HYPERTENSION

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by

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Abstract

Angiotensin II, generated either within the circulation, or within the vasculature has been suggested to have trophic effects on the vasculature. The present studies were designed to separate pressure-dependent effects on vascular structure from pressure-independent trophic effects of angiotensin II, in two experimental models of hypertension. Structural and functional studies were carried out on mesenteric and femoral resistance arteries from both Spontaneously Hypertensive rats and Goldblatt one-kidney, one-clip rats, using the Mulvany myograph technique.

Spontaneously Hypertensive rats were treated from three to twenty four weeks of age with the angiotensin-converting enzyme inhibitor perindopril. In addition, ACE inhibitor treatment was given in the presence of elevated dietary salt intake. This was found to attenuate the antihypertensive effects of perindopril and similar blood pressure-related structural alterations were observed, compared to the untreated SHR, in spite of the absence of Angiotensin II.

Goldblatt one-kidney, one clip rats were treated for either four, or eight weeks post clipping with either the ACE inhibitor perindopril, or the angiotensin II receptor antagonist losartan. Renin-angiotensin system blockade failed to prevent the development of hypertension in the one-kidney, one-clip rats, thus allowing specific effects of angiotensin II on vascular structure to be dissociated from those related to a reduction in blood pressure. Only slight alterations in vascular structure were observed in the absence of a reduction in blood pressure.

Overall, these results suggest that blood pressure elevation is a major determinant of structural vascular change. There was little evidence of a major direct angiotensin II mediated trophic effect, although slight effects of the renin-angiotensin system could not be ruled out. Endothelial dysfunction in these models also largely appeared to develop as a consequence of blood pressure elevation, although some improvement in endothelial function was observed as a result of drug treatment.

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CHAPTER ONE

INTRODUCTION

1.1 CHARACTERISTICS OF ESSENTIAL HYPERTENSION

The cardiovascular system allows cells a supply of nutrients, removal of waste products and gas exchange in most tissues and organs. It also plays a major role in the transport of secretory products from the endocrine system. It is therefore of primary importance and is one of the first organ systems to appear in the developing embryo. Primitive blood vessels can be observed as early as 15 days, and a beating heart is formed by three weeks in the human embryo.

Hypertension affects approximately 20% of the adult population. The majority of cases have no apparent cause and any abnormalities of the cardiovascular system appear to be a consequence of the disease, rather than an initial cause. This common type of hypertension is referred to as Primary or Essential Hypertension. In secondary hypertension, which is less common, an initiating mechanism can be identified, such as renal or endocrine disease. Secondary hypertension only accounts for a small proportion of cases, approximately 5-10%. The diagnosis of hypertension depends upon the definition, as there is no definitive cut-off for the disease. Hypertension in this country is currently defined by a finding of a systolic blood pressure (BP) ≥ 140mmHg and / or a diastolic pressure ≥ 90mmHg in a single assessment (World Health Organisation (WHO) Guidelines, 1999) (World.Health.Organisation, 1999).

1.1.1 WHO Guidelines for the diagnosis of Hypertension

Category	Systolic BP (mmHg)	Diastolic (mmHg)
Grade 1 (mild)	140-159	90-99
Subgroup : Borderline	140-149	90-94
Grade 2 (moderate)	160-179	100-109
Grade 3 (severe)	>180	>110

The incidence of hypertension can be overestimated using a single blood pressure measurement. Therefore, blood pressure values on subsequent occasions may lead to a different interpretation, and repeated readings are required. More recently ambulatory blood pressure monitoring has been introduced as a better predictor of hypertension. This involves 24 hour blood pressure profiling and has produced evidence of a smaller night-time decrease in mean blood pressure in hypertensive patients (Sihm *et al.*, 1995). However, even if the incidence has been overestimated it is still one of the most chronic diseases affecting the Western world today.

Blood pressure distribution in the population is univariate and forms a unimodal curve which has a skew in the high-pressure ranges (Swales, 1994). Hypertension can therefore be considered as a quantitative rather than qualitative deviation from the normal, rather than a distinct disease. It increases progressively with increasing age and tends to be consistently higher in men during early life, possibly due to their increased height. Individuals with a lower blood pressure tend to maintain a flatter blood pressure rise during their lifetime, whereas those with an initially higher blood pressure have a much steeper rise and are at increased risk from the disease. The prevalence of hypertension rises from 4% at ages 18-29, up to 65% at $\geq$ 80 years.

Essential hypertension is a product of both genetic and environmental factors. It is considered to be a polygenetic disorder and full expression may require the influence of environmental factors. The following environmental factors have been positively linked to hypertension in long-term studies of patients, such as the Framingham cohort, in the U.S.A. (Whelton *et al.*, 1994): -

a) Body weight and body fat distribution, overweight people are two to six times more likely to suffer hypertension. Waist: Hip ratio is used as an indicator of risk.

b) Excessive alcohol consumption

c) Excessive sodium intake

d) Smoking

e) Decreased physical activity

f) Nutritional abnormalities

Other disorders have also been found to have a common aetiology and pathology with hypertension such as Non-insulin dependent diabetes mellitus (NIDDM), hyperinsulinaemia, hypercholesterolaemia, obesity, and hypertriglyceridaemia.

1.2 HISTORICAL INTRODUCTION

Instruments used in the measurement of blood pressure in humans were developed in the second half of the 19th century. Before this time, estimates of increased blood pressure were carried out by palpation of the pulse. However, it was over a century earlier that the first demonstration of blood pressure measurement was made by Reverend Stephen Hales (Hales, 1733). His technique involved cannulation of a horse main artery with a short brass tube connected to a long glass tube. He found that removal of a ligature positioned around the artery caused blood to rise in the glass tube to a height greater than nine feet. This technique was refined by the use of a shorter tube containing mercury, thereby producing the mercury manometer in wide use today.

Richard Bright described hypertensive disease as early as 1836, although at this time blood pressure levels were undetermined. He noted from post mortem examinations that an increase in left ventricular weight was associated with pathological changes in the kidney. He speculated that a possible change in the smaller diseased vessels, causing increased resistance, would require an increased cardiac force to maintain an adequate circulation (Bright, 1836). A predecessor Samuel Schaarshmidt had also shown a limited understanding of the disorder in 1747, when he described a 'spastic contraction of the vascular bed' and that the circulation was in a state of 'vehement agitation'. The therapy

he recommended was similar to that used today as he recommended that the sufferer be kept calm, and gave sedatives and nitrites to cause vasodilatation (Folkow, 1982). In 1868, Johnson in England described thickening and narrowing of the walls of pre-capillary arterioles in the kidney, but found no thickening of the veins. He suggested that an obstruction of the capillaries would result in an increased blood pressure (Johnson, 1868). Further work in this area by Gull and Sutton provided evidence that structural changes were not limited to the kidney, although they concluded incorrectly that the widespread structural changes were the cause of the renal disease (Gull & Sutton, 1872). A few years later in 1877, Ewald proposed that an initial pressure increase in Bright's disease could induce widespread autoregulation and cause generalised hypertrophy with possible functional consequences (Folkow, 1982). Despite these suggestions, little was known about vascular haemodynamics and the functional consequences of these structural changes were overlooked. Therefore, alterations were generally considered to be late sclerotic changes, secondary to the disease.

The suggestion of the involvement of pressure change in Bright's disease renewed interest in the ability to determine levels of human blood pressure. The first successful arterial pressure measurements were made by Mahomed, and led to the realisation that high arterial pressure frequently occurred without renal disease (Mahomed, 1881). Techniques were further developed and Rocci described the Sphygmomanometer used today in 1886. It consisted of an inflatable rubber bag connected to a manometer, which could be used to reproducibly determine systolic blood pressure. Korotkoff described a later technique in 1905, allowing determination of both systolic and diastolic blood pressure. The occurrence of high arterial pressure with no apparent cause led the German clinicians Frank and Wiesbaden to rename it "Essentielle Hypertension" in 1911 (Paul, 1989).

Despite the lack of renal disease in many hypertensive patients' work still focused on the association between them. Research was enhanced by the discovery of the pressor substance renin in the rabbit renal cortex by Tigerstadt and Bergmann in 1898 (Swales, 1979). This led to the suggestion that humoral factors were of primary importance in hypertension. Research in the area remained limited, until a revival by Goldblatt in 1934. He produced secondary hypertension in animals by constriction of the renal arteries, thereby confirming the importance of kidney humoral factors. The rise in blood pressure was also observed after sympathectomy, excluding a role for neural factors in this form of hypertension (Goldblatt *et al.*, 1934).

Clinical studies during this period also provided much information concerning the aetiology of primary hypertension. In 1914 Franz Volhard with Fahr published a Classic systemisation of hypertension, and Volhard showed that uncomplicated primary hypertension was linked to the elastic properties of arteries and arterioles, and also to age and hereditary factors. The latter was suggested from studies indicating similar blood pressure levels in twins and in first degree relatives of hypertensive patients. The role of genetic factors was widely researched from the 1920's and the area became controversial as a number of different factors were implicated. It is now widely accepted that the disorder is polygenetically transferred (Folkow, 1982).

During the 1930's, studies were carried out to determine the nature and location of resistance to blood flow in the vascular system. Pickering demonstrated that it was not due to changes in blood viscosity and concluded that it was located in the smaller pre-capillary vessels. The sympathetic nervous system did not appear to be involved, as blockade had little effect on the resistance. He also reported an increase in the resistance to blood flow in hypertensive patients after vasodilatation. His original conclusions were similar to those of Goldblatt as he suspected the involvement of a stable humoral factor (Pickering, 1936).

There was also renewed interest in the involvement of structural alterations in hypertension. Pickering showed that elimination of the ischaemic kidney in rabbit renovascular hypertension only normalised blood pressure in the early, but not in the later stages of the disease. He concluded that renovascular hypertension was not fully explained by humoral factors and that a non-renal factor was important in the maintenance of increased pressure. He proposed that changes in vascular morphology were involved in the maintenance of hypertension (Pickering, 1945).

Later quantitative haemodynamic studies by Folkow and colleagues provided a greater insight into the role of structure in hypertension. Under maximal vasodilatation, blood vessels of hypertensive patients were found to maintain a greater level of resistance. It was shown that structural alterations leading to a reduced lumen, with virtually no alteration in smooth muscle activity, could entirely account for the increased resistance to flow in these vessels (Folkow *et al.*, 1958). Similar forearm blood flow experiments by Conway demonstrated an exaggerated vasoconstrictor response in essential hypertensive patients, unrelated to vascular smooth muscle activity and associated with a reduced lumen diameter. Suggestions of waterlogging of vessels or a change in the relaxed state of the muscle fibres or increased stiffness were proposed to explain the response (Conway, 1963). A detailed morphometric analysis of the hypertensive, human small intestine, perfused under pressure, was carried out to provide direct evidence of structural alterations. Short confirmed the presence of an increased wall to lumen ratio in pre-capillary vessels as small as 100μm, which tapered in the smaller vessels protected from the higher pressure upstream. In addition, significantly, fewer arterioles were seen in the hypertensive vascular bed. Alterations were thus an intrinsic feature of the vasculature as arterioles were fixed, ruling out nervous and humoral mechanisms (Short, 1966). Folkow proposed that the structural alterations could explain both the increased resistance at maximal

dilatation and the enhanced reactivity, due to increased wall thickness, of the hypertensive vascular bed to vasoconstrictors (Folkow *et al.*, 1973).

It is now accepted that hypertension results from a disturbance in the physiological homeostatic mechanisms, such as the hormonal and sympathetic nervous systems, as no single abnormality alone can explain hypertension. Investigations of the mechanisms involved have been greatly assisted by the introduction of genetically hypertensive animals in 1958. Spontaneously hypertensive rats have since played an important role in research as a model of hypertension, sharing many similarities to essential hypertension in man including hereditary factors, environmental influences, improvement by antihypertensive therapy and similar cardiovascular complications. Also the introduction of new techniques such as intravital microscopy and the small vessel wire myograph, in the early 1970's, has allowed detailed studies of the structure and function of both normal and hypertensive small arteries. The latter technique, which has been widely used, has recently been adapted as a perfusion myograph to enable the study of arteries under changing conditions of pressure and flow.

The study of arterial function was greatly influenced by the discoveries of Furchgott and Zadawski in the 1980's. They demonstrated that relaxation of pre-contracted rabbit arteries to acetylcholine was lost after endothelial cell removal by intimal rubbing (Furchgott & Zawadzki, 1980; Furchgott, 1981). This endothelial-dependent relaxation was attributed to a factor, which became known as endothelial-dependent relaxing factor (EDRF). Nitric oxide (NO) was suggested to be a candidate for EDRF by Furchgott and Ignarro, as there were many similarities between the behaviour of EDRF and endogenous nitrates (Furchgott, 1988). Many endothelial derived vasoactive factors have since been identified, indicating an important role for the endothelium in the control of vascular tone. Other work has shown that endothelial function is abnormal in the hypertensive state

(Luscher *et al.*, 1992; Luscher, 1993a; Vanhoutte & Boulanger, 1995; Vanhoutte, 1996). Therefore, research to date demonstrates the complexity of the vascular system, a very different view to the original concept of the blood vessel as a selective barrier and a conduit for blood.

1.3 HAEMODYNAMIC BALANCE IN HYPERTENSION

Arterial Pressure is divided into two components mean arterial pressure, the steady component, and pulse pressure, influenced mainly by the large arteries. The cardiac output and the total peripheral resistance determine mean arterial pressure, whereas pulsatility depends on large artery stiffness i.e. compliance. In the established phase, essential hypertension is characterised by an increase in total peripheral resistance (PR) with a normal cardiac output (CO) (Lund-Johansen, 1994).

Although, cardiac output was observed to be normal in early clinical investigations of hypertension. Later research in the 1960's indicated that cardiac output was increased in the early stages of hypertension. Ledingham and Cohen (Ledingham & Cohen, 1963) observed an increased cardiac output in experimental renal hypertension with a normal peripheral resistance in the initial phase. However, in the established phase the cardiac output fell to normal levels, whilst the peripheral resistance increased. Thus, it would appear that with time there is a shift in the vascular haemodynamics from a role for an increased cardiac output to a dependency on elevated vascular resistance. The contribution of cardiac output and peripheral resistance to hypertensive disease is dependent upon the progression of the disease, and with respect to the age of the patient. These changes have been characterised by the World Health Authority (WHO) (Lund-Johansen, 1980; Bohr *et al.*, 1991; Lund-Johansen, 1994).

1.4 PERIPHERAL VASCULAR RESISTANCE

1.4.1 Location of Peripheral Resistance

Resistance to blood flow occurs throughout the arterial system. The large or conduit arteries contribute little to the resistance to blood flow which resides in the smaller vessels. However, they have an important cushioning effect in smoothing out the pulsations, which occur with ventricular ejection, known as the Windkessel effect. As early as 1936 Pickering suggested that resistance was located in the smaller pre-capillary vessels (Pickering, 1936). Subsequent studies of the intra-vascular pressure distribution and vascular architecture confirmed that the main resistance to blood flow is located in the smaller arteries and arterioles. For example, a 50% drop in pressure was demonstrated in the small arteries and arterioles (10-25μm) of a normal hamster cheek pouch (Davis *et al.*, 1986) (Figure 1.1).

Intra-capillary pressure is suggested to be normal, from considerations of oncotic pressure, in both essential human and genetic animal models, suggesting that a drop in pressure occurs proximal to the capillary vessels (Mulvany, 1984). Similarly, studies on the spontaneously hypertensive rat (SHR) mesenteric vasculature suggest that 50% of the pre-capillary pressure reduction occurs proximal to vessels with an internal diameter of 100μm, (Mulvany, 1987; Bohlen, 1989). On the other hand, since techniques were carried out in unconscious animals the pressure profile may not be an accurate representation of the normal situation. A recent study in conscious animals, by Christensen provided confirmation that small arteries do contribute greatly to the peripheral resistance. A pressure drop, approximately 40%, was found at the base of mesenteric arcades in the conscious SHR (Christensen & Mulvany, 1993). The increased pre-capillary resistance appears to protect the exchange capillary vasculature, from increased pressure, thereby preventing over-perfusion of the tissues and oedema.

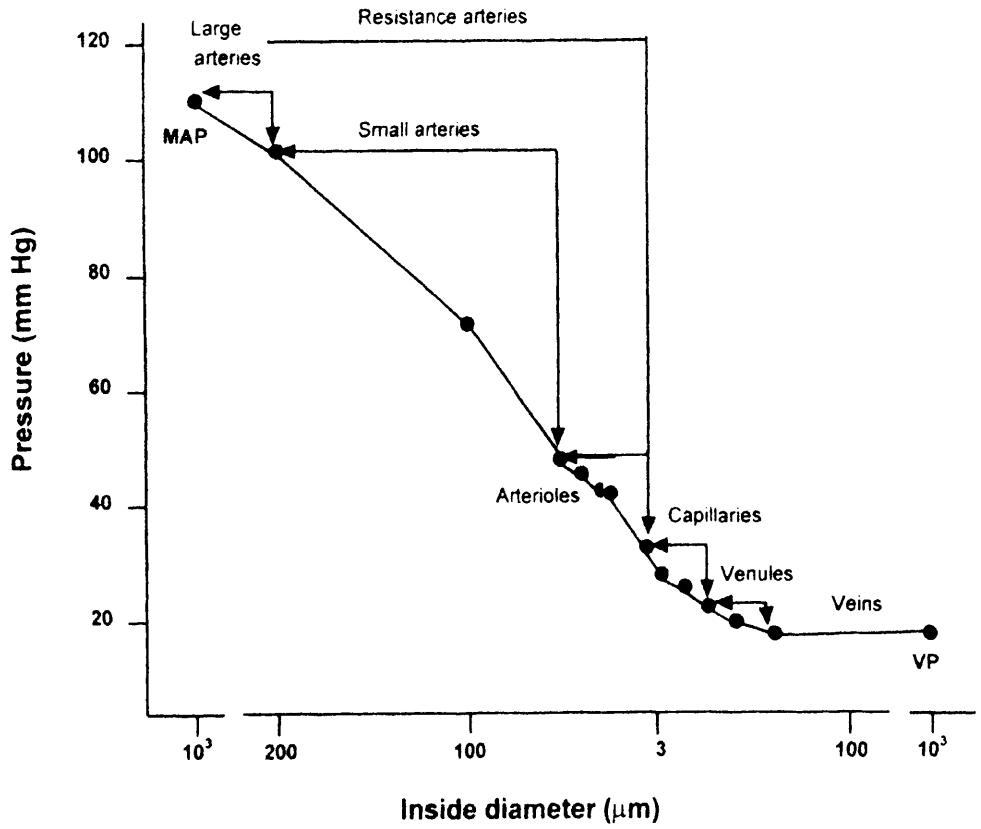


Figure 1.1 Vascular anatomy and hydrostatic pressure profile in the hamster cheek pouch. MAP, mean arterial pressure measured in the femoral artery; VP, venous pressure, measured near external mammary artery. Taken from (Davis, *et al.*, 1986)

Resistance artery is the term now given to the pre-capillary vessels, which contribute significantly to blood flow resistance.

1.4.2 Resistance artery structure

Resistance arteries are composed of three layers: - Outer tunica adventitia

- Central lumen media

- Inner tunica interna or Intima

The overall structure is similar to that seen in the larger conduit arteries with the proportion of each element varying according to the size and position of the artery in the vascular tree.

Tunica Adventitia

Outer arterial layer of loose connective tissue, acting as a fibrous support layer, comprising elastin, collagen, fibroblasts, mast cells and macrophages. In larger arteries it is involved in the transport of nutrients, as it contains arterioles, venules and lymphatic vessels, collectively known as the vasovasorum. Smaller arteries and the arterioles rely on diffusion of substances from the lumen, for their nutrition. The tunica adventitia also contains the nerve fibres, which penetrate the outer layers of the medial smooth muscle layer. There is little or no penetration into the central medial layers and response to nerve stimulation occurs through cell to cell excitation in the vascular smooth muscle layers.

Tunica Media

Central most prominent arterial layer consisting of a tight spiral of smooth muscle cells. Smooth muscle cells are approximately 25-50μm in length and 5μm wide and are arranged circumferentially around the lumen (Figure 1.2). They are embedded in a supporting matrix of proteoglycans and matrix proteins, primarily collagen and fibronectin, with few elastic fibres. In smaller arteries (200-300μm) this medial layer consists of up to six layers of cells, whereas only a single layer is found in arterioles (30-50μm). The outer layer of smooth muscle cells is separated from the adventitia, by a thin external elastic lamina. Few nerve endings are found to penetrate this arterial layer and it has been suggested that smooth muscle cells can as an electrical syncytium. This allows stimulation of the medial layer to occur by nerve endings present in the adventitia. The luminal side of the media is bounded by the internal elastic lamina. This is a thick fenestrated cylinder of elastic fibres in large arteries, but is much thinner in smaller arteries and absent in the arterioles.

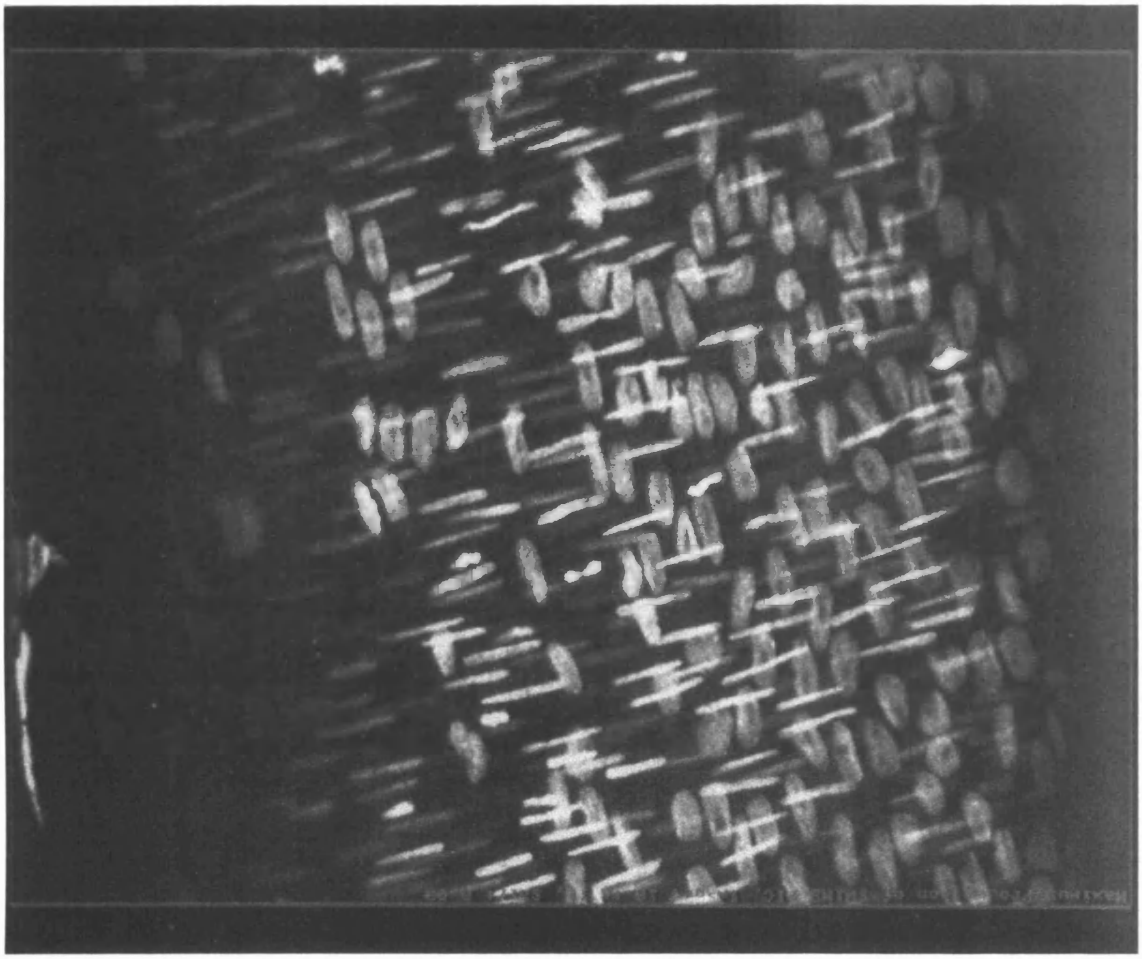


Figure 1.2. Confocal microscope image, using propridium iodide staining of cell nuclei, demonstrating smooth muscle cell nuclei arranged circumferentially around lumen and endothelial cell nuclei aligned parallel to direction of blood flow. Produced at University of Leicester by Helen Fish, 1994.

Tunica Intima

The innermost layer of the artery; describing the area from the lumen to the internal elastic lamina of the tunica media. It comprises two layers the innermost being the endothelial layer, which is always in contact with the blood. The endothelial layer consists of a monolayer of polygonal, elongated cells whose nuclei are aligned parallel to the direction of blood flow (Figure 1.2). These cells are approximately 25-50μm long and 10-15μm wide and are often observed to project through fenestration's in the internal elastic

lamina, allowing contact with the underlying medial smooth muscle cells. The endothelial cells are coupled to vascular smooth muscle cells via myoendothelial gap junctions (Chen & Cheung, 1992). Endothelial cells are metabolically active and release a variety of vasoconstrictor and vasodilator substances. In addition, the endothelium acts as a diffusion barrier regulating permeability between the intra-vascular and extra-vascular space. In the adult human, the endothelial layer weighs approximately 2.5Kg, making it one of the larger organs of the body. Mechanical support of the endothelium is provided by the underlying sub-endothelial layer, which is comprised of elastin and collagen fibres. In adult arteries, this layer may contain a variable amount of fine connective tissue and in addition, a small number of smooth muscle cells.

1.4.3 Blood flow haemodynamics

The major determinants of resistance to blood flow are the dimensions of the resistance vessel and the composition of blood flowing through the vessel. The flow of fluid through a blood vessel can be described by Poiseuille's formula, which was originally proposed to describe fluid flow through a tube.

$$\textbf{Flow} = r^4 (P_1 - P_2) / 8L\eta$$

Where r and L are the vessel length and radius respectively, P_1-P_2 is the pressure gradient along the vessel and η is the viscosity of the blood. This formula can be re-arranged to calculate resistance to blood flow, thereby bringing out the inverse relationship between resistance and the fourth power of the radius.

$$\text{Resistance to Flow } \textbf{(R)} = 8L\eta / \pi r^4$$

Blood viscosity, mainly due to erythrocytes, is thought to contribute to resistance in 20-100μm diameter vessels, but is of less significance in larger arteries. Vessel length is a

more important parameter as a pressure drop of 1 - 2.5mmHg per centimetre occurs. Therefore, small alterations in internal radius will result in large changes in the resistance to blood flow. The internal radius of an artery is determined by the structure and the smooth muscle cell activity or tone.

Pressure-flow determinations have also shown that SHR resistance vessels display a greater rise in resistance to vasoconstrictor agents, without an associated increase in the response threshold (Folkow, *et al.*, 1973). The increased pressor response was explained by an increased arterial wall mass and was considered to be indirect evidence in support of structural alterations.

1.4.4 Elevated peripheral resistance

In 1960 Fries suggested that the primary haemodynamic disturbance in hypertension was the increased peripheral resistance (Lund-Johansen, 1980). For many years the alteration in resistance was suggested to be a consequence of increased smooth muscle cell activity (Mendlowitz, 1973). Although, structural alterations had been recognised over 100 years earlier, they were originally overlooked as they were thought to represent a later deterioration of the vasculature. Instead the elevated peripheral resistance was attributed to either increased sympathetic nervous system activity (Abboud, 1982), or increased levels of circulating pressor hormones. However, despite extensive research, no common factor was found to explain the generalised increase in smooth muscle cell activity.

The functional significance of structural alterations was highlighted by forearm blood flow experiments in patients with essential hypertension compared to normotensive controls. An increased vascular resistance persisted in the presence of maximal dilatation, induced by hyperaemia or pharmacological means, in hypertensive patients (Folkow *et al.*, 1958; Conway, 1963; Takeshita *et al.*, 1980; Sivertsson, 1987). Since the increase was

observed despite maximal relaxation, it could not be accounted for by altered smooth muscle cell function. Folkow suggested that elevated resistance could be accounted for purely by vascular structural alterations resulting in a reduced lumen. Using Poiseuille's law he proposed that a 5% reduction in internal radius would produce a 20% increase in minimal vascular resistance (Folkow, *et al.*, 1973; Folkow, 1990 (review)). Later experiments in genetic and renovascular hypertensive rats confirmed that increased vascular resistance was associated with an increased wall to lumen ratio (Mulvany *et al.*, 1978; Korner *et al.*, 1989; Korner *et al.*, 1991; Folkow, 1993). The hypothesis was confirmed with studies of subcutaneous arteries, from gluteal biopsies, obtained from hypertensive patients. More recently, a close correlation was reported between forearm minimal vascular resistance and media to lumen ratio of subcutaneous small arteries (Rosei *et al.*, 1995).

1.4.5 Structural / Peripheral resistance relationship

Structural design is affected by the local tissue load, bulk and metabolic needs. Structural adaptation is a widely observed tissue response to sustained changes in load or activity. Skeletal muscles are a good example in that they adapt to physical training by hypertrophy, whilst lack of training may lead to atrophy. It is therefore not unexpected that arteries would respond to an increased pressure load by structural adaptation. Arterial adaptation can also be found in nature. For example, in the giraffe, the brain is situated three feet above its heart, and its feet are three metres below. It therefore requires a large arterial pressure, 250-300mmHg, to drive the blood into the brain, which is only exposed to a portion of the pressure, 80-90mmHg. However, this means that the legs are exposed to incredibly high-pressures, 400-500mmHg. The giraffe however has overcome this problem by developing much thicker leg arteries, 4-5 times that of the cranial arteries.

This structural adaptation allows normal levels of wall stress to be maintained throughout the vasculature (Folkow, 1993).

The relationship between tension per unit wall layer (T), regional transmural pressure (P) and vessel dimensions, radius (r) and wall thickness (w), is described by Laplace's law.

Tension (T) = P x r

A normal vascular wall tension only can be maintained in the presence of high blood pressure by a proportional increase in w/r. This can be achieved either by an increase in wall thickness, and/or by a decrease in luminal diameter. Structural adaptation overcomes the need for enhanced smooth muscle activity level to maintain an appropriate wall tension. There are two major consequences of arterial structural adaptation: -

1. A decrease in radius, which alone will increase vascular resistance for any given level of smooth muscle activity, even at maximal dilatation.

2. An increased wall to lumen ratio may act as a vascular amplifier. An increase in arterial wall thickness will enhance the degree of reduction in lumen diameter to vasoconstrictor substances. In addition, this results in a greater vascular resistance for any level of smooth muscle activity (Korner, *et al.*, 1989; Folkow, 1990; Korner, *et al.*, 1991; Folkow, 1993)

Small structural alterations may therefore produce a large increase in systemic resistance, even at normal levels of smooth muscle activation. Consequently, despite the important advantage in maintaining a constant wall tension, alterations may be detrimental to the cardiovascular system as they may compromise vascular reserve.

1.5 STRUCTURAL ABNORMALITIES IN HYPERTENSION

Arterial structural alterations were described early in the 19th century in post mortem examinations of patients dying of renal failure. However, the haemodynamic implications of these changes for the maintenance of hypertension were not recognised for many years. The fundamental significance of structural changes was recognised for the first time in the 1950's by studies of forearm blood flow in humans. Histological evidence confirming the existence of these alterations was also provided by detailed morphometric analysis of the small human intestine from hypertensive patients (Short, 1966). When perfusion fixed an increased media to lumen ratio was observed in the precapillary vessels. Subsequent histological studies report an increase in the media to lumen ratio in various arteries of the spontaneously hypertensive rat (SHR). These changes have been demonstrated in the mesenteric (Nordborg & Johansson, 1979; Lee, 1983), renal (Smeda & Lee, 1988), and cerebral arteries (Nordborg & Johansson, 1979). Similar findings have also been reported in other experimental models of hypertension, such as the one-kidney, one-clip renal hypertensive rat. Thus, Prewitt and colleagues reported an increased media to lumen ratio in arterioles of the gracilis muscle in anaesthetised rats, using *in vivo* video microscopy, trans-illumination techniques (Prewitt *et al.*, 1984).

In vitro evidence of these changes in isolated living vessels has been obtained by use of the Mulvany wire myograph technique, which was introduced in the early 1970's. Early reports by Mulvany and colleagues showed a large significant increase in media thickness of SHR mesenteric resistance arteries, varying between 30-50%, compared to the normotensive WKY rat. This was associated with a 15-22% reduction in lumen diameter and increased sensitivity to vasoconstrictors (Mulvany & Halpern, 1976; Mulvany & Halpern, 1977; Mulvany, *et al.*, 1978). The use of the Mulvany myograph technique has become widespread and similar findings were reported in resistance arteries from

subcutaneous gluteal biopsies of hypertensive patients (Aalkjaer *et al.*, 1987a; Korsgaard *et al.*, 1993).

Overall, the combined *in vivo* and *in vitro* evidence indicates that in established hypertension resistance vessels adapt to the increased systemic pressure with a decrease in the luminal diameter, and consequent increase in medial thickness to lumen diameter ratio (M:L). In addition, it has been suggested that rarefaction of resistance vessels may contribute to increased peripheral resistance observed in some vascular beds (Prewitt, *et al.*, 1984; Bohlen, 1989), although the major change appears to be the reduction in lumen diameter.

1.5.1 Nature of Structural alterations

The increase in media to lumen ratio could be the consequence of addition of material to either the luminal or the abluminal side of the vessel. Growth may occur via an increase in cell size (hypertrophy), by an increase in cell number (hyperplasia), or by a combination of both. The former, hypertrophy is often associated with an increased cell DNA content, polyploidy (Heagerty *et al.*, 1993a).

The development of the Mulvany myograph permitted the study of small mesenteric resistance arteries of approximately 150μm internal diameter. Mulvany and colleagues reported a large increase in the contractility to noradrenaline of 34-50%, along with a similar increase in the media thickness of 30-50%, compared to the Wistar Kyoto (WKY) control. Further analysis showed that the tension per unit wall thickness was unchanged, and there was no alteration in smooth muscle sensitivity to contractile agonists. They concluded that the alteration in structure alone could account for the increase in contractility. (Mulvany & Halpern, 1976; Mulvany & Halpern, 1977) In keeping with the Folkow hypothesis, later histological studies showed an increase in the number of smooth muscle cell layers in the hypertensive animal, the cells being of normal appearance

(Mulvany, *et al.*, 1978; Warshaw *et al.*, 1979). It was suggested that arterial remodelling was due to cellular hyperplasia, as had previously been described by Bevan in a rabbit model of experimental hypertension (Bevan *et al.*, 1976). Microchemical techniques confirmed hyperplasia by demonstrating a 29% increase in DNA content and increased amounts of actin and myosin in SHR mesenteric vessels (Brayden *et al.*, 1983; Owens *et al.*, 1988). The presence of arterial hyperplasia of the mesenteric resistance artery has since been confirmed by a variety of techniques, including thymidine incorporation studies (Yang *et al.*, 1989), and a novel three-dimensional dissector technique developed by Mulvany. This allowed an accurate estimation of the number of smooth muscle cells in the tissues (Mulvany *et al.*, 1985).

Although hyperplasia has been demonstrated in the smaller SHR mesenteric resistance arteries, smooth muscle cell hypertrophy underlies the change in the larger arteries of this model. (Owens, *et al.*, 1988). Morphometric studies have shown no increase in cell number, but demonstrated larger cells in the aorta of the hypertensive rat (Olivetti *et al.*, 1982; Owens & Schwartz, 1982). The increase in wall mass was also associated with a higher DNA content in as many as 50% of the cells, indicating cellular polyploidy (Owens and Schwartz, 1982; Rosen *et al.*, 1986). By contrast to the SHR, hypertrophy appears to be the major structural alteration reported in large and small arteries of Goldblatt hypertensive rats. Smooth muscle cell hypertrophy has been reported in the aorta of the two-kidney, one clip model (2K1C) (Owens & Schwartz, 1983), and in mesenteric resistance arteries from the one-kidney, one clip model (1K1C), by the dissector technique (Korsgaard & Mulvany, 1988).

In essential hypertension, the 19% increase in media to lumen ratio of subcutaneous resistance arteries was associated with no change in the number of vascular smooth muscle cells, thereby ruling out hyperplasia (Aalkjaer, *et al.*, 1987a; Sivertsson, 1987; Mulvany,

1991a). Thus, structural alterations are not necessarily associated with vascular growth. Therefore, it was proposed that they could be the consequence of a rearrangement of the vascular wall material around a smaller lumen, or remodelling (Baumbach & Heistad, 1989). Subsequent studies showed that a normal number of vascular smooth muscle cells are arranged more tightly around the lumen, in four layers rather than the normal three, explaining the increased media to lumen ratio (Mulvany, 1992a; Mulvany, 1993a). Further studies have confirmed remodelling as the predominant structural alteration in essential hypertension (Heagerty, *et al.*, 1993a; Korsgaard, *et al.*, 1993; Lee *et al.*, 1995). To avoid confusion Mulvany (Mulvany, 1993b) suggested a new classification of remodelling to accommodate the different types of alterations in structure found in hypertension, as shown in Figure 1.3.

Overall, there appears to be a wide heterogeneity in structural alterations in hypertension. Differences appear to depend on the size of the artery and it's location, but also on the species and model of hypertension studied

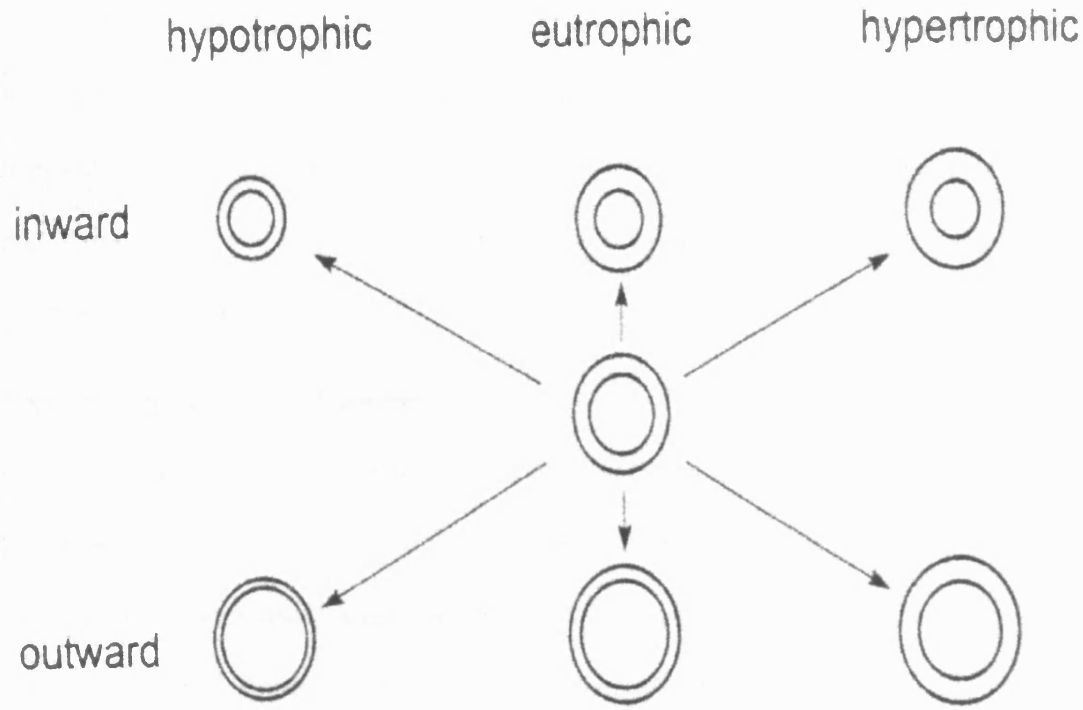


Figure 1.3. Remodelling can be hypertrophic (e.g. increase of wall cross-sectional area, vessels in right column, eutrophic (no change in wall cross-sectional area, vessels in centre column), or hypotrophic (e.g. decrease of wall cross-sectional area, vessels in left column). These forms of remodelling can be inward (i.e. reduction in lumen diameter, vessels in top row), or outward (i.e. increase in lumen diameter, vessels in lower row). Taken from (Mulvany, 1999)

1.5.2. Mechanisms involved in Structural Alteration

For many years, there has been an ongoing debate about the importance of structural alterations in hypertension i.e. their role as a primary cause or secondary consequence of hypertension. In 1956 Folkow proposed that essential hypertension was the consequence of a vicious cycle of events and that structural alterations develop in response to the elevated blood pressure. This implies that alterations are both a response to, and contribute long-term to the maintenance of hypertension (Folkow, 1956).

However, he later went on to suggest that the increased blood pressure itself was insufficient to cause the initial increase in structure and that other factors causing an initial haemodynamic disturbance were involved. In genetic models of hypertension, the initial disturbance may be a genetically determined predisposition to hypertension (Folkow, *et al.*, 1973; Lever, 1986).

Pressure-dependent mechanisms

A variety of studies implicates blood pressure in the development of structural alterations. One early study examined the response of an intact rabbit ear, arterial ring, exposed to an increased tangential load. After only three days an increase in protein synthesis and cell division was reported (Hume, 1980). Similarly, cyclical stretching of smooth muscle cells in culture, also has been used to imitate the response of cells during hypertension, and shows that cyclical stretching can stimulate vascular growth (Leung *et al.*, 1976; Wilson *et al.*, 1993; Williams, 1998). More recently, expression of early growth proto-oncogenes has been demonstrated upon exposue to high pressure in an isolated artery exposed to increased pressure (Allen *et al.*, 1996).

Many other studies show that there is a close relationship between blood pressure and structure. Saphenous vein grafts used in coronary bypass patients, hypertrophy when exposed to arterial blood pressure (Mulvany, 1984; Mulvany, 1987). On the other hand, hindlimb protection studies in the SHR showed that partial iliac artery ligature in one hindlimb prevented the development of structural change in vessels in which intravascular pressure remained normalised (Bund *et al.*, 1991). The aortic coarctation model of hypertension, where a ligature is placed between the renal artery origins leads to areas of both high and low pressure. At three days there was increased proto-oncogene expression above the ligature and the rats went on to develop left ventricular and aortic hypertrophy, but there was no alteration of the aorta below the ligature (Ollerenshaw *et al.*, 1988).

Thus, there is a volume of evidence, from both experimental models, and in human hypertensives, to suggest that structural vascular change is an adaptive response to the rise in blood pressure (Mulvany & Korsgaard, 1983; Aalkjaer, *et al.*, 1987a; Mulvany, 1987). Moreover, blood pressure restoration by antihypertensive treatment has been reported to reduce both structure, and blood pressure in both genetic and experimental hypertension (Owens, 1987; Bennett & Thurston, 1996; Thybo *et al.*, 1994).

Pressure-independent mechanisms

In contrast to the above reports, a number of studies suggest that mechanisms unrelated to pressure may be involved in the development of vascular change. The SHR may display a genetic predisposition for growth, as vascular smooth muscle cells, from this model have been shown to proliferate more rapidly, under cell culture conditions (Kanabe *et al.*, 1983; Mulvany, 1984; Yamori *et al.*, 1984; Folkow, 1986; Hadrava *et al.*, 1989). Also, thymidine incorporation studies in a one week old SHR demonstrated rapid proliferation of vascular smooth muscle cells in the superior mesenteric artery (Yang, *et al.*, 1989). Other studies have documented structural alterations in both the heart (Hallback-Nordlander, 1980; Mulvany, 1991b; Moteau *et al.*, 1997), and mesenteric arteries (Warshaw, *et al.*, 1979; Lee, 1985; Rizzoni *et al.*, 1994; Dickhout & Lee, 1997), during the prehypertensive stage in the SHR, prior to the rise in blood pressure. Similar early structural alterations have been reported in cases of borderline human hypertension, suggesting that structural alterations may be a primary cause of hypertension (Takeshita *et al.*, 1980). However, this evidence has been questioned since early structural alterations are not found in all studies (Hallback-Nordlander, 1980; Mulvany *et al.*, 1980; Gray, 1982; Owens & Schwartz, 1982; Aalkjaer *et al.*, 1987b). Moreover, the blood pressure levels in borderline hypertensives (Aalkjaer, *et al.*, 1987b), and immediately after birth in the SHR are higher than age-matched controls (Gray, 1982; Dickhout & Lee, 1998). Thus, early

vascular structural alteration is related to pressure. In addition, cross breeding experiments have yielded inconsistent results since SHR/WKY F_2-hybrids show either no correlation between mesenteric medial thickness and blood pressure (Mulvany & Korsgaard, 1983), or a positive correlation (Mulvany, 1986; Mulvany, 1988)

Pressure-independent effects also have been implicated in certain models of hypertension, such as nitric oxide synthesis inhibition, by the administration of L-NAME to the Brattleboro rat. Little evidence of structural alteration was found in this model, despite a severe hypertension (Dunn & Gardiner, 1995). Similarly, a dissociation of structure and blood pressure has been reported in therapeutic studies in both animal models (Jespersen *et al.*, 1985; Christensen *et al.*, 1989), and human hypertensive patients (Heagerty *et al.*, 1988; Aalkjaer *et al.*, 1989). Incomplete regression of vascular structure was found despite normalisation of blood pressure. However, these findings must be interpreted with caution and could relate to the type and duration of therapy.

Experimental work to date provides no definite answer as to whether structural change represents a primary cause or secondary consequence of hypertension. It is suggested that both pressure-dependent and -independent mechanisms may be important. Research to date has proposed roles for genetic, neural and humoral factors, in the initiation and maintenance of hypertension. The latter part of this introduction will discuss some of the neurohumoral mechanisms implicated in hypertension.

1.6 SYMPATHETIC NERVOUS SYSTEM

Sympathetic nerve fibres are the sole innervation for the majority of blood vessels. The smallest arteries and arterioles are the most densely innervated, indicating an important role of the sympathetic nerves in blood flow regulation within these vessels. The adrenergic nerve fibres are for the most part found in the adventitial layer of arteries and do not penetrate into the media. Noradrenaline is the main transmitter released by the postganglionic neurone. Response depends on the amount released from the presynaptic terminal into the synaptic cleft, between the nerve fibre and smooth muscle cell. This is regulated by the density of sympathetic innervation and by presynaptic mechanisms such as neuronal re-uptake of noradrenaline and presynaptic adrenergic stimulation, which can lead to autoinhbitory and stimulatory effects, as discussed below. Noradrenaline acts by activating postsynaptic adrenergic receptors of which there are two types, α and β, which are characterised by their relative orders of potencies for both agonists and antagonists. Blood vessel vasoconstriction is determined by the α-adrenoceptor and vasodilatation by the β-adrenoceptor and each of these exists as several subtypes α_1, α_2, β_1, β_2 (Raymond *et al.*, 1990; van Zwieten, 1991).

α-adrenoceptor

Agonist potency noradrenaline = adrenaline >> Isoprenaline

Both α-adrenoceptor subtypes are usually present in the postsynaptic nerve terminal in vascular smooth muscle. Stimulation leads to vasoconstriction, which may be blocked by selective α-adrenoceptor antagonists, such as prazosin. Receptor binding results in increased levels of intracellular calcium, which in turn induces smooth muscle cell contraction. Stimulation of the α_1-adrenoceptor is thought to be involved in the release of calcium from intracellular stores, thereby contributing to the increased intracellular levels.

The α_2-adrenoceptor is the only α subtype found in the pre-synapse, and is important in the conservation of noradrenaline. α_2 Receptor activation reduces the amount of noradrenaline released at any frequency of neuronal activity. This mechanism appears to be important when the nerve terminal is exposed to high noradrenaline concentrations (Ruffolo *et al.*, 1991).

β-adrenoceptor

Agonist potency Isoprenaline > adrenaline > noradrenaline

β-adrenoceptors are generally present at the postsynaptic site, although they may be found presynaptically. β_1-adrenoceptors are most commonly seen in the cardiac tissue, where they are involved in modulation of its electrical and contractile functions. β_2-adrenoceptors occur mainly in the respiratory tract, blood vessels, and skeletal muscle. Postsynaptic receptor activation causes relaxation of the smooth muscle by activating adenylate cyclase leading to increased generation of the second messenger cAMP. The β-adrenoceptor antagonist's propranolol, a non-selective antagonist, and atenolol, a cardioselective antagonist, may block this response. At presynaptic sites, the β_2-adrenoceptor has the opposite function to the α_2-adrenoceptor, and activation enhances noradrenaline release in situations of reduced concentrations of noradrenaline.

