	1.988 ± 0.0650
SHR quinapril-treated	1.801 ± 0.0516*	2.298 ± 0.0955*	3.321 ± 0.0949*§	2.010 ± 0.0513
SHR doxazosin-treated	1.709 ± 0.0282*	2.346 ± 0.0802*	3.177 ± 0.1015*	2.139 ± 0.0489
SHR diltiazem-treated	1.737 ± 0.0187*	2.299 ± 0.0485*	3.162 ± 0.1069*	2.062 ± 0.0486

Data represent the mean value for 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.
§ $p<0.05$ compared with SHR controls
a $p<0.05$ compared with SHRs treated with quinapril.

Table 4.3. Protein levels expressed as mg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
Normotensive WKY	0.212 ± 0.0055	0.153 ± 0.0061	0.132 ± 0.0046	0.128 ± 0.0017
SHR control	0.209 ± 0.0105	0.158 ± 0.0067	0.131 ± 0.0043	0.117 ± 0.0030*
SHR quinapril-treated	0.183 ± 0.0117	0.160 ± 0.0051	0.133 ± 0.0059	0.117 ± 0.0023*
SHR doxazosin-treated	0.219 ± 0.0066	0.173 ± 0.0059	0.135 ± 0.0068	0.119 ± 0.0025*
SHR diltiazem-treated	0.206 ± 0.0111	0.185 ± 0.0055*§	0.138 ± 0.0028	0.117 ± 0.0035*

Data represent the mean value for 8 rats ± s.e.m.

* $p<0.05$ compared with normotensive WKY rats.

§ $p<0.05$ compared with SHR controls.

Table 5.1. 3-Methylhistidine levels expressed as nmol per mg wet tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
SHR control	0.184 ± 0.0034*	0.206 ± 0.0099	0.457 ± 0.0281	0.436 ± 0.0138
Quinapril treated	0.139 ± 0.0048	0.187 ± 0.0113	0.441 ± 0.0361	0.432 ± 0.0195
Quinapril withdrawn	0.182 ± 0.0056*	0.212 ± 0.0124	0.477 ± 0.0153	0.448 ± 0.0238
Quinapril + doxazosin/ doxazosin	0.157 ± 0.0070*§	0.203 ± 0.0128	0.436 ± 0.0166	0.447 ± 0.0196
Quinapril/ doxazosin	0.166 ± 0.0044*§	0.210 ± 0.0160	0.474 ± 0.0193	0.435 ± 0.0201

Table 5.2. DNA levels expressed as µg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
SHR control	1.67 ± 0.0451	1.936 ± 0.0461	3.023 ± 0.2105	1.852 ± 0.0706
Quinapril treated	1.730 ± 0.0346	2.091 ± 0.0575	2.903 ± 0.1336	3.110 ± 1.206
Quinapril withdrawn	1.850 ± 0.1170	2.096 ± 0.0807	3.192 ± 0.1017	1.942 ± 0.0501
Quinapril + doxazosin/ doxazosin	1.838 ± 0.0305	2.030 ± 0.1026	2.948 ± 0.1315	1.958 ± 0.0906
Quinapril/ doxazosin	1.828 ± 0.0724	2.118 ± 0.0870	2.948 ± 0.1054	1.955 ± 0.0857

Data represents the mean value for 7 to 8 rats ± s.e.m.

Quinapril + doxazosin/doxazosin = SHRs treated with quinapril and doxazosin for 13 weeks then withdrawn from quinapril but continued on doxazosin for a further 4 weeks.
Quinapril/doxazosin = SHRs treated with quinapril for 13 weeks then withdrawn from quinapril but commenced on doxazosin for a further 4 weeks.

* $p<0.05$ compared with quinapril treated SHRs.
§ $p<0.05$ compared with SHR controls and quinapril withdrawn SHRs.

Table 5.3. Protein levels expressed as mg per mg of weight tissue weight

Treatment Groups	Aorta	Superior Mesenteric Artery	Mesenteric Branches	Vas Deferens
SHR control	0.237 ± 0.0075	0.158 ± 0.0080	0.136 ± 0.0111	0.112 ± 0.0039
Quinapril-treated	0.217 ± 0.0111	0.146 ± 0.0072	0.128 ± 0.0096	0.114 ± 0.0026
Quinapril-withdrawn	0.216 ± 0.018	0.151 ± 0.0082	0.141 ± 0.0081	0.116 ± 0.0038
Quinapril + doxazosin/ doxazosin	0.229 ± 0.0072	0.161 ± 0.0136	0.130 ± 0.0061	0.113 ± 0.0034
Quinapril/ doxazosin	0.233 ± 0.0077	0.150 ± 0.0093	0.137 ± 0.0064	0.116 ± 0.0056

Data represent the mean value for 7 to 8 rats ± s.e.m.

Quinapril + doxazosin/doxazosin = SHRs treated with quinapril and doxazosin for 13 weeks then withdrawn from quinapril but continued on doxazosin for a further 4 weeks.
Quinapril/doxazosin = SHRs treated with quinapril for 13 weeks then withdrawn from quinapril but commenced on doxazosin for a further 4 weeks.