1.6.1 Sympathetic Nervous System in Hypertension

If sympathetic drive were increased in hypertension, it could contribute to increased peripheral resistance by a variety of mechanisms. These include; vasoconstriction due to increased release of noradrenaline, increased sodium retention and renin release by the kidney (Brooks & Osborn, 1995), and increased growth of vascular smooth muscle (Abboud, 1982; Struijker-Boudier, 1994). Thus, surgical or chemical interventions in the central nervous system, leading to an increased sympathetic drive, have been shown to

cause labile or sustained hypertension (Brooks & Osborn, 1995). The opposite is found where chemical interventions are used to interrupt sympathoadrenal drive. Propranolol, guanethidine, prasozin, clonidine (Abboud, 1982; Zimmerman *et al.*, 1984) have been shown to reduce hypertension in some models of hypertension. Similarly, it has been shown that hypertension is more easily prevented by early intervention with sympatholytic drugs, rather than by later intervention, suggesting a prominent role for the sympathetic nervous system in the early stages of hypertension (Brown, 1985). Inhibition of sympathetic nerve development has also been shown to reduce or prevent hypertension. Folkow and colleagues partially prevented the rise in blood pressure in the young SHR by immunosympathectomy (Folkow *et al.*, 1972). Moreover, the combination of sympathectomy with α adrenergic blockade by prazosin, prevented the rise in blood pressure, left ventricular hypertrophy and increased hindquarter resistance properties (Lee *et al.*, 1987a; Korner *et al.*, 1993).

Increased plasma concentrations of circulating adrenaline have been reported in young spontaneously hypertensive rats, and in patients with borderline or mild hypertension (Brown, 1985; Floras, 1992). It has been suggested that increased adrenaline release from the adrenal medulla may be a primary abnormality, initiating hypertension in those with a genetic predisposition, by amplification of peripheral neurotransmission (Floras, 1992). Surgical resection of the adrenal medulla at 4-6 weeks was shown to attenuate hypertension in the young SHR, but was less effective in older rats, indicating involvement only in the early stages of disease (Borkowski, 1991). In essential hypertensive patients, increased sympathetic nervous system activity has been demonstrated using noradrenaline-radiolabelled techniques. Intravenous infusion of tritiated noradrenaline allows the tissue clearance of noradrenaline to be subtracted from plasma levels, thereby allowing determination of the remaining neurotransmitter

"spillover" from the neuroeffector junctions. An increase in noradrenaline spillover was found from the plasma of young hypertensive patients, in subjects with a familial history of hypertension (Brooks & Osborn, 1995), and more recently in human renovascular hypertension (Johansson *et al.*, 1999). Also direct recording techniques, such as microneurographic recordings of sympathetic nerve traffic, suggested that increased activity was correlated with cardiac disease (Grassi, 1998; Mancia *et al.*, 1999).

The sympathetic innervation of the vasculature has also been widely investigated. Increased sensitivity to noradrenaline, in the presence of cocaine, has been demonstrated in resistance arteries from the Spontaneously hypertensive rat (Mulvany, *et al.*, 1980; Stephens *et al.*, 1991), and in subcutaneous resistance arteries from essential hypertensives (Aalkjaer, *et al.*, 1987a), and their offspring (Aalkjaer, *et al.*, 1987b). Since responses were normal in the absence of cocaine it was proposed that neuronal re-uptake of noradrenaline was enhanced to compensate for the increased sensitivity (Whall *et al.*, 1980; Aalkjaer, *et al.*, 1987a; Head, 1989). Increased sensitivity may be due to either increased fractional noradrenaline release from individual sympathetic nerve fibres or to an increased sympathetic innervation, leading to greater absolute release of noradrenaline (Stephens, *et al.*, 1991). The latter explanation was thought more likely because it also explained the enhanced neuronal uptake of noradrenaline. Studies of the vasculature of the SHR have reported increases in sympathetic innervation (Head, 1989; Mulvany, 1994), density of α-adrenergic binding sites (Schiffrin, 1984) and α-adrenergic receptor binding affinity (Nyborg & Bevan, 1988; Head, 1989).

The sympathetic nervous system also has been implicated in trophic effects on the vasculature. Early sympathetic denervation of the rabbit ear artery prevented normal vascular growth and produced arteries with a smaller lumen diameter, although no effect was reported in the adult rabbit (Bevan, 1987). Later studies have confirmed the effects of

denervation on vascular growth (Lee & Gzik, 1991a), where neonatal sympathectomy which was shown to prevent hyperplastic changes in mesenteric vascular smooth muscle cells (Lee, *et al.*, 1987a). Thus, findings suggest the sympathetic nervous system is important in cell division and growth in the initial phase of hypertension. However, it is difficult to dissociate direct effects of the sympathetic nervous system on growth from the antihypertensive effects of sympathectomy. Catecholamines have been implicated in growth as they are reported to increase ornithine deoxycarboxylase activity, an indicator of cellular hypertrophy/hyperplasia, in cultured vascular smooth muscle cells (Kanabe, *et al.*, 1983; Yamori, *et al.*, 1984). However, results of *in vivo* studies are less clear because phenylepinephrine infusion into the intact rat failed to induce hypertrophy of the mesenteric arteries (Boonen *et al.*, 1993), but did increase expression of growth-related genes in aortic vascular smooth muscle (Struijker-Boudier, 1994).

Overall, the available evidence implicates the sympathetic nervous system only in the early stages of hypertension. However, its primary role is difficult to assess because of the interactions of the system with other systems, such as the renin-angiotensin cascade (Brooks & Osborn, 1995). It is possible that increased adrenergic activity is a secondary response to activation of the renin-angiotensin system, as angiotensin II exerts stimulatory effects on central and peripheral sympathetic outflow. Evidence supporting the involvement of the renin-angiotensin cascade in hypertension will be discussed in more detail in the next section.

1.7 RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system was traditionally viewed as a circulating endocrine system with an important role in maintaining cardiovascular homeostasis. Renin release by the kidney provides a rapid and efficient mechanism by which the body can respond to changes in blood pressure, fluid and electrolyte balance; i.e. short-term regulation of cardiovascular function. However, recent research points to the existence of a tissue renin-angiotensin system, acting in an autocrine/paracrine manner which may be involved in the long-term regulation of the cardiovascular system.

1.7.1 Classical Renin-Angiotensin System

In 1836 Bright made the first association between left ventricular hypertrophy and renal disease (Bright, 1836). This work also was the first to suggest a role for a circulating humoral factor in the structural alterations observed with renal disease. Many years later Tigerstadt and Bergmann, working at the Karolinska Institute in 1898, first demonstrated the presence of a pressor substance, in a saline extract from the renal cortex of rabbits. This pressor substance, which they named renin, was found to cause a rapid, sustained increase in blood pressure in normotensive rabbits (Inagami, 1998). Further research was hampered by methodological problems and interest in the area fell into abeyance, until Goldblatt showed that renal artery narrowing could induce hypertension. He and his colleagues developed a technique by which sustained hypertension could be produced in dogs by bilateral renal artery constriction using adjustable silver clamps. They proposed that this caused the accumulation or formation of a substance with pressor activity (Goldblatt, *et al.*, 1934). This stimulated further investigation into the nature of the pressor substance involved, and subsequently the discovery of the renin-angiotensin cascade. Braun-Menedez and colleagues showed that renin acted upon a plasma substrate to release

a heat stable, short-lived pressor substance, which they named hypertensin (Braun-Mendez *et al.*, 1940). At the same time Page and Helmer reported that renin only produced a vasoconstrictor response in the rabbit ear in the presence of fresh blood perfusate. They named the vasoconstrictor substance generated angiotonin (Page & Helmer, 1940). Therefore, both groups determined that renin was an enzyme which acted on a plasma substance to produce a vasoconstrictor substance. Subsequently, with the realisation that both groups had discovered the same substance they came to the agreed name angiotensin. In addition, the plasma substrate, which was cleared by renin to give angiotensin, was named angiotensinogen. Later studies by Skeggs in 1954 showed that a lower molecular weight potent pressor substance angiotensin II (AII) was formed from Angiotensin I (AI) in the presence of chloride ions. This lead to the discovery of Angiotensin converting enzyme (ACE), a chloride dependent enzyme, which was later also identified as kininase II by Erdos (Inagami, 1998). This research led to the classical concept of the blood-bourne renin-angiotensin cascade.

1.7.2 Components of the Renin-Angiotensin System

Renin

The juxtaglomerular apparatus (JGA) of the kidney is the major site of synthesis and storage of renin. Pure renin was first isolated from the hog kidney in 1977 by Inagami and Murakami. It was found to be a glycoprotein with a molecular weight of 36,400 with an pH optimum 5.5-7. Renin is synthesised in the kidney as a 45kDa pre-prorenin, which is converted into the inactive prorenin form by removal of a single peptide and glycosylation. Some conversion of prorenin occurs immediately in the kidney although much is excreted. In humans 90% of renin is present in the inactive form suggesting that prorenin is the more abundant circulating form of renin. A number of stimuli can cause renin release by the kidney, such as a decrease in blood pressure or extracellular volume in

the afferent arteriole, as sensed by JGA baroreceptors and macula densa chemoreceptors. The juxtaglomerular cells are specialised myoepithelial cells, mainly situated in the afferent arteriole media, which act as miniature pressure transducers. Many other factors influence renin secretion including, renal nerve activity, several hormones such as AII, Adenosine diphosphate (ADP), Atrial natriuretic peptide (ANP), catecholamines, via β receptors, and histamine (Hackenthal & Nobiling, 1994; Rosendorff, 1996). AII has a strong inhibitory influence on renin release by negative feedback mechanisms acting via the AT_1 receptor (Abdelrahman *et al.*, 1993).

Angiotensinogen

Angiotensinogen is a large α2 globulin synthesised and secreted at a variable rate mainly by the pericentral zone of the liver lobules. It is the only precursor of the angiotensin cascade of peptides, but may be converted to AI by enzymes other than renin, including cathepsin D, pepsin and various aspartyl proteases and renin-like enzymes. In addition, certain enzymes may also cleave angiotensinogen directly to AII, examples of these enzymes are cathepsin G, tonin, trypsin, kallikrein, elastase and tissue plasminogen activator.

Angiotensin I (AI)

Angiotensin I (AI) is an inactive decapeptide prohormone, produced by the action of renin on its substrate angiotensinogen in the circulation and other tissues. AI is hydrolysed by ACE, a primarily membrane bound dipeptidyl-carboxypeptidase, to the octapeptide AII. However, ACE independent pathways for the conversion of AI to AII have been described in many tissues. For example, ACE inhibitor studies indicate that only 30-40% of the conversion of AI into AII in human arteries appears dependent on ACE (Okunishi *et al.*, 1993). Several other AII generating enzymes have been described including; chymostatin-sensitive AII generating enzyme (CAGE), in human gastroepiploic

arteries, and chymase in the human heart (Okunishi, *et al.*, 1993; Nishimura *et al.*, 1996; Urata *et al.*, 1996; Roks *et al.*, 1997; Balcells *et al.*, 1997; Akasu *et al.*, 1998). Many animals also possess the chymase pathways (Wei *et al.*, 1999), although the role of this pathway varies depending on the species studied (Akasu, *et al.*, 1998). Moreover, it is unclear whether these pathways contribute significantly to AII formation under physiological conditions, as there is conflicting evidence. In view of the evidence from both rodent arteries and hearts, AII formation, even when hypertrophied appears to be totally dependent on ACE, since its production is totally abolished by ACE inhibition (Okunishi, *et al.*, 1993; Nishimura, *et al.*, 1996).

Angiotensin Converting Enzyme (ACE)

Two forms of ACE have been found to exist, one in the endothelium and the second in developing spermatozoa. In the vascular system ACE is a primarily membrane bound dipeptidyl-carboxypeptidase, which removes the carboxy-terminal dipeptide fragment from the C terminal end of AI, thereby generating the vasoactive octapeptide AII. It is a relatively non-specific enzyme, which acts on several peptide substrates including Bradykinin, enkephalins, neurotensin, substance P, and luteinising hormone-releasing hormone. ACE degrades bradykinin (BK) to an inactive peptide by removal of the terminal dipeptide (Vane, 1993). Vascular endothelium appears to be one of the major sites of AII production by ACE. AII production occurs on the luminal surface of the endothelial layer, where converting enzyme is present at high concentrations bound to the cell membrane. A significant amount of conversion occurs in the pulmonary vascular endothelium, a site rich in ACE. Under physiological conditions, 3-5 seconds of blood flow, through the pulmonary circulation, leads to conversion of approximately 80% of AI. The enzyme is also present in the plasma, although conversion at this site is insufficient to account for the total conversion normally observed *in vivo* (Morton, 1993).

Angiotensin II (AII)

Angiotensin II is the main effector hormone of the renin-angiotensin system, which acts on specific receptors, and has an important role in the direct, indirect regulation of blood pressure and fluid electrolyte homeostasis. Angiotensin II is a powerful vasoconstrictor which is 10 times more potent than the equivalent weight of noradrenaline (Peach, 1977). An increase in the level of AII can result in an elevated peripheral resistance via constrictor action on arterial beds (Oliver & Sciacca, 1984). It also plays an important role in the maintenance of a normal blood pressure, in the face of depletion in extracellular volume, or during cardiac failure, via endocrine actions including medial smooth muscle contraction, and release of other vasoactive substances, such as endothelin. It also has both direct and indirect effects, via aldosterone release, on the kidney leading to decreases in renal blood flow, glomerular filtration rate (GFR) and to sodium and water retention. It may also act to stimulate pituitary vasopressin release and induce thirst to restore fluid levels (Swales, 1979).

Angiotensin II has many other functions in the vascular system, via autocrine and paracrine actions. It may cause local vasoconstriction, increase vascular permeability (Williams *et al.*, 1995), and enhances sympathetic nervous system activity, by releasing catecholamines from the adrenal medulla and by facilitating central and peripheral adrenergic neurotransmission (Zimmerman, 1981; Timmermans *et al.*, 1993). Angiotensin II stimulates the release of noradrenaline from prejunctional sympathetic nerve terminals, whilst at the same time inhibiting noradrenaline re-uptake at these sites (Collis & Keddie, 1981; Zimmerman, 1981). Angiotensin II has also been reported to have a direct inotropic action on the heart (Dzau, 1993a). More recently, studies suggest that AII may have actions in the brain, influencing memory and retention performance, and also may have a modulatory action on the processing of genetic information.

At present there are three known angiotensin receptor subtypes AT_1, AT_2 and AT_4. Of which the AT_1 receptor subtype mediates most of the known biological actions of angiotensin II, although both AT_1 and AT_2 receptors have similar affinities for AII. The AT_1 receptor is coupled to G-proteins, and receptor stimulation initiates the classical intracellular messenger systems. These include activation of the intracellular enzymes, adenylate cyclase and the phospholipases A_2, C and D. These interactions stimulate many intracellular events including a reduction in intracellular cAMP, and the generation of inositol triphosphate (IP_3) and diacylglycerol (DAG), via phospholipase C, leading to increased concentrations of intracellular calcium. Much less is known about the role of the AT_2 receptor, which has a similar affinity for AII as the AT_1 receptor. It has been suggested that it may be involved in early regulation of growth and differentiation, because it is found in abundance in foetal rats. Moreover, this receptor subtype was also found to disappear shortly after birth. Little is known about the pathways involved in AT_2 receptor signalling, but it has been suggested that a novel signalling mechanism may depend on phosphotyrosine phosphatase stimulation (Levens *et al.*, 1992; Timmermans, *et al.*, 1993; Unger *et al.*, 1996).

AII has a short half-life of approximately 10 seconds *in vivo*. It is rapidly degraded by plasma and tissue peptidases and endopeptidases to smaller fragments which may themselves be biologically active end-products. Two of the fragments are angiotensin III (AIII), a relatively weak pressor agent with only 30% of the activity of AII, which is formed by aminopeptidase, and Angiotensin- (1-7) formed by endopeptidase carboxypeptidase (Ferrario, 1990a). Recently, the latter compound has been more recently shown to mimic some of the effects of AII including prostanoid and vasopressin release, but displays no vasoconstrictor, central pressor or thirst stimulating activity. In addition, it appears to have vasodilator and antihypertensive actions, which may oppose the actions of

AII. These include, stimulation of the synthesis and release of prostaglandins and nitric oxide and augmentation of the response to bradykinin (Ferrario *et al.*, 1998). Increased formation of Angiotensin-(1-7) occurs where tissue or plasma levels of AI are increased, being formed directly from AI, and may be important in the inactivation of AII. The effects of the end-products similar to those of AII appear to be mediated through the AT_1 receptor. However, the actions of Angiotensin-(1-7) which appear to oppose those of AII , such as its vasodilatory effects, appear to be mediated through a novel AT_1/AT_2 receptor subtype (Ferrario *et al.*, 1997; Ferrario, *et al.*, 1998).

1.7.3 Tissue Renin-Angiotensin System

Some years ago, it was postulated that AII generation must occur at a site other than the circulatory system to explain the *in-vivo* findings of Swales and colleagues. Rats in a renin-angiotensin suppressed state, induced by high salt diet or nephrectomy, required larger doses of AII antibodies than expected to block the exogenous pressor response to AII. In addition, it was shown that renin infusion lowered the blocking requirement. They suggested that AII was generated from renin bound to the blood vessels and that this site of generation was inaccessible to AII antibodies (Swales & Thurston, 1973). Other evidence suggested that peripheral sites were a major site of AII generation as the concentrations in arterial blood and the effective metabolic clearance of AII were too high to be explained by plasma generation of AII (Campbell, 1987). Arterial tissue homogenates have since been shown to generate AI and AII from semi-purified angiotensinogen (Dzau, 1993a). Similar findings have been reported using whole tissue preparations, the response being suppressed renin and ACE inhibitors (Mizuno *et al.*, 1988; Saito *et al.*, 1989; Unger *et al.*, 1989; Higashimori *et al.*, 1991). Oliver and Sciacca (Oliver & Sciacca, 1984) showed AII-mediated vasoconstriction in an isolated rat hindlimb artery by perfusion with physiological saline solution containing a renin substrate, and this could be prevented by a

renin inhibitor peptide. Synthesis of AII also has been demonstrated in cultured vascular smooth muscle cells (Dzau *et al.*, 1987), as has ACE synthesis in endothelial cells (Swales & Heagerty, 1987), suggesting renin-angiotensin system components are present in adequate concentrations in individual cells. The actions of ACE inhibitors in the circulatory system also point to the existence of a tissue renin-angiotensin system as they reduce blood pressure in normal or low-renin states. Furthermore, it has since been shown that these effects correlate with inhibition of the tissue system, rather than that of the circulation (Dzau, 1988; Swales & Samani, 1989; Unger, *et al.*, 1989).

The existence of local synthesis of renin in the vascular tissues has been the subject of many debates. Arterial tissue extracts contain an enzyme with similar kinetics to renal renin, which releases a pressor substance from the plasma, and its activity can be inhibited by antirenin antibody treatment (Dzau, *et al.*, 1987). Higashimori and colleagues in 1991 demonstrated AII production in both the isolated perfused rat mesenteric and hindlimb arterial beds, in the absence of renin substrate and ACE components in the perfusate. Dose-dependent blockade of the response was shown using renin inhibitors, in a manner suggesting local renin activity (Higashimori, *et al.*, 1991). In order to exclude the kidney as a source for tissue renin many studies have been performed after bilateral nephrectomy. However, these studies also have provided conflicting results. Some workers have reported the persistence of aortic renin-like activity in the tissues despite a reduction in plasma renin activity, within one hour after bilateral nephrectomy (Asaad & Antonaccio, 1982). However, Thurston and colleagues reported that the activity was significantly reduced by 24 hours, suggesting it was mainly derived from the kidney (Thurston *et al.*, 1979). Loudon *et al* (Loudon *et al.*, 1983) also noted a dissociation between plasma and aortic renin concentrations in nephrectomised rats injected with exogenous renin. However, it was suggested that the presence of renin in the aortic wall be due to uptake of

plasma renin by the wall, rather than local synthesis. Other later reports suggested that cardiovascular renin disappeared completely after bilateral nephrectomy (von Lutterotti *et al.*, 1994), thereby supporting the involvement of renal renin. These discrepancies may be explained by either the presence of circulating prorenin remaining after nephrectomy (von Lutterotti, *et al.*, 1994), or by the presence of other enzymes capable of converting angiotensinogen (Dzau, *et al.*, 1987). At present, it is unclear if non-renin enzymes contribute to AII production during normal *in vivo* conditions (Swales & Heagerty, 1987). Renin mRNA has also been shown to be expressed in the heart, adrenal, brain, and other vascular tissues (Dzau, et al., 1987; Swales & Samani, 1989), although it is uncertain if the level of expression is of physiological relevance (von Lutterotti, *et al.*, 1994; Pinto *et al.*, 1995).

An overwhelming mass of evidence now suggests that the kidney is the major source of renin within the blood vessel wall. (Thurston, *et al.*, 1979; Loudon, *et al.*, 1983; Unger *et al.*, 1991; von Lutterotti, *et al.*, 1994; Pinto, *et al.*, 1995). Immunoflourescence studies suggest that most of the vascular renin in-situ is trapped and taken up from the circulation (von Lutterotti, *et al.*, 1994), and that renin is bound to the luminal surface of the vasculature (Inagami, 1998)

Despite the involvement of renal renin there is evidence suggesting that a separate renin-angiotensin system exists in many organs and tissues, which contain the genes and mRNA which encode for the requisite polypeptides and enzymes. Expression of renin-angiotensin system mRNA and protein products has been demonstrated by a variety of techniques including; northern Blotting, in-situ hybridization immunochemistry and by biochemical analysis (Dzau, 1993a; Inagami, 1998). These components also have been demonstrated in individual blood vessels, including the aorta, coronary, renal, carotid, mesenteric artery and veins and in vascular smooth muscle and endothelial cells in culture

(Swales & Samani, 1989; Unger, *et al.*, 1991). Thus, tissues are a major site of angiotensin generation (Admiraal *et al.*, 1993). The angiotensin I, which is formed by renin of renal origin, being cleaved by ACE in the luminal surface of the vascular endothelial cell (Campbell, 1987). These present evidence of a local renin-angiotensin system, which may contribute to blood pressure control by local effects in the wall of arteries or other tissues. Therefore, alterations in this system may play an important role in the pathogenesis of vascular disease (Pinto, *et al.*, 1995).

1.7.4 Role of the Renin-Angiotensin System in Hypertension

The association between hypertension and renal disease has long been recognised and involvement of renal pressor systems was demonstrated by the experiments of Goldblatt and colleagues, as previously described. On the other hand the role of the renin-angiotensin system in the majority of patients, with essential hypertension, who display normal plasma renin, and angiotensin levels, is still unclear. Despite plasma renin levels being normal, there is a great deal of evidence, which indicates the involvement of the renin-angiotensin system in the pathogenesis of hypertension, and the tissue system may be of particular importance.

The resistance of hypertension to immunisation against AII by antibodies was powerful early evidence, suggesting a role for AII independent of its circulating levels, in hypertension (Swales & Thurston, 1973). Later studies with injected renin in nephrectomised rats demonstrated a rise in blood pressure, which correlated with tissue, but not plasma renin activity (Loudon, *et al.*, 1983; Dzau, *et al.*, 1987). Observations were supported by renin inhibitor studies which showed that plasma renin did not contribute to BP regulation to the same extent as bound renin (Miyazaki *et al.*, 1988). The hypotensive action of ACE-inhibitors was also reported to correlate more closely with changes in tissue, rather than plasma AII (Swales & Samani, 1989).

Increased levels of tissue renin-angiotensin components and activity have been reported by various studies, although there are inconsistencies. The levels are reportedly increased in the arterial, renal and pulmonary beds, and in the brain of the SHR (Ferrario, 1990b; Okunishi *et al.*, 1991; Pesquero *et al.*, 1992; Johnston, 1994; Samani, 1994). Also, it was reported that the hypotensive action of hydralazine was associated with a reduction in arterial renin in the SHR (Swales & Heagerty, 1987). However, not all studies are in agreement with reports of normal, or reduced activity, in the brain (Okunishi, *et al.*, 1991; Jandeleit *et al.*, 1992), mesenteric vasculature (Morton *et al.*, 1990; Jandeleit, *et al.*, 1992), and other tissues (Welsch *et al.*, 1987; Johnston, 1994; Campbell *et al.*, 1995; Grima *et al.*, 1997). It is possible that these discrepancies are due to the use of different animal strains and the age at which the animals were studied. In addition, some reports suggest that the renin-angiotensin system may be overactive in the young SHR, prior to the development of hypertension (Harrap, 1991). Similarly, increased activity has been reported in the aorta, and mesenteric artery, from the goldblatt one-kidney, one-clip (1K1C) (Miyazaki, *et al.*, 1988; Yu *et al.*, 1993), and in the aorta of the two-kidney, one clip (2K1C) hypertensive models, during the early stages of development (Morishita *et al.*, 1992). It has been suggested that the increase in pressure and concomitant increase in wall tension may activate mRNA expression and subsequently tissue levels (Dzau, 1993a).

Direct evidence has also been obtained by gene transfer studies. Transfer of the Mouse ren-2-gene into a rat produced a hypertensive model with low circulating levels of renin, but increased tissue expression of both renin and related proteins. Furthermore, the increased tissue expression directly correlated with the level of hypertension (Samani, 1994). Polymorphisms in the genes encoding for the components of the renin-angiotensin system also exist in the human population (Roks, *et al.*, 1997), and have been found to

correlate with hypertension and left ventricular hypertrophy (LVH). Thus, suggesting differences in component levels may be linked to the genetic abnormalities.

Overall, evidence suggests abnormalities in the tissue, rather than the circulating renin-angiotensin system play an important role in the development and maintenance of hypertension.

1.7.5 Role of Angiotensin II in vascular growth.

AII generated either within the circulation or in the local tissue has been widely implicated in structural alterations observed in hypertension.

Evidence from cell culture experiments

A large majority of the work supporting a role for AII as a vascular smooth muscle mitogen has been obtained from cell culture studies. Early studies of human smooth muscle cell culture showed an increase in both growth rate and cell size with AII, in the presence of serum (Campbell-Boswell & Robertson, 1981). Similar dose-dependent growth has since been reported in many studies of animal vascular smooth muscle cells (Geisterfer *et al*, 1988; Lyall *et al.*, 1988), and these responses could be abolished by saralasin, indicating the involvement of AT receptor operated second messenger systems. There has been controversy about the nature of the growth response. Thus, Geisterfer reported that AII stimulated protein synthesis and hypertrophy, in the absence of an overall change in cell number. (Geisterfer *et al*, 1988). In addition, Berk reported that AII was not mitogenic, but that it increased protein synthesis by as much 80% (Berk *et al.*, 1989). This may be due to the effects of AII on extracellular matrix production, since it has been shown to have stimulatory effects on collagen, glycoproteins, proteoglycans and fibronectin production (Scott-Burden *et al*, 1990; Kato *et al.*, 1991). Guicheney also reported that AII was unable to stimulate DNA synthesis in SHR vascular smooth muscle

cells in culture, despite leading to high levels of inositol phosphates (Guicheney *et al.*, 1991). By contrast, Campbell and Boswell (Campbell-Boswell & Robertson, 1981), showed that AII induced both hypertrophy and hyperplasia in human aortic cell culture. Similarly, a six-day AII infusion *in vivo* led to a dose-dependent increase in cell number in the rat mesenteric artery, suggesting hyperplasia It was later shown that a dose-dependent increase, in expression of the early growth response gene c-jun occurred (Lyall *et al.*, 1991), which could be blocked by the AII antagonist saralasin. Reports of increased expression of c-fos mRNA (Naftilan, 1992; Lever, 1993) and c-myc (Kato, *et al.*, 1991) indicate that these proto-oncogenes may play a role in this cell proliferation and growth.

Some of the conflicting experimental reports can be explained by the presence of serum factors. In the absence of serum, hypertrophy occurs as a result of increased expression of platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and transforming growth factor beta-1 ($TGF_{\beta\text{-}1}$), in the vascular smooth muscle cells. The PDGF and bFGF act to stimulate cell growth (Itoh *et al.*, 1993), but $TGF_{\beta\text{-}1}$ has inhibitory effects on proliferation (Gibbons *et al.*, 1992). However, the antiproliferative effect of TGF_{β} is inactivated in the presence of serum, thereby unmasking that full mitogenic response of AII. Thus, other factors are required, in cell culture conditions, for AII to produce a hyperplastic response.

In vivo Evidence

Dickinson and Lawrence demonstrated that administration of low, subpressor doses of AII can produce an increase in arterial pressure (Dickenson & Yu, 1967). Since then, this model of hypertension has since been studied in rats, rabbits, dogs, and humans. In the rat subpressor AII infusion, via minipumps, produced a slow progressive rise in systemic BP and associated increase in mesenteric medial volume and cardiac weight. Co-administration of hydralazine prevented the increase in cardiac structure and blood

pressure, suggesting mediation by pressor mechanisms. However, the mesenteric structure was only partially reduced suggesting the involvement of a non-pressor AII mediated mechanism (Griffin *et al.*, 1991). Later studies confirmed these findings and demonstrated that noradrenaline infusion produced a similar rise in BP, without the structural effects observed with AII (Black *et al.*, 1993; Black *et al.*, 1995). Subsequently the pressor AII infusion has been shown to stimulate c-fos expression in the mesenteric artery (Lever, 1993).

Several other studies indicated direct growth effects of AII. Implants of AII into the avascular rabbit cornea stimulated growth of pre-existing vascular pathways, and also new vessel formation (Fernandez & al, 1985). In addition, a similar technique also showed neovascularisation in the chorioallantoic membrane of the chick embryo (Lever *et al.*, 1992). Also, *in vivo* transfer of the human ACE gene selectively increased ACE activity in the rat carotid and led to medial hypertrophy, which could be prevented by AII antagonists (Morishita *et al.*, 1994)

Indirect evidence has been obtained from studies involving ACE inhibitors, which not only lower BP, but also to prevent development of vascular and cardiac hypertrophy (Nagano & al, 1991). These findings have been demonstrated in both genetic (Harrap *et al.*, 1990; Lee *et al.*, 1991b; Thybo, *et al.*, 1994) and Goldblatt two-kidney, one-clip hypertensive rats (Bennett *et al.*, 1996; Bennett & Thurston, 1996). Treatment was reported to prevent the increase in smooth muscle cell content, medial weight and polyploidy in the myocardial and aortic wall of the SHR (Owens, 1987). Similar findings have been reported by blockade of the actions of AII using AII receptor antagonists (Siegl *et al.*, 1995). Some studies suggest that these antihypertensive agents are more effective in the regression of structure than other drug types, such as Hydralazine (Berk, *et al.*, 1989) and propranolol (Owens, 1985), despite similar reductions in blood pressure. Restoration

of blood pressure and structure after therapy was obtained by AII infusion (Harrap, *et al.*, 1990; Bunkenburg *et al.*, 1991). Studies of this nature are difficult to interpret, because direct effects of AII are difficult to distinguish from that dependent on increased pressure *per se*. However, low doses of ACE inhibitors, which do not lower blood pressure, also have been reported to reduce structure (Linz *et al.*, 1989; Simon & Altman, 1992; Rizzoni *et al.*, 1995), indicating a direct action of AII. On the other hand, these effects could be related to specific drug effects such as the potentiation of bradykinin and angiotensin (1-7).

1.8 ENDOTHELIUM

Endothelium was the term used by a German anatomist His, in 1865 to describe the innermost cellular lining of the circulatory system. The word was derived from "endom" meaning within and "thele" meaning nipple. The endothelial lining was originally thought to be an biologically inactive barrier, enabling the exchange of molecules by passive diffusion from the blood into the tissues. This concept was challenged by the observation of Ernst Brücke in 1857 that blood remained fluid in living vessels but coagulated in dead ones, suggesting production of vasoactive substances (Gryglewski *et al.*, 1988). The endothelium is now known to be highly metabolically active regulatory organ, with a weight of 2.5Kg, making it one of the largest in the body. This is equivalent to that of the liver, a highly metabolically active organ. It forms a continuous monolayer throughout the circulatory system and is ideally situated to detect chemical signals within the bloodstream and to respond to any changes in blood flow and pressure. The endothelial cell membrane behaves as a mechano-transducer, thereby responding to changes in pressure and flow by the release of vasoactive substances (Gardiner *et al.*, 1990a; Khayutin *et al.*, 1995). Endothelial cells are also involved in the synthesis of a variety of paracrine substances and metabolism of blood born compounds. The enzymes ACE and ecto-ATPase present on the endothelial surface are responsible for angiotensin II synthesis or degradation of bradykinin and adenine nucleotide metabolism, respectively (Vane *et al.*, 1990a). Luminal and abluminal release of substances from the endothelium interacts with a variety of cells, including platelets, leukocytes, mast cells, and vascular smooth muscle cells.

Destruction of the endothelial layer produces a range of adverse effects. These include; a 70% reduction in cellular cyclase activity (Gryglewski, *et al.*, 1988), enhanced contractile response to adrenergic nerve stimulation and to exogenous vasoconstrictors (Criscione *et al.*, 1984; Urabe *et al.*, 1991; Sunano *et al.*, 1996), increased DNA synthesis

(Schiffers *et al.*, 1992; Schiffers *et al.*, 1994a) and adhesion and aggregation of platelets to the luminal surface (Radomski *et al.*, 1987). It is clear therefore, that due to the release of a variety of vasoactive substances discussed in the next section, the endothelium is of major importance in the regulation of vascular homeostasis, vascular contractility, cell proliferation, and inflammatory mechanisms.

1.8.1 Endothelium-derived Relaxing Factors

1.8.1.i Prostacyclin

Prostacyclin (PGI_2) was identified in 1976, by Moncada and colleagues, as a product of arachidonic acid metabolism in the wall. They showed that prostaglandin endoperoxides were transformed by a microsomal enzyme from the rabbit and pig aorta, into an unstable product which relaxed arterial strips and inhibited platelet aggregation. (Moncada *et al.*, 1976). It was therefore considered a physiological antagonist to the previously described thromboxane A_2 (Hamberg *et al.*, 1975).

Arachidonic acid is synthesised from the unsaturated fatty acid, linoleic acid, which is liberated from membrane phospholipids, to which it is bound, by the enzyme phospholipase A_2. Arachidonic acid then is converted to the unstable products prostaglandin G_2 (PGG_2) and Prostaglandin H_2 (PGH_2) by the enzyme cyclo-oxygenase. These unstable prostaglandins are finally converted to stable end products. The enzyme prostacyclin synthase, present in abundance in the intima, acts upon the endoperoxide PGH_2 to generate prostacyclin (Vane, 1993).

Prostacyclin, the major vasodilatory product of the cyclo-oxygenase pathway, produced primarily by endothelial cells, with some evidence of production by smooth muscle cells (Vane, 1993; Luscher, 1994a). It causes relaxation by activating cellular adenylate cyclase in vascular smooth muscle cells, leading to a rise in the intracellular messenger cAMP (Moncada & Vane, 1979). In platelets the same mechanism inhibits

aggregation, and prostacyclin is one of the most potent inhibitors known (Vane, 1993). Prostacyclin production is stimulated by many of the same substances known to stimulate EDRF production, such as bradykinin, acetylcholine, substance P, arachidonic acid, PDGF, AII, adenine nucleotides, thrombin, trypsin interleukin-1 and shear stress. It has a short half life of approximately three minutes, being rapidly degraded into 6-keto $PGF\alpha_1$ which is excreted in the urine (Gryglewski, *et al.*, 1988). Due to the short half-life, the actions of prostacyclin are generally localised. Although prostacyclin has been shown to stimulate relaxation, its release does not appear to have a significant effect on vascular tone, or on acetylcholine induced relaxation. Thus, removal using the cyclo-oxygenase inhibitor indomethacin has been shown to have little effect on blood flow in the forearm (Kiowski, 1991).

1.8.1.ii Endothelium-dependent relaxing factor (EDRF)

Discovery of EDRF

For many years physiologists had observed considerable variability in the relaxant response to the muscarinic agonist, acetylcholine. Although it was well established that direct intravenous infusion into an arterial bed, or organ resulted in vasodilatation, in many experiments using isolated arterial tissue demonstrated vasoconstriction. Furthermore, relaxation occurred in response to acetylcholine in pre-constricted aortic rings, whereas helical strip preparations often contracted. In 1978 it was discovered that in many of the arterial preparations the endothelial cell layer was destroyed during the procedure, by either rubbing, or by the introduction of air bubbles into perfusion preparations (Furchgott, 1993). The importance of the endothelial layer was confirmed by silver staining of the intimal surface by Zadawzki in 1979, relaxation being absent if the endothelial layer was removed (Cohen, 1995). Furchgott and Zadawzki investigated the role of the endothelial cells further using the sandwich technique of Sir John Vane. The technique made use of an

acetylcholine stimulated donor aorta possessing endothelium and an opposing recipient artery, preconstricted and denuded of endothelium. They confirmed that the vasodilatory response was endothelium-dependent, and postulated that acetylcholine acted on muscarinic receptors to stimulate the release of a diffusible non-prostanoid substance. They called this evanescent substance Endothelium-dependent relaxing factor (EDRF) (Furchgott & Zawadzki, 1980; Furchgott, 1993). This exciting work stimulated major research efforts and worldwide interest into the properties and identity of EDRF. Vascular sandwich techniques and bioassay perfusion techniques demonstrated that EDRF was likely to act locally on the surrounding tissues, because of its short half-life (Griffith *et al.*, 1984a; Rubanyi, 1985a). Others showed that canine or bovine endothelial cells cultured on microcarrier beads were able to release EDRF in response to agonist stimulation (Cocks & Angus, 1985; Palmer *et al.*, 1987)

***Identification* of EDRF**

In the early 1980's, many substances were proposed to account for the activity of EDRF. Early investigations by Furchgott indicated possible roles for products of arachidonic acid via the lipoxygenase pathways, or that it could be a free radical, since free radical scavengers inhibited relaxation (Furchgott, 1981). Products of the cytochrome P450 pathway (Singer *et al.*, 1984; Bredt *et al.*, 1991) and carbonyl containing compounds (Griffith *et al.*, 1984b; Murray *et al.*, 1986) also were considered. Finally, in the late 1980's that Furchgott and Ignarro, after careful comparison of the properties of EDRF and nitric oxide, proposed that it was nitric oxide or a chemically related species (Furchgott, 1988; Ignarro *et al.*, 1987a). Both EDRF and nitric oxide activated guanylate cyclase, and acted as inhibitors of platelet aggregation in humans. In addition, haemoglobin and methylene blue could inhibit the actions of both EDRF and NO. (Ignarro, 1989) Further evidence was obtained from perfusion studies of cultured endothelial cells on microcarrier

beads in a chromatography column. Oxyhaemoglobin was used to capture NO and quantities were determined by spectrophometric assay (Palmer, *et al.*, 1987). The quantities of NO released in the perfusate under basal conditions and to bradykinin stimulation was later shown to be sufficient to relax denuded rabbit aortic strips (Palmer, *et al.*, 1987; Kelm *et al.*, 1988; Kelm & Schrader, 1990). In addition, confirmation was obtained from chemiluminescence techniques, as nitric oxide may be measured directly as the chemiluminescent product of its own reaction with ozone. Studies using this technique reported a correlation between NO measured by bioassay and that detected by chemiluminescence. EDRF and nitric oxide were also shown to have similar half-lives (Moncada *et al.*, 1991a). However, despite this mass of evidence other studies cast doubt on NO as the identity of EDRF. Thus, ion exchange column studies reported differences in binding properties (Greenberg *et al.*, 1990; Long *et al.*, 1987), and some bioassay and culture experiments have reported differences in half life (Griffith, *et al.*, 1984b; Cocks & Angus, 1985; Rubanyi, 1985a) and response in vascular tissues. (Dusting *et al.*, 1988; Gryglewski, *et al.*, 1988; Furchgott *et al.*, 1990; Marshall & Kontos, 1990). It is widely accepted now that NO is responsible for the tissue EDRF observed, although it may not exist in the free form (Ignarro, *et al.*, 1987a; Palmer, *et al.*, 1987; Moncada, *et al.*, 1991a). It has been suggested that NO may be liberated from a stable NO precursor, possibly the S-nitrosothiols (Ignarro, *et al.*, 1987a; Furchgott, *et al.*, 1990; Marshall & Kontos, 1990; Vedernikov *et al.*, 1990).

Synthesis of EDRF

Studies by Palmer and colleagues (Palmer, *et al.*, 1987), were the first to show that porcine endothelial cells stimulated with either bradykinin or calcium ionophore synthesised NO from L-arginine. In the absence of L-arginine EDRF decreased, and could be restored by reintroducing L-arginine, but not D-arginine, indicating a stereospecific

reaction. In addition, spectrophometric analysis also demonstrated the production of radiolabelled [N^{15}] NO from the terminal, guanidino nitrogen atom of radiolabelled [N^{15}] L-arginine added to the cells. In addition, they showed that the EDRF production was enhanced by the addition of L-citrulline suggesting an involvement in the reaction (Palmer & Moncada, 1988; Moncada *et al.*, 1991b). These observations led to the development of L-arginine analogues, which inhibit EDRF formation, and have greatly contributed to research in this area. The inhibitory action could be overcome by the addition of excess L-arginine substrate or L-citrulline, providing further confirmation that L-arginine was the precursor for EDRF (Sakuma *et al.*, 1988; Rees, *et al.*, 1989a; Vallance *et al.*, 1989a).

It is now clear that EDRF/NO is synthesised from the terminal, guanidine nitrogen atom, or atoms of the L-arginine, with the formation of the biproduct L-citrulline. L-citrulline is rapidly recycled back to L-arginine, by an enzyme present in the vascular smooth muscle cells, thereby maintaining adequate cellular L-arginine levels (Schini *et al.*, 1994; Schini-Kerth & Vanhoutte, 1995). The enzyme responsible for the conversion of L-arginine was identified as nitric oxide synthase (NOS), which exists as three isoforms. Two of these are constitutive forms, the first being located in the plasma membrane of endothelial cells (eNOS), and the second in the peripheral and central neuronal cells (nNOS) (Forstermann *et al.*, 1993; Pollock *et al.*, 1993; Fleming & Busse, 1995). Endothelial NOS is active during both basal conditions (Christie *et al.*, 1989) and in response to a variety of stimuli (Rubanyi, 1993a; Rubanyi, 1985a; Vane, 1993; Vanhoutte, 1989a). Synthesis of NO depends on calcium binding and is modulated by calmodulin (Singer & Peach, 1982; Mayer *et al.*, 1989; Busse *et al.*, 1991). A third inducible calcium-independent NOS isoform has been identified in various cell types in response to immunological stimuli, such as bacterial lipopolysaccharides, cytokines (Hecker *et al.*, 1999) and Tumour Necrosis Factor (TNF) (Rubanyi, 1993a). Although, this isoform has

been implicated in pathological conditions such as autoimmune disease and septic shock (Forstermann *et al.*, 1994), its presence has been demonstrated under normal conditions (Marin & Rodriguez-Martinez, 1997). NOS isoforms utilise nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen as cosubstrates and require the presence of a number of cofactors; 5,6,7,8 tetrahydrobiopterin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Vane, *et al.*, 1990a; Dinerman *et al.*, 1993; Schini-Kerth & Vanhoutte, 1995; Marin & Rodriguez-Martinez, 1997).

Release of EDRF

EDRF is released from endothelial cells in large and small blood vessels from most mammalian species (De Mey & Gray, 1985; Peach *et al.*, 1985; Sakuma *et al.*, 1990; Yang *et al.*, 1990), and often is co-released with prostacyclin. Continuous basal release of EDRF has been suggested in some tissues because endothelial removal was observed to reduce tissue levels of cGMP (Ignarro *et al.*, 1987b). It would appear that arteries of smaller diameter produce large basal quantities of EDRF (Aalkjaer *et al.*, 1987c; Ignarro, 1989; Galle *et al.*, 1993), which may contribute to peripheral resistance and blood flow regulation (Vallance *et al.*, 1989b)

Either agonist receptor interactions or physical stimuli may stimulate release of EDRF. The many substances reported to stimulate EDRF release, include acetylcholine, bradykinin, catecholamines, angiotensin II (Boulanger *et al.*, 1995), histamine, arachidonic acid and platelet-derived products such as thrombin, serotonin and adenine nucleotides (Busse *et al.*, 1993). The vasodilatation produced depended on the presence of extracellular calcium (Singer & Peach, 1982), and could be attenuated by calcium antagonists (Griffith *et al.*, 1985). Therefore, a receptor mediated increase in intracellular calcium in the endothelial cell is an important initial step in synthesis and release of EDRF (Peach, *et al.*, 1985). Agonist-receptor interactions stimulate an increase in inositol

triphosphate (IP_3) production, which in turn triggers calcium release from intracellular stores. A sustained influx of calcium from the extracellular space also occurs due to opening of potassium channels, causing membrane hyperpolarization (Johns *et al.*, 1988; Busse, *et al.*, 1991; Busse, *et al.*, 1993). Receptor independent synthesis of EDRF has also been demonstrated by stimulation with calcium ionophores (Furchgott, *et al.*, 1990; Schroder *et al.*, 1990).

Physical stimuli such as shear stress, pulsatile stretch (Lamontagne *et al.*, 1992) and hypoxia (Pohl & Busse, 1989) may also trigger EDRF release. Schretzenmeyer was the first to observe flow-mediated dilatation in 1933, in the canine femoral artery. This was followed in 1966, by the suggestion of Robard that shear stress, and viscous forces exerted on the wall by the flowing blood, might act via the endothelium to cause relaxation of the underlying smooth muscle cells (Khayutin, *et al.*, 1995). The involvement of EDRF was confirmed by sandwich experiments, in which low flow stimulated EDRF release from canine femoral arteries (Rubanyi *et al.*, 1986). Subsequently, increased flow has been shown to release both EDRF/NO and prostacyclin from the perfused arteries (Smeisko *et al.*, 1989; Gardiner, *et al.*, 1990a; Kuo *et al.*, 1990). Shear stress on the vascular endothelium is thought to be to be the most important physiological stimulator of release (Lamontagne, *et al.*, 1992; Fleming & Busse, 1995). This view is supported by studies of EDRF release from endothelial cells, cultured on microcarrier beads in columns, to differing perfusion pressures and flow rates. Increased flow enhanced release of basal EDRF/NO flow rates, there was no change with hydrostatic pressure (Kelm *et al.*, 1991). Prolonged increases in shear stress have been found to increase eNOS expression (Schini-Kerth & Vanhoutte, 1995; Bassenge, 1996; Marin & Rodriguez-Martinez, 1997). It is thought that endothelial cells sense the magnitude of shear stress and pulsatile flow by the degree of deformation. This is detected by stretch-sensitive cation channels which in turn

induce cell membrane hyperpolarization, thereby stimulating synthesis of EDRF (Lamontagne, *et al.*, 1992; Ohno *et al.*, 1993).

More recently, it was shown that blood vessels can be innervated by novel nitrergic nerves which release and utilise nitric oxide as a neurotransmitter. Nitric oxide release from these nerves may act to decrease vascular tone and oppose the actions of sympathetic nerves (Forstermann, *et al.*, 1994).

Actions of EDRF

EDRF acts mainly as a paracrine hormone because it decays rapidly in the bloodstream to nitrate/nitrite. It has a very short half-life, approximately 6 seconds (Griffith, *et al.*, 1984b; Rubanyi, 1985a; Vane, 1993), due to inactivation by superoxide anions and other antioxidants, and by rapid binding to free haemoglobin in the bloodstream. Free radical scavengers e.g. superoxide dismutase (SOD), have been shown to potentiate the actions of EDRF under certain experimental conditions (Ignarro, *et al.*, 1987a; Furchgott, *et al.*, 1990).

EDRF released from endothelial cells acts either upon the endothelial cells, or diffuses to act on nearby vascular smooth muscle cells, or platelets. It is able to diffuse freely in the cellular environment because it is a small molecule which is soluble in both water and lipids (Ignarro, 1990). EDRF binds to the heme moiety of cellular guanylate cyclase in target cells, initiating a conformational change at the porphyrin binding site (Ignarro, *et al.*, 1987b; Ignarro, 1991a). This conformational change triggers an increase in the levels of the intracellular messenger cyclic GMP. In turn this stimulates a series of intracellular events including increased calcium extrusion, re-uptake of calcium into the internal stores and inhibition of receptor operated calcium influx (Ignarro, 1990; Ignarro, 1991b; Vane, 1993; Cohen *et al.*, 1999). These processes cause a rapid reduction in intracellular calcium and consequently vascular smooth muscle cell relaxation (Marin &

Rodriguez-Martinez, 1997). However, a calcium-independent mechanism of relaxation has also been described (Chabaud *et al.*, 1994).

Nitric oxide synthesis inhibitors have confirmed the importance of EDRF in the regulation of vascular tone during both basal and stimulated conditions. They have been shown to produce long-lasting reductions in blood flow, around 50%, in the brachial artery of healthy volunteers, and attenuated the vasodilator response with acetylcholine infusion (Collier & Vallance, 1991; Vane, 1993). Similar reductions in the response to acetylcholine were observed in the perfused rat mesenteric bed pre-treated with L-NMMA (Ralevic *et al.*, 1991). Systemic intravenous administration of N^G-monomethyl-L-arginine (L-NAME) into the systemic circulation causes a notable increase in blood pressure in anaesthetised rats (Gardiner *et al.*, 1990b; Rees, *et al.*, 1990a), and rabbits (Rees *et al.*, 1989b). L-NAME animal models of hypertension are widely used as research tools to investigate the role of nitric oxide in hypertension. The effects of nitric oxide synthesis inhibitors can be reversed by the administration of L-arginine (Gardiner, *et al.*, 1990b). More recently, experiments using knockout mice confirm the importance of EDRF/NO in blood pressure regulation. Removal of the gene encoding for eNOS leads to the development of hypertension (Huang *et al.*, 1995).

Nitric oxide also may be important in the control of vascular growth as it acts as a growth inhibitor in smooth muscle cells (Raij, 1991; Dzau, 1993b; Lee, *et al.*, 1995; Nakaki & Kato, 1996). NO generating vasodilators display an antiproliferative effect on cultured mesangial cells stimulated with PDGF or thrombin (De Mey *et al.*, 1991a), and inhibit total protein and collagen synthesis in rabbit aortic smooth muscle cells in a dose-dependent manner (Kolpakov *et al.*, 1995). Endothelial cells in culture suppress thymidine incorporation into the vascular smooth muscle cells indicating reduced DNA synthesis, not protein synthesis. Similar findings were reported using isolated arterial preparations

(Schiffers, *et al.*, 1992). Some studies indicated that the antiproliferative response is likely to be partially mediated by increased levels of cyclic GMP in the smooth muscle cells (Scott-Burden & Vanhoutte, 1993). However, more recently studies have implicated other endothelial factors, such as TGF-ß, in the inhibition of DNA synthesis (Schiffers *et al.*, 1994b; Schiffers, *et al.*, 1994a). These observations make it tempting to consider the endothelium as an inhibitor of vascular growth, but it is important to remember that the endothelium is also capable of releasing several potential growth promoters.

Finally, the data which has amassed over the past decade clearly shows that the contribution of EDRF to vascular structure and function varies widely according to the arterial bed and species studied (Christie, *et al.*, 1989).

1.8.1.iii EDHF

Discovery of EDHF

Acetylcholine induced vasorelaxation has been associated with hyperpolarization of vascular smooth muscle cells. This led to the suggestion that two endothelium-derived relaxing factors may be involved in the response to acetylcholine (Chen *et al.*, 1988; Feletou & Vanhoutte, 1988; Chen & Cheung, 1992). The presence of a diffusible endothelial-derived substance was proposed based on experiments using sandwich techniques and endothelial denudation (Feletou & Vanhoutte, 1988; Vanhoutte, 1993a). Nitric oxide itself has been shown to cause hyperpolarization in some vascular beds (Tare *et al.*, 1990; Plane & Garland, 1993). This involves activation of ATP-sensitive potassium channels, which can be inhibited by glibenclamide (Murphy & Brayden, 1995; Murphy & Brayden, 1996). However, many studies suggest that the response to certain agonists is only partially dependent on the L-arginine/NO pathway. Thus, a component of relaxation remained after blockade of NO synthesis by agents such L-NMMA, methylene blue or oxy-haemoglobin (Fujii *et al.*, 1992; Feletou & Vanhoutte, 1996; Edwards & Weston,

1998a; Sunano *et al.*, 1999). Similar observations have been made using acetylcholine in rat mesenteric arteries (Fujii, et al., 1992; Garland & McPherson, 1992; Hwa *et al.*, 1994; Parsons *et al.*, 1994; Kamata *et al.*, 1995; Mantelli *et al.*, 1995), and in human arteries (Woolfson & Poston, 1990; Petersson *et al.*, 1997; Urakami-Harasawa *et al.*, 1997).

Hyperpolarization in response to acetylcholine depends on the opening of potassium channels in the vascular smooth muscle cells. Studies show that hyperpolarization is markedly inhibited by non-specific potassium channel blockade, using tetraethylammonium (TEA), or by increasing the physiological potassium concentration (Fujii, *et al.*, 1992; Adeagbo & Triggle, 1993; Kamata, *et al.*, 1995; Mantelli, *et al.*, 1995; Chen & Cheung, 1997). Also, potassium channel activating agents appear to have similar properties to EDHF in the rat mesenteric artery (White & Hiley, 1998). Calcium-activated potassium channels have been implicated in some vascular beds, because the inhibitor apamin was found to reduce vasodilatation to bradykinin in porcine coronary arteries (Hecker *et al.*, 1994), and that to acetylcholine in rat mesenteric arteries (Adeagbo & Triggle, 1993; Kahonen *et al.*, 1995; Wu *et al.*, 1997). Research by Chen and Cheung reported that the inhibitor apamin provided the most effective blockade of EDHF in rat mesenteric arteries whilst charybdotoxin also produced a small significant reduction in hyperpolarization. Combination of the two completely abolished the response suggesting the involvement of both large and small conductance calcium-activated potassium channels, with the small conductance channels playing the greater role (Chen & Cheung, 1997). However, the literature on potassium channel involvement in hyperpolarization is complex since the channels involved vary dependent on the vascular bed and species studied (Waldron & Cole, 1999).

Hyperpolarization of smooth muscle cells is thought to induce relaxation by reducing the open probability of voltage-dependent calcium channels thereby reducing

calcium entry into the cells (Mombouli & Vanhoutte, 1995). There is evidence also that it may inhibit IP_3 synthesis (White and Hiley, 1998), and both these processes contribute to a decrease in vascular smooth muscle intracellular calcium, initiating relaxation.

Identity of EDHF

A number of candidate molecules have been proposed to act as EDHF. A role for prostacyclin was ruled out by studies since blockade with indomethacin did not alter the response (Chen & Cheung, 1992; Hecker, et al., 1994; Hansen & Olesen, 1997; Feletou & Vanhoutte, 1988). A considerable mass of evidence pointed to the involvement of epoxyeicosatrienoic acids, metabolites of arachidonic acid formed by the cytochrome P450 mono-oxygenase pathway (Fleming & Busse, 1999). Thus, in the microcirculation and carotid artery of the rabbit, P450-derived arachidonic acid metabolites display similar characteristics to EDHF and cause relaxation by activation of potassium channels. In addition, EDHF induced vasodilatation could be strongly reduced by phospholipase A_2 inhibitors, cytochrome P450 inhibitors and tetraethylammonium (Hecker, *et al.*, 1994; Adeagbo, 1997; Dong *et al.*, 1997; Weintraub *et al.*, 1997). On the other hand, the metabolites ought to be more easily detectable. In addition, the pharmacological responses and potassium channels involved have been reported to be different from those of EDHF (Eckman *et al.*, 1998; Edwards & Weston, 1998a; Campbell & Harder, 1999). A role for a second group of arachidonic acid derivatives, the cannabinoids has been proposed more recently because anandamide produces effects very similar to EDHF (Randall *et al.*, 1997a; Randall & Kendall, 1997b). However, other studies raise doubts about the co-identity of anandamide as EDHF (Plane *et al.*, 1997).

It has been suggested that hyperpolarization is due to electrical coupling through the gap junctions, which would allow the spread of endothelial hyperpolarization to the underlying smooth muscle cells. However, this appears unlikely because inhibitors of gap

junctions do not appear to modify the EDHF response to ACh in the rat hepatic artery. In addition, myoendothelial gap junctions only appear to involve conductance of electrical signals from the smooth muscle cells to the endothelial cells (Feletou & Vanhoutte, 1999). Recently, Weston and colleagues recently proposed that EDHF could simply be the potassium ion, as ACh causes hyperpolarization, together with an increased potassium concentration in the myoendothelial space (Edwards *et al.*, 1998b). However, it is not known if endothelial cells could release a large enough quantity of the ions. Moreover, recent evidence from our laboratories showed that potassium is unlikely to be EDHF, because the response to potassium was different to that of acetylcholine in rat small mesenteric arteries (Lacy *et al.*, 2000).

The identity of EDHF is still unclear and recently Busse described EDHF as the "eternally deceptive hyperpolarizing factor" (Vanhoutte, 1998). A variety of factors may be involved in producing tissue hyperpolarization, depending on the species and tissue studied (Triggle *et al.*, 1999). EDHF is a more important component to endothelial-dependent relaxation, particularly in the smaller arteries (Wu, *et al.*, 1997), consequently a small change in membrane potential could produce a substantial change in vessel diameter. (Brayden *et al.*, 1991; Nelson & Quayle, 1995).

1.8.2 Endothelium-derived Contracting Factors

Vanhoutte and colleagues showed that endothelial contracting factors were produced by several stimuli such as hypoxia (Rubanyi & Vanhoutte, 1985b), physical forces and receptor stimulation (Vanhoutte, 1987a; Vanhoutte, 1987b; Vanhoutte, 1993a). The major mediators implicated in vascular smooth muscle contraction are vasoconstrictor metabolites of arachidonic acid, superoxide anions, and the vasoconstrictor polypeptide endothelin.

1.8.2.i Cyclo-oxygenase-dependent Contracting Factors

In 1936, Van Euler used the term prostaglandin's to describe vasodilator smooth muscle stimulating acid lipids found in the seminal fluid. In 1947, Bergstrom began work to determine the structure of these relatively stable prostaglandins showing they derived predominantly from arachidonic acid (for review, see (Pickard, 1981)). Many cyclo-oxygenase derivatives of arachidonic acid metabolism have since been described, their synthesis was shown to be inhibited by non-steroidal, anti-inflammatory agents such as aspirin and indomethacin (Vane, 1971) (Figure 1.4).

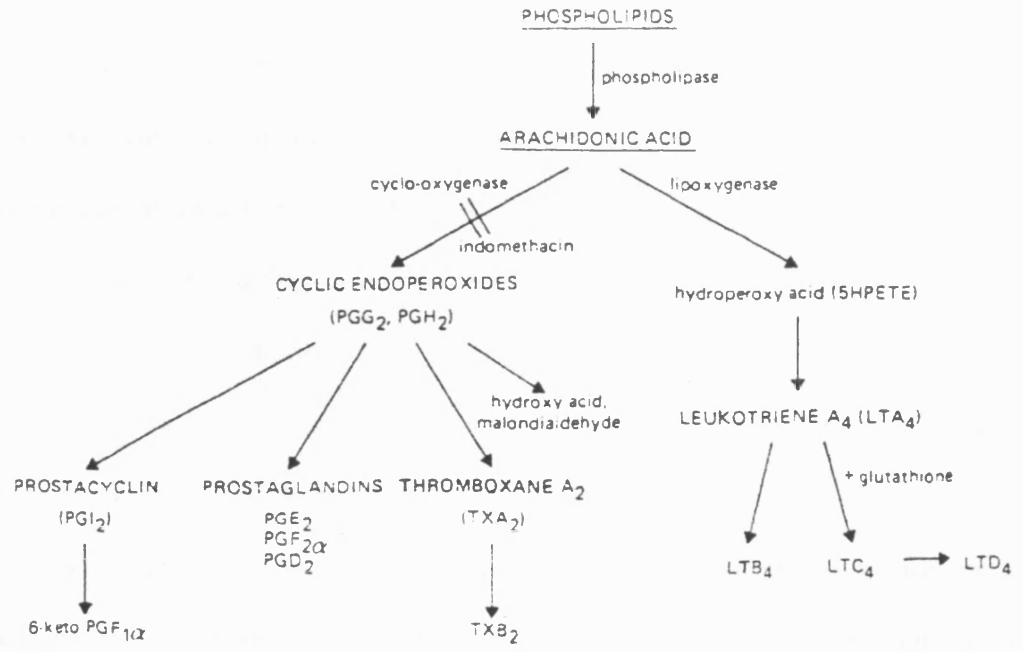


Figure 1.4. Pathways of arachidonic acid metabolism; PG, prostaglandin; PGI_2, prostacyclin; TX, thromboxane; LT, leukotriene. Taken from (Pickard, 1981).

Early studies demonstrated that the application of exogenous arachidonic acid to canine basilar arteries caused a potent contractile response (Katusic *et al.*, 1988; Katusic & Shepherd, 1991). Similar contractions to high concentrations of acetylcholine and to the calcium ionophore (A23187) were reported in the canine basilar artery (Katusic, *et al.*,

1988; Shirahase *et al.*, 1988). Subsequently, cyclo-oxygenase-dependent contractions have been demonstrated in a variety of vascular beds after stimulation by acetylcholine, rapid stretch and calcium ionophores (Vanhoutte, 1993a).

The most potent cyclo-oxygenase derived products of arachidonic acid, formed by platelets and the vascular tissues include; Thromboxane A_2, PGG_2, and PGH_2. (Cohen, 1995). Thromboxane A_2 (TxA_2) was characterised by Bergt Samuelsonn in 1975 and was shown to be a potent aggregator of platelets and vasoconstrictor substance (Vane, *et al.*, 1990a; Vane, 1993). It is synthesised mainly in the platelets, but may also be produced by the blood vessels. Thromboxane A_2 is rapidly broken down to the more stable metabolite TxB_2 (Bassenge, 1996). The contractile response in cerebral arterioles, to calcium ionophore, can be blocked by a thromboxane synthetase inhibitor, suggesting the involvement of thromboxane (Katusic & Shepherd, 1991). PGH_2 and PGG_2 also may act as vasoconstrictors as they have been shown to interact with the same receptor type as thromboxane. Also, addition of PGH_2 at threshold concentrations to an artery was shown to produce a vasoconstrictor response, which was great enough to inhibit endothelium-dependent relaxation (Cohen, 1995)

Research suggests that different cyclo-oxygenase products are involved in vasoconstriction, depending upon the vascular bed studied and agonist applied (Luscher, 1994a).

In the last decade prostaglandin-like compounds, derived from arachidonic acid were identified (Morrow *et al.*, 1990). These compounds were produced by free radical-catalysed peroxidation of arachidonic acid, independent of cyclo-oxygenase, and differ from the prostaglandins as a result of the *cis* orientation of their side chains (Elmhurst *et al.*, 1996). Several families of free radical catalysed products of arachidonic acid have been described including; E_2, F_2 and D_2 isoprostanes (Morrow *et al.*, 1992; Morrow *et al.*,

1994), isothromboxanes (Pratico *et al.*, 1995) and isoleukotrienes (Harrison & Murphy, 1995). Of particular interest is 8-Epi, $PGF_{2\alpha}$, a member of the F_2 isoprostane family. This is reported to be a potent vasoconstrictor of the renal vasculature (Takahasi *et al.*, 1992) and to stimulate vascular smooth muscle cell DNA synthesis (Natarajan *et al,* 1996). Fukunaga and colleagues reported the existence of distinct F_2 isoprostane receptors exist in rat vascular smooth muscle cells (Fukunaga *et al.*, 1993). In addition, more recent research using human platelets demonstrated the formation of 8-Epi, $PGF_{2\alpha}$, via cyclo-oxygenase dependent pathways (Pratico *et al.*, 1995).

1.8.2.ii Oxygen-derived free radicals

In arterial preparations, endothelial-dependent contractions of the canine basilar artery have been found to be inhibited selectively by the free radical scavenger superoxide dismutase (Katusic & Vanhoutte, 1989). Similar observations have been made in both the rat mesenteric artery and human subcutaneous arteries, although results are inconsistent (Sunman *et al.*, 1993). In addition, superoxide anion generation, by addition of xanthine plus xanthine oxidase, produced a dose-dependent contraction in the rat aorta, which could be blocked cyclo-oxygenase inhibitors (Auch-Schwelk *et al.*, 1989). Superoxide anions also may play an indirect vasoconstrictor role by chemical inactivation of EDRF/NO in the vascular wall. Such reactions have been demonstrated in cultured endothelial cells, where superoxide anion production was shown to inhibit both NO and prostacyclin production (Katusic & Shepherd, 1991; Vanhoutte & Boulanger, 1995). A variety of different stimuli have been reported to produce superoxide anions in cultured endothelial cells including; anoxia, calcium ionophore, interleukin-1 and excessive stimulation of the arachidonic acid cascade (Katusic & Shepherd, 1991)

1.8.2.iii Endothelin

Early in the 1980's, the conditioned media from cultured endothelial cells was shown to contain a vasoconstrictor substance (Hickey *et al.*, 1985). Masaki and his team in 1988 went on to isolate a novel peptide vasoconstrictor from cultured pig aortic endothelial cells, which they called endothelin (Yanagisawa *et al.*, 1988). This peptide is to date one of the most potent naturally occurring vasoconstrictors known, which is 10 times more potent than angiotensin II. In addition, it was shown to produce greater contraction in veins than arteries. Later work revealed the existence of three different isopeptides, each differing by a few amino acids. These were designated, ET_1, ET_2 and ET_3, and endothelin-1 was shown to be the main peptide produced by the endothelial cells. Endothelin-1 is a 21 amino acid peptide formed from the 200 amino acid precursor preproendothelin. An intermediate step is involved where preproendothelin first is broken down to the less active intermediate proendothelin or "big endothelin", consisting of 38 amino acids, from which endothelin is released. Endothelin-1 binds to two specific receptors ET_A and ET_B. It was originally thought that constriction depended on endothelin binding to ET_A receptor in the vascular smooth muscle cells. However, later studies showed that in certain vascular beds ET_B receptors also mediated vasoconstriction (Vane, 1990b; Rubanyi & Botelho, 1991; Vane, 1993; Nava & Luscher, 1995).

Intravenous administration of endothelin has been shown to cause a long-lasting increase in blood pressure in both rats and rabbits (Yanagisawa, *et al.*, 1988), and in man, an increased peripheral resistance after local administration in the forearm circulation (Luscher *et al.*, 1991). *In vivo* infusion of endothelin peptide into healthy volunteers or experimental animals was shown initially to produce a small but significant increase in blood flow with vasodilatation, followed by vasoconstriction, suggesting a dual mechanism of action. The initial vasodilatation appears due to stimulation of ET_B receptors on the

endothelium stimulating prostacyclin and PGE_2 release, which can be blocked by indomethacin (Kiowski, 1991; Luscher, *et al.*, 1992). Evidence accumulating in both human and animal studies now suggests that the pressor activity of endothelin is self-limiting because it stimulates of both EDRF and prostacyclin production (Vane, *et al.*, 1990a; Wu & Bohr, 1990; Luscher *et al.*, 1993b). The release of endothelin in porcine arteries was enhanced by the introduction of L-NMMA, confirming an inhibitory role for NO (Boulanger & Luscher, 1990).

The majority, approximately two thirds, of endothelin is rapidly removed by the lungs, since the response to intra-arterial infusion is more marked than with intravenous administration, indicating that it acts as a basal vasodilator (Raij, 1991; Vane, 1993; Vanhoutte, 1993a). It is uncertain whether endothelin has a physiological role in the human because circulating levels appear to be very low. However, such concentrations may be sufficient to potentiate the actions of other vasoactive substances such as noradrenaline and serotonin (Luscher, 1993a). Several receptor-dependent and independent stimuli have been shown to stimulate endothelin release in endothelial cell culture including thrombin, TGF-ß, adrenaline, AII, calcium ionophore A23187, stretch and hypoxia (Boulanger & Luscher, 1990; Vane, *et al.*, 1990a; Rubanyi & Botelho, 1991). Endothelin has been implicated as a vascular mitogen causing proliferation and transcription of the proto-oncogenes c-fos and c-myc in culture smooth muscle cells (Vane, 1993).

1.8.3 Interactions of platelets and the vascular wall

Blood cells, especially the aggregating platelets, are known to release a variety of vasoactive substances, including adenosine nucleotides, serotonin, thromboxane A_2, platelet activating factor and in some species vasopressin and histamine. In addition,

thrombin is formed at sites of aggregating platelets due to activation of the coagulation cascade. Many of these substances cause endothelial-dependent relaxation. However, where endothelium is absent or dysfunctional several platelet-derived products, such as serotonin and thromboxane, stimulate vasoconstriction by acting directly on vascular smooth muscle cell receptors (Luscher *et al.*, 1990a).

Several endothelial cell characteristics are important for the maintenance of normal vascular tone. Endothelial cells synthesise and release heparin-like anticoagulant glycosaminoglycans (Scott-Burden & Vanhoutte, 1993). They release EDRF and prostacyclin, which protect the wall against the vasoconstrictor platelet-derived products, thereby reducing vasospasm. Nitric oxide is a naturally occurring leukocyte inhibitor (Lefer, 1997). In the hamster cheek pouch, at sites of platelet activation, it has been demonstrated to be a potent inhibitor of thrombus formation (Rees *et al.*, 1989b). Platelet function is profoundly affected by the release of these vasodilators, for example aggregation is inhibited by both NO (Vallance *et al.*, 1992a) and prostacyclin (Radomski, *et al.*, 1987; Ignarro, 1989). In addition, EDRF and prostacyclin act synergistically to inhibit aggregation. However, inhibition of platelet adhesion appears to be the province solely of NO (Radomski, *et al.*, 1987; Ignarro, 1989; Marin & Rodriguez-Martinez, 1997). Platelets are capable of NO synthesis and release, which may act via negative feedback systems to modulate their own activity (Rubanyi, 1993a).

1.8.4 Endothelial Dysfunction

1.8.4.i Morphological alterations

Morphological changes occur despite preservation of endothelial integrity in many cases of hypertension. Alterations in the number, size (Arribas *et al.*, 1997), shape, replication rate of endothelial cells, and permeability of the endothelial layer have all been

reported (Luscher, 1990b; Vallance *et al.*, 1992b). More detailed examination using transmission electron microscopy shows endothelial cells have augmented numbers of their cytoplasmic organelles (Luscher, 1990b). In addition, endothelial cells are reported to bulge into the vascular lumen and subintimal space. The interaction of the endothelial layer with other cells also is altered, with studies showing increased adhesion of monocytes, macrophages and platelets and infiltration into the sub-endothelial layer (Raij, 1991). These alterations may lead to enhanced vascular smooth muscle contraction because of the release of platelet-derived constrictor substances.

1.8.4.ii Functional Alterations

Once appreciated that the vascular wall could produce many vasoactive substances, considerable research was undertaken to compare their activity during both normal conditions, and vascular disease. These studies quickly demonstrated that endothelium-dependent relaxation to certain agonists, was reduced in various animal models of hypertension (Konishi & Su, 1983; Luscher & Vanhoutte, 1986a; Lockette *et al.*, 1986; Watt & Thurston, 1989; Fu-Xiang *et al.*, 1992; Luscher, *et al.*, 1992; Falloon *et al.*, 1993). Similar observations have been reported in both primary, and secondary human hypertension (Panza *et al.*, 1990; Luscher, *et al.*, 1992; Taddei *et al.*, 1993; Marin & Rodriguez-Martinez, 1997). An important second consequence of the changes was an enhanced constrictor response to exogenous agonists, such as noradrenaline (Dohi *et al.*, 1990; Woodman, 1995; Dohi *et al.*, 1996). These findings suggest that abnormalities in the balance between the release of contracting and relaxing factors could play a role in producing the increased peripheral resistance and vascular complications found in hypertension (Luscher, 1990b).

Several mechanisms have been proposed to explain endothelial dysfunction. These include; a reduced synthesis and release of vasodilators, increased levels of scavengers of

EDRF or enhanced release of constrictor factors, impaired diffusion to the smooth muscle cells, and decreased responsiveness of the vascular smooth muscle cells. Mechanisms involved also vary depending on the species, model, severity of hypertension, vascular bed studied, and on the agents used to induce constriction (Bund, 1998), and relaxation (Lockette, *et al.*, 1986; Luscher, 1990b; Li & Bukoski, 1993; Mitchell *et al.*, 1992).

Human Hypertension

Endothelial-dependent relaxation to acetylcholine is reported to be impaired in studies of blood flow in the human brachial artery (Vanhoutte & Boulanger, 1995; Li *et al.*, 1997a; Lyons, 1997), and in myograph studies of subcutaneous resistance arteries (Falloon & Heagerty, 1994; Deng *et al.*, 1995). However, others have failed to demonstrate the impairment (Cockcroft *et al.*, 1994; Bruning *et al.*, 1995), and it has been suggested this discrepancy may be due to the use of different dilator agents. The relaxation to nitric oxide donors, such as sodium nitroprusside, was unchanged, ruling out an impaired responsiveness of the vascular smooth muscle to NO (Panza, *et al.*, 1990; Panza *et al.*, 1993a; Marin & Rodriguez-Martinez, 1997). Various studies suggest a reduction in the nitric oxide component because of a reduced vasoconstrictor response to nitric oxide synthesis inhibitors, in the chronic hypertensive forearm of patients with essential hypertension. In addition, L-NMMA infusion was shown to have no significant effect on the relaxation to ACh in hypertensive patients, confirming a diminished nitric oxide response (Vallance, et al., 1989b; Luscher, et al., 1993b; Panza, et al., 1993a; Calver *et al.*, 1994; Cosentino & Luscher, 1995). Currently, the majority of evidence supports a nitric oxide deficiency in human hypertension, and indicates a reduction in both basal and stimulated release. (Cohen, 1995; Dominiczak & Bohr, 1995; Kelm *et al.*, 1996; Panza, 1997; Cardillo & Panza, 1998). More recently, evidence has emerged of accelerated inactivation of NO, which could contribute to the impaired response (Kelm, *et al.*, 1996).

The importance of this mechanism remains uncertain because no improvement occurred following exposure to superoxide dismutase (Vanhoutte, 1996). However, a defective NO pathway may not be the only abnormality present. Indomethacin administration improved endothelial function in human hypertension, implying that enhanced release of vasoconstrictor prostanoids also could be involved (Taddei *et al.*, 1997).

Animal models of Hypertension

Endothelial dysfunction, mainly using the vasodilator ACh, has been reported in a variety of animal models of hypertension (Angus, 1996). This has been demonstrated in both the aorta, and mesenteric arteries of the SHR, (Luscher & Vanhoutte, 1986a; Carvalho *et al.*, 1987; Watt & Thurston, 1989; Fu-Xiang, *et al.*, 1992), and in goldblatt models of hypertension (Lockette, *et al.*, 1986; Bell & Bohr, 1991; Dohi *et al.*, 1991; Bennett *et al.*, 1993). Relaxation to nitric oxide donor substances was normal suggesting that the ability of vascular smooth muscle to respond to nitric oxide was not impaired. Bioassay studies using an endothelium denuded recipient artery showed that acetylcholine stimulated release of both NO and prostacyclin from the hypertensive aorta was normal (Luscher *et al.*, 1986b). These findings are at variance with reports of impaired nitric oxide production in both human hypertension and the Dahl salt-sensitive hypertensive rat (Vanhoutte & Boulanger, 1995). A large number of studies have since confirmed a normal, or enhanced activity of the nitric oxide production pathway in the SHR (Tschudi *et al.*, 1991; Sawada *et al.*, 1994; Cosentino & Luscher, 1995; Nava & Luscher, 1995; Hayakawa & Raij, 1997; Noll *et al.*, 1997;)

Later studies suggested that the reduced endothelium-dependent relaxation in the SHR did not reflect a reduction in EDRF, but the increased release of endothelium-dependent contracting factors. Thus, as high concentrations of ACh were shown to produce an endothelium-dependent contraction in the aorta (Luscher & Vanhoutte, 1986a;

Koga *et al.*, 1989), and mesenteric arteries of the SHR (Watt & Thurston, 1989; Luscher, 1990b). Normal responses were restored in the presence of a phospholipase A_2 inhibitor or the cyclo-oxygenase inhibitor, indomethacin, indicating the involvement of vasoconstrictor prostanoids. Thromboxane A_2 and PGH_2 were suggested as possible candidates because the constrictor response was abolished by a receptor antagonist of both cyclo-oxygenase products (Auch-Schwelk *et al.*, 1990; Ito *et al.*, 1991). Superoxide anions did not appear to be involved as free radical scavengers had little effect on the contractile response (Auch-Schwelk, *et al.*, 1989). However, some reports have suggested the involvement of superoxide radicals in endothelial dysfunction in the young SHR (Jameson *et al.*, 1993) and Stroke-prone SHR (Nava & Luscher, 1995; Kerr *et al.*, 1999).

Therefore, it would appear that the impaired endothelial relaxation may be due to the concomitant release of EDRF and contractile factors. However, more recent research suggests that this is not the complete story, and that reduced release of EDHF may explain the abnormal relaxation in various beds of the SHR (Van De Voorde *et al.*, 1992; Li *et al.*, 1994; Vanhoutte & Boulanger, 1995). The reduced release of EDHF results in the balance between contracting and relaxing factors being altered in hypertension, favouring vasoconstriction.

Cause or consequence of Hypertension

The majority of studies indicate that endothelial dysfunction occurs as a consequence of the altered haemodynamics in hypertension. Several reports have shown that the degree of endothelial impairment to ACh correlates positively with the level of blood pressure (Luscher, 1990b; Iwama *et al.*, 1992; Luscher, 1993a), and that reduced relaxation in the SHR becomes progressively impaired as the blood pressure rises (Rizzoni, *et al.*, 1994). In addition, it is commonly observed that improved endothelial function with antihypertensive therapy in animals is related to the reduction in blood pressure (Lockette,

et al., 1986; Luscher, 1990b; Van Riper & Bevan, 1992). More recently, perfusion studies demonstrated that the impairment in mesenteric arteries was only present during intraluminal acetylcholine application and not extraluminal application. Thus, suggesting that prolonged exposure of the endothelium to high blood pressure is an important factor in endothelial dysfunction (Dohi *et al.*, 1994). Protection of a vascular bed, and therefore the endothelium from high pressure, has also been shown to preserve function. This was demonstrated first in the rabbit aortic coarctation model of hypertension where endothelial-dependent relaxation was preserved in areas distal to the aortic stenosis (Miller *et al.*, 1987). Ageing causes similar abnormalities to those found in hypertension, leading to the suggestion that the impairment observed in hypertension is due to premature ageing of arteries. Ageing has been shown to be associated with a reduced relaxation to ACh, increased production of endothelial-derived contracting factors and reduced EDHF production (Fujii *et al.*, 1993; Mantelli, *et al.*, 1995).

1.9. ANTIHYPERTENSIVE THERAPY

Major breakthroughs in pharmacological therapy occurred with the discovery of rauwolfia, hydralazine, autonomic blocking agents, and chlorthiazide in the 1950's. Six main classes of drugs now are widely used in the worldwide treatment of hypertension. These are diuretics, β-blockers, calcium antagonists, ACE inhibitors, Angiotensin II receptor antagonists and α adrenergic blockers. Randomised placebo control studies with antihypertensive therapy show favourable effects on morbidity and mortality. Many drugs used in the treatment of hypertension have mechanisms of action involving the inhibition of the processes underlying the increase in intracellular calcium, or inhibition of the responses to the increased calcium levels.

The past two decades have witnessed the recognition that structural and functional abnormalities play an important role in the pathogenesis of hypertension. These may contribute to both the increased peripheral resistance, and vascular damage observed as a consequence of disease. Therefore, normalisation of cardiovascular structure and function, as well as blood pressure reduction is an important objective in the treatment of hypertension.

1.9.1 Effects of Antihypertensive Treatment on Structure

Early investigations involving haemodynamic measurements in the SHR reported a reduction in blood pressure with both beta blockade, and a cocktail of guanethidine and hydralazine. However, a similar reduction in vascular structure, as a result of these treatments, was not observed (Weiss & Lundgren, 1978). Subsequently, it was suggested that the response may depend on the drug type used, and the severity and duration of hypertension (Owens, 1985). It was also generally observed that early treatment was more effective in the prevention of structural alterations (Lee, *et al.*, 1991b; Gohlke *et al.*, 1993a). In the SHR early long-term ACE inhibition prevented the development of structural alteration (Lee, *et al.*, 1991b), whereas vasodilators, calcium antagonists and β-blockers, were found to be less effective (Jespersen, *et al.*, 1985; Owens, 1985; Struyker-Boudier *et al.*, 1990). The beneficial effects of ACE inhibitors may be due to their greater antihypertensive effects, or alternatively to a specific drug effect. These may include effects related to the reduction in AII, or alternatively to the potentiation of bradykinin (Heagerty *et al.*, 1993b; Lee, 1983; Hackenthal & Nobiling, 1994). The involvement of specific drug effects has been suggested both by the observation of a persistent effect on structure following treatment withdrawal (Unger, *et al.*, 1989; Thybo, *et al.*, 1994), and also by a reduction in structure following treatment with sub-antihypertensive doses of these drugs (Castellano *et al.*, 1995). Reversal of established cardiovascular structural

alterations has been demonstrated after antihypertensive treatment with amlodipine, and enalapril in the SHR model of hypertension (Sharifi *et al.*, 1998). It has been recently reported that blockade of the renin-angiotensin system by AT_1 receptor antagonists have similar effects on structure to those reported with ACE inhibition (Levens, *et al.*, 1992; Rizzoni *et al.*, 1998).

Normalisation of structural vascular change in human hypertension has proved more difficult to achieve (Aalkjaer, *et al.*, 1989; Struyker-Boudier, *et al.*, 1990). Studies of subcutaneous resistance arteries from essential hypertensive patients treated for one year showed a regression in the media to lumen ratio, but complete normalisation was not observed (Heagerty, *et al.*, 1988). Similarly, a three-year study, using lisinopril, also reported incomplete normalisation (Rizzoni *et al.*, 1997). Although, ACE inhibitors were reported to have greater effects on structure than other agents, with β-blockers having little effect, or no effect (Thybo *et al.*, 1995; Mulvany, 1996). In contrast, more recent reports suggest that ACE inhibitors can normalise the abnormal structure of human resistance arteries, under conditions where the blood pressure is properly controlled (Schiffrin, 1995; Schiffrin, 1996; Mulvany, 1998)

1.9.2 Effects of Antihypertensive Treatment on Endothelial Function

Early studies in Dahl rats with salt induced hypertension reported that a combination of reserpine, hydrochlorthiazide and hydralazine normalised both the blood pressure and relaxation to ACh, adenosine diphosphate and thrombin (Luscher *et al.*, 1987). Since that time, other studies have reported an improvement, or normalisation of endothelial function to ACh, with therapy, in a variety of experimental models (Luscher, 1994b; Kahonen *et al.*, 1996). Although, not all drugs appear to normalise impaired endothelial function, despite causing a reduction in blood pressure. The non-specific vasodilator, hydralazine, and the calcium antagonist, amlodipine have been shown to have

little effect in some studies (Clozel *et al.*, 1990; Bennett, *et al.*, 1996), whereas others have reported an improvement in endothelial function (Shultz & Raij, 1989; Sharifi, *et al.*, 1998). ACE-inhibitor drugs are reported to completely normalise relaxation to acetylcholine, in various arterial beds (Clozel, 1991; Rubanyi *et al.*, 1993b; Dohi, *et al.*, 1994), leading to the suggestion that antihypertensive agents may have direct effects on the endothelium, thus augmenting relaxation to ACh (Clozel, 1991). In addition, AT_1 receptor antagonists are reported to result in amelioration of endothelial dysfunction, similar to the effect observed with ACE inhibitors (Rodrigo *et al.*, 1997).

Improvement in endothelial function is less well documented in human hypertension and failure may be due to the long-duration of the disease (Panza *et al.*, 1993b). Lyons demonstrated that enalapril and amlodipine normalised the response to L-NMMA in the forearm of newly diagnosed patients, reversal appeared mainly due to reversal of blood pressure rather than to direct drug effects (Lyons, 1997). In addition, Schriffin demonstrated some improvement in endothelial function in human hypertensive subcutaneous resistance arteries after treatment with cilazapril (Schiffrin, 1995; Schiffrin, 1996). It has been suggested that early antihypertensive therapy may reverse endothelial dysfunction, but that once established endothelial abnormalities may become irreversible (Luscher, 1994b). This may explain why treatment is effective in normalising function in animal models, as hypertension is of shorter duration and treatment is usually initiated in the early stages of disease. However, in human hypertension treatment is only initiated once hypertension is established, when abnormalities may already be irreversible.

AIMS OF THE EXPERIMENTAL STUDIES

The studies described in this thesis were designed to examine the role of angiotensin II on the structural alterations of small resistance arteries in hypertension. Previous studies suggest AII may play an important role in the development of vascular hypertrophy. However, they were not able to dissociate direct effects of AII from effects due to a concomitant rise in blood pressure. Experiments were designed to allow blockade of angiotensin II, whilst maintaining a raised blood pressure, in two distinct animal models of hypertension.

The first section of the thesis describes experiments in the spontaneously hypertensive rat (SHR), a genetic model of hypertension. This model is susceptible to salt loading, and therefore allows the study of ACE inhibition, whilst maintaining a high blood pressure by an increase in dietary salt intake. In addition, studies were carried out in this model to investigate the nature of the impaired endothelial function, resulting from hypertensive disease

The second section of the thesis describes experiments in the Goldblatt 1K1C renovascular model of hypertension. It has been shown that administration of ACE inhibitors or AII receptor antagonists are ineffective in reducing blood pressure in this model. This permitted studies of the effect of blockade of the renin-angiotensin system, in the absence of a blood pressure reduction

CHAPTER TWO

MATERIALS AND METHODS

2.1 THE MULVANY-HALPERN MYOGRAPH.

The development of this technique has been beneficial in hypertension research as it allows *in vitro* investigation of small resistance arteries. These arteries are important as they contribute greatly to the increased peripheral resistance seen in hypertension.

The Mulvany-Halpern myograph was first described in 1976 (Mulvany & Halpern, 1976). The method was developed from a technique used by Bevan and Osher in 1972 (Bevan & Osher, 1972) to investigate arteries with internal diameters greater than 100μm. The technique involved ring preparation of arteries mounted on two fine wires, which could be clamped at each end to allow isometric responses measurement. Further modifications of the myograph have been described and have contributed to the development of the current Mulvany-Halpern myograph, widely used in vascular laboratories today. The myograph used in the current studies is the double myograph, adapted by John Pedersen (Myograph trading Ltd) in consultation with the original authors. This permits the study of two small arteries to be carried out simultaneously. The myograph allows the determination of many small artery characteristics including; determination of normalized internal lumen diameter, measurements of artery wall parameters and measurements of isometric responses to agonists. The technique is most suited to arteries with internal diameters of 100-400 μm.

The myograph used in these studies consisted of a 12ml capacity, stainless steel, organ bath chamber containing two separate pairs of mounting heads. One mounting head of each pair was attached to a micrometer, to enable measurement of the distance between the mounting heads, and adjustment of the passive tension. The opposite mounting head was attached to a pre-calibrated force transducer in the body of the myograph. Changes in

tension, detected by the force transducer, were amplified and recorded on a two-channel, flatbed recorder. The organ bath was heated to 37°C by means of water from a heidolph, circulated through heating blocks positioned on either side of the organ bath. A later model was also used in which the water heating system was replaced by a heater in the myograph block. Ports into the myograph chamber allowed oxygenation and addition of solutions during experimentation. Solutions were removed from the chamber by a vacuum system consisting of a vacuum pump and solution trap. (Figure 2.1)

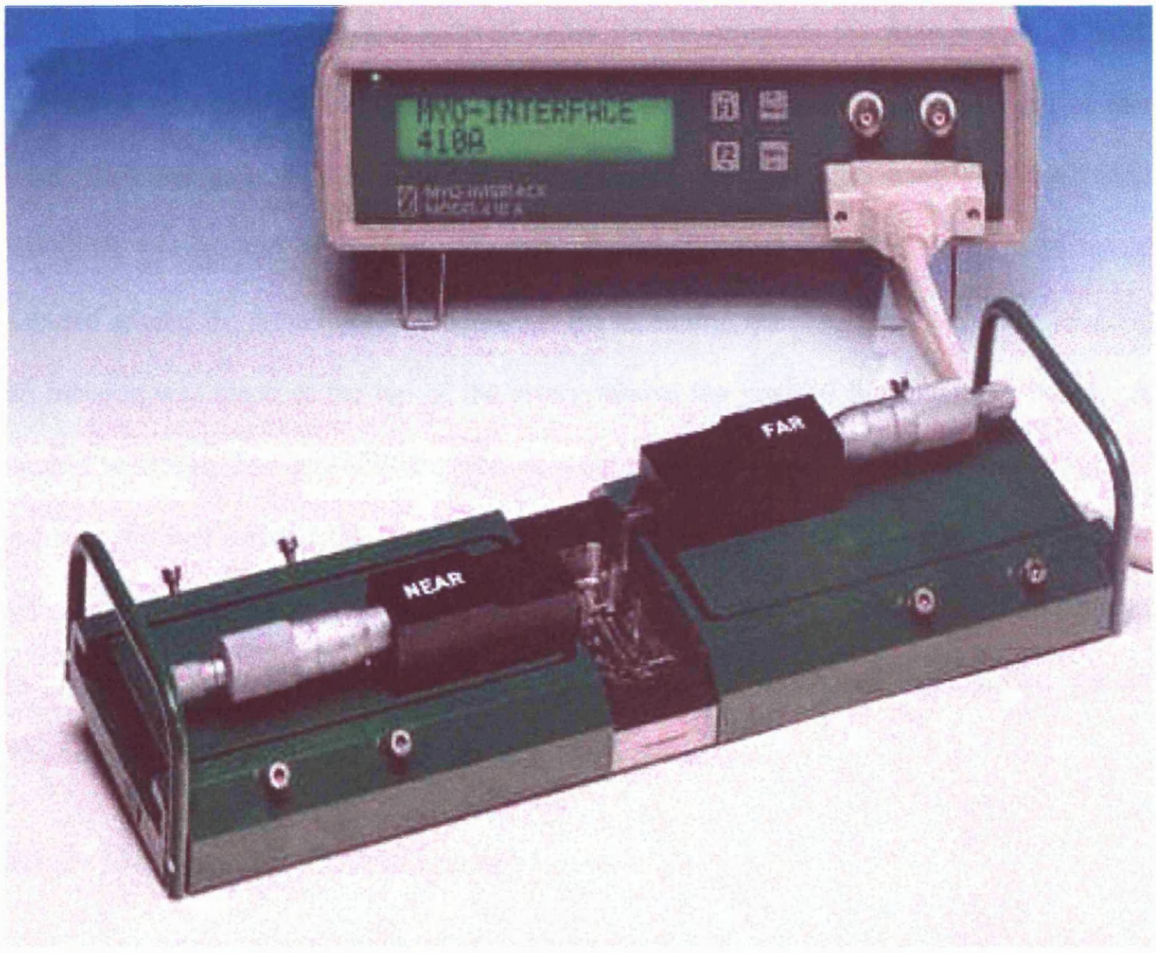


Figure 2.1. Mulvany-Halpern myograph model 410A supplied by Myograph Trading Ltd, Aarhus, Denmark

2.1.1 Technique for mounting arterial segments

Resistance arteries were threaded onto two 40μm stainless steel wires in the myograph. One wire was connected to the mounting head attached to the force transducer and the second to that of the micrometer. The wires were secured onto the mounting heads by two screws on the mounting head. The technique was carried out using a binocular microscope.

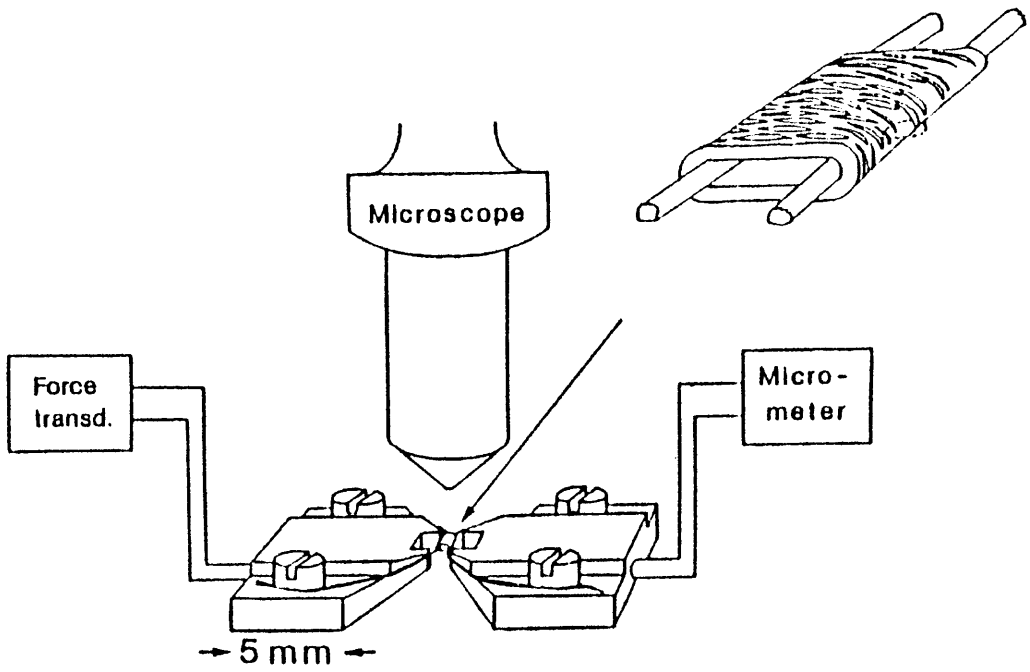
Initially a length of stainless steel wire was trapped between the jaws of the two mounting heads. The wire was secured under the far screw on the right mounting head. The artery was carefully threaded, using watchmakers forceps, along the length of this wire. This was carried out with the mounting heads apart, until the artery was positioned above the bottom end of the jaw. Once in position the free end of the wire was tightly secured around the remaining near screw on the mounting head. Using trabecular scissors, an incision was made in the top of the artery, above the jaws of the mounting heads. A second wire was then carefully threaded along the arterial lumen, using the first wire as a guide. The free ends of the second wire were secured at both ends by the near and far screws on the remaining mounting head. The wires were then levelled so that both sides of the artery were in a horizontal plane. The mounting procedure was carried out in Physiological saline solution (PSS) at room temperature.

2.1.2 Morphology measurements

The Mulvany myograph permits measurements of dimensions of arterial segments *in vitro* by light microscopy. This was made possible by glass windows situated in the base of the organ chamber, directly below the mounting heads. The myograph was carefully transferred onto the stage of a light microscope, fitted with a water immersion lens and calibrated filar micrometer eyepiece, for measurements of media thickness and lumen diameter (Figure 2.2). A disadvantage of this technique is that these measurements

are made before the vessels are set at their normalised diameter. Direct measurements are not possible using this technique as once normalised the force of stretching the artery over the wires causes the wall to be thinned at these points. To overcome this problem the arterial measurements were carried out under minimal tension (0.2mN). This was sufficient to ensure that the wall was flat between the wires whilst avoiding thinning of the wall.

Figure 2.2. Morphology measurements using the Mulvany-Halpern microscope



Under minimal tension measurements of lumen, media and wire thickness were made, the latter two on both sides of the arterial segment. These measurements were repeated at three individual points along the length of the artery, giving a total of six

readings of media (m) and wire thickness (d), and three readings of lumen diameter (f). The average values of these readings were used to calculate arterial dimensions, when the vessel is set to its normalised diameter. Arterial segment length (s) was also measured using the filar eyepiece in an ordinary pre-calibrated stereomicroscope.

Arterial dimensions were calculated using the following equations:-

Internal circumference (μm)

$IC = (2+\pi)d + 2f$

Lumen diameter (μm)

$l_1 = IC/\pi$

Media cross sectional area (μm^2)

$MCSA = s(\pi.m^2 + l_1.m)$

Arterial segments were normalised using the procedure described below. This allowed determination of the media thickness corresponding to the normalised lumen diameter. This estimation of medial thickness is dependent on the assumption that segment length is unaltered when the artery is stretched during the normalisation procedure. If this assumption is correct then the cross sectional area of media will also remain constant. (Patel & Fry, 1969)

2.1.3 Determination of normalised lumen diameter

The development of this technique enabled investigations of *in vitro* arteries to be carried out under standardised conditions. This is important firstly because previous experiments in arterial segments were carried out at a fixed tension, irrespective of arterial size, leading to a great variability in experimental results. Smaller arteries at a fixed force would therefore be subjected to a much greater wall tension than large arteries under the

same conditions. Secondly, studies in large arteries have shown that agonist sensitivity and the maximum response are dependent on the passive wall tension. Finally, the active tension-internal circumference relationship must be considered. Active force generation by the artery increases with stretch i.e. increased internal circumference. This relationship holds until an optimal internal circumference is reached. If the artery is stretched beyond this optimal point, active tension generation is reduced despite an increased passive tension. The majority of studies have been carried out under conditions where the resting tension is set to achieve the maximum active tension. Most studies involve *in vitro* measurement under isometric conditions, the lumen diameter being constant whilst changes in wall tension are recorded.

Many different techniques for standardising conditions to achieve maximal tension have carried out. A basic technique involved setting the stretch until a maximum response to the agonist applied was seen. Mulvany and Halpern described a technique by which the point of maximum active tension could be calculated from the passive diameter-tension relationship of the artery. They reported that maximum active tension development, in the mesenteric artery, occurred at 0.8-0.9 times the internal circumference of the artery, when relaxed and subjected to an intraluminal pressure of 100mmHg (Mulvany & Halpern, 1977).

Normalisation in the myograph was carried out according to the procedure described by Mulvany and Halpern (Mulvany & Halpern, 1977). Initially the micrometer was moved until the two wires just touched, shown by a negative displacement on the chart recorder. The jaws were moved slightly apart resulting in no tension on the artery. In this position a micrometer reading was taken and the position of the pen on the chart recorder noted. Movement of the micrometer, at two-minute intervals then progressively stretched the artery. Readings of micrometer setting and displacement of the pen on the chart

recorder were recorded at each time interval. Data was entered, along with the previously recorded segment length of the artery, into a computer programmed to determine the length tension characteristics of small arteries. Force generated by the artery was calculated from the following:.

Force = (displacement reading - zero tension reading). Force transducer calibration. Calculations of internal circumference (μm), described previously and wall tension (mN/mm), i.e. force measured divided by the arterial length, were performed. Arterial length was taken as twice that of the measured segment length, as both and upper and lower artery walls were present. Effective pressure was derived using the Laplace relationship, which states that in a hollow tube wall tension (T) is a product of pressure (P) and radius (r). This law can be applied to blood vessels, which are essentially distensible cylinders. Therefore, for any given internal circumference and wall tension:

$$\text{Transmural P (kPa)} = T / (\text{internal circumference}/(2.\pi))$$

The stretching procedure was stopped when the calculated effective pressure was greater than 13.3KPa (100mmHg). An exponential curve was fitted to the internal circumference/pressure data, by the computer programme, and the internal circumference (IC) corresponding to a pressure of 100mmHg determined. A value for 0.9 times the value of the internal circumference ($IC_{0.9}$) was calculated which corresponded to the maximal active tension of the artery. A micrometer reading was also generated allowing adjustment of the myograph to this normalised setting. A normalised lumen diameter value was calculated from the above results using the following

$$L_{0.9} = IC_{0.9} / \pi$$

Media thickness corresponding to the normalised lumen diameter was then calculated, based on the assumptions previously discussed.

2.2 ANIMALS

2.2.1 Spontaneously Hypertensive rat

Female spontaneously hypertensive rats (SHR) and Wistar-Kyoto normotensive controls were obtained from the Biomedical Services Unit inbred colony at the University of Leicester. Animals were given standard laboratory chow and water *ad libitum*, and were maintained on a 12-hour light/dark cycle. Room temperature and humidity were maintained at suitable levels throughout the studies.

2.2.2 Goldblatt One-Kidney, One Clip (1K1C) Hypertension

Goldblatt 1K1C hypertension was induced in female Wistar rats, weighing 170-190g, also obtained from the Biomedical Services Unit at the University of Leicester. Hypertension was induced by nephrectomy of the right kidney, followed by the application of a constricting silver clip to the left renal artery. The left renal artery was clipped in this procedure, as it is more accessible. Control rats were prepared using the same general procedure, except that a non-constricting silver clip was placed between the renal artery and vein. All surgical operations were performed under halothane, oxygen and nitrous oxide anaesthesia, using sterile techniques.

Constricting clips were made from annealed silver ribbon, approximately 2.30mm in width and 0.15mm thickness. Bending a small piece of ribbon around a set width of the feeler gauge allowed individual clips to be made. The ribbon was bent so that the clip had unequal arm lengths, this was done to enable easier placement of the clip around the renal artery. A clip width of 0.2mm was generally used in this study. However, a smaller clip size was occasionally used in lower weight animals. The size of clip was decided during surgery by testing the movement of the clip around the artery. Too much movement

suggested that the clip was too loose to induce hypertension. Clips were sterilised along with the other equipment used in the surgical technique.

A Wistar of the appropriate size was placed into a Perspex box filled with a mixture of halothane, oxygen, and nitrous oxide. Oxygen and nitrous oxide were introduced at a ratio of 2:1 respectively. Once unconscious the animal was transferred onto a heated pad on an operating table, anaesthesia was maintained via a facemask. The animals breathing rate was monitored throughout and anaesthetic levels were adjusted as necessary. Each side of the rat above the kidney was shaved. An incision was made through the skin and muscle layers of the right abdomen, just below the border of the rib cage. The position of the kidney was determined by the presence of a layer of peri-renal fat. The kidney was exposed and lifted clear of the body cavity. The exposed artery, vein and ureter were carefully cleaned with a cotton bud. These were tightly tied, using nurolon thread, below the kidney, which was then removed. After nephrectomy the fat was replaced and the wound sutured. Prolene and mersilk sutures were for the muscle layer and skin respectively.

The procedure was repeated on the left abdominal side, up to the point of exposure of the renal artery and vein. At this point the renal artery and vein were separated using a fine pair of surgical forceps. A constricting silver clip was placed around the exposed renal artery in a position such that the open ends were facing away from the spleen. The kidney and fat were replaced and wound sutured as previously described. The animals were allowed to recover from the anaesthetic after this final part of the procedure. Control rats underwent the same procedure with a non-constricting clip.

Blood pressure was determined in these animals at weekly intervals using the technique described below. Animals were killed immediately if the pressure was not significantly greater than the controls 3-4 weeks after surgery.

2.3 ANTIHYPERTENSIVE THERAPY

Antihypertensive drugs were administered to the animals as follows: Perindopril and Losartan were dissolved in drinking water to which animals had free access. Hydralazine was administered by oral gavage, because administration by drinking water reduced the amount of water consumed each day. Drug doses in solution were adjusted according to body weight, and water intake, which were monitored regularly. Hydralazine dosage was calculated according to individual animal body weights. All solutions were freshly supplied on a weekly basis. Dosages used in these studies were perindopril $1mgday^{-1}kg^{-1}$, losartan $10mgday^{-1}kg^{-1}$ and hydralazine $50mgday^{-1}kg^{-1}$.

2.4 INDIRECT MEASUREMENT OF SYSTOLIC BLOOD PRESSURE

Indirect blood pressure was measured using the light plethysmographic technique described by Swales and Tange in 1970. The equipment consists of a photoelectric sensor used to detect light changes in light translucency in the rat tail. The signal from this sensor can be amplified, and displayed on a chart recorder.

Rats were placed in pre-warmed Perspex restraint tubes in a heating cabinet set to 30°C. A suitable period of time was allowed for the rat to become calm. Once achieved an inflatable cuff was placed around the tail and secured onto the Perspex tube. The cuff contained a built in photoelectric sensor and opposing light source, positioned immediately behind the cuff. The cuff was also connected to a mercury sphygmomanometer to allow inflation. An amplifier attached to a double channel chart recorder received the output from the photoelectric sensor. One channel, received details of the rats pulse wave pattern from the photoelectric sensor, the other, previously calibrated using a mercury sphygmomanometer, recorded the pressure inside the cuff. The systolic blood pressure of the animal was determined in a fully relaxed animal by inflation of the cuff until the pulse

wave pattern was undetectable. Inflation of the cuff was stopped just above this point to avoid over-inflation leading to damage of the rat's tail. The cuff was allowed to slowly deflate until the pulse wave returned. The pressure at which this occurred could be read from the pressure channel on the chart recorder, and also determined by eye from the sphygmomanometer. The latter measurement was used to confirm the reading obtained from the chart recorder. Systolic blood pressure measurements were repeated three times to allow determination of an average reading.

2.5 DISSECTION TECHNIQUES

All rats were killed by stunning followed by cervical dislocation. A midline laparotomy was used to exteriorise the mesenteric bed. This was cut free, and was immediately placed in cold (4°C) PSS for later dissection of the mesenteric arcade. The chest cavity was opened and the heart removed. This was gently blotted dry, cleaned of fat and connective tissue, and weighed. Both hind limbs were removed and were also placed in cold (4°C) PSS for later dissection of femoral arteries. After removal of the femoral arteries both hindlimbs were cleaned of muscular tissue and the tibial length measured using a vernier calliper. The average tibial length was used to determine the heart weight to tibial length ratio. This ratio was used to assess the degree of cardiac hypertrophy as previously described (Yin *et al.*, 1982).

2.5.1 Mesenteric Artery Dissection

The mesenteric arcade was placed into a Petri-dissecting dish in cold PSS. The dissecting dish contained a layer of sylgaard in the base, enabling fixing pins to be secured. Fixing pins were used to display the feeding vasculature of the mesenteric arcade, and a portion of the artery 8-10cm from the pylorus was identified. Vessels branching directly

from the superior mesenteric were designated as first order vessels. Third order mesenteric arteries were selected from the site according to the branching pattern. It was important to identify arteries from the same site in each animal so that accurate comparisons could be made. Trabecular scissors and watchmakers forceps were used to clean the connective tissue and associated vein away from the artery. The cleaned artery was then cut free of the mesenteric arcade.

2.5.2 Femoral Artery Dissection

The hind limb was pinned in the dissecting dish. Skeletal muscle layers were dissected using large scissors to display the vasculature. An artery was identified branching from the main femoral artery down into the hindlimb., designated as first order. This then branched into the tissues, towards a fat pad in the hindlimb. Third order arteries running into the fat pad were cleaned and were cut free from the vasculature. This identification allowed the same arterial branch to be used in each experiment.

2.6 MYOGRAPH STUDIES

Femoral and mesenteric arteries were dissected from each animal and were mounted in the Mulvany myograph as described in section 2.1.1. Arterial segments were heated to 37°C and gassed with 5%CO_2 / 95%O_2. An equilibration period of thirty minutes was allowed before arterial dimensions were determined, as described in section 2.1.2. A second equilibration period was allowed after which the artery was normalised by the procedure described in section 2.1.3.

After a further 60 minutes arterial reactivity was assessed using high potassium PSS solution (124mM). Activation was carried out by the addition of 12ml high potassium PSS to the bath. The resulting contraction was maintained for at least two minutes or until

a stable plateau was reached. The artery was rinsed at least three times, using normal PSS, allow relaxation of the artery to a baseline condition. This potassium activation was repeated after two to three minutes at baseline conditions. A final activation was carried out using high potassium PSS containing 10μM noradrenaline. Arteries, which did not respond to activation by high potassium PSS, were excluded from the study. Arteries were left to recover for 10 minutes before dose response studies were performed.

2.6.1 Vascular contractility

A cumulative dose response curve was performed to noradrenaline (NA) to assess vascular contractility. This was achieved by addition of increasing doses of noradrenaline, to achieve a range of concentrations from 0.01μM to 300μM. Each dose was added after the previous dose response had reached a plateau, approximately two minutes. Responses were assessed in the presence of 1μM cocaine to prevent neuronal re-uptake of noradrenaline. Cocaine was added to the bath twenty minutes prior to the addition of noradrenaline. A dose response curve in the absence of cocaine was also carried out in some studies. Arteries were rinsed three times after every dose response curve and were allowed a ten-minute recovery period.

2.6.2 Endothelial Function

Endothelial-dependent relaxation was assessed by observing the response to cumulative doses of acetylcholine (ACh) and bradykinin (BK). Dose relaxation response curves were performed after maximally contracting the arteries with noradrenaline. Cumulative doses were added to the bath once a stable contraction of the artery had been achieved. Doses of acetylcholine and bradykinin were added to produce a range of concentrations from 0.0001μM to 100μM and 0.001μM to 10μM, respectively. Doses were added to the bath and the plateau relaxation response observed. The direct relaxation

response of the vascular smooth muscle was determined using a nitric oxide donor sodium nitroprusside (SNP). Doses were added to produce a range of concentrations from 0.001μM to 100μM.

2.6.3 Drugs and solutions

During initial dissection and mounting the arteries were maintained in cold (4°C) physiological saline solution. Solutions added to the chamber were maintained at 37°C in a water bath and were constantly gassed with $5\%CO_2$ / $95\%O_2$. Composition, of solutions is described in appendix B. Chemicals used in this study were dissolved in distilled water, unless otherwise stated. A list of suppliers of chemicals, drugs and equipment used in this study is contained in appendix C. Solutions were freshly prepared each day.

2.7 EXPRESSION OF RESULTS

Results are expressed as means ± SEM. Arterial contractility was expressed as active tension (mN/mm). This was calculated by division of contraction force (mN) by twice the arterial segment length. Force (mN) was calculated as described previously by multiplying pen displacement by force transducer calibration. Results were also expressed as active media stress (mN/mm^2) i.e. forces per unit media area. Relaxation responses were expressed as a percentage decline of the maximal contractile response, and the maximal arterial relaxation (E max) reported. Sensitivity was expressed in terms of the EC_{50} i.e. the concentration required to produce a half-maximal contraction or relaxation. EC_{50} values were calculated using Dose-Effect Analysis with Microcomputers, published and distributed by Biosoft.

CHAPTER THREE

Angiotensin II and Vascular Hypertrophy in Genetic Hypertension.

3.1 INTRODUCTION

Hypertension is associated with thickening of small blood vessels and left ventricular hypertrophy in both essential hypertension in humans (Short, 1966; Cook & Yates, 1972; Mulvany, 1987; Sihm, *et al.*, 1995), and in genetic hypertension in rats (Folkow, *et al.*, 1973). Recent work has implicated growth factors, particularly angiotensin II (AII), as having mitogenic effects on vascular smooth muscle cells. Therefore, it has been suggested that AII, either within the circulation, or at the local tissue level may play a pathophysiological role in hypertension.

The Spontaneously hypertensive rat is a widely used model of human hypertension, sharing many similarities to the disease in humans. These include; a genetic predisposition, aggravation of the condition by environmental influences on the disease such as stress, and increased dietary sodium, and a similar improvement in response to antihypertensive agents. In addition, these animals develop similar complications during the latter stages of hypertension, such as haemorrhaging, brain softening, myocardial lesions, and arteriolar nephrosclerosis. The frequency and severity of these complications generally relates to the degree of hypertension (Azizi *et al.*, 1994).

Historically, the spontaneously hypertensive rat (SHR) was derived by Okamoto and Aoki in 1963 by selecting offspring with a high systolic blood pressure (SBP) from a rat colony. These animals were then brother-sisters mated to produce an inbred strain. The colony was fully established as an inbred strain by 1969, at the Department of Pathology in the University of Kyoto. After 20 generations of this type of inbreeding, the offspring should be homozygous, although hypertension appeared to be fixed after only a few generations. Animals from the original colony were distributed world-wide, at the F13 generation, prior to full inbreeding, and used to establish inbred strains, separate from the Japanese strain. Consequently, there is genetic variability between SHRs obtained from

different colonies across the world. The normotensive control WKY strain was derived from descendants of the original rat colony, with relatively normal systolic blood pressures, some years after the SHR colony was developed.

The SHR model of hypertension displays normal levels of the components of the renin-angiotensin system, during the established phase of hypertension. Therefore, hypertension has been considered independent of the renin-angiotensin system, despite some reports of raised levels in early hypertension (Harrap, 1991). Although considered a renin-independent model of hypertension, perindopril has been widely reported to have beneficial effects. It is reported to both reduce systolic blood pressure in the SHR, and to normalise cardiac, and vascular structure (Short, 1966; Cook & Yates, 1972; Mulvany, 1987; Sihm, *et al.*, 1995). The dosage used in this study has previously been shown to have antihypertensive effects in both the SHR, and 2K1C models of hypertension inhibitors (Christensen, *et al.*, 1989; Bennett, *et al.*, 1996; Bennett & Thurston, 1996). ACE inhibitors are reported to have secondary effects, other than blockade of the renin-angiotensin system, which may contribute to their antihypertensive action. A major secondary effect, which may influence vascular dilatation, is potentiation of bradykinin. ACE acts by degrading BK, thus system blockade will result in increased BK levels and may cause increased vasodilatation via nitric oxide release (Mombouli *et al.*, 1991; Feletou, *et al.*, 1992; Mombouli *et al.*, 1992). ACE inhibitors are also reported to have secondary effects on the sympathetic nervous system (Vanhoutte *et al.*, 1989b).

Moreover, the blood pressure of the SHR is highly sensitive to an increase in dietary sodium intake, resulting in an elevated blood pressure. The rise in blood pressure is due to water, and consequently an expansion of the intra-vascular volume, because of an abnormal excretion of sodium and water. It is also possible that increased sympathetic nervous system activity may be involved in the salt sensitivity of the SHR (Nickenig *et al.*,

1998; Wyss *et al.*, 1994). Previous studies have shown that the antihypertensive effects of ACE inhibitors can be prevented by the co-administration of saline in this model (Wyss, *et al.*, 1994). Therefore, administration of saline, in combination with ACE inhibitor treatment will allow us to investigate the effects of AII blockade on arterial structure and function, without the confounding influence of a reduction in blood pressure. Thus, allowing us to dissociate the direct effects of AII on vascular growth, from those related to alterations in systolic blood pressure.

3.2 METHODS

The SHR and WKY rats used in this study were obtained from the Biomedical Services Unit inbred colony at the University of Leicester. They were randomly allocated at three weeks of age to one of six groups, no treatment, treatment with perindopril ($1mgday^{-1}kg^{-1}$), or treatment with perindopril ($1mgday^{-1}kg^{-1}$) in a 1% sodium chloride (NaCl) solution. Perindopril and NaCl were dissolved in drinking water, to which rats had free access. The drug concentrations in solution were adjusted according to the regularly monitored body weight and water intake, to maintain a constant dose per 100g body weight. Drugs were administered at three weeks of age and were continued for the duration of the study. Systolic blood pressure (SBP) was recorded at weekly intervals in all groups, using the photoplethysmograph technique, in warmed (30°C), conscious restrained animals.

Rats were killed by stunning followed by cervical dislocation at 16 weeks. Heart weight and tibial lengths were recorded as indicators of cardiac hypertrophy. At 16 weeks, measurements were recorded only in a representative sample, because remaining hearts were frozen in liquid nitrogen for use in other studies. Femoral arteries and third order mesenteric arteries, approximately 200µm internal diameter, were dissected and mounted in the Mulvany myograph, as previously described. After making morphological observations, arteries were normalised and left to equilibrate for one hour. Following the equilibration period, arteries were subjected to a standard activation procedure, after which cumulative contraction response curves to noradrenaline were performed in the presence of 10^{-6}M cocaine. This was followed by a series of cumulative dose-relaxation response curves. Arteries were maximally contracted with noradrenaline, and when the contraction reached a plateau, relaxation response curves were performed to acetylcholine (10^{-10} to 10^{-4}mol/l) and bradykinin (10^{-11} to 10^{-5}mol/l). Finally, the relaxation response to the

endothelium-independent vasodilator, sodium nitroprusside (10^{-9} to 10^{-4}mol/l) was observed.

A second study was carried out using rats killed at 24 weeks, to determine the longer-term effects of hypertension on vascular structure and endothelial function. The initial data showed structural alterations in the SHR arteries, although there was no reduction in internal lumen diameter of the SHR mesenteric arteries, as previously reported (Mulvany, *et al.*, 1978). In addition, the initial data failed to show marked endothelial dysfunction in the mesenteric arteries, as shown in previous studies (Watt & Thurston, 1989). Therefore, the second study was performed to determine the development of more marked structural change and endothelial dysfunction in the untreated SHRs at 24 weeks. Also, it would provide an opportunity to establish if longer-term treatment were more effective in the prevention of structural alterations in hypertension, because perindopril treatment was only partially effective, in the mesenteric arteries, at 16 weeks.

Additional myograph studies were performed to assess the role of EDRF, EDHF and endothelium-derived vasoconstrictor substances in the relaxation response of the mesenteric and femoral arteries. Cumulative dose-relaxation response curves to acetylcholine and bradykinin, relaxation curves were performed in mesenteric and femoral arteries from 24 week SHR and WKY, following a thirty minute pre-incubation period with 10^{-5}M indomethacin, dissolved in absolute ethanol. In addition, relaxation responses to both acetylcholine and bradykinin were repeated after incubation with the nitric oxide synthesis inhibitor, L-NOARG (10^{-4}M for one hour), or with the PGH_2/TxA_2 receptor antagonist SQ29548 (10^{-5}M for thirty minutes).

3.3 STATISTICAL ANALYSIS

Differences in the physical characteristics, arterial morphology, and dose-responses between groups were determined using one way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. A non-parametric ANOVA was performed using the Dunn's correction, a variation of the Bonferroni correction, in situations where the standard deviations of the populations were unequal. Comparisons of parameters between 16- and 24-week old animals were determined using the Student's unpaired t-test. $P<0.05$ was considered significant. Numbers in brackets denote a reduced sample size.

3.4 SUMMARY OF 16 WEEK RESULTS

3.4.1 Physical Characteristics

The systolic blood pressure (SBP) was significantly raised in the untreated SHRs. There was a gradual rise in SBP until approximately 12 to 14 weeks, after which the blood pressure maintained a plateau. Treatment with perindopril prevented the development of hypertension in the SHRs, and reduced the SBP of the normotensive rats. Hypertension developed in the perindopril and saline treated SHRs, with the saline treatment overcoming the antihypertensive effects of perindopril, despite a slight delay in the rise in SBP (Figure 3.1 & Table 3.1)

The body weight was lower in all the SHRs studied. Perindopril treatment reduced the body weight of the WKY rats, but had little effect on the body weight of the SHRs (Table 3.1).

There was only slight cardiac hypertrophy in the untreated SHRs in this study. Perindopril treatment reduced the cardiac structure of both the SHR, and WKY rats. This effect was not observed in the presence of 1% saline, suggesting that the reduction in cardiac structure may be related to the antihypertensive actions of perindopril (Table 3.1).

3.4.2 Resistance Artery Structure

The vascular structure was altered in both the mesenteric (Table 3.2), and femoral arteries (Table 3.3), from the untreated SHRs. Treatment with perindopril prevented the development of structural alterations in the SHR femoral arteries. Similarly, perindopril prevented the increase in mesenteric medial cross-sectional area. However, treatment did not completely prevent the increase in media thickness or media to lumen ratio in the SHR mesenteric artery. The combination treatment of perindopril with 1% saline failed to prevent the development of structural alterations, in both the mesenteric and femoral

arteries from the 16 week SHRs. At 16 weeks, there was a positive correlation between SBP and both mesenteric artery structure (Figures 3.2 - 3.4), and femoral artery structure (Figures 3.5-3.7).

3.4.3 Resistance Artery Contractility

The active tension generated to contractile agents was increased in the untreated SHR mesenteric arteries. Expression of the results as active media stress showed this to be a consequence of the structural alterations present, revealing a slight reduction in the smooth muscle contractility in the hypertensive mesenteric artery. Treatment with perindopril prevented the increase in tension in the SHR mesenteric artery, a likely consequence of the reduction in structure of the mesenteric artery, because there was little alteration in active media stress. Treatment with perindopril and 1% saline failed to prevent the increased contractile response in the mesenteric artery.

When expressed as active media stress, there was found a significant reduction in the contractile response of the SHR femoral resistance arteries at 16 weeks. This may suggest a reduction in the contractile ability of the smooth muscle cells in the SHR arteries at this age. Treatment with perindopril, and the combination of perindopril with 1% saline had little effect on the active media stress response of the SHR femoral arteries.

3.4.4 Resistance Artery Relaxation

There was heterogeneity in the response of the arteries to the endothelial-dependent relaxing agents' acetylcholine and bradykinin. The mesenteric arteries relaxed to both acetylcholine and bradykinin, whilst the femoral arteries displayed re-contraction with higher concentrations of acetylcholine, the maximum relaxation being observed at 10^{-7}M

ACh. Also, femoral arteries failed to relax in response to the increasing concentrations of bradykinin with, a slight contraction being observed at the high concentrations.

There was no significant alteration in the endothelium-dependent relaxation of arteries from the untreated SHR, although some re-contraction was observed at the highest doses of ACh, in the mesenteric artery. Perindopril treatment had no significant effect on the maximum relaxation, but did prevent the re-contraction response to high doses of ACh in the mesenteric artery. Similarly, 1% saline and perindopril treatment failed to alter the response of the SHR resistance arteries. There was no significant change in the endothelium-independent relaxation of the resistance arteries with the development of hypertension, suggesting the ability of the smooth muscle cells to respond to nitric oxide is unchanged

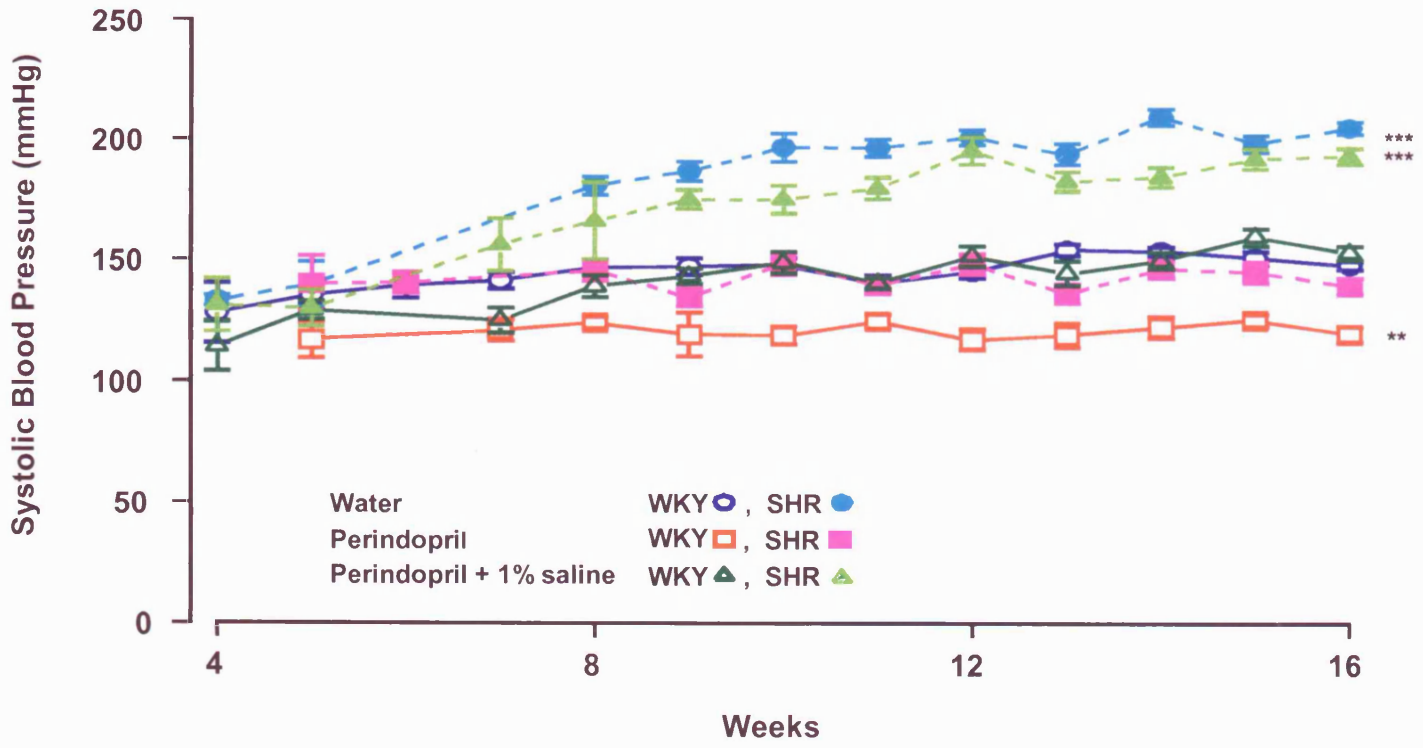


Figure 3.1. Systolic blood pressure (SBP) profile of untreated, perindopril treated, or 1% saline with perindopril treated 16 week old SHR and WKY rats.

** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

Table 3.1. Physical characteristics of untreated, perindopril treated, or perindopril with 1% saline treated 16 week SHR and WKY rats

Rats	Treatment	N	Systolic blood pressure (mmHg)	Body Weight (g)	Heart Weight(HW) (g)	HW / Weight (%)	HW / Tibia (g/mm)
WKY	Water	31	147 ± 2	234 ± 3	0.79 ± 0.01 (15)	0.353 ± 0.004 (15)	2.282 ± 0.039 (15)
SHR	Water	28	204 ± 3 ***	199 ± 3 ***	0.79 ± 0.02 (13)	0.396 ± 0.005 * (13)	2.349 ± 0.054 (13)
WKY	Perindopril **(P)**	25	119 ± 2 **	219 ± 2 **	0.65 ± 0.01 *** (15)	0.291 ± 0.001 ** (15)	1.890 ± 0.023 *** (15)
SHR	Perindopril **(P)**	23	139 ± 3 +++	191 ± 2 ***	0.62 ± 0.02 *** (14) +++	0.323 ± 0.008 +++ (14)	1.852 ± 0.048 *** (14) +++
WKY	1% NaCl + **P**	26	152 ± 3	231 ± 3	0.82 ± 0.24 (13)	0.350 ± 0.006 (13)	2.360 ± 0.063 (13)
SHR	1% NaCl + **P**	25	192 ± 3 ***	200 ± 3 ***	0.78 ± 0.02 (12)	0.388 ± 0.005 (12)	2.263 ± 0.040 (12)

** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

++ P<0.01, +++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

Table 3.2. Mesenteric resistance artery morphology of untreated, perindopril treated, or perindopril with 1% saline treated 16 week SHR and WKY rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
WKY	Water	24	250 ± 5	11404 ± 348	13.81 ± 0.34	5.62 ± 0.19
SHR	Water	22	234 ± 8	15989 ± 646 ***	20.38 ± 0.97 ***	9.18 ± 0.70 ***
WKY	Perindopril	18	288 ± 8 **	11086 ± 476	11.77 ± 0.26	4.15 ± 0.10 **
SHR	Perindopril	16	249 ± 10 (17)	12765 ± 480 +++	15.82 ± 0.48	6.70 ± 0.38
WKY	1% NaCl + Perindopril	18	261 ± 7	11525 ± 459	13.40 ± 0.40	5.25 ± 0.22
SHR	1% NaCl + Perindopril	17	244 ± 8	14741 ± 398 ***	18.05 ± 0.49 ***	7.67 ± 0.45 *

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

+++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

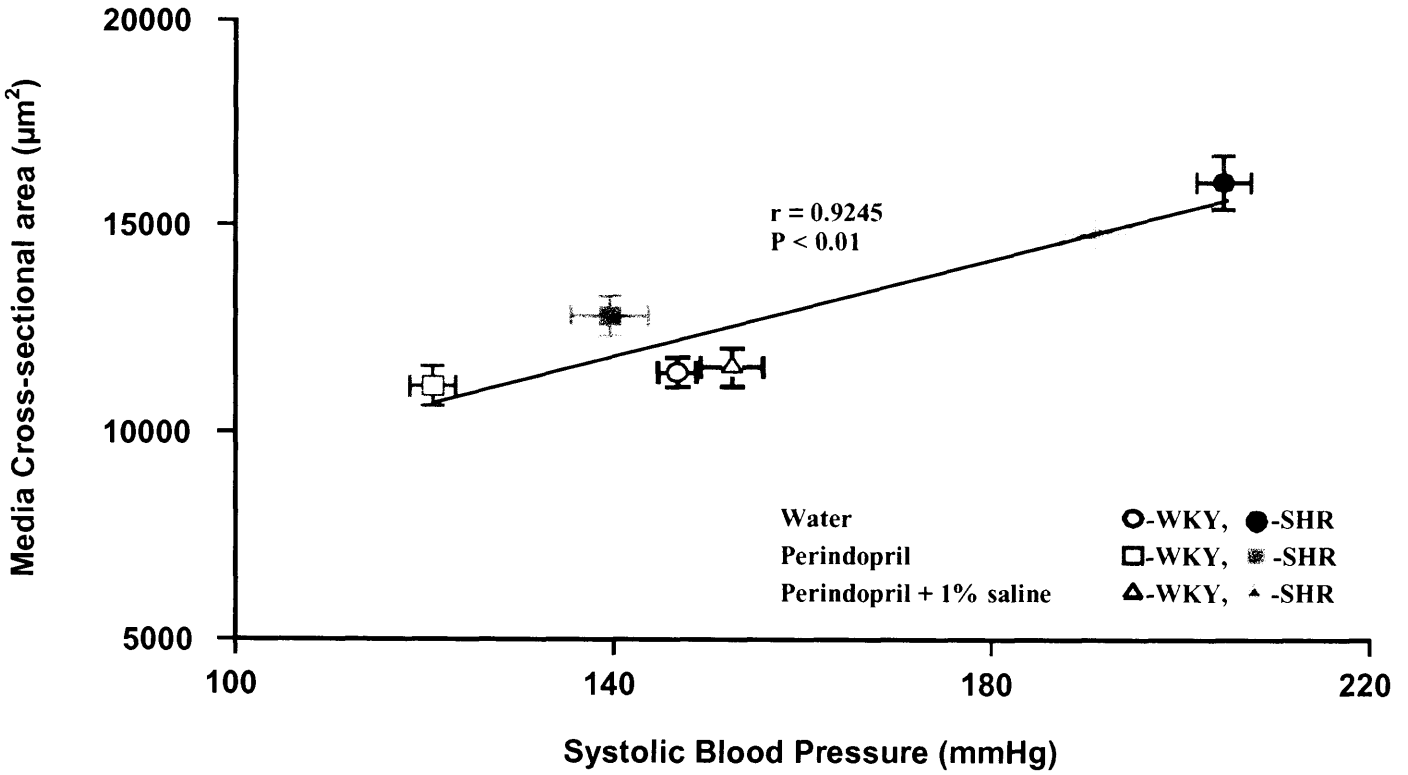


Figure 3.2. Line-graph showing media cross-sectional area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

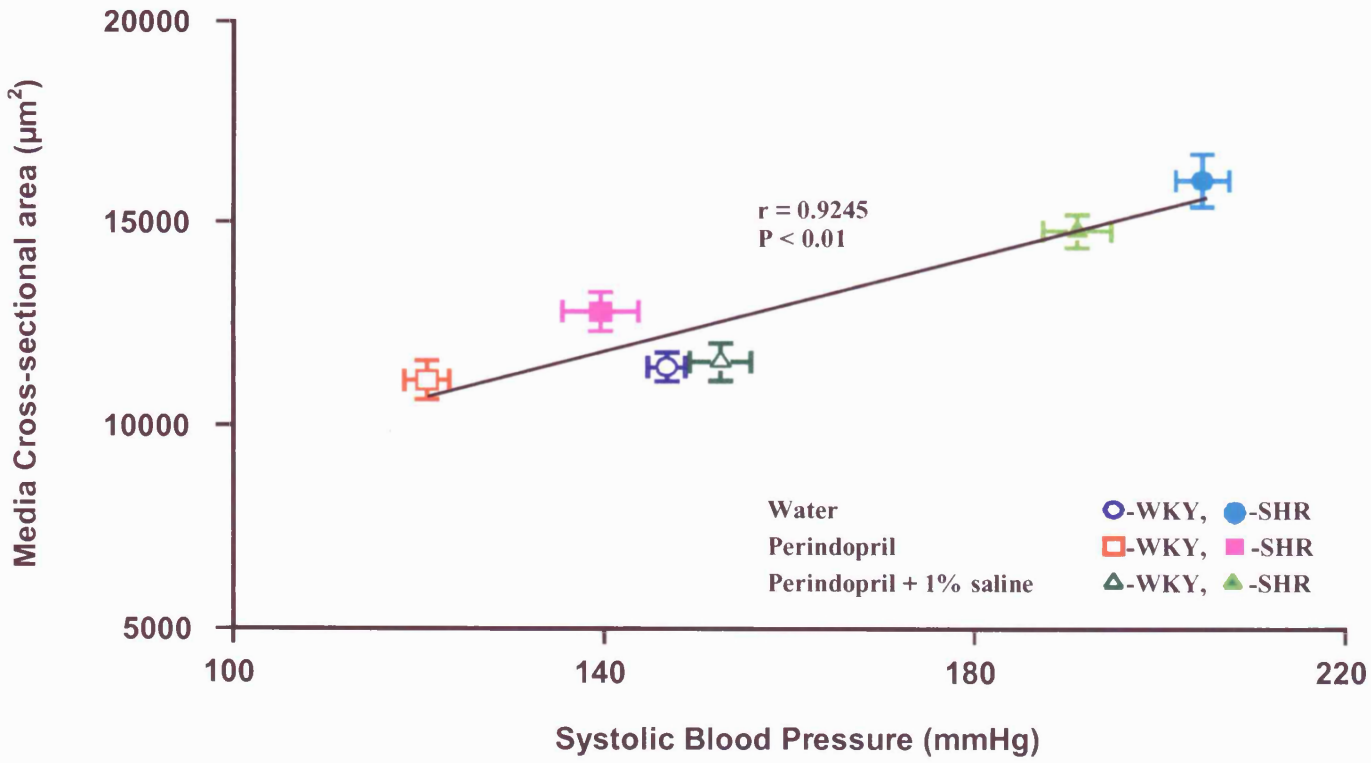


Figure 3.2. Line-graph showing media cross-sectional area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

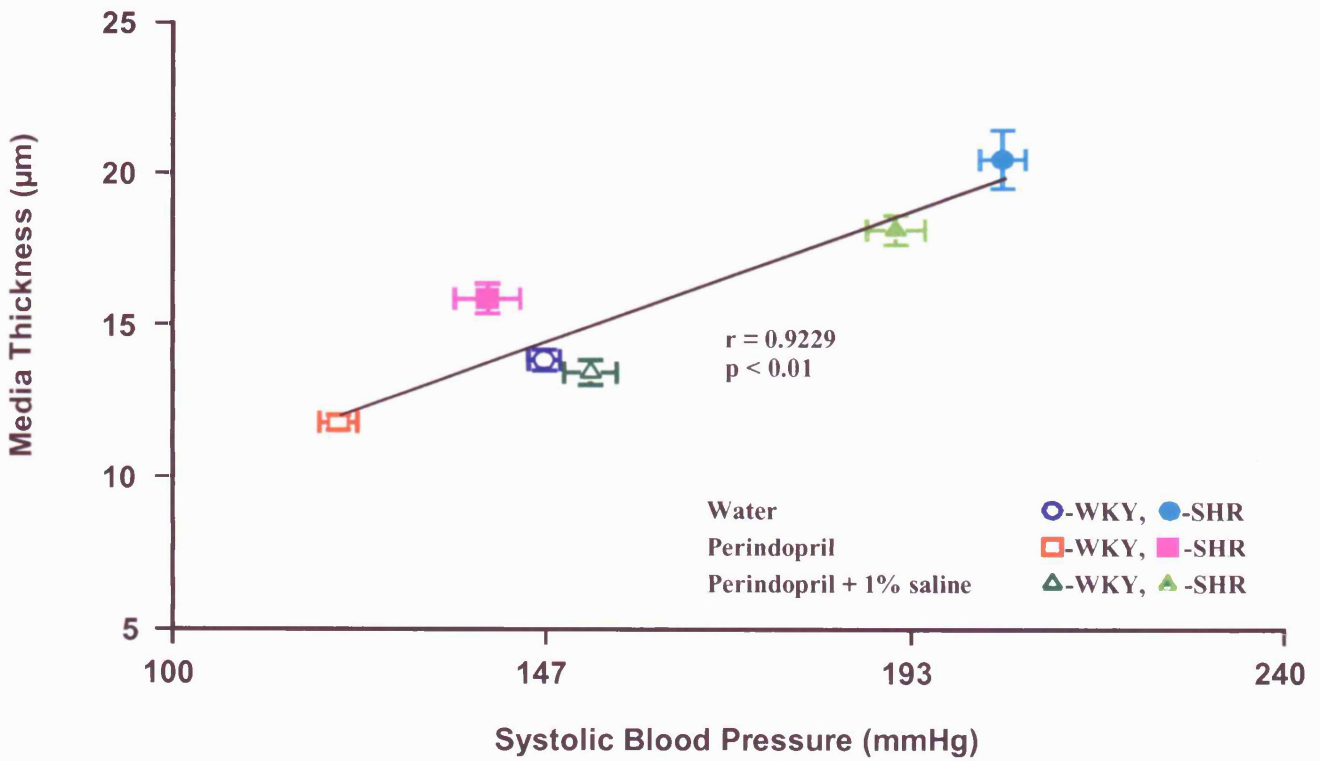


Figure 3.3. Line-graph showing media thickness area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

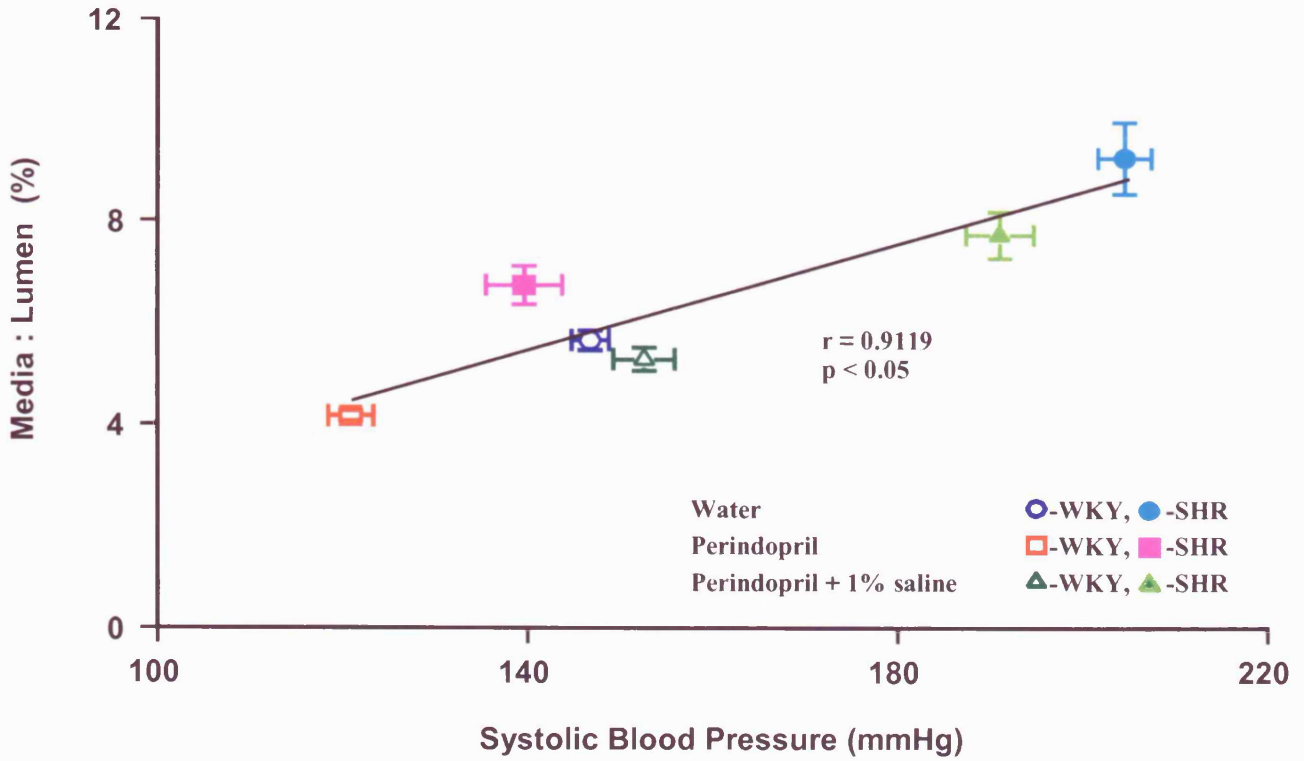


Figure 3.4. Line-graph showing media to lumen ratio area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

Table 3.3. Femoral resistance artery morphology of untreated, perindopril treated, or perindopril with 1% saline treated 16 week SHR and WKY rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
WKY	Water	22	246 ± 6	15336 ± 316	18.62 ± 0.50	7.79 ± 0.38
SHR	Water	21	227 ± 10	17948 ± 502 ***	23.18 ± 0.61 ***	10.84 ± 0.68 *
WKY	Perindopril	18	253 ± 6	13226 ± 392 *	15.76 ± 0.49	6.32 ± 0.29
SHR	Perindopril	17	239 ± 5	15366 ± 484 +++	18.85 ± 0.41 ++	7.92 ± 0.25
WKY	1% NaCl + Perindopril	18	254 ± 8	15280 ± 628	18.16 ± 0.90	7.54 ± 0.65
SHR	1% NaCl + Perindopril	18	221 ± 7	18104 ± 642 ***	23.73 ± 0.80 ***	11.13 ± 0.66 **

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

++ P<0.01, +++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

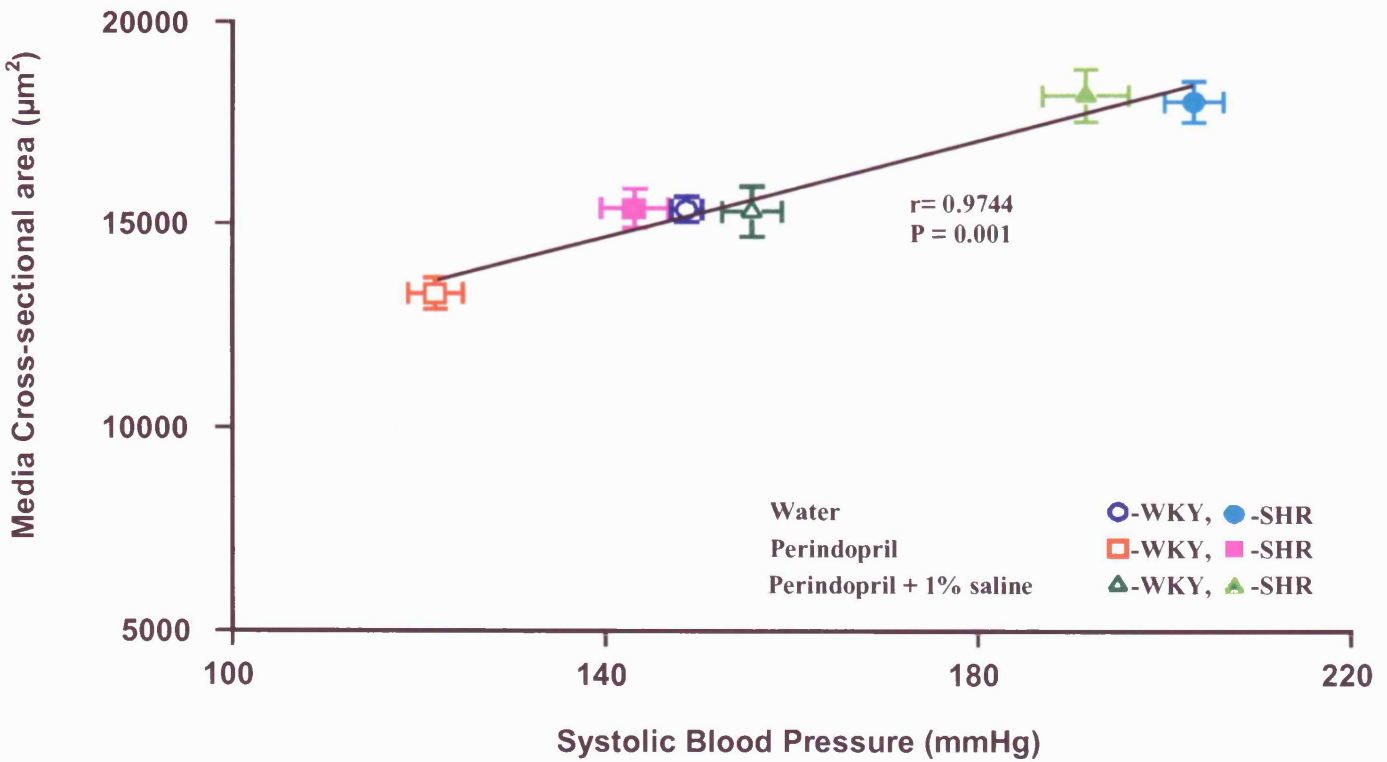


Figure 3.5. Line-graph showing media cross-sectional area versus systolic blood of femoral resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

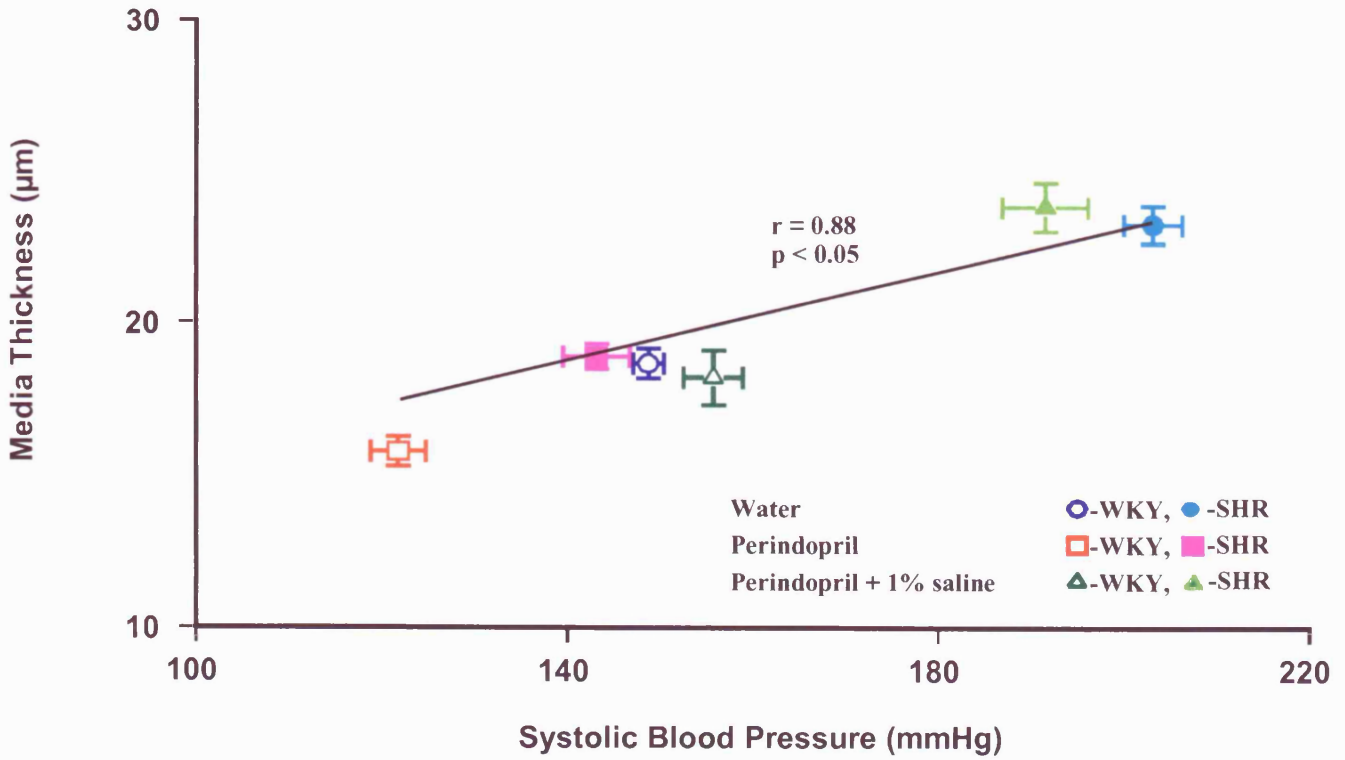


Figure 3.6. Line-graph showing medial thickness versus systolic blood of femoral resistance arteries from untreated, perindopril treated or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

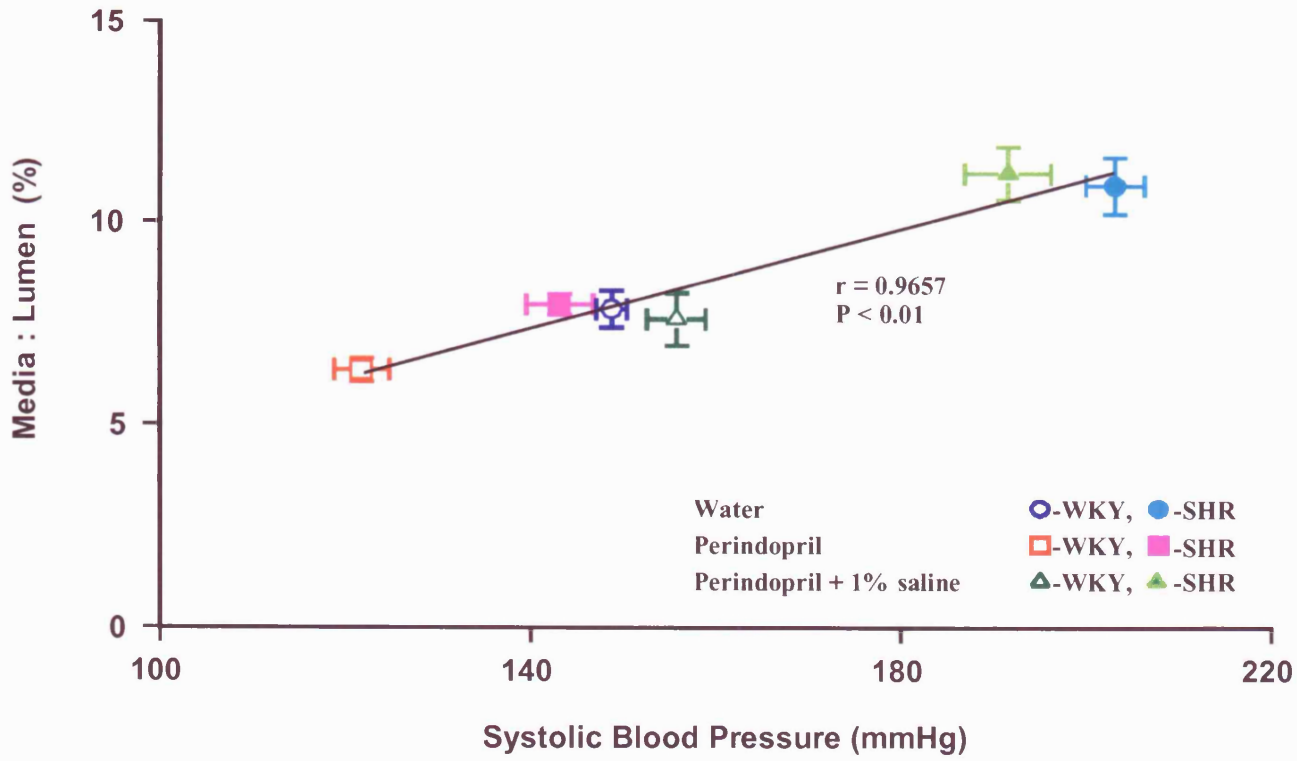


Figure 3.7. Line graph-showing media to lumen ratio versus systolic blood of femoral resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 16 weeks.

3.5 RESULTS OF 24 WEEK STUDY

3.5.1 Physical Characteristics

The systolic blood pressure profile at 24 weeks (Figure 3.8) followed a similar pattern to that observed at 16 weeks (Figure 3.1). The systolic blood pressure (SBP) was significantly raised in the untreated SHRs at 24 weeks (192 ± 3mmHg), compared to the untreated WKY rats (143 ± 2mmHg) ($P<0.001$) (Table 3.4). Treatment with perindopril prevented the development of hypertension in the SHRs (Figure 3.8 & Table 3.4), reducing the SBP at 24 weeks of both the SHRs to 127 ± 3mmHg ($P<0.01$), and the WKY rats to 110 ± 3mmHg ($P<0.001$), compared to the untreated WKY rats (Table 3.4). Treatment with perindopril and 1% saline failed to prevent the rise in SBP in the SHR, although the SBP of these rats did not quite reach the level obtained in the untreated SHRs (Figure 3.8). As a result, the final SBP in these rats was lower (181 ± 2mmHg), than the untreated SHRs (192 ± 3mmHg) ($P<0.01$), but remained significantly greater than the untreated WKY rats (143 ± 2mmHg) ($P<0.001$) (Table 3.4).

The body weight of the SHRs at 24 weeks was significantly lower, compared to the untreated WKY rats (Table 3.4). Perindopril treatment resulted in a significant reduction in body weight (207 ± 2g), compared to the untreated SHRs at 24 weeks (223 ± 3g) ($P<0.01$), but there was no significant change in the body weight of the WKY rats. This is different to that reported in the 16 week study suggesting the longer-term perindopril treatment may cause a reduction in body weight. The combination of perindopril and 1% saline treatment had no effect on the body weights of either the SHRs, or WKY rats.

The heart weight and heart weight to tibial length ratio was similar in the untreated SHRs and WKY rats, at 24 weeks (Table 3.4). Treatment with perindopril significantly reduced the heart weight, heart weight to body weight ratio, and heart weight to tibial

length ratio in both the SHRs and WKY rats at 24 weeks. However, treatment with perindopril and 1% saline had no significant effect on the heart weight, heart weight to body weight ratio, or heart weight to tibial length ratio of either the SHRs or WKY rats.

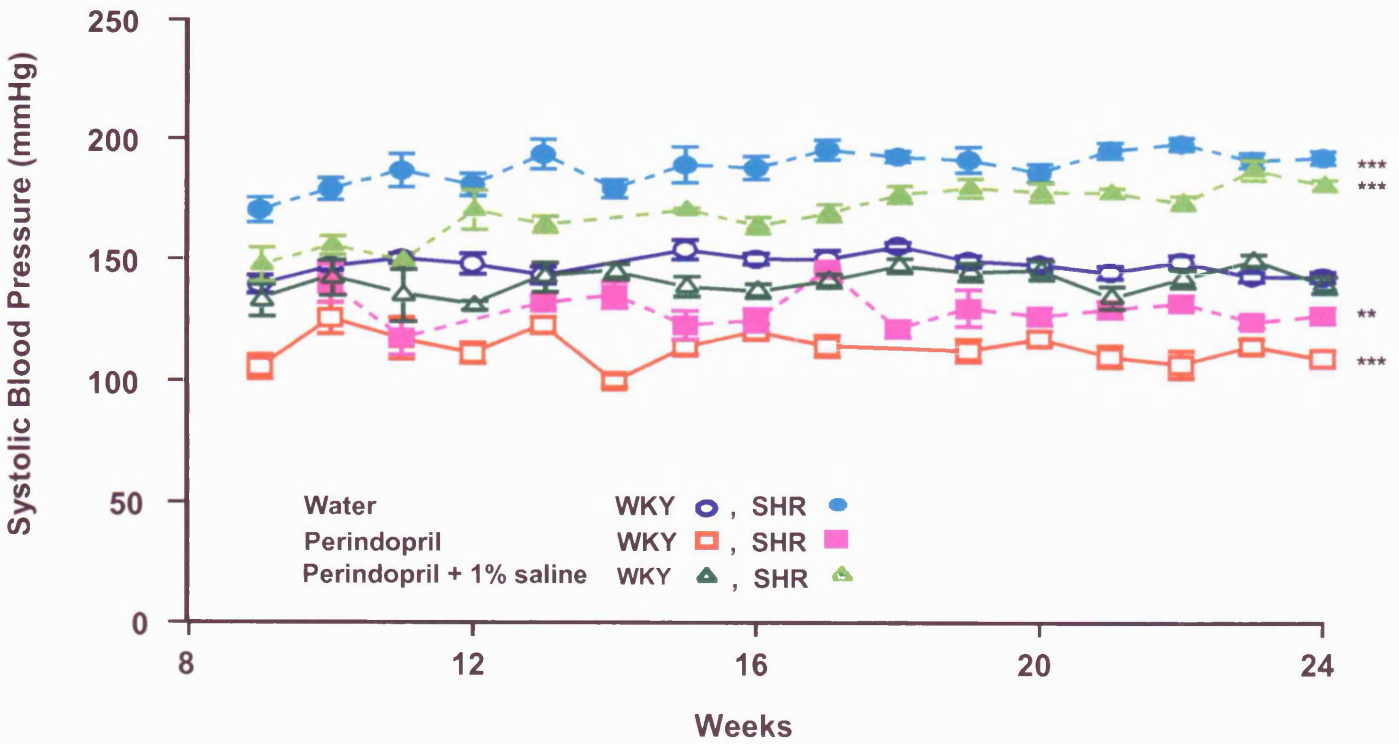


Figure 3.8. Systolic blood pressure (SBP) profile of untreated, perindopril treated, or perindopril with 1% saline treated 24 week old SHR and WKY rats.

**, P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

Table 3.4. Physical characteristics of untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Systolic blood pressure (mmHg)	Body Weight (g)	Heart Weight(HW) (g)	HW / Weight (%)	HW / Tibia
WKY	Water	16	143 ± 2	240 ± 4	0.87 ± 0.02 (15)	0.364 ± 0.010	2.454 ± 0.062
SHR	Water	19	192 ± 3 ***	223 ± 3 **	0.89 ± 0.02	0.398 ± 0.004	2.451 ± 0.038
WKY	Perindopril **(P)**	20	110 ± 3 ***	229 ±4 (19)	0.66 ± 0.02 ***	0.290 ± 0.004 ***	1.884 ± 0.041 ***
SHR	Perindopril **(P)**	16	127 ±3 ** +++	207 ± 2 *** ++	0.67 ± 0.01 *** +++ (15)	0.324 ± 0.005 +++	1.874 ± 0.039 *** +++
WKY	1% NaCl + **P**	19	140 ± 3	242 ± 3	0.86 ± 0.02	0.354 ± 0.007	2.400 ± 0.056
SHR	1% NaCl + **P**	16	181 ± 2 *** +	226 ± 3 *	0.89 ± 0.02	0.392 ± 0.006	2.477 ± 0.059

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

\+ P<0.05, ++ P<0.01, +++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

3.5.2 Mesenteric Resistance Artery Study

3.5.2.i Morphological Characteristics

The internal lumen diameter was significantly smaller in all SHR mesenteric arteries at 24 weeks (Table 3.5). Treatment with perindopril, or perindopril and 1% saline did not alter the lumen diameter of either the SHRs or WKY rats.

The media cross-sectional area (MCSA) of the untreated WKY mesenteries was significantly greater at 24 weeks (13,439 ± 632μm^2) (Table 3.5), than at 16 weeks (11,404 ± 348μm^2) (Table 3.2). Because of this increase, the MCSA (14,281 ± 531μm^2) of the untreated SHR mesenteric arteries was no longer significantly greater than that of the WKY (13,439 ± 632μm^2) (Table 3.5). However, both the MT and M:L were significantly increased in the untreated SHRs, compared to the untreated WKY rats at 24 weeks ($P<0.001$) (Table 3.5).

Treatment with perindopril prevented the development of structure in the SHR mesenteric arteries. In addition, perindopril treatment significantly reduced the MCSA of the WKY mesenteries (11,522 ± 438μm^2), compared to the untreated WKY rats (13,439 ± 632μm^2) (Table 3.5), the resulting value being similar to that observed in the untreated WKY at 16 weeks (Table 3.2). A similar reduction was observed in the MT of the perindopril treated WKY mesenteries (12.13 ± 0.35μm), compared to the untreated WKY rats (14.43 ± 0.65) ($P<0.05$). The combination treatment of perindopril and 1% saline failed to prevent the development of structural change in the SHR mesenteric arteries, and also had no effect on the structure of the mesenteric arteries from the WKY (Table 3.5).

At 24 weeks, there were positive correlations between SBP and mesenteric MCSA ($r = 0.88$, $P<0.05$) (Figure 3.9), MT ($r = 0.99$, $P<0.001$) (Figure 3.10), and M:L ($r = 0.94$, $P<0.01$) (Figure 3.11).

Table 3.5. Mesenteric resistance artery morphology of untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
WKY	Water	16	284 ± 10	13439 ± 632	14.43 ± 0.65	5.25 ± 0.39
SHR	Water	19	230 ± 7 ***	14281 ± 531	18.55 ± 0.73 ***	8.42 ± 0.62 ***
WKY	Perindopril	16	291 ± 9	11522 ± 438 *	12.13 ± 0.35 *	4.26 ± 0.22
SHR	Perindopril	16	239 ± 8 **	11258 ± 412 * +++	13.98 ± 0.63 +++	5.97 ± 0.52 +
WKY	1% NaCl + Perindopril	18	283 ± 10	13406 ± 442	14.58 ± 0.52	5.39 ± 0.37
SHR	1% NaCl + Perindopril	16	239 ± 7 **	14276 ± 496	17.42 ± 0.60 **	7.51 ± 0.45 *

* P<0.05, ** P<0.01 ANOVA compared to untreated WKY rats

+ P<0.05, +++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

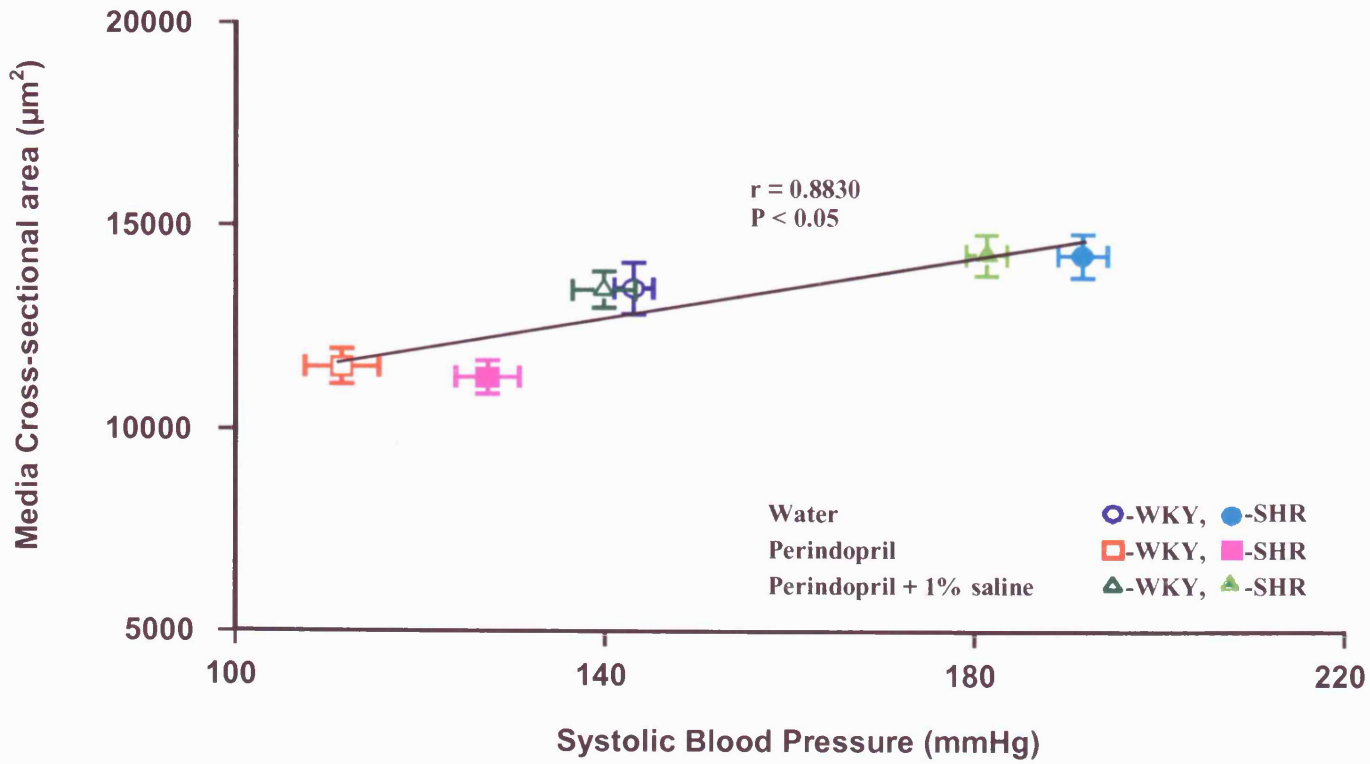


Figure 3.9. Line-graph showing media cross-sectional area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks

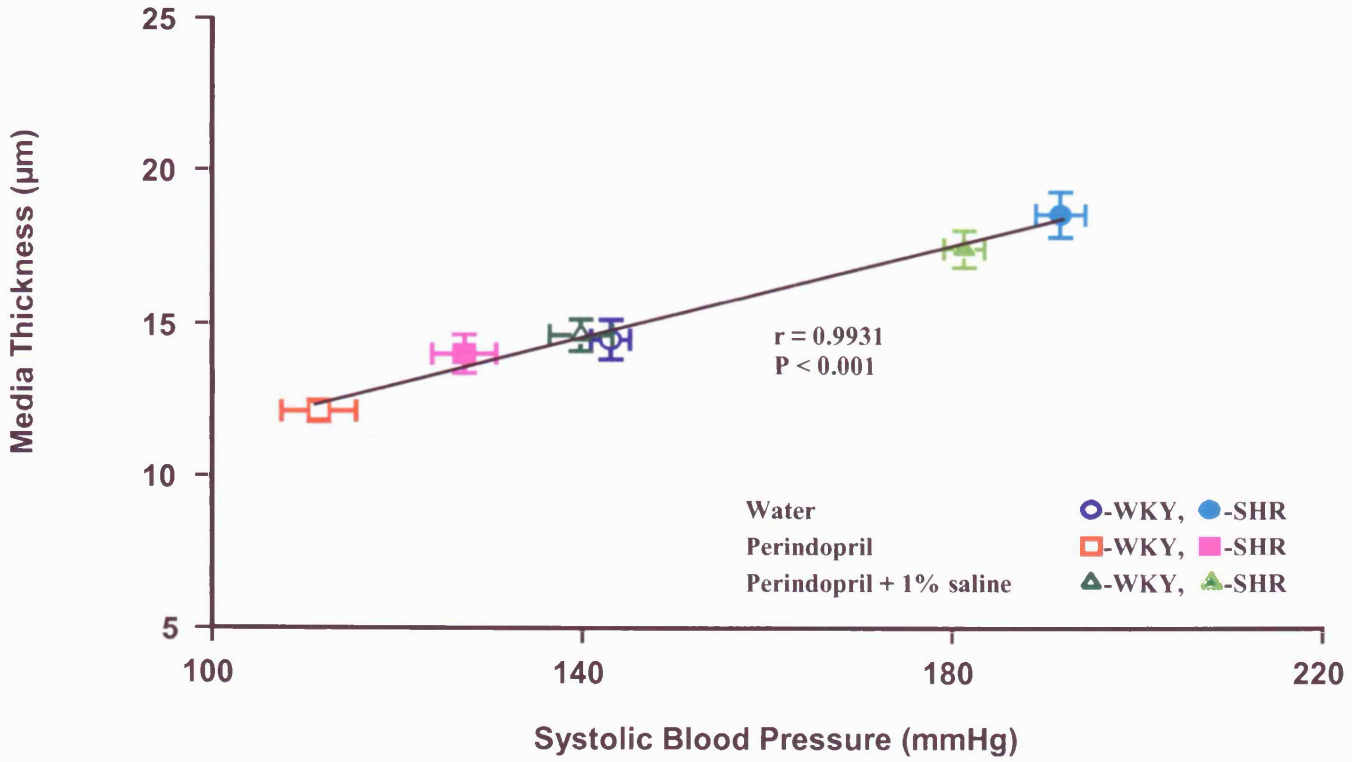


Figure 3.10. Line-graph showing media thickness area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks

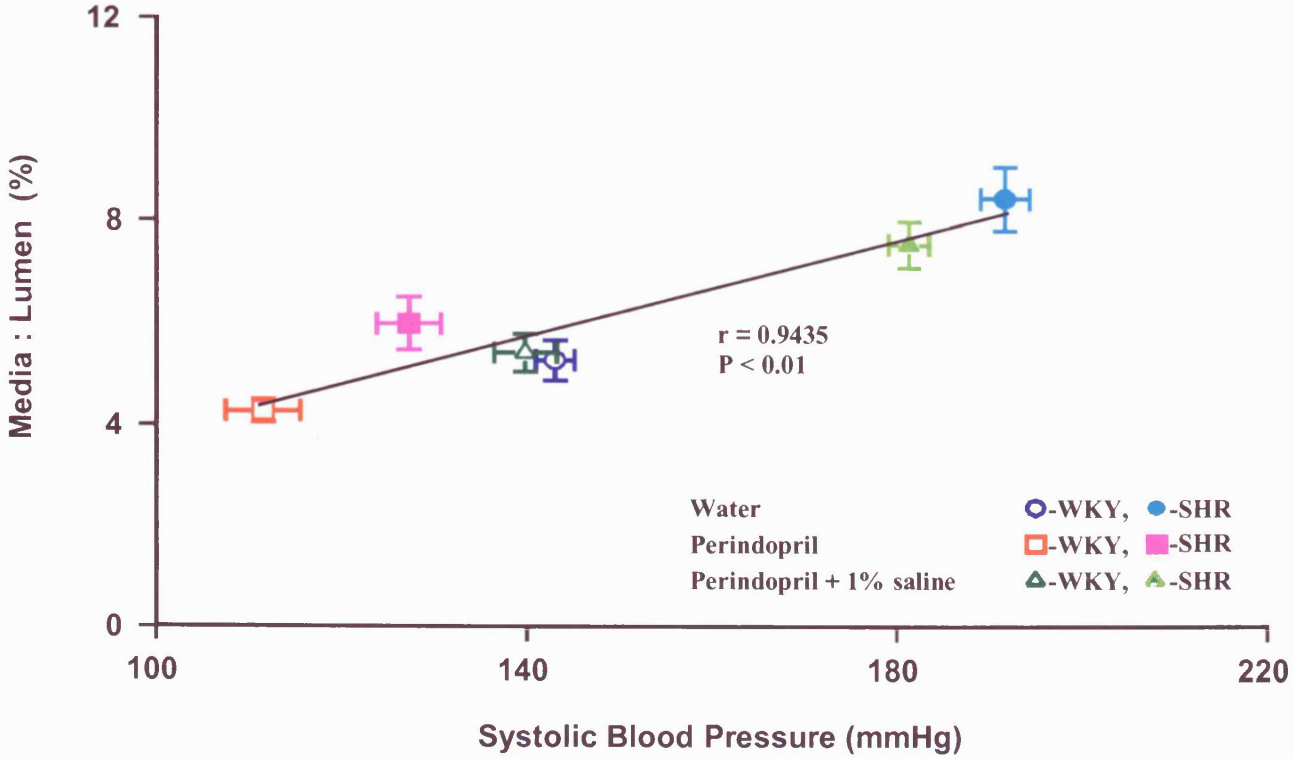


Figure 3.11. Line-graph showing media to lumen ratio area versus systolic blood of mesenteric resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks.

3.5.2.ii Mesenteric Resistance Artery Contractility

Activation procedure

There was no significant difference between the contractile responses generated to high potassium, and high potassium containing 10^{-5}M noradrenaline (NAK), in the untreated SHRs and WKY mesenteric arteries, despite a slight increase in contractility of the SHR mesenteric arteries (Table 3.6). Treatment with perindopril prevented the slight increase in contractility, to both high potassium and NAK in the SHR mesenteries, compared to the untreated SHRs, but had little effect on the response of the WKY mesenteric arteries. Treatment with perindopril and 1% saline did not prevent the slight increase in contractility in the SHRs, or alter the response of the WKY rats (Table 3.6).

When expressed as active media stress, the responses to high potassium and NAK were similar in the untreated SHR and WKY mesenteric arteries, despite a slight reduction in the contractility of the untreated SHR mesenteries. Treatment with perindopril, or perindopril and 1% saline did not effect the active media stress response of either the SHR, or WKY mesenteries (Table 3.6).

Table 3.6. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline of mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
WKY	Water	16	3.74 ± 0.18	4.84 ± 0.22	265 ± 14	341 ± 15
SHR	Water	19	4.33 ± 0.24	5.17 ± 0.29	240 ± 15	286 ± 19
WKY	Perindopril	17	3.50 ± 0.22	4.14 ± 0.24	292 ± 20 (16)	346 ± 22 (16)
SHR	Perindopril	16	3.26 ± 0.18 ++	3.71 ± 0.21 * +++	240 ± 14	272 ± 16 *
WKY	1% NaCl + Perindopril	18	3.74 ± 0.21	4.69 ± 0.23	260 ± 15	328 ± 18
SHR	1% NaCl + Perindopril	16	4.52 ± 0.28	5.59 ± 0.39	260 ± 13	321 ± 13

* $P<0.05$ ANOVA compared to untreated WKY rats

++ $P<0.01$, +++ $P<0.001$ ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

Noradrenaline (NA) contractility

At 24 weeks, there was no significant difference between the active tension generated to noradrenaline (NA), which was similar in both the untreated SHR and WKY mesenteric arteries, despite a slight increase in contractility of the SHR mesenteric arteries (Table 3.7 & Figure 3.12). Treatment with perindopril significantly reduced the active tension to NA in the SHRs (Table 3.7 & Figure 3.12), but did not alter the response of the WKY. Combination of perindopril and 1% saline treatment had no effect on the active tension to noradrenaline, in either group of rats. The sensitivity to noradrenaline was similar in all mesenteric arteries studied (Table 3.7).

When expressed as active media stress the response to NA was similar in the untreated SHR, and WKY mesenteric arteries (Table 3.7 & Figure 3.13). Treatment with perindopril, or perindopril and 1% saline did not alter the active media stress response of SHR, or WKY mesenteric arteries at 24 weeks (Table 3.7 & Figure 3.13).

Table 3.7. The maximum contractile response and sensitivity (EC_{50}) to noradrenaline of mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Maximum NA (mN/mm)	NA EC_{50} (μM)	Maximum NA active media stress (mN/mm^2)
WKY	Water	16	4.54 ± 0.19	2.00 ± 0.31 (14)	320 ± 13
SHR	Water	19	5.13 ± 0.30	1.99 ± 0.30	284 ± 19
WKY	Perindopril	17	3.93 ± 0.28	1.72 ± 0.17	333 ± 25 (16)
SHR	Perindopril	16	3.69 ± 0.22 +++	2.10 ± 0.30 (13)	271 ± 17
WKY	1% NaCl + Perindopril	18	4.49 ± 0.22	1.57 ± 0.20	310 ± 16
SHR	1% NaCl + Perindopril	16	5.31 ± 0.31	1.96 ± 0.25 (15)	306 ± 14

+++ P<0.001 ANOVA treated SHRs compared to untreated SHRs with Bonferroni correction for multiple comparisons.

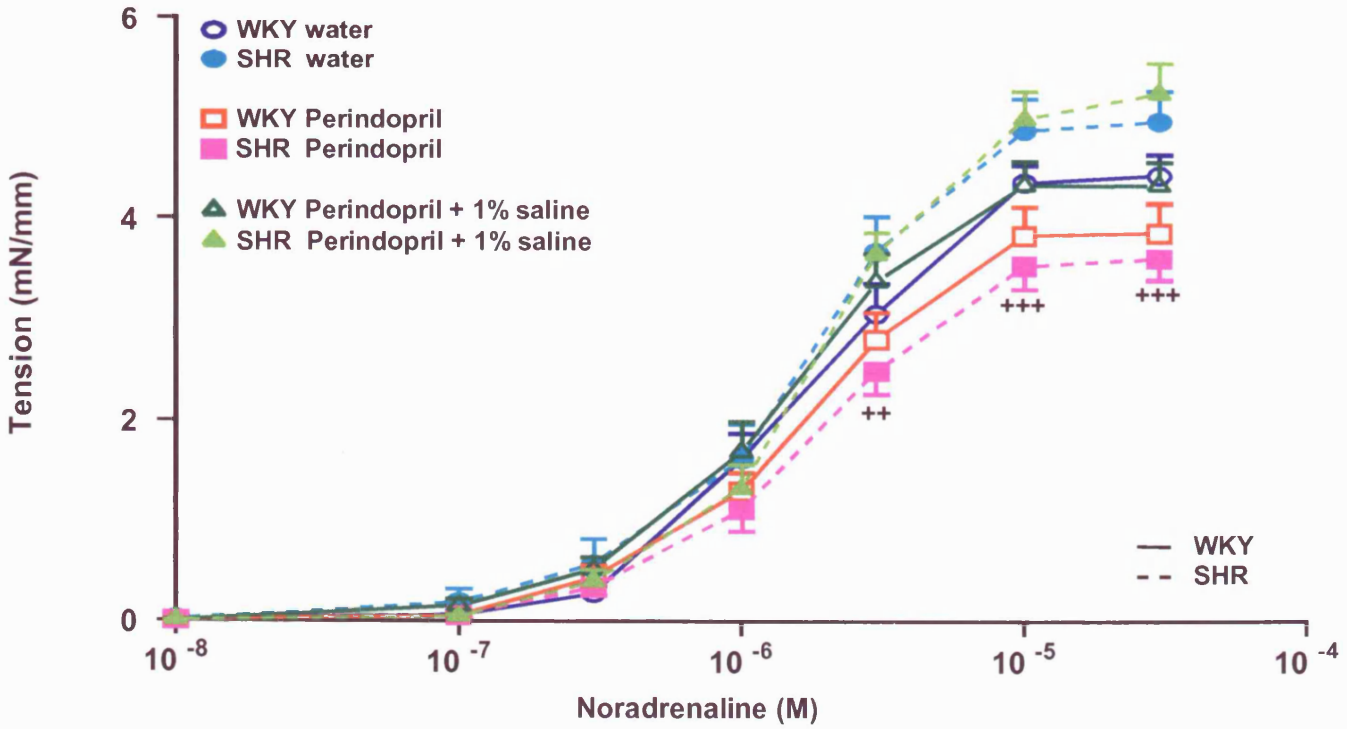


Figure 3.12. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of mesenteric resistance arteries from 24 week SHR and WKY rats.

$_{+}$ P<0.05, $_{++}$ P<0.01, $_{+++}$ P<0.001 ANOVA treated SHRs compared to untreated SHRs

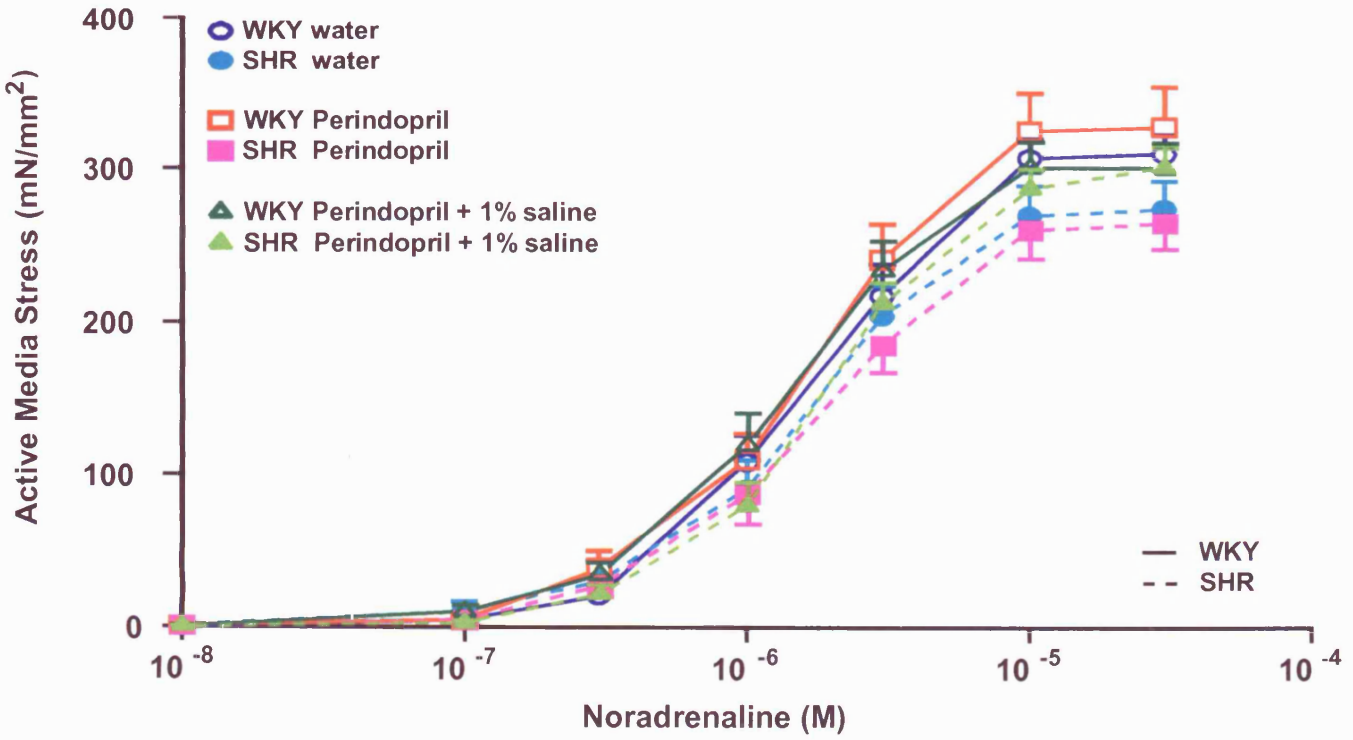


Figure 3.13. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2), in the presence of cocaine, of mesenteric resistance arteries from 24 week SHR and WKY rats.

3.5.2.iii Endothelium-dependent relaxation

Acetylcholine (ACh)

The relaxation response to ACh was significantly reduced in the 24 week untreated SHR and WKY mesenteric arteries (Table 3.8, Figure 3.14), compared to the relaxation responses of these groups observed at 16 weeks (Table 3.5 & Figure 3.8). This reduction was significant for the maximum relaxation of the SHR (81 ± 3.4%), and WKY rats (93 ± 0.9%), compared to the maximum relaxation of the 16 week untreated SHR (92 ± 1.9%) ($P<0.01$), and WKY rats (96 ± 0.6%) ($P<0.01$). The sensitivity to ACh was also reduced in the untreated SHR (0.12 ± 0.05μM), compared to the sensitivity in the 16 week untreated SHR (0.02 ± 0.01μm) ($P<0.001$). A similar reduction in sensitivity was found in the untreated WKY (0.14 ± 0.05μM), compared to the 16 week untreated WKY rats (0.03 ± 0.01μM) ($P<0.01$).

At 24 weeks, there was a significant reduction in the relaxation response of the untreated SHRs at 10^{-5}M ACh, compared to the untreated WKY rats at 24 weeks ($P<0.05$) (Figure 3.14). This was the result of a re-contraction in response to higher concentrations of ACh. Treatment with perindopril prevented the impaired relaxation to ACh in the SHR mesenteric arteries (Figure 3.14). Treatment with perindopril and 1% saline prevented some of the re-contraction observed in the SHR mesenteric arteries, but this did not reach significance when compared to the response of the untreated SHR mesenteric arteries (Figure 3.14).

Bradykinin (BK)

At 24 weeks, the maximum relaxation to bradykinin (BK) was significantly reduced in the untreated SHR mesenteric arteries (63 ± 5.2%) (Table 3.8), compared to the maximum relaxation of the same group studied at 16 weeks (82 ± 3.6%) ($P<0.01$).

At 24 weeks, the relaxation response to BK was similar in the untreated SHR and WKY mesenteric arteries (Table 3.8 & Figure 3.15). Treatment with perindopril significantly improved the relaxation response to bradykinin in the SHR mesenteries, compared to the untreated SHRs at 24 weeks, but had little effect on the WKY rats. Treatment with perindopril and 1% saline did not alter the relaxation response to BK in either the SHR, or WKY mesenteric arteries.

3.5.2.iv Endothelium-independent relaxation

Sodium nitroprusside (SNP)

The maximum relaxation to SNP of the untreated SHR mesenteric arteries was significantly reduced at 24 weeks (90 ± 2.1%) (Table 3.8), compared to the maximum relaxation of the same group studied at 16 weeks (96 ± 0.7%) ($P<0.01$).

At 24 weeks, the relaxation response, and sensitivity to sodium nitroprusside (SNP) were similar in all mesenteric arteries (Table 3.8 & Figure 3.16).

Table 3.8. The maximum relaxation responses and sensitivity (EC_{50}) to acetylcholine (ACh), bradykinin (BK) and sodium nitroprusside (SNP), of mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Maximum ACh (%)	ACh EC_{50} (μM)	Maximum BK (%)	Maximum SNP (%)	SNP EC_{50} (μM)
WKY	Water	16	93 ± 0.9	0.14 ± 0.05	66 ± 5.6	93 ± 1.4	0.67± 0.12 (14)
SHR	Water	19	81 ± 3.4	0.12 ± 0.05 (18)	63 ± 5.2	90 ± 2.1	0.60 ± 0.16
WKY	Perindopril	17	86 ± 3.0	0.33 ± 0.19	59 ± 6.2	94 ± 1.2 (16)	0.83 ± 0.14 (15)
SHR	Perindopril	16	88 ± 3.1	0.10 ± 0.03	81 ± 3.9 +	90 ± 1.6	0.27 ± 0.10
WKY	1% NaCl + Perindopril	18	92 ± 2.1	0.24 ± 0.10	52 ± 5.3	91 ± 2.2 (16)	0.92 ± 0.37 (16)
SHR	1% NaCl + Perindopril	16	85 ± 3.8 (15)	0.21 ± 0.07 (13)	72 ± 5.3 (15)	91 ± 1.6 (15)	0.52 ± 0.11 (15)

$_{+}$ $P<0.05$ ANOVA treated SHRs compared to untreated SHRs with Bonferroni correction for multiple comparisons.

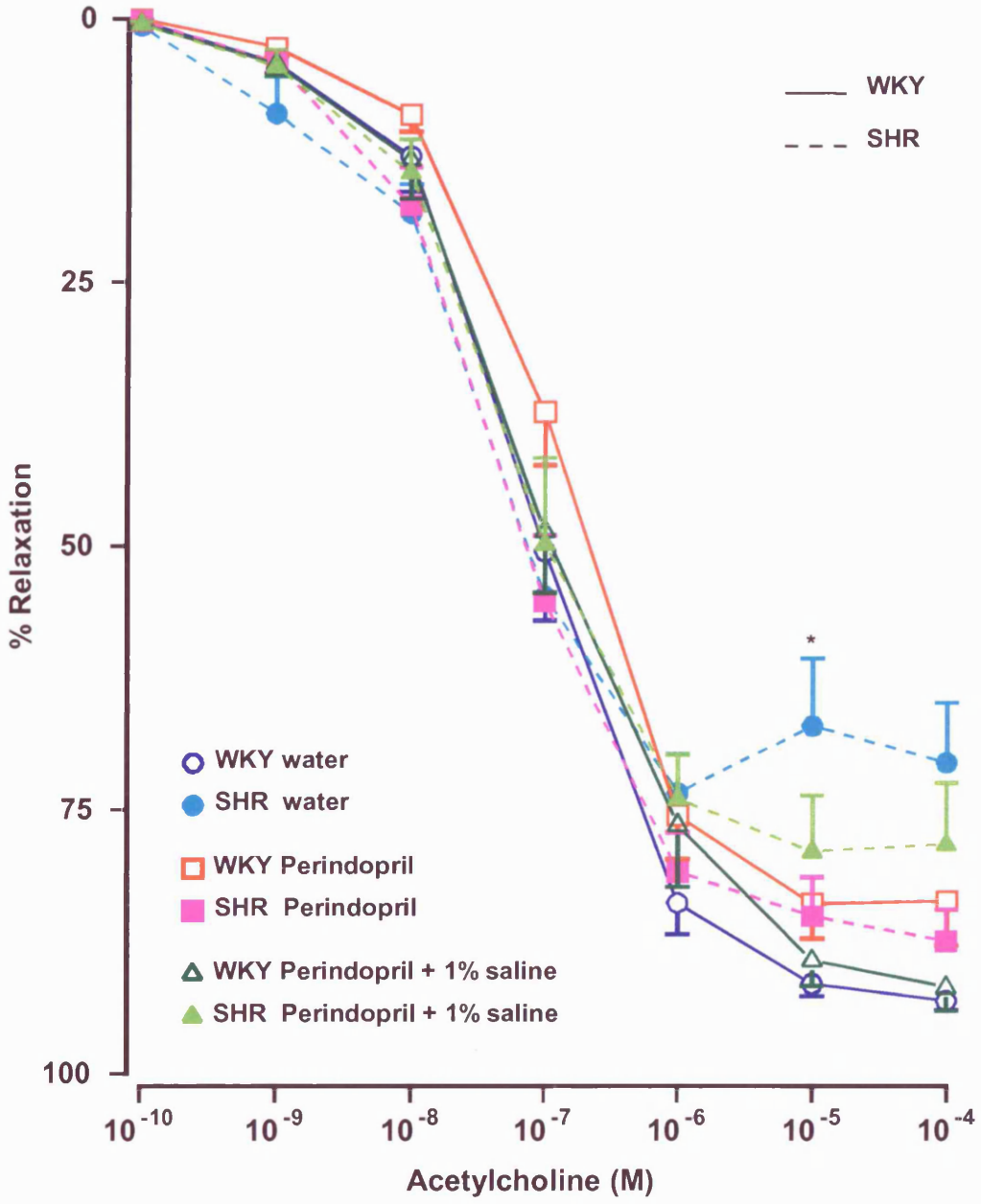


Figure 3.14. The relaxation response to acetylcholine of mesenteric resistance arteries from 24 week SHR and WKY rats.

* P<0.05 ANOVA compared to untreated WKY rats

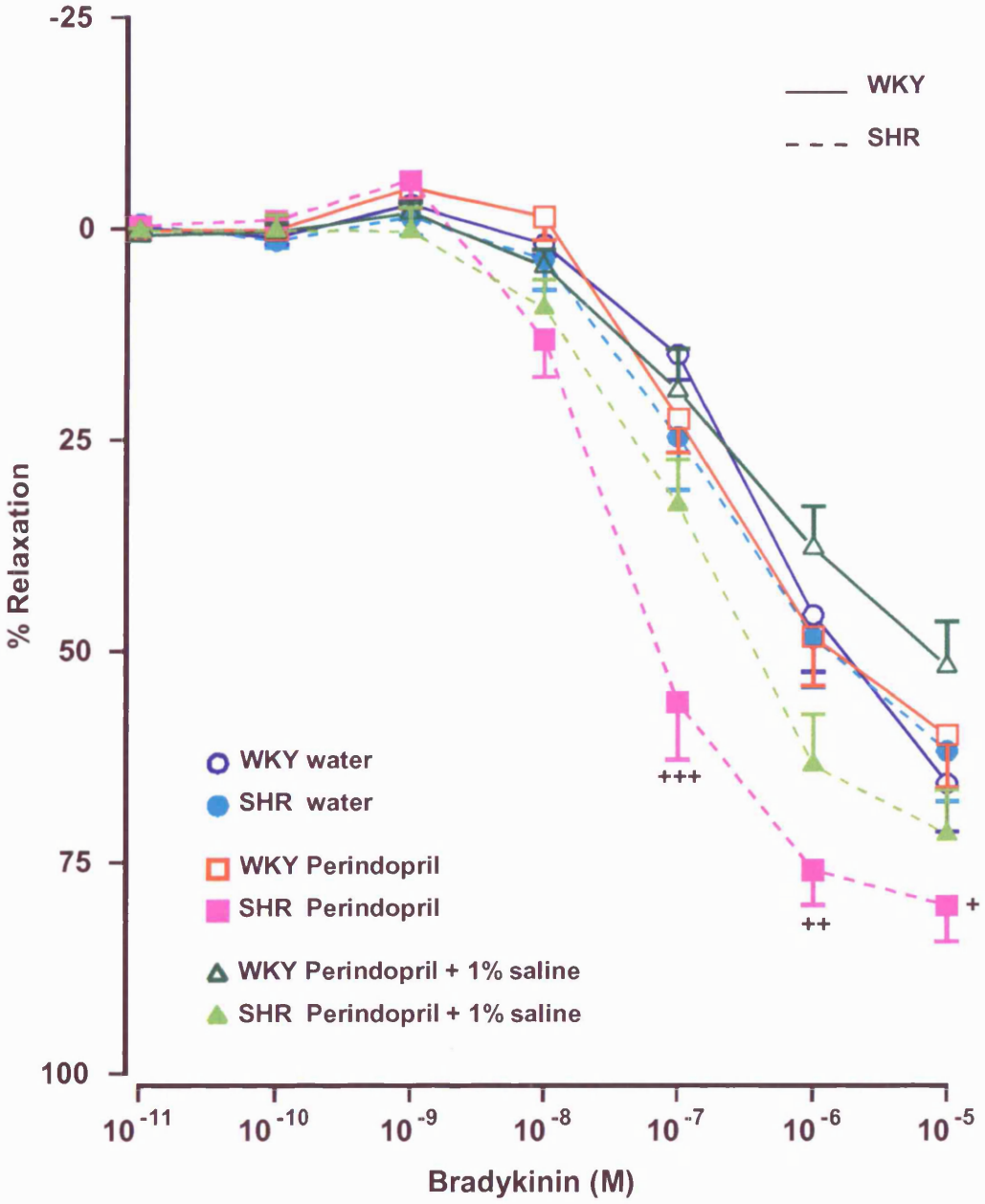


Figure 3.15. The relaxation response to bradykinin of mesenteric resistance arteries from 24 week SHR and WKY rats

$_{+}$ P<0.05, $_{++}$ P<0.01, $_{+++}$ P<0.001 ANOVA treated SHRs compared to untreated SHRs

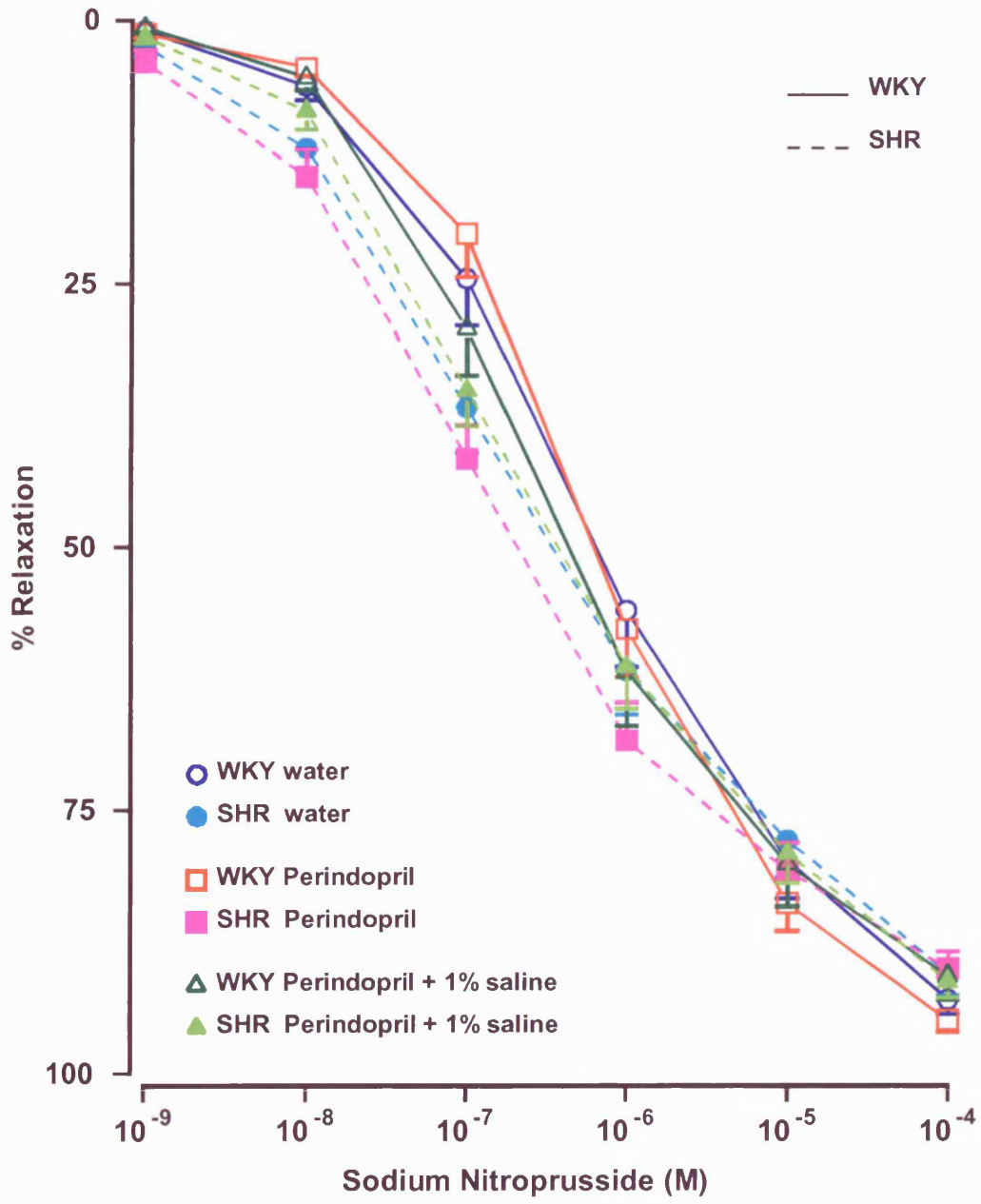


Figure 3.16. The relaxation response to sodium nitroprusside of mesenteric resistance arteries from 24 week SHR and WKY rats.

3.5.3 Mesenteric Resistance Artery EDRF Characterisation

3.5.3.i Indomethacin

Incubation with indomethacin improved the endothelial-dependent relaxation, to both acetylcholine (Table 3.9 & Figure 3.17) and improved the relaxation to bradykinin (Table 3.10 & Figure 3.18), in the untreated SHR mesenteric arteries. Although, the improvement in response to bradykinin observed with indomethacin did not quite reach significance. In addition, there was a slight increase in the sensitivity to acetylcholine in the mesenteric arteries after incubation with indomethacin. These results suggest that impaired relaxation may be either the result of an increased level of cyclo-oxygenase vasoconstrictor prostanoids, or due to the lack of an endothelium-derived relaxing factor resulting in a shift towards vasoconstriction. There was also a significant improvement in the relaxation of the WKY mesenteric artery after incubation with indomethacin, suggesting the involvement of vasoconstrictor prostanoids in the normal relaxation in the 24 week WKY rats.

3.5.3.ii SQ29548

Incubation with SQ29548 did not significantly improve the relaxation response to either acetylcholine (Table 3.11 & Figure 3.19), or bradykinin (Table 3.12 & Figure 3.20), in any of the mesenteric arteries studied. The lack of improvement with SQ29548 suggests that the vasoconstrictor prostanoids thromboxane A_2, and prostaglandin H_2 are unlikely to be involved in the reduced response to acetylcholine and bradykinin observed in the present study.

3.5.3.iii L-NOARG

The response to acetylcholine was slightly reduced in the WKY mesenteric arteries after incubation with L-NOARG, although to a lesser extent than that observed in the SHR, due to the lack of re-contraction (Table 3.13 & Figure 3.21). This suggests that a large

component of relaxation to ACh is not dependent upon nitric oxide, and that other endothelium-derived relaxing factors, such as EDHF and the prostaglandins are likely to be involved. Incubation with L-NOARG significantly reduced the relaxation response of the SHR mesenteric arteries to acetylcholine (Table 3.13 & Figure 3.21). Consequently, a re-contraction was observed to higher concentrations of acetylcholine, in the presence of L-NOARG. This suggests that there is a significant reduction in the EDRF-independent component of relaxation in the SHRs. In addition, it may suggest a greater contribution of nitric oxide to relaxation in the hypertensive artery.

Incubation with L-NOARG failed to improve bradykinin relaxation in the mesenteric arteries, with the exception of a significant reduction in the perindopril treated SHRs (Table 3.14 & Figure 3.22). However, there was a reduction in the maximum relaxation to bradykinin in the presence of L-NOARG, which achieved significance in the untreated SHRs, perindopril treated rats, and perindopril with 1% saline treated WKY rats. Thus, suggesting the majority of relaxation to bradykinin is not dependent on nitric oxide. However, there did appear to be a slight contribution of nitric oxide to relaxation, in response to the highest concentrations of bradykinin. Treatment with perindopril improved the relaxation to bradykinin, likely due to an increased production or release of EDNO, as the improvement was not observed after nitric oxide synthesis blockade.

Table 3.9. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh EC_{50}(μM)		ACh Mean R (%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
WKY	Water	8	92 ± 2.5	97 ± 0.6	0.10 ± 0.04	0.03 ± 0.01 (6)	48 ± 3.5	59 ± 2.7 *
SHR	Water	7	80 ± 4.8	96 ± 1.8 *	0.07 ± 0.04 (5)	0.05 ± 0.02 (6)	38 ± 5.8	56 ± 3.3 *
WKY	Perindopril **(P)**	6	86 ± 5.1	92 ± 3.3	0.10 ± 0.02 (5)	0.04 ± 0.01 (4) *	45 ± 4.2	52 ± 2.7
SHR	Perindopril **(P)**	7	88 ± 6.2	92 ± 4.9	0.10 ± 0.03 (6)	0.03 ± 0.01 (7)	46 ± 4.2	55 ± 4.3
WKY	1% saline & **P**	8	96 ± 1.2	99 ± 0.5 *	0.12 ± 0.03	0.03 ± 0.01 (7)	51 ± 2.8	58 ± 2.2
SHR	1% saline & **P**	6	92 ± 2.5	97 ± 0.6	0.04 ± 0.02 (5)	0.02 ± 0.01 (6)	49 ± 5.3	62 ± 2.0

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$ T-test of ACh relaxation compared to the control in the absence of indomethacin.

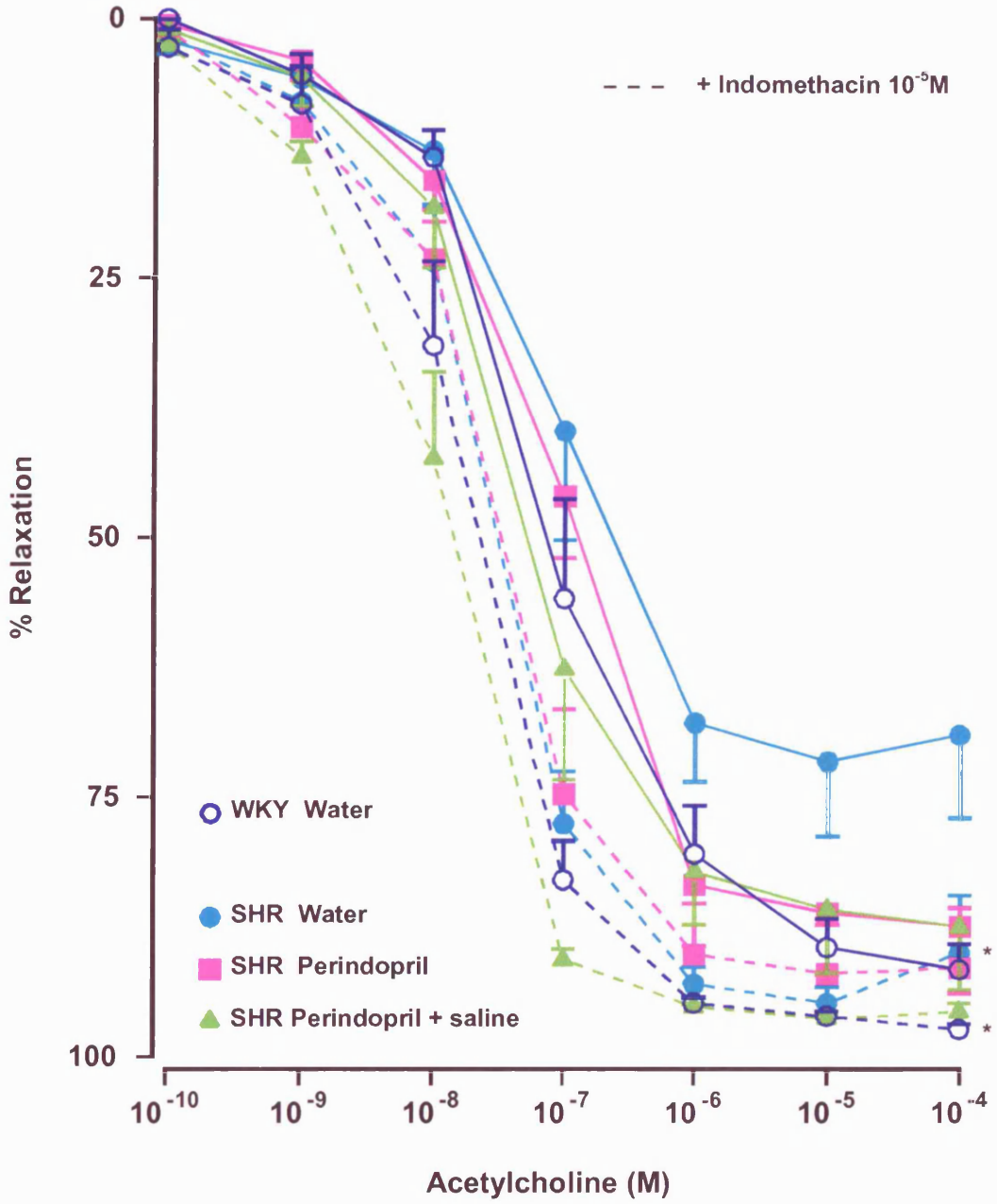


Figure 3.17 The relaxation response to acetylcholine, in the absence and presence of indomethacin 10^{-5}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

* $P<0.05$ T-test of mean relaxation compared to that in the absence of indomethacin.

Table 3.10. The effect of indomethacin 10^{-5}M on the relaxation to bradykinin (BK), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	BK E max (%)		BK Mean R (%)	
			Control	Indomethacin	Control	Indomethacin
WKY	Water	8	76 ± 8.9	87 ± 2.8	27 ± 4.7	33 ± 3.2
SHR	Water	7	66 ± 7.5	83 ± 5.9	20 ± 1.2	32 ± 5.1
WKY	Perindopril **(P)**	5	80 ± 10.9	81 ± 4.5	24 ± 7.2	30 ± 4.0
SHR	Perindopril **(P)**	7	79 ± 12.5	93 ± 1.4	30 ± 5.1	40 ± 5.6
WKY	1% saline & **P**	7	76 ± 7.4	91 ± 2.1	26 ± 4.9	31 ± 4.8
SHR	1% saline & **P**	5	76 ± 8.9	87 ± 2.8	32± 8.8	44 ± 3.9

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

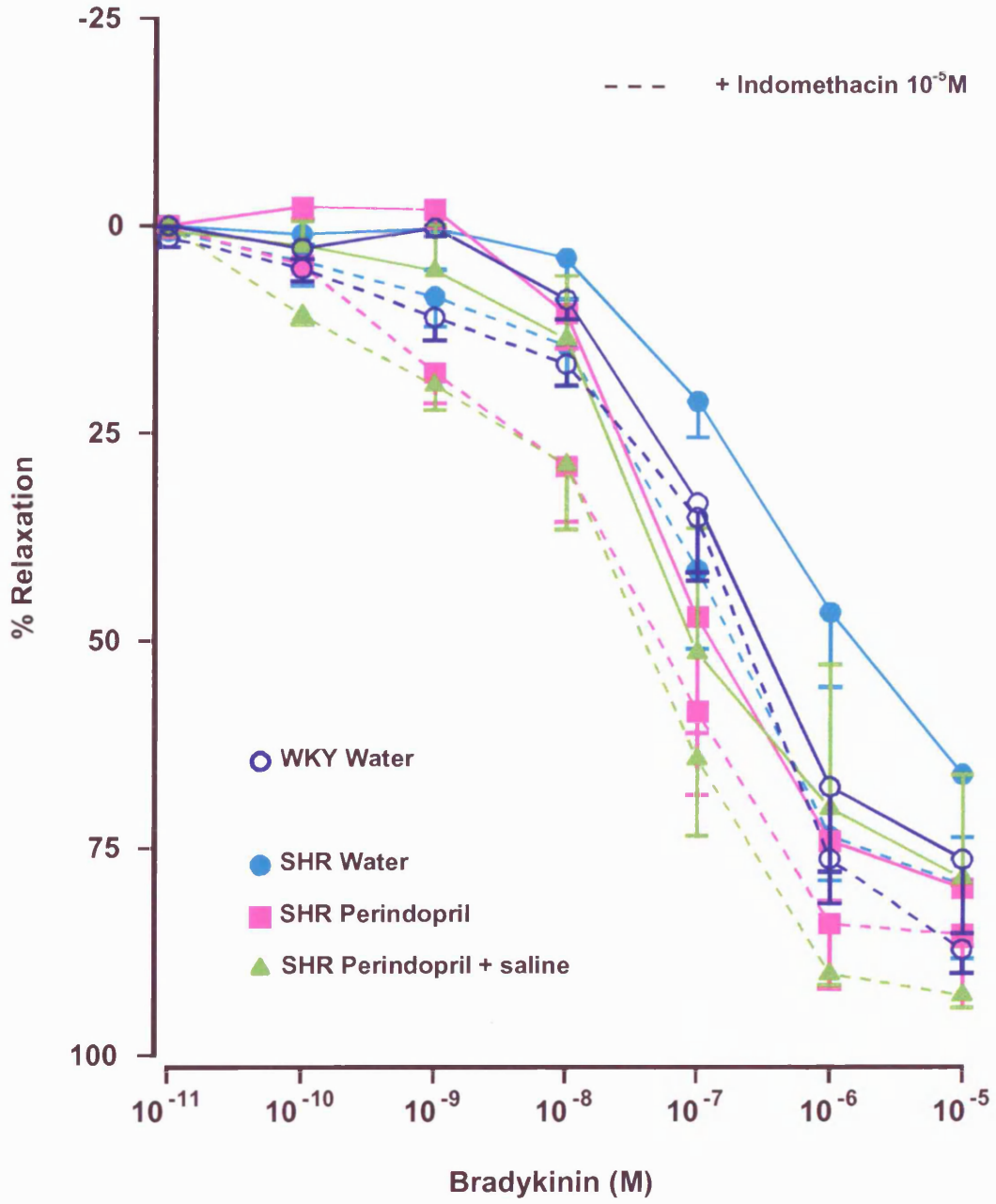


Figure 3.18 The relaxation response to bradykinin, in the absence and presence of indomethacin 10^{-5}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

Table 3.11. The effect of SQ29548 10^{-5}M on the relaxation to acetylcholine (ACh), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh EC_{50}(μM)		ACh Mean R (%)	
			Control	SQ	Control	SQ	Control	SQ
WKY	Water	8	95 ± 1.0	98 ± 0.9 *	0.02 ± 0.01 (6)	0.05 ± 0.01 (7)	51 ± 2.9	49 ± 2.5
SHR	Water	9	77 ± 8.0	77 ± 7.9	0.03 ± 0.01 (7)	0.03 ± 0.02 (5)	42 ± 8.0	39 ± 6.5
WKY	Perindopril **(P)**	7	91 ± 1.3	93 ± 2.8	0.10 ± 0.05		49 ± 2.7	45 ± 3.9
SHR	Perindopril **(P)**	8	82 ± 8.2	87 ± 9.0	0.03 ± 0.01 (4)	0.07 ± 0.05 (5)	49 ± 4.7	51 ± 7.2
WKY	1% saline & **P**	8	96 ± 0.8	98 ± 0.9	0.06 ± 0.01	0.09 ± 0.03 (6)	53 ± 1.8	49 ± 3.1
SHR	1% saline & **P**	7	92 ± 2.0	94 ± 2.2	0.08 ± 0.03	0.11 ± 0.04 (5)	55 ± 3.1	56 ± 5.1

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

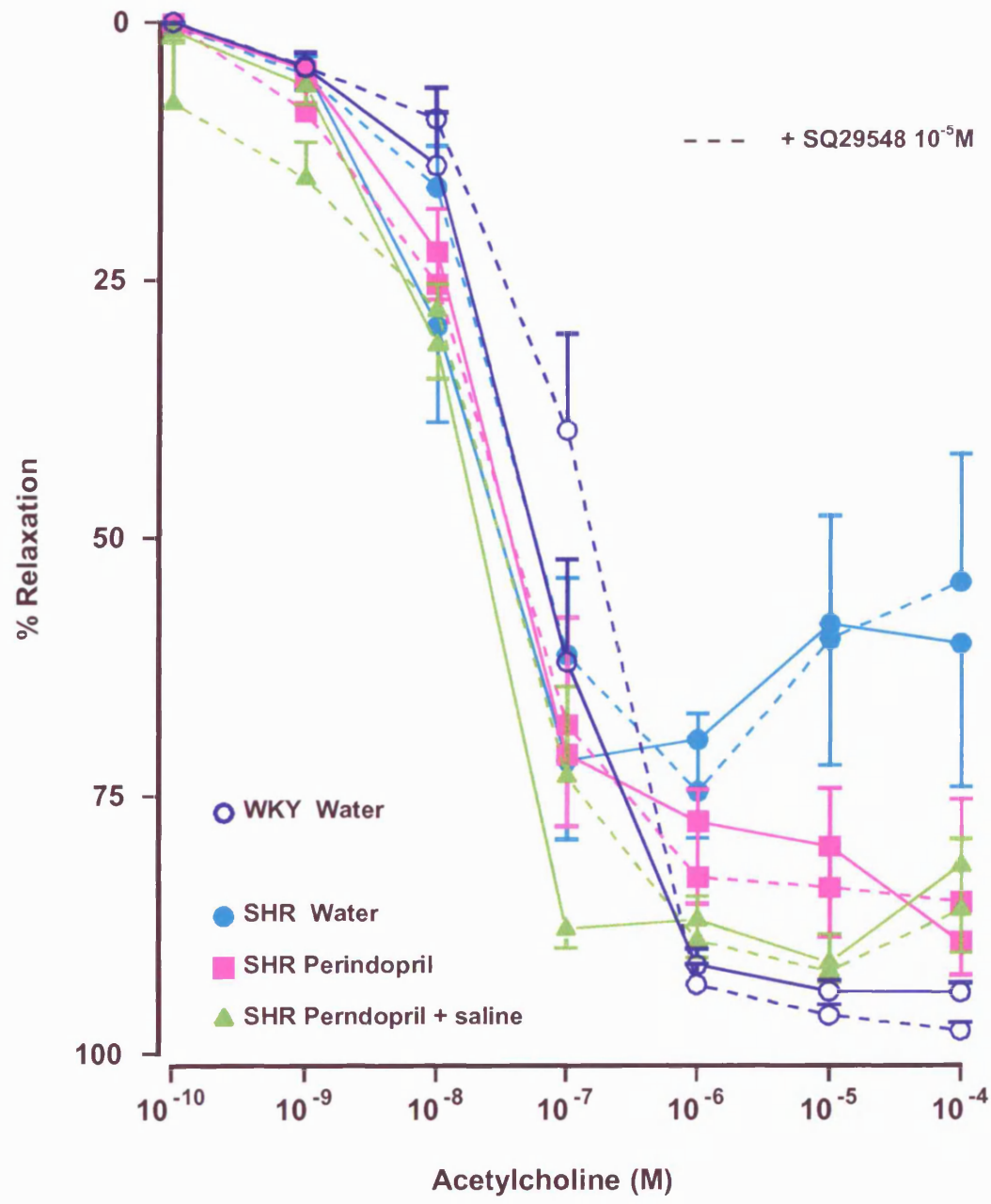


Figure 3.19 The relaxation response to acetylcholine, in the absence and presence of SQ29548 10^{-5}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

Table 3.12. The effect of SQ29548 10^{-5}M on the relaxation to bradykinin (BK), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	BK E max (%)		BK Mean R (%)	
			Control	SQ	Control	SQ
WKY	Water	8	54 ± 9.3	74 ± 7.7	11 ± 3.9	14 ± 7.2 *
SHR	Water	8	51 ± 9.1	48 ± 10.7	16 ± 6.2	5 ± 6.8 *
WKY	Perindopril **(P)**	7	63 ± 9.4	67 ± 5.6	18 ± 3.8	16 ± 4.2
SHR	Perindopril **(P)**	7	78 ± 8.9	73 ± 10.0	30 ± 5.4	26 ± 9.0
WKY	1% saline & **P**	8	45 ± 7.5	66 ± 7.5	9 ± 4.2	16 ± 5.1
SHR	1% saline & **P**	7	81 ± 3.2	78 ± 6.4	28 ± 3.6	20 ± 6.8

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$ T-test of BK relaxation compared to that in the absence of SQ29548.

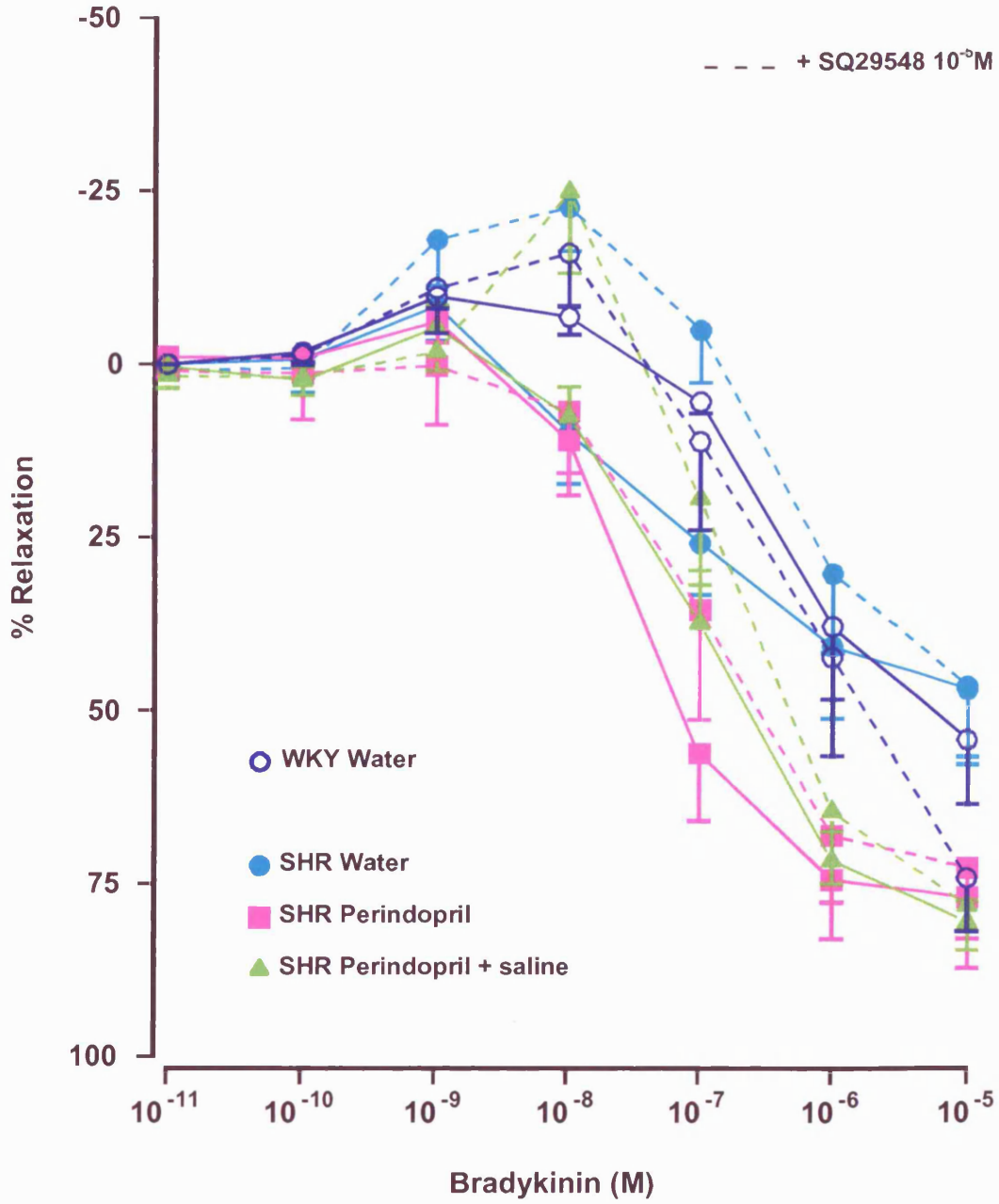


Figure 3.20 The relaxation response to bradykinin, in the absence and presence of SQ29548 10^{-5}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

Table 3.13. The effect of L-NOARG 10^{-4}M on the relaxation to acetylcholine (ACh), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)	
			Control	L-NOARG	Control	L-NOARG
WKY	Water	8	93 ± 1.3	87 ± 3.4	46 ± 3.8	36 ± 4.1
SHR	Water	10	87 ± 2.6	62 ± 8.0 *	44 ± 5.8	20 ± 6.4 *
WKY	Perindopril **(P)**	8	91 ± 3.2	68 ± 9.3 *	40 ± 3.4	24 ± 5.8 *
SHR	Perindopril **(P)**	8	89 ± 3.1	62 ± 9.8 *	47 ± 4.4	25 ± 7.3 *
WKY	1% saline & **P**	10	89 ± 3.6	64 ± 9.4 *	42 ± 4.8	27 ± 6.0
SHR	1% saline & **P**	9	80 ± 6.5	51 ± 8.9 *	35 ± 5.5	18 ± 7.2

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$ T-test of ACh relaxation compared to that in the absence of L-NOARG.

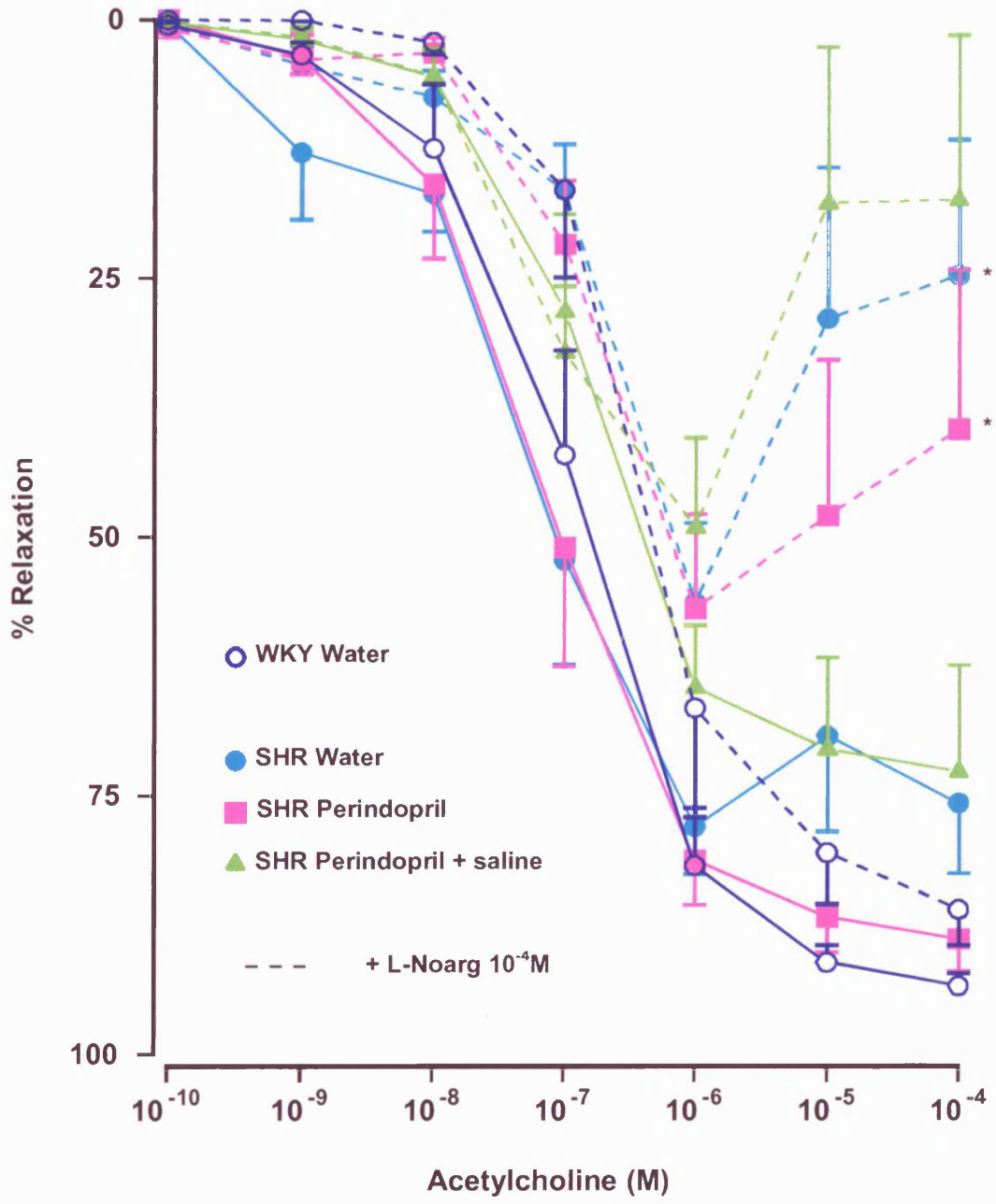


Figure 3.21 The relaxation response to acetylcholine, in the absence and presence of L-NOARG 10^{-4}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

* $P<0.05$ T-test of mean relaxation compared to that in the absence of L-NOARG.

Table 3.14. The effect of L-NOARG 10^{-4}M on the relaxation to bradykinin (BK), in mesenteric resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	BK E max (%)		BK Mean R (%)	
			Control	L-NOARG	Control	L-NOARG
WKY	Water	8	66 ± 8.0	56 ± 10.3	17 ± 3.9	11 ± 3.7
SHR	Water	10	67 ± 7.3	40 ± 9.5 *	20 ± 5.4	10 ± 4.4
WKY	Perindopril **(P)**	8	56 ± 8.8	32 ± 5.4 *	16 ± 4.6	6 ± 3.0
SHR	Perindopril **(P)**	8	81 ± 4.6	48 ± 11.4 *	33 ± 4.8	14 ± 5.5 *
WKY	1% saline & **P**	10	48 ± 8.6	24 ± 5.6 *	15 ± 4.4	6 ± 2.7
SHR	1% saline & **P**	8	64 ± 8.7	38 ± 10.0	22 ± 5.4	9 ± 4.8

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$ T-test of BK relaxation compared to that in the absence of L-NOARG

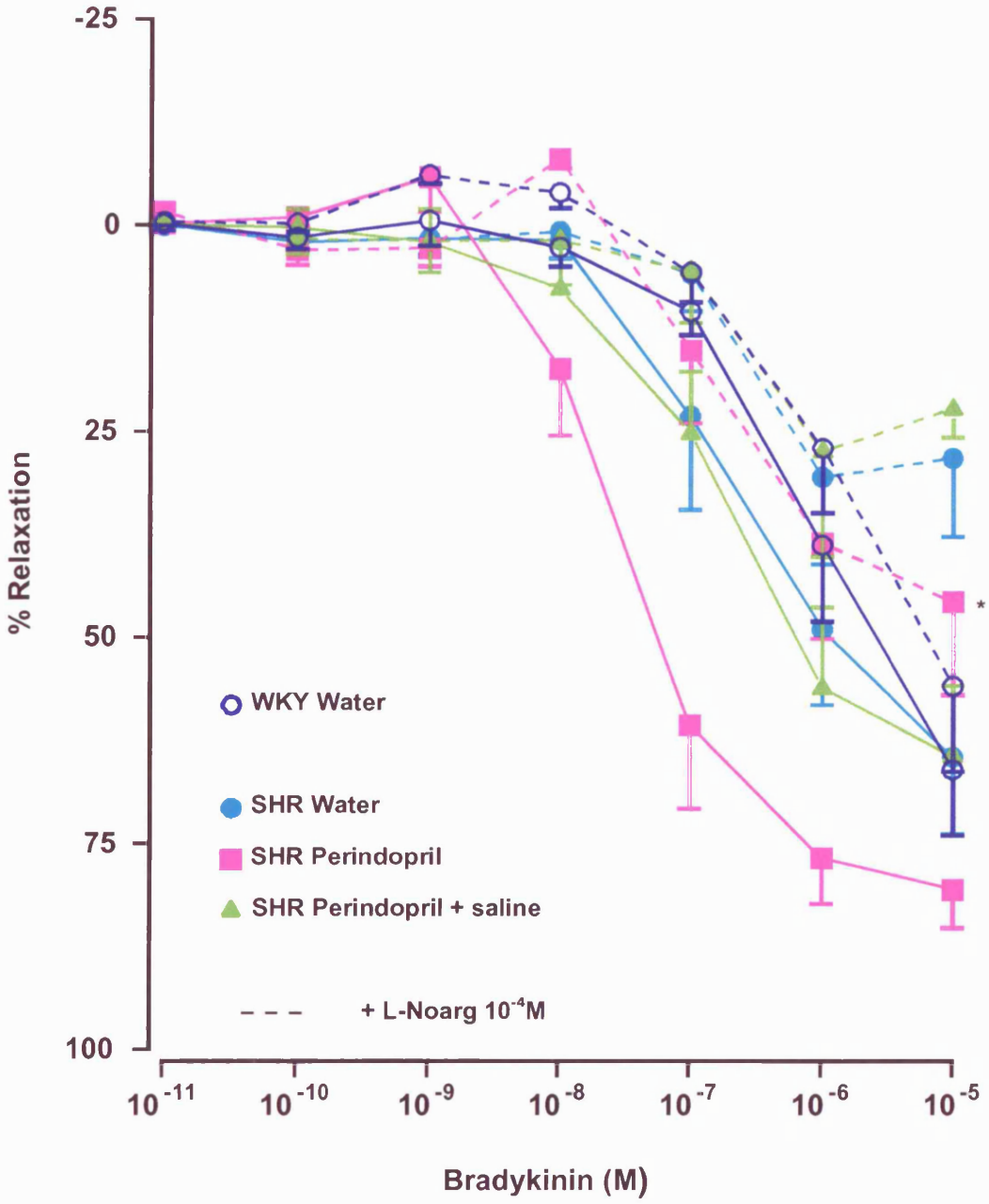


Figure 3.22 The relaxation response to bradykinin, in the absence and presence of L-NOARG 10^{-4}M, of mesenteric resistance arteries from 24 week SHR and WKY rats.

* P<0.05 T-test of mean relaxation compared to that in the absence of L-NOARG.

3.5.4 Femoral Resistance Artery Study

3.5.4.i Morphological Characteristics at 24 weeks

The normalised internal lumen diameter (LD) was similar in all femoral arteries at 24 weeks, although there was a trend towards a smaller LD in the SHRs (Table 3.15). The MCSA of both the untreated SHR (19,768 ± 599μm^2), and WKY femoral arteries (18,213 ± 760μm^2) (Table 3.15), were significantly greater than those observed in the 16 week untreated SHRs (17,948 ± 502μm^2) ($P<0.05$), and WKY rats (15,336 ± 316μm^2) ($P<0.01$) (Table 3.3). Similarly, at 24 weeks, the MT was significantly greater in the untreated SHR (25.49 ± 0.87μm), and WKY femoral arteries (20.87 ± 0.76μm), compared to the 16 week untreated SHRs (23.18 ± 0.61μm) ($P<0.05$), and WKY rats (18.62 ± 0.50μm) ($P<0.05$), at 16 weeks (Table 3.3). Because of the increased thickening in the 24 week WKY femoral artery, there was no significant difference between the MCSA and MT of the untreated SHR, compared to the untreated WKY femoral arteries (Table 3.15). However, there was a significant increase in the M:L of the femoral arteries from the 24 week untreated SHR , (11.86 ± 0.77%), compared to the untreated WKY rats (8.44 ± 0.54%) ($P<0.05$) (Table 3.15).

Perindopril treatment prevented the increase in MCSA and MT of the SHR femoral arteries, although the latter change in MT just failed to achieve significance compared to the untreated SHR. Perindopril treatment did not prevent the increase in M:L of the SHRs, because perindopril treatment had little effect on the LD of these rats. In addition, perindopril treatment reduced the MCSA and MT of the WKY femoral arteries, but did not alter the M:L of these rats. Treatment with perindopril and 1% saline had no influence of the development of vascular change in the SHR femoral arteries. However, there was a significant reduction in the MCSA of the WKY femoral arteries (15,231 ± 433μm^2), compared to the untreated WKY rats (18,213 ± 760μm^2) ($P<0.01$) (Table 3.15).

There were positive correlations between SBP and femoral artery MCSA ($r = 0.90$, $P<0.05$) (Figure 3.23), MT ($r = 0.93$, $P<0.01$) (Figure 3.24) and M:L ($r = 0.88$, $P<0.05$) (Figure 3.25), in all groups at 24 weeks.

Table 3.15. Femoral resistance artery morphology of untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Lumen Diameter (µm)	MCSA (μm^2)	Media Thickness (µm)	Media : Lumen (%)
WKY	Water	16	248 ± 7	18213 ± 760	20.87 ± 0.76	8.44 ± 0.54
SHR	Water	19	224 ± 7	19768 ± 599	25.49 ± 0.87	11.86 ± 0.77 *
WKY	Perindopril	18	264 ± 5	14436 ± 408 *** (19)	16.50 ± 0.44 *	6.41 ± 0.26
SHR	Perindopril	16	222 ± 7	16119 ± 672 +++	21.40 ± 0.90	10.11 ± 0.68
WKY	1% NaCl + Perindopril	18	236 ± 8	15231 ± 433 **	19.23 ± 0.49	8.39 ± 0.43
SHR	1% NaCl + Perindopril	16	216 ± 6 **	18829 ± 439	25.47 ± 0.64 *	12.45 ± 0.67 **

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated WKY rats

+++ P<0.001 ANOVA treated SHRs compared to untreated SHRs

with Bonferroni correction for multiple comparisons.

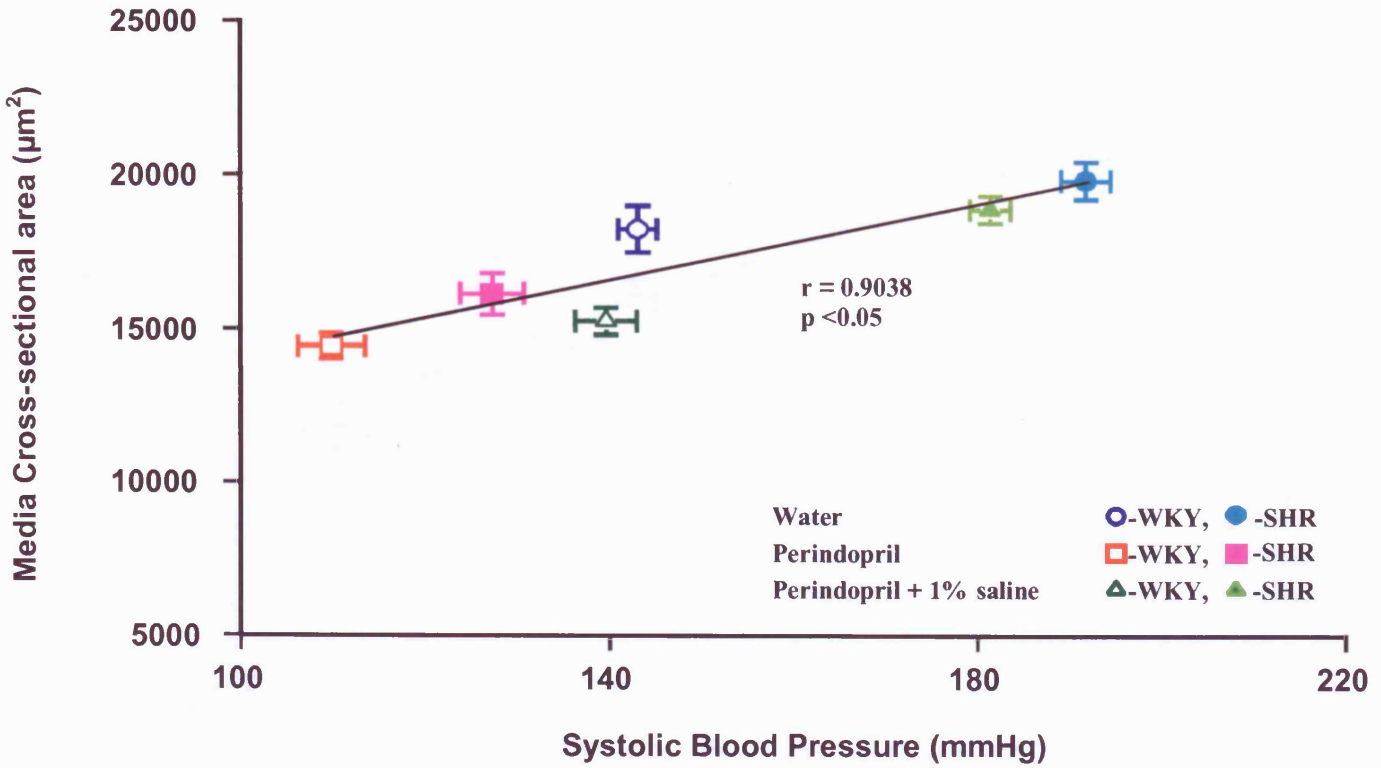


Figure 3.23. Line-graph showing media cross-sectional area versus systolic blood of femoral resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks.

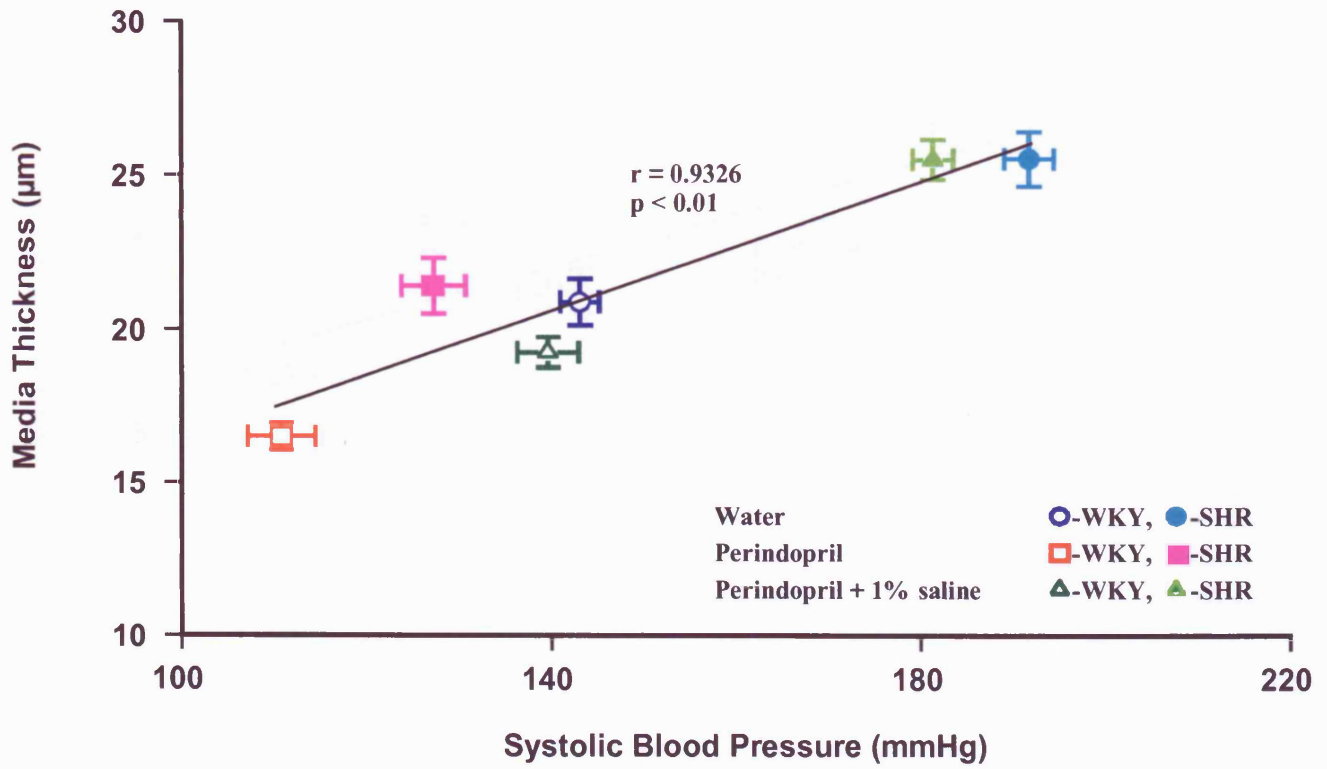


Figure 3.24. Line-graph showing medial thickness versus systolic blood of femoral resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks

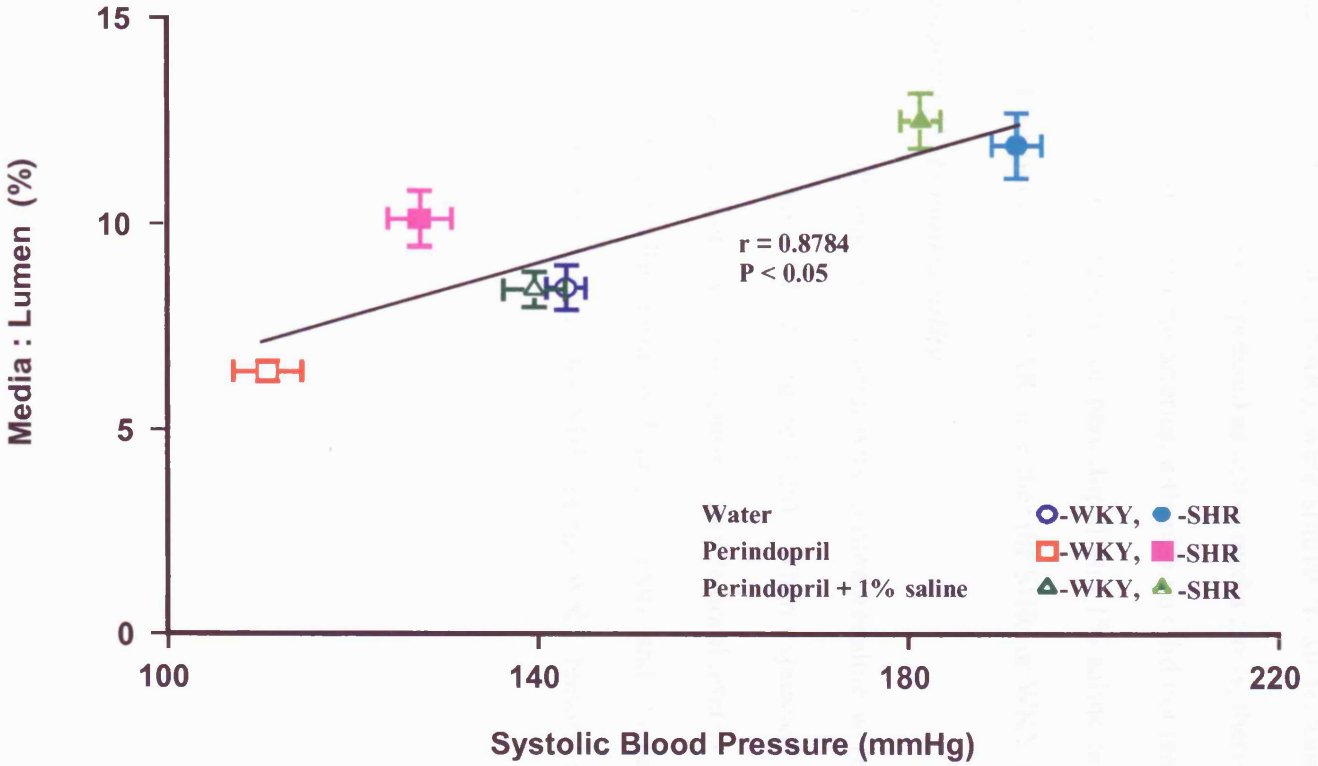


Figure 3.25. Line graph-showing media to lumen ratio versus systolic blood of femoral resistance arteries from untreated, perindopril treated, or perindopril and 1% saline treated SHR and WKY rats, at 24 weeks

3.5.4.ii Femoral Resistance Artery Contractility at 24 weeks

Activation procedure

The contractile responses generated to high potassium, and high potassium containing 10^{-5}M noradrenaline (NAK), were similar in all femoral arteries at 24 weeks (Table 3.16). However, when expressed as active media stress, there was a slight reduction in contractility of the SHR femoral arteries, although this did not reach significance (Table 3.16). Treatment with perindopril, or perindopril and 1% saline failed to alter the active media stress to high potassium or NAK, in either the SHR, or WKY femoral arteries.

Noradrenaline (NA) contractility

The active wall tension and sensitivity to noradrenaline were similar in all femoral arteries at 24 weeks (Table 3.17 & Figure 3.26). When expressed as active media stress, the response to noradrenaline was also similar in all femoral arteries studied, (Table 3.17 & Figure 3.27). Treatment with perindopril, or perindopril and 1% saline failed to alter the active media stress to NA of either the SHR, or the WKY femoral arteries (Table 3.17 & Figure 3.27).

Table 3.16. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline, of femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
WKY	Water	16	5.00 ± 0.29	5.79 ± 0.32	244 ± 16	281± 18
SHR	Water	19	4.63 ± 0.34	4.99 ± 0.36	187 ± 16	200 ± 15
WKY	Perindopril	18	4.23 ± 0.19	4.73 ± 0.20	260 ± 11	287± 11
SHR	Perindopril	16	4.44 ± 0.25	4.99 ± 0.26	212 ± 14	238 ± 15
WKY	1% NaCl + Perindopril	18	3.82 ± 0.42	4.31 ± 0.46	200 ± 22	225 ± 24
SHR	1% NaCl + Perindopril	16	4.61 ± 0.28	5.31 ± 0.29	183 ± 12	211 ± 13

Table 3.17. The maximum contractile response and sensitivity (EC_{50}), to noradrenaline of femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Maximum NA (mN/mm)	NA EC_{50} (µM)	Maximum NA active media stress (mN/mm^2)
WKY	Water	16	5.27 ± 0.26	4.02 ± 0.94	256 ± 14
SHR	Water	19	5.19 ± 0.38	3.76 ± 0.49	210 ± 17
WKY	Perindopril	18	4.15 ± 0.18	3.85 ± 0.59	255 ± 10 [18]
SHR	Perindopril	16	4.85 ± 0.27	3.88 ± 0.73	231 ± 14
WKY	1% NaCl + Perindopril	18	4.19 ± 0.43	2.63 ± 0.59	218 ± 23
SHR	1% NaCl + Perindopril	16	5.27 ± 0.27	3.44 ± 0.55	209 ± 12

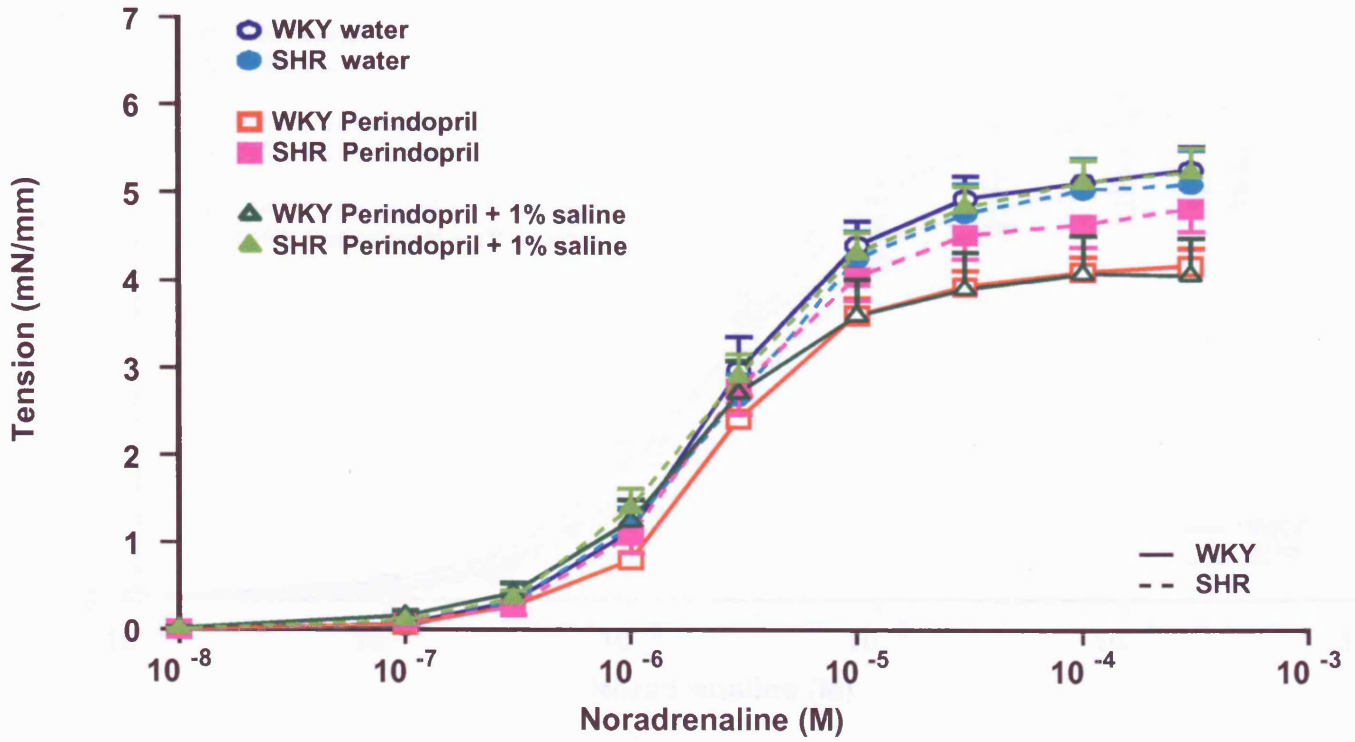


Figure 3.26. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of femoral resistance arteries from 24 week SHR and WKY rats.

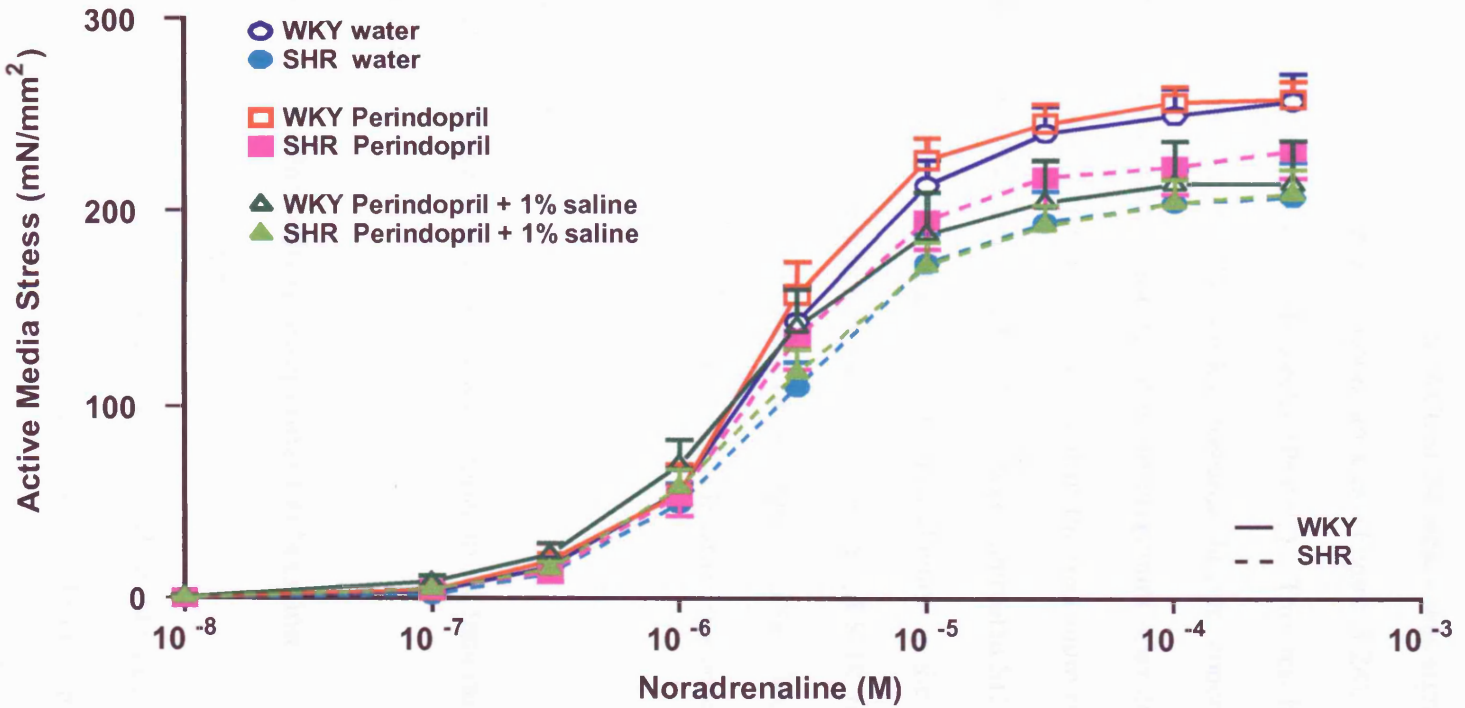


Figure 3.27. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2), in the presence of cocaine, of femoral resistance arteries from 24 week SHR and WKY rats.

3.5.4.iii Endothelium-dependent relaxation

Acetylcholine (ACh)

The relaxation response to ACh at 24 weeks was significantly reduced in both the untreated SHR, and WKY femoral arteries (Figure 3.28), compared to the responses observed in these groups at 16 weeks ($P<0.01$). This resulted a rightwards shift in the maximum relaxation at 24 weeks, towards higher concentrations of ACh (10^{-6}M), compared to the concentration resulting in maximum relaxation at 16 weeks (10^{-7}M). In addition, there was a significant reduction in the maximum relaxation of both the SHR at 24 weeks (28 ± 3.1%), compared to the 16 week untreated SHR (60.5 ± 5%) ($P<0.001$).

At 24 weeks, there was a significant reduction in the maximum relaxation of both the untreated, and perindopril with 1% saline treated SHR femoral arteries, compared to the untreated WKY rats (Table 3.18 & Figure 3.28). Treatment with perindopril, or perindopril and 1% saline did not alter the relaxation response of either the SHR, or WKY femoral arteries (Figure 3.28).

Bradykinin (BK)

The response to bradykinin was similar in all femoral arteries studied at 24 weeks (Figure 3.29).

3.5.3.iv Endothelium-independent relaxation

Sodium Nitroprusside (SNP)

The maximum relaxation response to SNP at 24 weeks was significantly reduced in the untreated SHR (74 ± 3.7) (Table 3.18), compared to the untreated 16 week SHRs (96 ± 1.4%) ($P<0.001$). A similar reduction was found in the maximum relaxation of the untreated WKY femoral arteries (86 ± 3.2) ($P<0.05$), compared to the untreated 16 week WKY rats (95 ± 1.1%). The sensitivity to SNP was also reduced at 24 weeks in the

untreated SHR femoral arteries (0.39 ± 0.10μm) (Table 3.18), compared to the sensitivity observed in this group at 16 weeks (0.14 ± 0.06μm) ($P<0.01$).

At 24 weeks, there was a significant reduction in the relaxation response to SNP in the untreated SHR femoral arteries, compared to the untreated WKY femoral arteries (Table 3.18 & Figure 3.30). Treatment with perindopril, or perindopril and 1% saline did not alter the relaxation response to SNP in either the SHR, or WKY femoral arteries.

Table 3.18. The maximum relaxation response to acetylcholine (ACh), and maximum relaxation and sensitivity (EC_{50}) to sodium nitroprusside (SNP), of femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	Maximum ACh (%)	Maximum SNP (%)	SNP EC_{50} (μM)
WKY	Water	16	43 ± 4.4	86 ± 2.3 (13)	0.27± 0.06 (13)
SHR	Water	19	28 ± 3.1 *	74 ± 3.7 * (16)	0.39 ± 0.10 (15)
WKY	Perindopril	18	39 ± 3.5	88 ± 1.9 (19)	0.22 ± 0.03 (19)
SHR	Perindopril	16	30 ± 5.2	82 ± 2.4	0.24 ± 0.05 (15)
WKY	1% NaCl + Perindopril	18	32 ± 4.5	83 ± 2.2 (15)	0.46 ± 0.18 (15)
SHR	1% NaCl + Perindopril	16	25 ± 4.0 * (15)	82 ± 3.1 (12)	0.40 ± 0.09 (12)

* $P<0.05$ ANOVA compared to untreated WKY rats with Bonferroni correction for multiple comparisons.

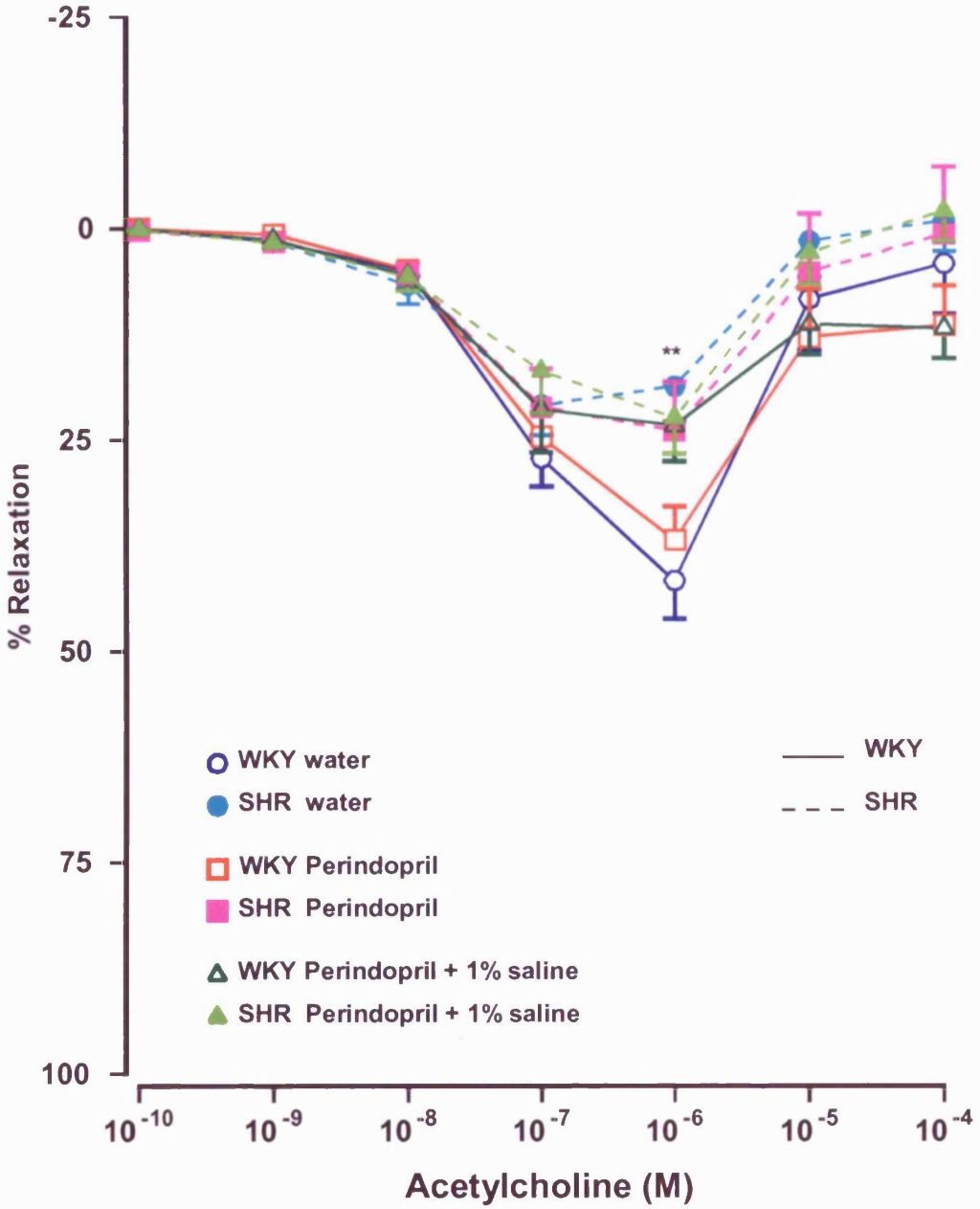


Figure 3.28. The relaxation response to acetylcholine of femoral resistance arteries from 24 week SHR and WKY rats.

** P<0.01 ANOVA compared to untreated WKY rats

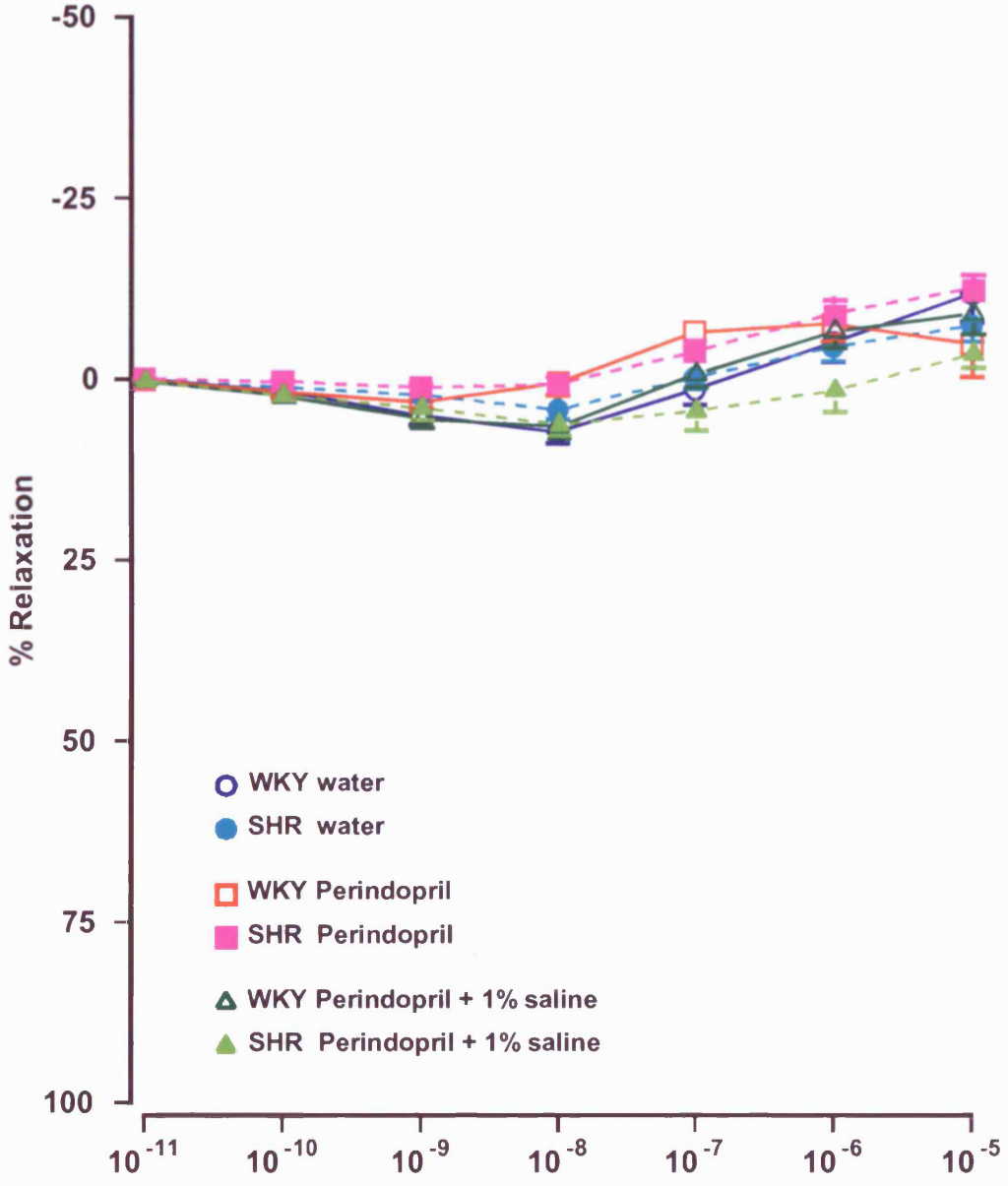


Figure 3.29. The relaxation response to bradykinin of femoral resistance arteries from 24 week SHR and WKY rats.

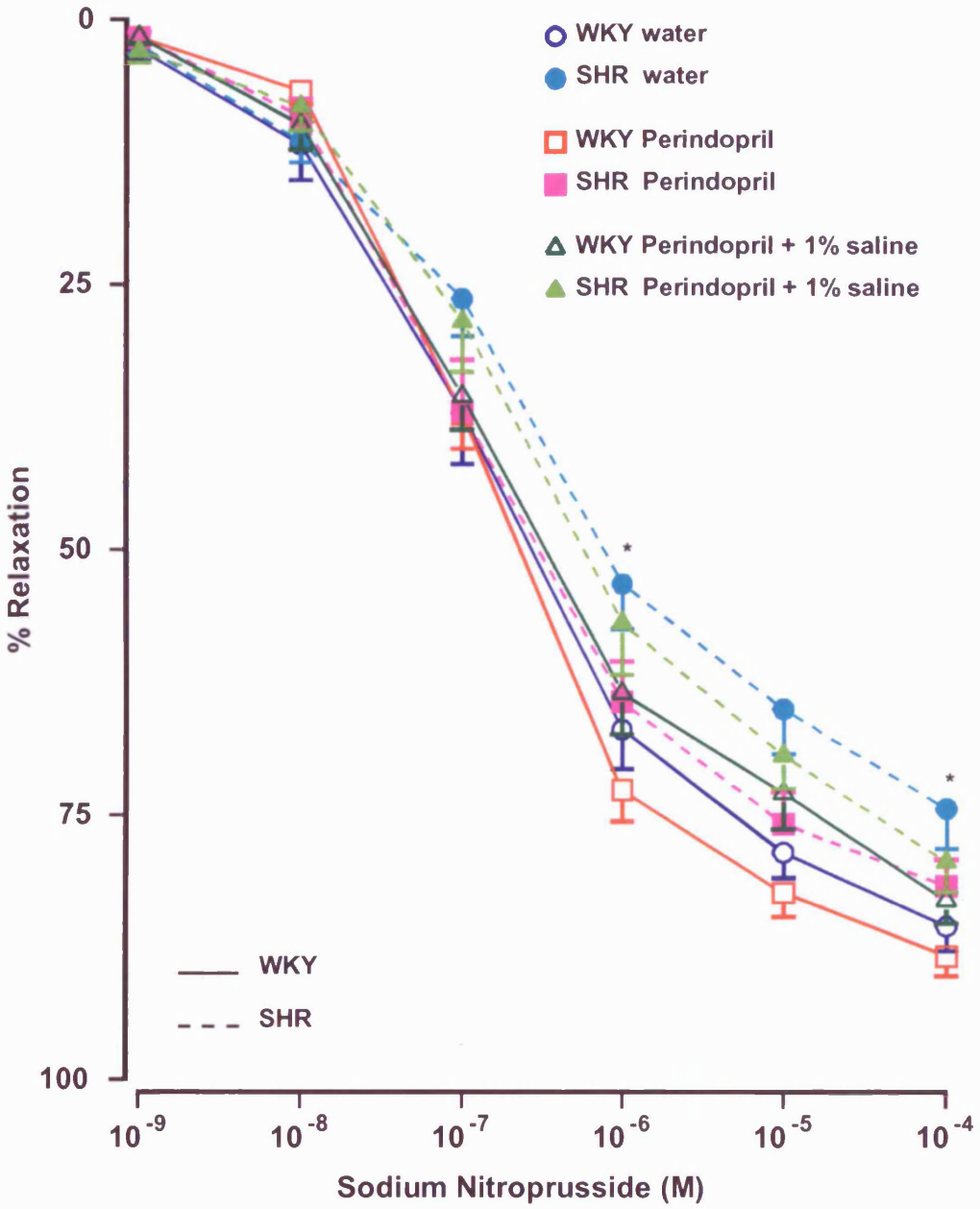


Figure 3.30. The relaxation response to sodium nitroprusside of femoral resistance arteries from 24 week SHR and WKY rats.

* $P<0.05$ ANOVA compared to untreated WKY rats

3.5.5. Femoral Resistance Artery EDRF Characterisation

3.5.5.i Indomethacin

Incubation with indomethacin significantly improved the relaxation response to acetylcholine (Table 3.19 & Figure 3.31) in all femoral arteries studied, but had little effect on the response to bradykinin in these arteries (Table 3.19 & Figure 3.32). Thus, suggesting that the re-contraction observed in the femoral artery is due to the action of cyclo-oxygenase vasoconstrictor prostanoids. However, there appeared to be little contribution of these prostanoids in the response of the femoral artery to bradykinin.

3.5.5.ii SQ29548

Incubation with SQ29548 improved the relaxation response to acetylcholine in all arteries, although this did not quite reach significance in the untreated, or saline treated SHRs (Table 3.20 & Figure 3.33). Thus, suggesting that thromboxane A_2 and prostaglandin H_2 are likely to be involved in the normal re-contraction response to ACh in the femoral artery. Incubation with SQ29548 had little effect on the femoral artery response to bradykinin, with the exception of the perindopril treated SHR femoral arteries. There was a significant reduction, as a result of treatment, in the contractile response of these arteries to the highest concentrations of bradykinin (Table 3.20 & Figure 3.34).

3.5.5.iii L-NOARG

Incubation with L-NOARG reduced the maximum relaxation of the untreated WKY rats, and the perindopril treated SHRs to acetylcholine, although this did not reach significance (Table 3.21 & Figure 3.35). The contractile response to higher concentrations of bradykinin was found to be reduced after NO synthesis blockade (Table 3.21 & Figure 3.36), possibly indicating a reduction in the ability of the femoral arteries to contract in the presence of L-NOARG. This reduced contractile ability is supported by a reduction in the re-contraction to ACh in the femoral artery after incubation with L-NOARG. These results

may suggest that relaxation of the WKY femoral artery is partially dependent upon EDNO, whilst there could be less contribution of EDNO to relaxation in the untreated SHR femoral arteries.

Table 3.19. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh) or bradykinin (BK), in femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)		BK Mean R (%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
WKY	Water	8	30 ± 5.8	71 ± 4.4 ***	6 ± 3.7	41 ± 3.6 ***	1 ± 1.9 (7)	6 ± 4.0 (7)
SHR	Water	7	17 ± 4.1	59 ± 8.7 **	2 ± 3.8	32 ± 5.9 **	-3 ± 3.7	-2 ± 4.9
WKY	Perindopril **(P)**	7	35 ± 7.1	79 ± 5.1 ***	13 ± 4.6	45 ± 3.6 ***	-1 ± 2.6	-1 ± 3.1
SHR	Perindopril **(P)**	8	15 ± 4.4	65 ± 4.7 ***	2 ± 3.1	35 ± 3.5 ***	-4 ± 2.0	-2 ± 2.4
WKY	1% saline & **P**	9	31 ± 7.5	71 ± 3.8 ***	9 ± 3.8	39 ± 4.4 ***	-3 ± 2.3	-1 ± 3.6
SHR	1% saline & **P**	7	24 ± 6.4	59 ± 5.5 **	7 ± 4.1	31 ± 4.7 **	3 ± 2.2	1 ± 4.5

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* P<0.05, ** P<0.01,*** P<0.001 T-test of ACh or BK relaxation compared to that in the absence of indomethacin.

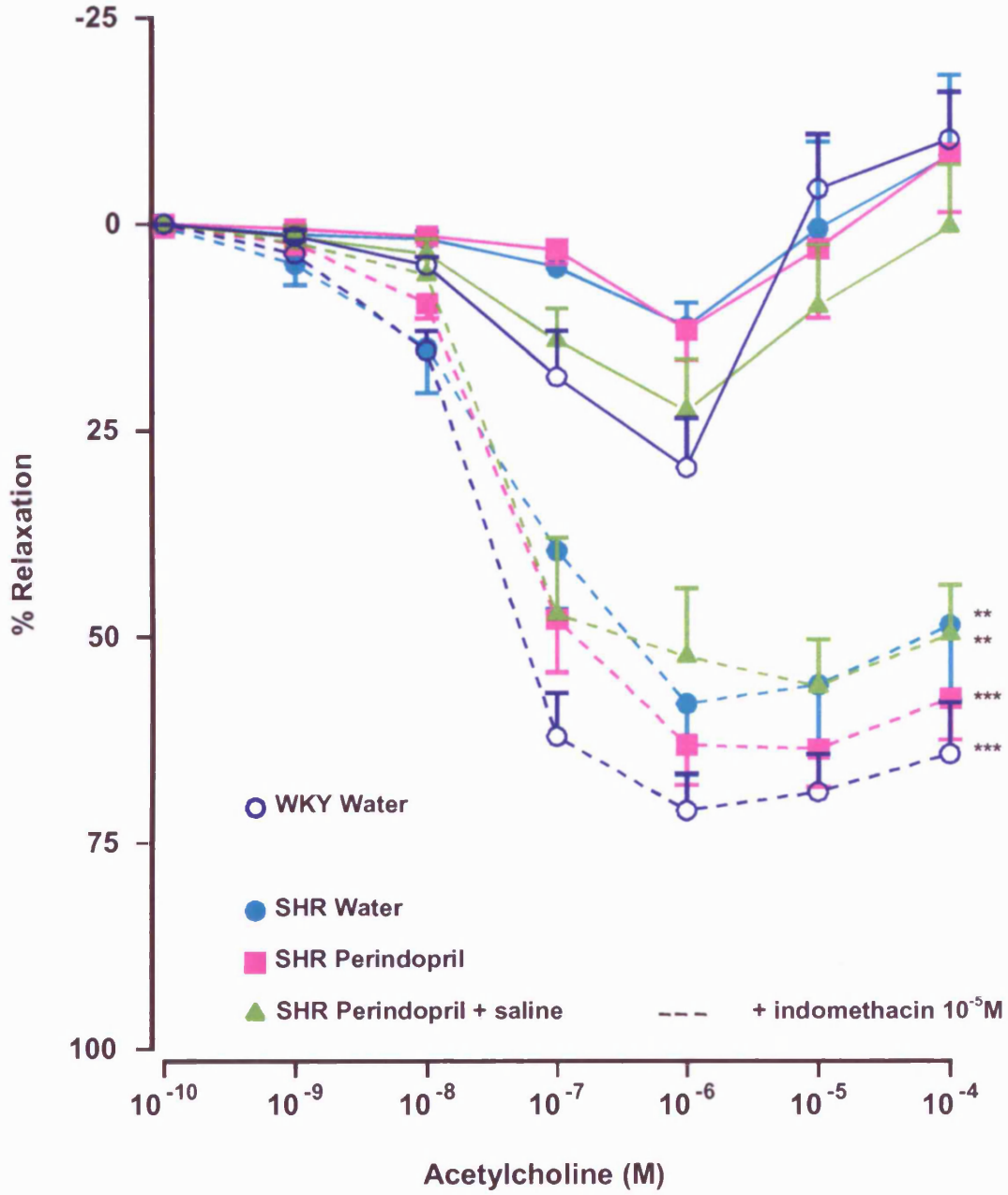


Figure 3.31 The relaxation response to acetylcholine, in the absence and presence of indomethacin 10^{-5}M, of femoral resistance arteries from 24 week SHR and WKY rats.

** P<0.01, *** P<0.001 T-test of mean relaxation compared to that in the absence of indomethacin.

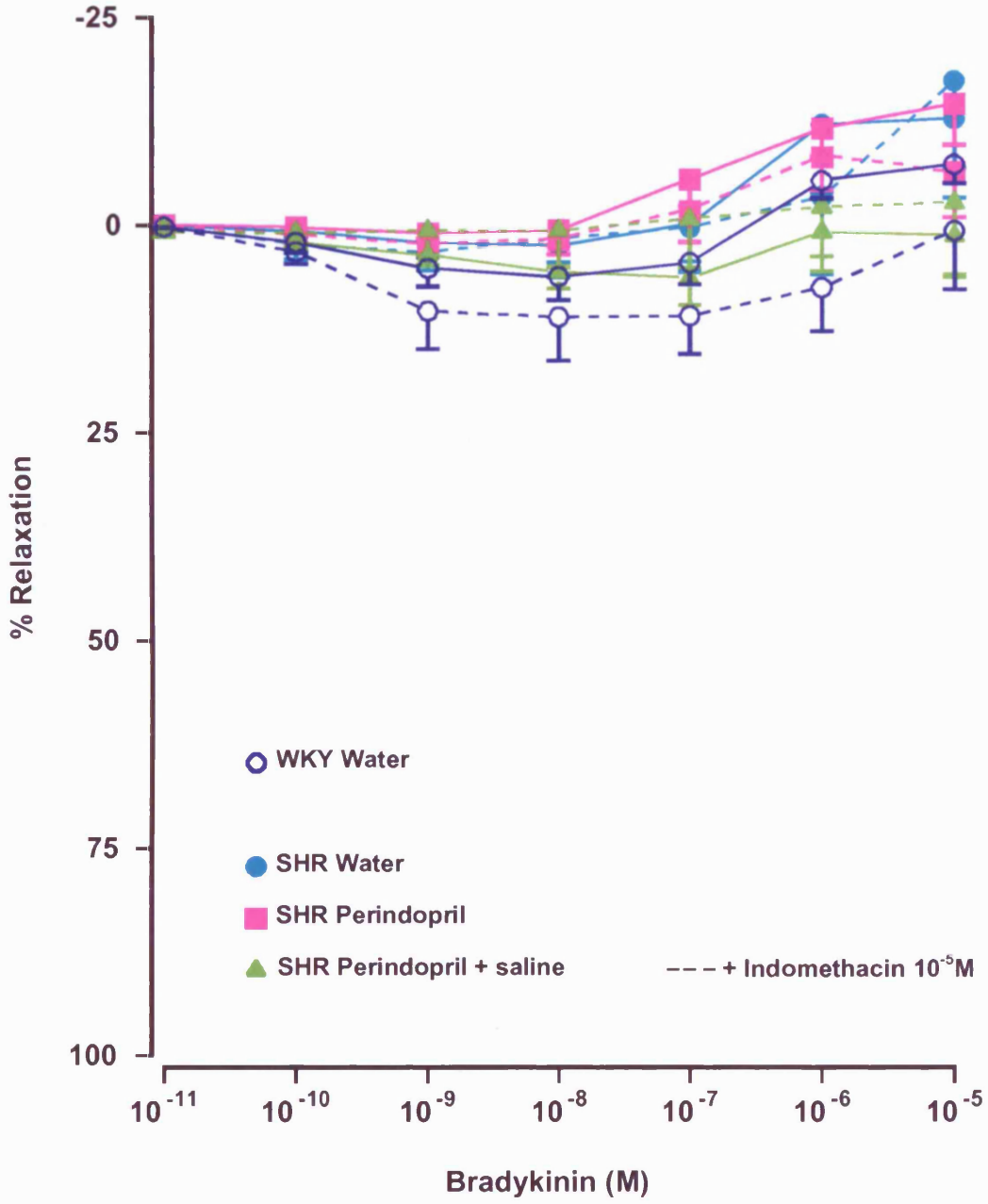


Figure 3.32 The relaxation response to bradykinin, in the absence and presence of indomethacin 10^{-5}M, of femoral resistance arteries from 24 week SHR and WKY rats.

Table 3.20. The effect of SQ29548 10^{-5}M on the relaxation to acetylcholine (ACh) or bradykinin (BK), in femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)		BK Mean R (%)	
			Control	SQ	Control	SQ	Control	SQ
WKY	Water	7	38 ± 7.2	63 ± 4.2 *	18 ± 3.9	32 ± 2.8 **	2 ± 2.1	5 ± 3.0
SHR	Water	9	50 ± 6.3	53 ± 6.5	13 ± 5.6	23 ± 5.2	1 ± 2.6	-3 ± 4.7
WKY	Perindopril **(P)**	9	43 ± 3.7	68 ± 4.9 **	18 ± 3.2	34 ± 3.4 **	-4 ± 2.1 (8)	-1 ± 3.1 (8)
SHR	Perindopril **(P)**	8	36 ± 3.0	55 ± 4.0 **	8 ± 3.4	29 ± 3.0 ***	-4 ± 2.1	4 ± 2.7 *
WKY	1% saline & **P**	9	49 ± 5.7	65 ± 3.3 *	20 ± 4.6	34 ± 3.7 *	-2 ± 2.6	2 ± 3.4
SHR	1% saline & **P**	7	41 ± 8.4	48 ± 6.8	11 ± 3.8	23 ± 4.4	1 ± 2.0	3 ± 4.1

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ T-test of ACh or BK relaxation compared to that in the absence of SQ29548.

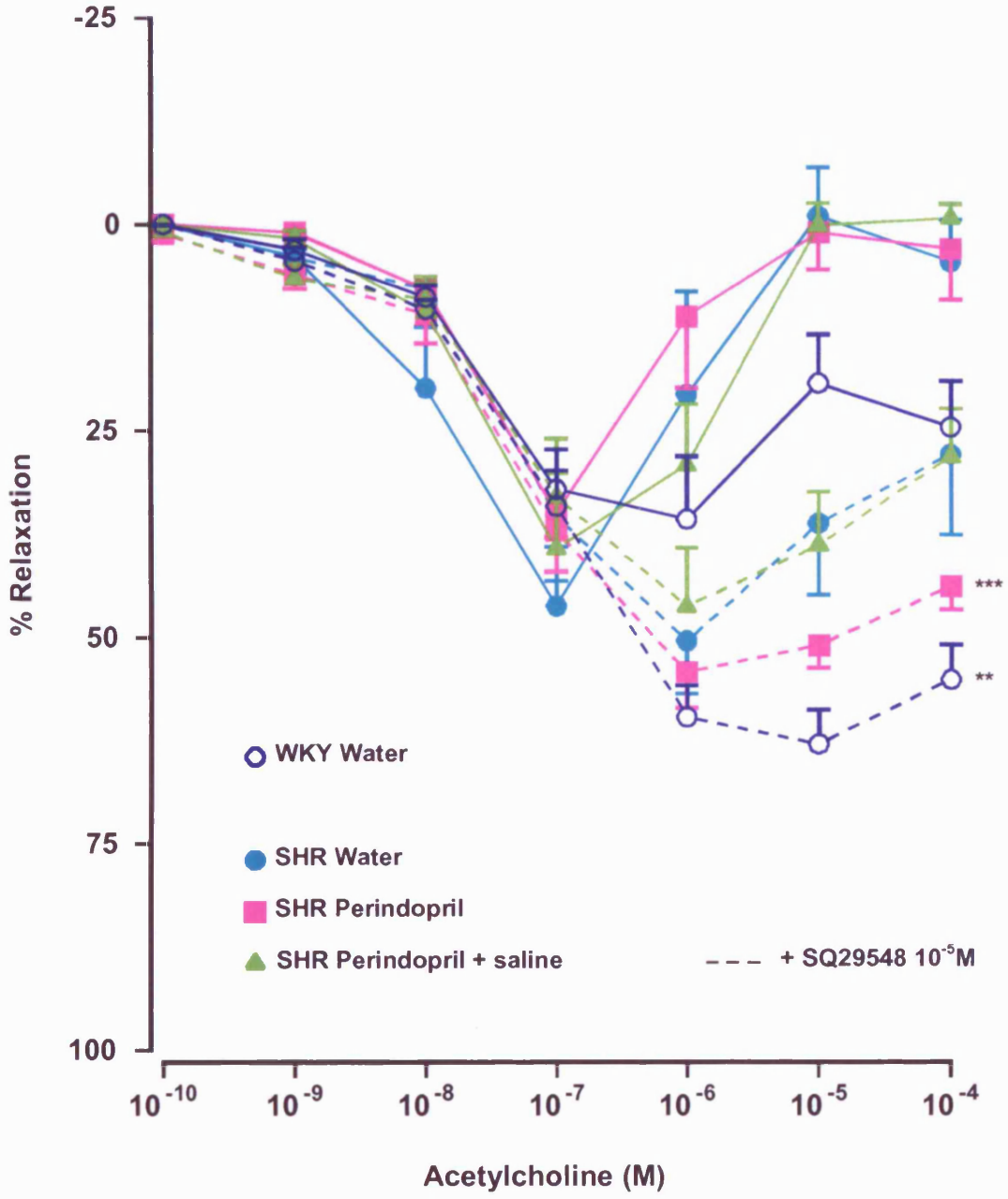


Figure 3.33 The relaxation response to acetylcholine, in the absence and presence of SQ29548 10^{-5}M, of femoral resistance arteries from 24 week SHR and WKY rats.

** P<0.01, *** P<0.001 T-test of mean relaxation compared to that in the absence of SQ29548.

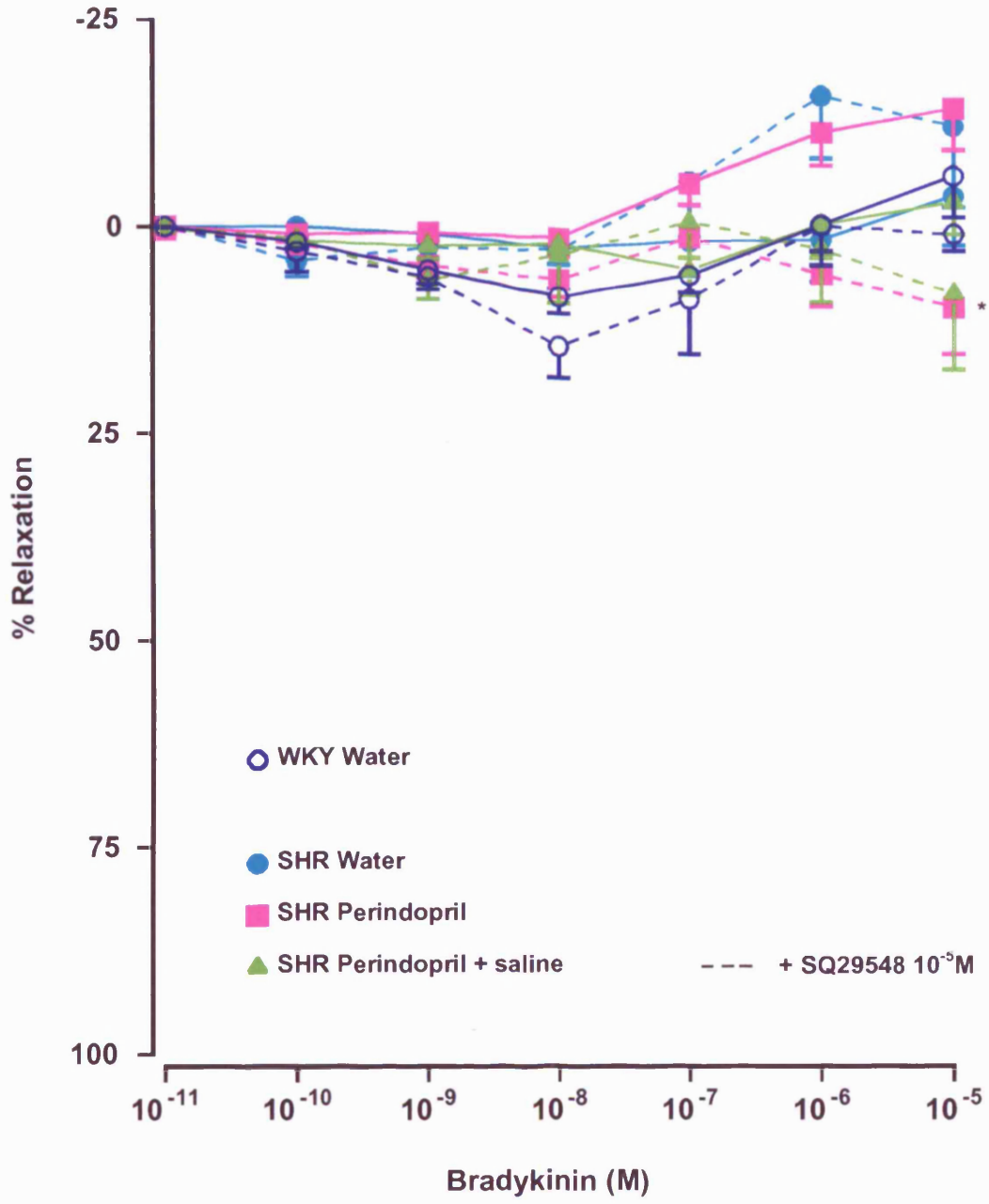


Figure 3.34 The relaxation response to bradykinin, in the absence and presence of SQ29548 10^{-5}M, of femoral resistance arteries from 24 week SHR and WKY rats.

* P<0.05, *** P<0.001 T-test of mean relaxation compared to that in the absence of SQ29548.

Table 3.21. The effect of L-NOARG 10^{-4}M on the relaxation to acetylcholine (ACh) or bradykinin (BK), in femoral resistance arteries from untreated, perindopril treated, or perindopril with 1% saline treated 24 week SHR and WKY rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)		BK Mean R (%)	
			Control	L-NOARG	Control	L-NOARG	Control	L-NOARG
WKY	Water	5	50 ± 7.2	30 ± 6.4	8 ± 4.7	14 ± 3.6	-1 ± 3.3 (4)	9 ± 5.0 (4)
SHR	Water	8	24 ± 4.5	28 ± 7.5	6 ± 2.9	11 ± 3.5	-1 ± 1.6	8 ± 3.0 (7) *
WKY	Perindopril **(P)**	10	42 ± 5.9	31 ± 5.4	9 ± 3.8	11 ± 2.7	-3 ± 2.3	-1 ± 3.8
SHR	Perindopril **(P)**	8	38 ± 9.5	17 ± 3.4	12 ± 7.4	6 ± 2.3	-2 ± 1.6	4 ± 2.0 (7) *
WKY	1% saline & **P**	9	26 ± 7.1	23 ± 8.8	7 ± 3.9	10 ± 4.3	1 ± 2.7	3 ± 2.6 (7)
SHR	1% saline & **P**	5	16 ± 2.4	20 ± 8.2	1 ± 3.2	8 ± 5.7	-1 ± 2.2	5 ± 3.6 (4)

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$ T-test of ACh or BK relaxation compared to that in the absence of L-NOARG.

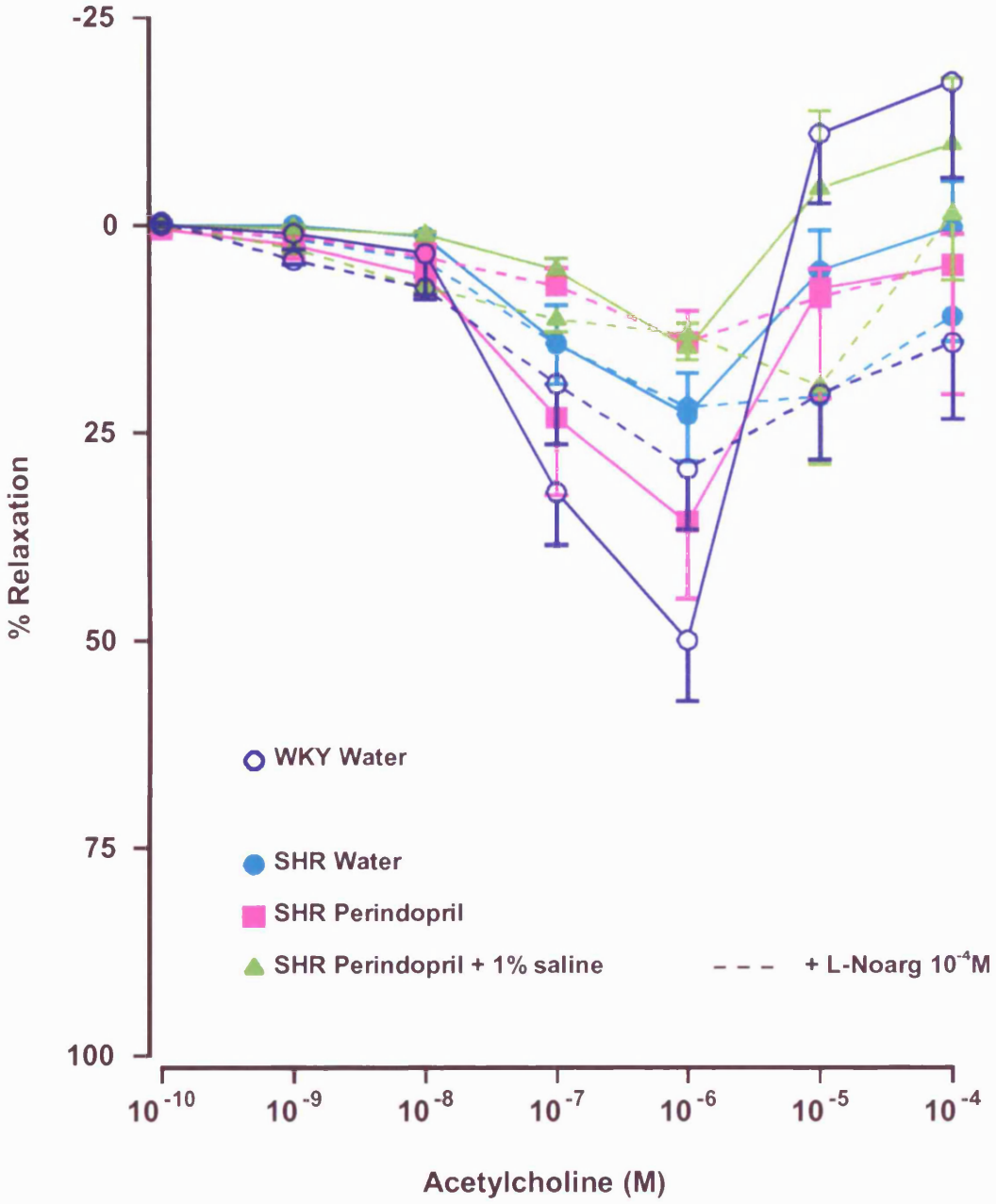


Figure 3.35 The relaxation response to acetylcholine, in the absence and presence of L-NOARG 10^{-4}M, of femoral resistance arteries from 24 week SHR and WKY rats.

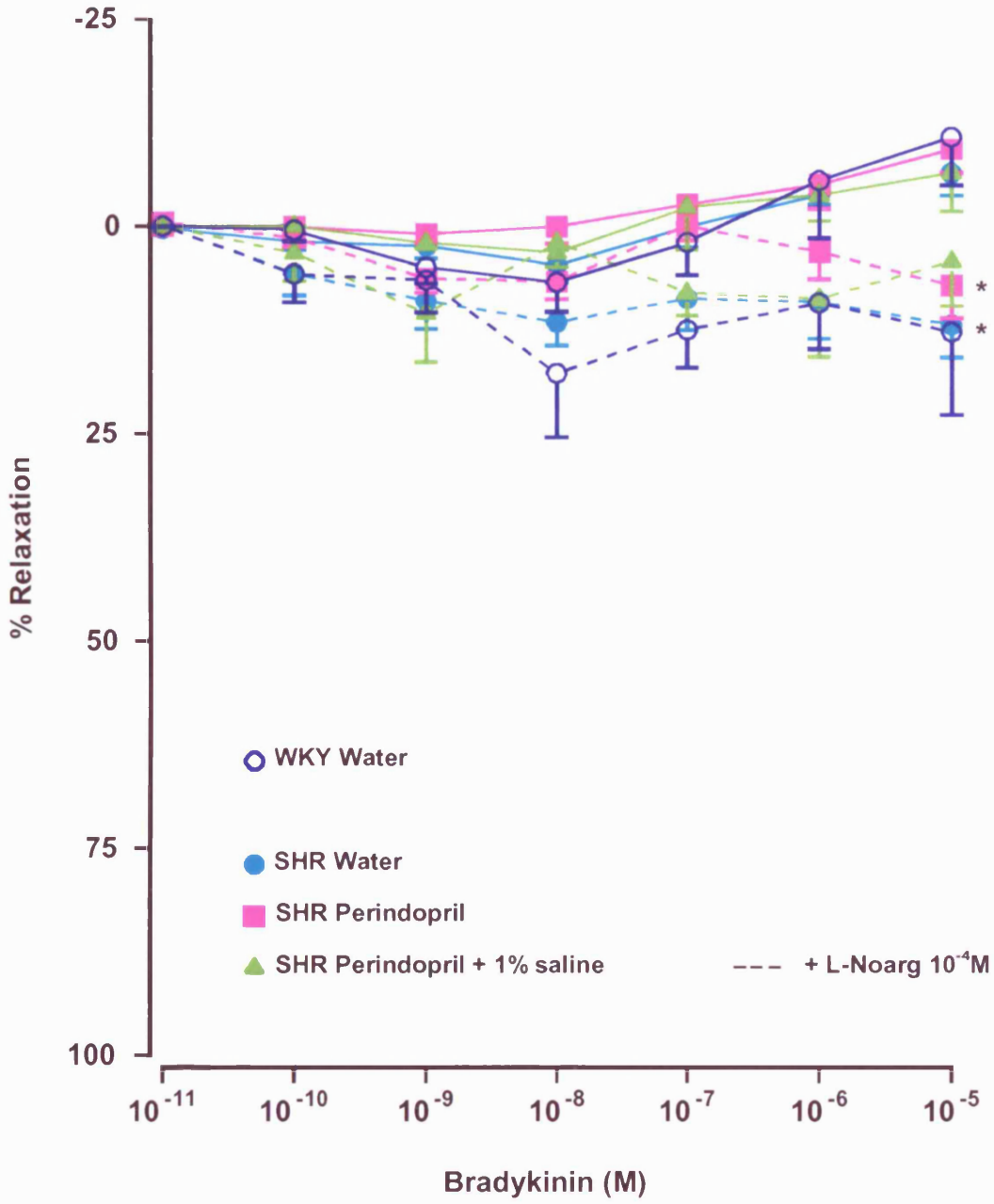


Figure 3.36 The relaxation response to bradykinin, in the absence and presence of L-NOARG 10^{-4}M, of femoral resistance arteries from 24 week SHR and WKY rats.

* $P<0.05$ T-test of mean relaxation compared to that in the absence of L-NOARG.

3.5.6 Summary of 24 week results

3.5.6.i Physical Characteristics

The systolic blood pressure was significantly raised in the untreated SHRs at 24 weeks. Treatment with perindopril prevented the development of hypertension in the SHRs, and reduced the SBP of the SHR and WKY rats to levels below that of the normotensive WKY rats. Treatment of the SHRs with 1% saline overcame the effect of perindopril treatment and resulted in a significantly elevated blood pressure in these rats.

The body weight was lower in all SHRs, and was further reduced by perindopril treatment.

There was no evidence of cardiac hypertrophy in the untreated SHRs at 24 weeks. Perindopril treatment reduced the cardiac structure in both the SHRs and WKY rats. As also observed at 16 weeks, this reduction was not found with perindopril in the presence of 1% saline.

3.5.6.ii Resistance Artery Structure

The internal lumen diameter was smaller in all SHR resistance arteries at 24 weeks, although this only gained significance in the mesenteric arteries. Treatment with perindopril or perindopril and 1% saline did not alter the lumen diameter of the resistance arteries.

The resistance artery structure was increased in the untreated SHRs at 24 weeks, although this increase was not significant for the media cross-sectional area of both arteries, or for the media thickness of the femoral artery. This lack of significance could be the result of further increases in structure at 24 weeks, in both the SHRs, and WKY rats, compared to the structure at 16 weeks. Treatment with perindopril prevented the development of structural alterations in the SHR resistance arteries, and reduced the

structure of the normotensive WKY rats. Treatment with perindopril and 1% saline failed to prevent the development of structure in the SHR resistance arteries.

3.5.6.iii Resistance Artery Contractility

The response of the resistance arteries to contractile agents was not significantly altered in the untreated SHRs at 24 weeks. However, there was a slight increase in the active tension of the hypertensive mesenteric arteries, which was prevented by perindopril treatment.

3.5.6.iii Resistance Artery Relaxation

The endothelial-dependent relaxation to acetylcholine and bradykinin was reduced in the untreated SHR and WKY resistance arteries at 24 weeks, compared to the relaxation responses at 16 weeks. Similarly, there was a reduction in the endothelial independent relaxation to sodium nitroprusside at 24 weeks, compared to the response at 16 weeks.

The endothelial-dependent relaxation to acetylcholine was reduced in the untreated SHR mesenteric, and femoral arteries. The reduced response in the hypertensive mesenteric artery was likely due to increased release or the preponderance of vasoconstrictor prostanoids . In contrast the reduced response to ACh in the femoral artery appeared related to a reduction in the EDNO component of relaxation. Treatment with perindopril prevented the impaired relaxation response to ACh in the mesenteric arteries, but had little effect on the impaired ACh response of the femoral arteries. In addition, treatment with perindopril and 1% saline slightly improved the relaxation to acetylcholine in the mesenteric artery. The endothelial-dependent relaxation to bradykinin was unaltered by hypertension, although perindopril treatment improved the response to bradykinin in the SHR mesenteric arteries. This was likely due to increased production or release of EDNO.

The endothelial-independent relaxation to sodium nitroprusside was unaltered in the mesenteric arteries. However, the response to sodium nitroprusside was reduced in the untreated SHR femoral artery, suggesting a possible reduction in the ability of the femoral artery smooth muscle cells to respond to nitric oxide. Treatment with perindopril prevented this reduced response, whereas perindopril and 1% saline had little effect.

3.6 DISCUSSION

The present study confirmed the gradual rise of systolic blood in the spontaneously hypertensive rat (SHR) (Smeda & Lee, 1988; Bennett, *et al.*, 1996), reaching a plateau at between 12 and 16 weeks. Perindopril administration not only prevented the development of hypertension in the SHR, but also resulted in the blood pressure of the treated SHR falling to levels below that of the untreated normotensive WKY rats. These observations complement those of Christensen et al (1989), using a similar perindopril treatment regime of 1.5mg/kg per day, in the SHR from 4 to 24 weeks (Christensen, *et al.*, 1989). Also, similar results have been obtained after treatment of SHRs with other ACE inhibitors (Heagerty, 1991; Korner & Bobik, 1995a; Safar *et al.*, 1995). However, it is of interest that the present study showed that perindopril administration caused a significant blood pressure fall in the normotensive WKY rats.

The antihypertensive response to perindopril was overcome by salt administration in the drinking water. However, there was an initial delay in the development of hypertension, and the final systolic blood pressure did not achieve that of the untreated SHR. This is in keeping with the view that there is an initial phase, in the development of hypertension, during which the SHR is resistant to salt loading (Ledingham & Olaf Simpson, 1984). However, an increased salt appetite and susceptibility to high dietary salt are inherent features of this genetic model of hypertension (Ledingham & Olaf Simpson, 1984; Ledingham, 1989; Yamori & Swales, 1994). The systolic blood pressure of the WKY rats was unaffected by salt administration, confirming that the WKY was less susceptible to salt loading (Ledingham & Olaf Simpson, 1984).

The body weight of the SHRs used in this study was lower compared to the WKY strain, a commonly reported finding with this model of hypertension (Sharifi, *et al.*, 1998).

However, by 24 weeks, the difference between the body weight of the two strains was smaller, suggesting that SHRs continued to grow slowly achieving their final adult body weight at a later age. Although, perindopril had a beneficial effect on blood pressure, it had an adverse effect on the rate of weight gain in both the SHR and WKY rats. Black and colleagues observed that ACE inhibitor treatment attenuated weight gain in the WKY rat (Black *et al.*, 1997), and this was attributed to differences in drug metabolism in the SHR, and WKY, with increased bioavailability in the WKY rat. However, this suggestion cannot account for the reduced weight gain observed in both strains in the present study. As a consequence, the mechanism by which perindopril inhibited growth is at present unclear. Also, other studies report little alteration in body weight after treatment with perindopril (Thybo, *et al.*, 1994; Bennett, *et al.*, 1996). The inconsistencies between the studies may relate to variations in dosage, the age at which treatment was initiated, and the length of treatment. Alternatively, they could reflect slight genetic variations between different WKY and SHR colonies (Johnson *et al.*, 1992; St.Lezin *et al.*, 1992).

In contrast to other studies (Clozel *et al.*, 1989; Morton, *et al.*, 1990; Lee, *et al.*, 1991b; Rizzoni, *et al.*, 1994), the present study revealed only modest evidence of cardiac hypertrophy in the SHR. The heart weight to body weight ratio appeared to be elevated, but was largely the result of the reduction in body weight. When heart weight to tibial length ratio was used, which has been suggested to be a better predictor of cardiovascular hypertrophy, because it eliminates effects due to fluctuation in body weight occurring naturally with ageing (Yin, *et al.*, 1982), there was no evidence of cardiac hypertrophy in the SHR.

Despite this finding, treatment with perindopril reduced the cardiac mass in both the SHR and WKY rats, which is in agreement with the work of others (Clozel, *et al.*, 1989; Linz, *et al.*, 1989; Black *et al.*, 1996; Black, *et al.*, 1995). This reduction may be

related to a reduction in the pressure load on the heart, resulting from the fall in pressure observed with perindopril treatment, in both strains. On the other hand, a number of studies have implicated AII as an important factor in the early development of cardiac structure (Korner & Bobik, 1995a; Klingbeil *et al.*, 1999). Thus, early blockade of the renin-angiotensin system may reduce cardiac structure in later life. Moreover, many studies suggest a drug specific effect leading to improved cardiac structure and function with non-hypertensive doses of ACE inhibitors (Benetos *et al.*, 1994; Johnston, 1994; Black, *et al.*, 1996; Kaneko *et al.*, 1997). Also, Clozel and colleagues have demonstrated improved coronary reserve and increased capillary density (Clozel, *et al.*, 1989). Many of these studies conclude that these effects are likely due to AII suppression, or to the potentiation of bradykinin, leading to an increased EDRF and prostacyclin release (Linz, *et al.*, 1989; Gavras *et al.*, 1995; Black, *et al.*, 1996). However, not all studies support a role for EDRF in the beneficial effects of ACE inhibitors on cardiac structure (Black, *et al.*, 1996; Pitt, 1998). Other mechanisms such as AII stimulation of catecholamine production in the adrenal medulla may also influence cardiac structure (Teravainen *et al.*, 1997).

The prevention of cardiac hypertrophy with perindopril in the present study was unlikely to be related to the effects of renin-angiotensin system blockade, or to a specific drug effect. Thus, ACE inhibitor treatment was ineffective when salt loading overcame the blood pressure lowering effect of perindopril. It is therefore more likely that the reduction in pressure, to below normotensive levels, was responsible for the reduction in cardiac structure. However, although blood pressure may be the most important determinant of cardiac structure, a small trophic effect of angiotensin II cannot be excluded. It is also possible that saline per se may have had a pressure-independent effect on cardiac structure. Saline administration may induce alterations in the myocardial alpha-receptors, and by stimulating IP_3 and DAG production, may lead to cardiac hypertrophy (Linz *et al.*, 1997).

In contrast, Teravainen and colleagues reported little alteration in left ventricular mass in 3 month and 18 month WKY rats, after administration of a high-sodium diet (Teravainen *et al.*, 1997). The present findings are supported by Gillies and colleagues, who recently showed that the reduction in cardiac weight correlated to the blood pressure achieved with ACE inhibitor treatment (Gillies *et al.*, 1998).

By contrast to the absence of cardiac hypertrophy in the untreated SHRs, marked structural alterations developed in the small arteries of both the mesenteric and femoral vascular beds. These structural alterations closely paralleled the pattern previously described in the genetically hypertensive rat. The lumen diameters of both mesenteric and femoral arteries were reduced, but the change in the femoral artery just failed to attain significance. Also, there was evidence of an increased media thickness, with a consequent increase in the media to lumen ratio of both vascular beds. However, despite the marked alteration of vascular structure, the media cross-sectional area was found to be elevated only at 16 weeks, and not at 24 weeks. The loss of significance appeared to be related to an increase in MCSA in the older untreated WKY rats. Since these alterations were observed in the normotensive animal, they probably reflected an age dependent change in arterial structure. Similar increases in MCSA and MT of the normotensive rat mesenteric artery have also been reported by Moreau, although the animals were 32 months old (Moreau *et al.*, 1998).

The present study was not designed to elucidate the underlying mechanisms concerned in the structural adaptation of the mesenteric and femoral arteries in response to hypertension. It is generally accepted that vascular growth *i.e.* an increase in vascular smooth muscle mass, in the genetically hypertensive rat, in response to increased blood pressure, is dependant upon hyperplasia. This view is supported both by DNA synthesis (Owens, et al., 1988; Yang, et al., 1989), and cell counting studies (Mulvany, *et al.*, 1985).

Studies utilising the nuclear incorporation of the thymidine analogue 5-bromo-2'-deoxyuridine have confirmed hyperplasia. Increased incorporation was reported in a variety of arteries, including the femoral, and mesenteric arteries from the SHR. By contrast, vascular remodelling may make a more important contribution to structural alterations in the SHR femoral artery (Bund, *et al.*, 1991; Bund, 1996). Although these changes can lead to encroachment it is thought unlikely that structural alterations lead to an increase in arterial stiffness in the femoral artery (Bund, 1996).

There has been much debate as to whether structural vascular alterations are a primary cause or secondary consequence of hypertension (Korner, 1995b; Mulvany, 1995). Many studies have reported a genetic predisposition for increased smooth muscle growth, and it has been suggested that these changes occur in early life as the major starting point in hypertension (Folkow, 1986; Folkow *et al.*, 1997). Thus, the young SHR may respond to initial elevations in pressure with pronounced growth (Folkow, 1986; Ledingham, 1989). However, others found little evidence of genetically exaggerated growth, and suggested that alterations occur as an adaptive response to the rise in blood pressure (Mulvany & Korsgaard, 1983; Bund, et al., 1991).

Also, there is some doubt as to whether structural alterations, identified by measurement using the Mulvany myograph, are a true reflection of the situation *in vivo*. None the less, increased structure has been widely reported in many laboratories. Moreover, studies using the perfusion myograph system, thought a better reflection of *in vivo* conditions, revealed a close correlation between arterial measurements, and those previously reported in the Mulvany myograph (Lew & Angus, 1992). A similar correlation was observed upon comparison of the mesenteric artery in a single study involving measurement of structural parameters, using both the Mulvany and perfusion myograph (Sharifi, *et al.*, 1998). Moreover, similar structural alterations have been

observed in mesenteric arteries from the SHR, mounted in a perfusion myograph system, using the confocal microscopy technique (Dickhout & Lee, 1997).

Treatment with the ACE inhibitor perindopril brought about an increase in the lumen diameter of the mesenteric artery from the WKY. This is in agreement with previous studies reporting a dose-dependent increase in lumen diameter with perindopril (Thybo, *et al.*, 1994), and cilazapril (Li & Schiffrin, 1996). Thybo and colleagues (1994) concluded that the perindopril-induced change in lumen diameter was likely to be related to the marked hypotensive action of the drug (Thybo, *et al.*, 1994). The present study supports this view, because the blood pressure also was significantly reduced in the WKY rats, to below normal levels. Perindopril treatment in the 16 week SHR did not completely attenuate the development of structural change in the mesenteric artery, despite a marked reduction compared to the untreated SHR. Thybo and colleagues also reported that normalising the blood pressure, failed to bring complete structural normalisation, during perindopril treatment (Thybo, *et al.*, 1994). However, in the present study perindopril treatment completely prevented the development of mesenteric artery structural change, at 24 weeks, where an even more marked antihypertensive effect of perindopril was observed. A number of investigators have reported that long-term ACE inhibitor treatment can reverse both the structural, and functional changes induced in hypertensive disease (Scalbert *et al.*, 1991; Linz, *et al.*, 1997). In addition, reports suggest that adequate supression of the tissue renin-angiotensin system occurs as a result of ACE inhibitor treatment (Mendelsohn *et al.*, 1991; Higashimori *et al.*, 1991; Chai *et al.*, 1992; Kawamoto *et al.*, 1997; Zhuo *et al.*, 1997).

The marked response of the mesenteric artery structure to long-term treatment with perindopril contrasted to the to the disappointing less marked change in the media thickness and media to lumen ratio of the femoral artery at 24 weeks. This was surprising

in view of the effective reduction in structure of the femoral artery structure in the 16 week treatment study. It is possible that structural alterations developed in this vascular bed with increasing age, and that such changes could not be easily prevented by long-term ACE inhibition. These discrepancies may reflect variability in the structural alterations of different vascular beds (Lacy & Thurston, 1994; Daemen & De Mey, 1995). However, femoral artery structure morphological measurements made during the present study may also be less accurate than measurements of the mesenteric artery, because of the greater number of cell layers. This made it more difficult to distinguish the boundary between the thicker adventitial, and medial cell layers in the femoral arteries.

The prevention of the increased MCSA and MT, in both arterial beds from the 24 week SHR and WKY rats was another interesting feature of perindopril treatment in the present study. This resulted in these parameters remaining similar to that observed in the respective arteries, at 16 weeks of age. Thus, treatment may have prevented some of the age-related changes observed in these resistance arteries, although the mechanisms involved are unclear. The study of Li and colleagues concluded that the reverse remodeling of small arteries, including the femoral and mesenteric artery, as a result of treatment, depended on the degree of blood pressure reduction (Li & Schiffrin, 1996). This may explain the findings of the present study, where blood pressure was lower in rats, after long-term perindopril treatment, compared to the untreated SHR and WKY rats. Alternatively, there may be a specific effect of perindopril on age-related vascular alterations, because some reports suggest that ACE inhibitors may affect the synthesis, degradation, or disposition of extracellular matrix components (Sharifi, *et al.*, 1998).

The literature yields conflicting evidence about the mechanisms involved in the cardiovascular structural alterations observed during hypertension. A large number of studies support a role for pressure-dependent mechanisms (Leung, *et al.*, 1976; Hume,

1980; Mulvany, 1984; Mulvany, 1987; Ollerenshaw, *et al.*, 1988; Bund, et al., 1991; Wilson, *et al.*, 1993; Allen, *et al.*, 1996; Williams, 1998), while others emphazised a role for angiotensin II (Dickenson & Yu, 1967; Campbell-Boswell & Robertson, 1981; Geisterfer *et al*, 1988; Lyall, *et al.*, 1988; Berk, *et al.*, 1989; Linz, *et al.*, 1989; Kato, *et al.*, 1991; Lyall, *et al.*, 1991; Naftilan, 1992; Simon & Altman, 1992; Black, *et al.*, 1993; Lever, 1993; Black, *et al.*, 1995; Rizzoni, *et al.*, 1995). For a fuller discussion of this evidence, see chapter one of this thesis. Much of the evidence supporting an important role for AII in the development of structural change stems from ACE inhibitor studies, which suggested that prevention of structural change was due to blockade of direct effects of AII on the vasculature. However, unlike the present study, many of these studies failed to distinguish between the direct effects of renin-angiotensin system blockade, and the blood pressure lowering effect of these drugs. In addition, ACE inhibitor treatment is known to potentiate bradykinin, which is reported to display antigrowth properties, thus further complicating these studies (Farhy *et al.*, 1993). The present experiments indicate that structural alterations were more likely to be the consequence of an elevation in blood pressure, rather than a direct trophic effect of AII, although small effects of drug treatment could not be ruled. Thus, mesenteric, and femoral arteries of perindopril and 1% saline treated SHRs showed a similar development of structural change to the untreated SHR, despite inhibition of the renin-angiotensin (Fish *et al*., 1995). These findings are in agreement with those of Harrap and colleagues using combinations of ACE inhibition with either saline or aldosterone, to elevate blood pressure (Harrap *et al.*, 1993). They found similar increases in mesenteric and cardiac structure, in response to the elevated blood pressure, in the presence of renin-angiotensin system blockade. However, a role for indirect effects of the renin-angiotensin system, could not be ruled out by these studies.

Also, studies in the young SHR have suggested little evidence of a direct trophic effect of the renin-angiotensin system at three weeks of age. Renin-angiotensin system activity was normal, despite an increased blood pressure, structure and decreased reactivity (Morton, *et al.*, 1990). This lack of increased renin-angiotensin system during established hypertension has been reported by more than one study (Miyazaki, *et al.*, 1988). In addition, reports failed to demonstrate elevated vascular RAS activity in the hypertensive mesenteric artery (Jandeleit, *et al.*, 1992). These reports may lend support to the hypothesis that the renin-angiotensin system is unlikely to contribute to hypertension in the SHR. However, more recently the interpretation of these results has been questioned as variability is reported to occur in the levels of ACE from different SHR/WKY colonies (Michel *et al.*, 1993). This could explain some of the variation in studies of renin-angiotensin system levels, in this genetically hypertensive strain, and may cast doubt upon the interpretation of these studies.

Thus, in the present study the beneficial effect of perindopril was largely dependent upon the reduction of blood pressure, perhaps leading to a lower wall stress, rather than low AII levels. This view was supported by the close correlation between structure, and blood pressure in all of the resistance arteries in the present study. Similarly, a close correlation between structure and blood pressure has been observed by many research groups, using a variety of techniques (Mulvany, 1986; Tofovic *et al.*, 1991; Mulvany, 1992b; Mulvany, 1995; Lundie *et al.*, 1997). Increased pressure was reported to be a stimulus of vascular hypertrophy in many studies, and was likely to be consequent to hypertension (Morton, *et al.*, 1990; Negoro *et al.*, 1995; Allen, *et al.*, 1996; Williams, 1998). It has been proposed that alterations in wall stress, which arise due to the rise in blood pressure, are a likely stimulus for the structural alterations observed in hypertension. Allen and colleagues reported a correlation between wall stress and the expression of

proto-oncogenes, and suggested that the resultant changes in wall structure may be an adaptive response in an attempt to maintain a constant wall stress (Allen *et al.*, 1997). Cyclic stretching of vascular smooth muscle cells has been shown to increase the growth response of vascular smooth muscle cells (Wilson, *et al*, 1993; Williams, 1998). This appears related to an increased PDGF expression, thus suggesting the involvement of growth factors, other than AII during hypertension (Wilson, *et al.*, 1993). Negoro and colleagues demonstrated a correlation between blood pressure levels and PDGF-A expression. They showed a reduction in blood pressure and aortic hypertrophy after antihypertensive therapy in the SHR, along with an associated reduction in PDGF-A expression (Negoro, *et al.*, 1995). Angiotensin II itself may also stimulate PDGF-A expression as demonstrated in both cell culture studies (Naftilan, 1992; Berk & Rao, 1993), and in the AII infusion model of hypertension (Wang *et al.*, 1995; Parker *et al.*, 1998)

However, SHRs also display a genetic predisposition for growth, with an increased replication rate of vascular smooth muscle cells in culture (Kanabe, *et al.*, 1983; Folkow, 1986; Hadrava, *et al.*, 1989; Kuriyama *et al.*, 1992; Saltis & Bobik, 1992). This growth predisposition may reinforce the structural adaptation to the increased pressure load in hypertension (Heagerty, *et al.*, 1993a), or alternatively, could be involved in initiating the rise in blood pressure in the SHRs (Korner, *et al.*, 1991).

The contractile response to high potassium, NAK, and noradrenaline, was found to be increased in the untreated, and perindopril with saline treated SHR mesenteric arteries, although this was less marked in the older rats. Hyper-reactivity has been commonly reported in the SHR mesenteric vascular bed, in studies using a variety of techniques, and was suggested to be largely a consequence of the altered vascular structure (Head, 1989; Leenen *et al.*, 1994; Dickhout & Lee, 1997). The increased contractility demonstrated by the present study also appeared to be largely a consequence of the structural alterations

present in the SHR, because there was no difference between the SHR, and WKY, when expressed as the media stress values, and the EC_{50} was unchanged. Thus, there was little alteration in the mesenteric artery smooth muscle cell sensitivity to noradrenaline, in this model of hypertension.

Treatment with perindopril prevented the development of an enhanced response to vasoconstrictor substances, and mesenteric artery hypertrophy. This may just depend on the lack of structural change, but may reflect other functional changes produced by perindopril treatment. It has been reported that ACE inhibitors enhance the release of endothelial-derived relaxing factors, which modulate the vascular contractile response, leading to reduced contractility (Atkinson, 1995). Also, the increased bradykinin levels, and reduced AII levels, as a result of ACE inhibitor treatment, may alter noradrenergic transmission (Collis & Keddie, 1981; Vanhoutte *et al.*, 1989b);. Thus, a reduction in AII levels, because of ACE inhibition, would downregulate sympathetic nervous system activity.

There was little alteration in the contractile ability of the smooth muscle cells in all SHR femoral arteries, compared to the increase observed in the mesenteric artery. This could be related to different forms of structural alteration in this vascular bed. It is possible that there could be a greater contribution of remodelling to structural alterations in the femoral artery (Bund, *et al.*, 1991). Although, this is only speculation, as the contribution of remodelling could not accurately be determined in the present study, suggesting that further analysis would be required to elucidate the mechanisms of structural change involved.

The response to acetylcholine in the SHR mesenteric artery did not become significantly reduced until 24 weeks of age, and was less marked than that reported by other studies (Lee *et al.*, 1987b; Luscher, *et al.*, 1991; Fu-Xiang, *et al.*, 1992; Bennett, *et*

al., 1996). Impaired relaxation with hypertension, could be the result of functional changes in the endothelium, smooth muscle cells, or alternatively to morphological changes in the arterial wall. The latter would lengthen the diffusion pathway for vasoactive substances from the endothelium to the underlying smooth muscle cells. However, in these studies the response to the nitric oxide donor SNP was similar in both the 24 week untreated SHR, and WKY rats, suggesting no change in the diffusion pathway, or the ability of the smooth muscle cells to respond to nitric oxide.

In contrast to the full relaxation response observed to ACh in the WKY mesenteric artery, the SHR displayed a re-contraction in response to the highest concentrations of ACh. It has been suggested that the re-contraction response may be the result of a shift towards vasoconstriction in the hypertensive artery. Possible mechanisms for this shift include enhanced production of endothelial-derived contracting factors, decreased synthesis and/or release of endothelium-derived relaxing factors (EDRF's), or increased degradation of EDRF's. The re-contraction observed in the SHR mesenteric artery in the present study was improved after incubation with indomethacin. Also, there was a slight improvement in the relaxation response to ACh in the 24 week WKY mesenteric arteries, after incubation with indomethacin, making the response similar to that in 16 week WKY rats. This suggests that increased release or the preponderance of prostanoids may contribute to the deterioration in relaxation observed with hypertension, and ageing of the vasculature. These vasoconstrictor prostanoids have been shown to be co-released in response to ACh stimulation of the noradrenaline-constricted artery (Luscher, *et al.*, 1993b). Previous studies have shown that the contractile response is likely due to either thromboxane A_2, or to prostaglandin H_2. Blockade of the TxA_2/PGH_2 receptor subtype prevented the contractile response in the SHR mesenteric artery (Watt & Thurston, 1989; Fu-Xiang *et al.*, 1992). However, the present study failed to show the involvement of

either thromboxane A_2, or prostaglandin- H_2, as blockade of the receptor subtype with SQ29548 failed to improve relaxation. Others have suggested that superoxide anions may be involved in the re-contraction, since these may be generated as a result of cyclo-oxygenase activation (Auch-Schwelk, *et al.*, 1989). The superoxide anions could stimulate re-contraction by inactivating EDNO (Katusic & Vanhoutte, 1989; Katusic & Shepherd, 1991; Vanhoutte & Boulanger, 1995), and increased nitric oxide degradation has been reported to occur in the SHR mesenteric artery (Tschudi *et al.*, 1996). However, there was a greater reduction in relaxation to the highest concentration of ACh after incubation with L-NOARG, in the SHR mesenteric artery, suggesting a greater contribution of EDNO to relaxation in the hypertensive arteries. More recently, studies have suggested that isoprostanes may be formed via cyclo-oxygenase-dependent pathways. Thus, these vasoconstrictor substances could be involved in the re-contraction observed in the present study, although further work would be required to determine their role.

Many studies have been carried out to investigate abnormalities in the synthesis, release, or activity of EDNO, during hypertensive disease. Different techniques, such as porphyrinic electrode detection, and nitric oxide synthase inhibition have been employed, with wide variations in the results. Some studies have suggested reduced release of the nitric oxide component of relaxation in mesenteric arteries (Sunano, *et al.*, 1999), and also in cultured endothelial cells from the SHR (Malinski *et al.*, 1993). However, others reported little, or no abnormality in EDNO activity in the SHR (Angus *et al.*, 1992a; Ito & Carretero, 1992; Sawada, et al., 1994).

More recent studies indicate that a reduction, or loss of the endothelium-dependent hyperpolarizing factor may underlie the absent relaxation response of these arteries. Bolton first reported the EDHF component of relaxation in 1984, when nitric oxide synthesis inhibitors failed to abolish a large component of the relaxation to ACh in the

mesenteric artery (Vanhoutte, 1993a; Vanhoutte, 1996). Since this time, a number of studies have implicated EDHF as an important component of the response to ACh. The final relaxation being determined by the balance of EDNO, EDHF and EDCF's (Garland & McPherson, 1992; Adeagbo & Triggle, 1993; Parsons, *et al.*, 1994; Hansen & Olesen, 1997). However, previous studies suggest that the contribution of hyperpolarization to acetylcholine relaxation varies dependent upon the vascular bed, arterial size, species and endothelial stimulant used (Fujii, *et al.*, 1992; Shimokawa *et al.*, 1996; Triggle, *et al.*, 1999). Studies from our laboratories indicate that EDHF is the major component of endothelium-dependent relaxation in the WKY mesenteric artery, because of a significant contribution of an indomethacin/ODQ resistant component to relaxation (Hanvesakul, 1998). However, since arteries were not incubated with L-NOARG in combination with indomethacin in the present study, it proved impossible to distinguish between the EDHF component of relaxation, and that due to cyclo-oxygenase-derived vasodilator substances.

A reduction in the EDHF component of relaxation has been described, in both the SHR and SHR-SP hypertensive rats (Mantelli, *et al.*, 1995; Sunano, *et al.*, 1999; Goto *et al.*, 2000a). Several studies also have shown that the ACh relaxation response in the WKY was inhibited by nitric oxide synthase blockade, in the presence of high potassium, implicating both EDNO and EDHF in the relaxation response. On the other hand, high potassium had little effect on the ACh induced relaxation in the SHR, whereas L-NNA reduced the response. This suggested that the EDHF component of relaxation was reduced or absent in the hypertensive SHR (Fujii, *et al.*, 1992; Kahonen, *et al.*, 1995). However, the mechanisms underlying arterial hyperpolarization are still not fully understood, and we can only speculate about the mechanisms responsible for the endothelial dysfunction in hypertension.

Interestingly, in the present study, there appeared to be an increased contribution of EDNO to ACh relaxation in the hypertensive mesenteric artery, because a greater proportion of the response was blocked, after incubation with L-NOARG. This suggests that a compensatory rise in EDNO production, or release may have occurred during hypertension, to counteract some of the adverse effects of hypertension A similar finding was reported by Mantelli, who found that relaxation of older WKY rats, and hypertensive rats, appeared largely dependent on EDNO. However, the activation of potassium channels appeared to be the main response in the young WKY rats (Mantelli, *et al.*, 1995). There are also reports which suggest that upregulation of inducible nitric oxide synthase (iNOS), in the vascular smooth muscle cells (Folkow, 1993; Singh *et al.*, 1996; Chou *et al.*, 1998), may occur as a result of hypertension. This appeared to be a protective mechanism as it was not apparent before the development of hypertension.

It is possible that alterations in endothelial function are consequent to the raised blood pressure, as there is evidence of a deterioration in endothelial function with the progression of disease (Rubanyi, 1993a; Rizzoni *et al.*, 1997; Rizzoni *et al.*, 1998b). This was also observed in the present study, as there was little reduction in the endothelium-dependent relaxation at 16 weeks, despite the presence of marked structural alterations, whereas a significant reduction was observed at 24 weeks. Thus, endothelial dysfunction appears to be related to the rise in blood pressure and it is likely that the reduced response is dependent upon the severity of hypertension. It is also possible that differences in the level of endothelial dysfunction reported may be explained be differing amounts of endothelial disruption, occurring as a result of experimental techniques.

Treatment with perindopril prevented the development of a reduced response to ACh, in the SHR. This appeared partially due to the prevention of blood pressure elevation in the SHR, because the impairment in the response to ACh was less marked in

the perindopril and 1% saline treated SHRs. This suggests that perindopril treatment may have a beneficial effect on endothelial function, unrelated to either the blood pressure lowering response, or its ability to prevent the development of structural change. This may be a drug specific effect, because a number of studies reported beneficial effects even with low doses of ACE inhibitors (Gohlke *et al.*, 1993b; Gohlke, *et al.*, 1993a). This may involve reduced breakdown of bradykinin, which can potentiate both EDNO, and prostacyclin release, via it's actions upon the β_2 kininergic receptors (Vanhoutte, *et al.*, 1989b; Waeber *et al.*, 1989; Campbell *et al.*, 1994; Hutri-Kahonen *et al.*, 1997a). Many of the early studies reported that ACE inhibitor treatment led to increased production of nitric oxide, and prostacyclin (Waeber, *et al.*, 1989; Berkenboom, 1998; Lee & Struthers, 1996). ACE inhibitors may also potentiate the actions of bradykinin via the increase in Angiotensin (1-7), formed from the abundant AI levels during treatment (Ferrario, *et al.*, 1997; Lima *et al.*, 1997; Iyer *et al.*, 1998). However, recent work has questioned the contribution of bradykinin-stimulated nitric oxide release to the enhanced relaxation observed with ACE inhibitor treatment. This was suggested by a similar improvement in relaxation with enalapril after selective bradykinin receptor blockade (Rizzoni *et al.*, 1998a). More recently, ACE inhibitors have been reported to improve the EDHF component of relaxation in hypertension. Thus, a marked improvement in relaxation to ACh, after ACE inhibition, was observed in the presence of indomethacin, or methylene blue, in combination with L-NNA (Kahonen, *et al.*, 1995; Hutri-Kahonen, *et al.*, 1997a; Onaka *et al.*, 1998; Kahonen *et al.*, 1999).

There was no significant reduction in the relaxation response to bradykinin, in the mesenteric arteries from the 24 week SHRs. However, results from the EDRF characterisation studies demonstrated a slight improvement in the relaxation to bradykinin in the untreated SHRs, after incubation with indomethacin. This may suggest a slight

increase in the contribution of vasoconstrictor prostanoids, in response to bradykinin stimulation, in these arteries. Again, the vasoconstrictor prostanoids TxA_2 and PGH_2 did not appear to be involved. However, since only slight alterations were observed no definite conclusions can be made about the role of these vasoconstrictor prostanoids. In agreement with the other results from our laboratory, the relaxation of the mesenteric arteries to bradykinin was not nitric oxide dependent, because there was little change in relaxation after incubation with L-NOARG. However, results did suggest evidence of a slight contribution from nitric oxide, to the relaxation response with the highest concentrations of bradykinin.

Treatment with perindopril improved the relaxation response to bradykinin in the SHR mesenteric arteries, resulting in a leftwards shift in the dose-response curve, in agreement with the finding of Vanhoutte (Vanhoutte *et al.*, 1993b). This appeared to be in part related to a specific drug effect, because some improvement was observed in the perindopril and 1% saline treated SHRs, in which the blood pressure, and mesenteric artery structure remained similar to that of the untreated SHR. It would appear that some of the improvement in the present study was related to an increase in the EDNO component of relaxation, because blockade of nitric oxide synthesis with L-NOARG, markedly reduced the relaxation of the SHR mesenteric artery to bradykinin. This probably reflected enhanced release of EDRF, as a result of potentiation of bradykinin by ACE inhibition (Clozel, 1991; Feletou *et al.*, 1992). It has also been suggested that ACE inhibitors may augment the EDHF effects of kinins (Vanhoutte, *et al.*, 1993b). However, in the present study whether specific drug effects were involved was unclear, as there was little evidence of an improvement in the WKY rats. This suggests that the blood pressure lowering effect of perindopril was an important factor in the improved relaxation to bradykinin.

The pattern of relaxation in the femoral arteries to ACh was quite different from that of the mesenteric artery, indicating that there is heterogeneity in the responses of different vascular beds to vasodilator agents. Re-contraction at higher concentrations of acetylcholine was observed in both femoral arteries from both the SHR and WKY rats. Thus, indicating re-contraction to be the normal response to high concentrations of ACh. However, there was a reduction in the maximum relaxation response of the 24 week untreated SHR femoral artery, compared to the untreated WKY rats.

Re-contraction of both the SHR and WKY femoral arteries was abolished by incubation with the cyclo-oxygenase inhibitor indomethacin, suggesting the involvement of vasoconstrictor prostanoids, probably TxA_2 or PGH_2, since the re-contraction was attenuated following incubation with SQ29548. Inhibition of nitric oxide synthase using L-NOARG reduced the ACh relaxation in the WKY femoral arteries, suggesting contribution from EDNO, in these vessels. Moreover, incubation with L-NOARG had little effect on the already reduced ACh relaxation in the hypertensive artery. It appeared therefore, that the reduced relaxation response to ACh in the 24 week SHRs, may depend on the loss of the EDNO component of relaxation. Alterations in the ability of the smooth muscle cells to respond to nitric oxide are likely to be involved because the relaxation response to the nitric oxide donor, SNP was reduced in the hypertensive femoral arteries at 24 weeks.

The marked improvement in ACh relaxation observed in the mesenteric artery following perindopril treatment contrasted with little, or no change in the femoral artery relaxation. On the other hand, the later L-NOARG study showed a slight improvement in the EDNO component of relaxation in the perindopril treated SHR femoral arteries, likely related to specific drug effects. However, these findings must be interpreted with caution because femoral artery contractile ability appeared to be reduced following incubation with

L-NOARG. This was suggested by a reduction in the re-contraction response to higher concentrations of ACh in the femoral artery, in the presence of L-NOARG. Also, a slight reduction in the normal contraction, in response to bradykinin was observed.

Bradykinin failed to produce any relaxation of the pre-constricted femoral arteries, possibly related to a difference in receptor subtypes in this vascular bed. The normal response to bradykinin was contraction in this vascular bed, and was largely unaffected by the development of hypertension. Vasoconstrictor prostanoids may be involved, because there was a slight reduction in the contractile response to bradykinin, following incubation with indomethacin. Perindopril treatment had no significant effects on the response to bradykinin in these arteries.

In addition to a reduced response to endothelium-dependent vasodilator substances during hypertension, previous studies also demonstrate a marked reduction in the response of arteries to both endothelium (Luscher, *et al.*, 1992), and endothelium-independent vasodilator substances (Lee, *et al.*, 1987b; Dohi & Luscher, 1990), with increasing age. This was observed in both mesenteric and femoral arteries from the SHR and WKY rats , in the present study, in agreement with other reports in the literature (Ibarra *et al.*, 1995; Mantelli, *et al.*, 1995; Marin, 1995). The reduction in the ability of the smooth muscle cells to relax to the nitric oxide donor, SNP with increasing age, clearly indicated that an age related smooth muscle cell abnormality was involved. However, other mechanisms also may have contributed to the deterioration of the relaxation response with increasing age, including increased production of vasoconstrictor prostanoids and nitric oxide (Matz *et al.*, 2000), and a reduction in the EDHF components of relaxation (Mantelli, *et al.*, 1995; Goto *et al.*, 2000b). In the present study, there was a greater reduction in response to the endothelium-independent vasodilator SNP in the 24 week untreated SHRs, compared to that of the untreated WKY rats at 24 weeks. This is in agreement with other reports that

the relaxation response was more impaired in arteries from old hypertensive rats, compared to those from normotensive rats of the same age (Kung & Luscher, 1995; Marin, 1995). Thus, premature ageing of the arteries during hypertension may accelerate the onset of functional abnormalities of the endothelium.

Overall, the results from this study support the view that structural vascular change develops in hypertension largely as a consequence of the raised blood pressure. Blockade of the renin-angiotensin system with the ACE inhibitor perindopril prevented the rise in systolic blood pressure, and structural change, but this effect was largely overcome by administration of a high salt intake. The major beneficial effect of ACE inhibition therefore was related to the lowering of blood pressure and there was little evidence of a direct trophic effect of AII on either the femoral, or mesenteric arteries in the hypertensive SHR.

These experiments also support the view that endothelial-dependent relaxation in the normotensive mesenteric artery, is largely dependent on an EDNO-independent component of relaxation. Reduced endothelium-dependent relaxation was observed in the present study with increasing age, and in hypertension. This was the result of an increased release, or preponderance of cyclo-oxygenase derived vasoconstrictor prostanoids in the hypertensive mesenteric artery. In addition, a compensatory rise in the EDNO component of relaxation was observed in the hypertensive mesenteric artery, whilst in the hypertensive femoral artery the EDNO component of relaxation was slightly reduced. This appeared related to a reduction in the ability of the femoral artery smooth muscle cells to respond to nitric oxide.

Perindopril treatment prevented the development of an impaired ACh relaxation response in the SHR mesenteric artery. This was partially dependent on the blood pressure

lowering effect of perindopril, although the drug also appeared to have a specific beneficial effect on endothelial function. This specific drug effect appeared unrelated to pressure, as there was some improvement in endothelial function after perindopril treatment, in the presence of an elevated blood pressure due to salt loading. Perindopril treatment did prevent the reduced response to SNP in the femoral artery, suggesting an improvement in the ability of the femoral artery smooth muscle cells to respond to NO. By contrast, there was little effect of perindopril treatment on the endothelial dysfunction to ACh, which developed in the femoral artery, although it was not possible to exclude a slight increase in the EDNO component of relaxation.

CHAPTER FOUR

Angiotensin II and Vascular Hypertrophy in Renovascular Hypertension.

4.1 INTRODUCTION

Angiotensin II has been widely suggested to be an important growth factor in the development of vascular structural alterations during hypertension. The antihypertensive treatments used for blockade of the renin-angiotensin system are widely reported to be beneficial in the prevention of hypertension. Thus, ACE inhibitors have been shown to reduce systolic blood pressure, and to normalise cardiac and vascular hypertrophy, in both genetic, and experimental two-kidney, one-clip models of hypertension (Levy *et al.*, 1988; Christensen, *et al.*, 1989; Lee, *et al.*, 1991b). Similar benefits on cardiac and vascular hypertrophy, and endothelial dysfunction have been reported in the Goldblatt two-kidney, one-clip rat model of hypertension, with the specific ACE inhibitor perindopril (Bennett & Thurston, 1996). More recently, studies have shown that the angiotensin receptor antagonist, losartan, reduces cardiac and vascular hypertrophy in the SHR (Oddie *et al.*, 1993; Dahlof, 1995; Li *et al.*, 1997b; Rizzoni, *et al.*, 1998). It was also reported that losartan treatment was associated with amelioration of both aortic (Rodrigo, *et al.*, 1997), and mesenteric artery endothelial dysfunction (Kahonen, *et al.*, 1999).

The present study used the 1K1C hypertensive rat, which is a low circulating renin model of hypertension. The raised blood pressure in this model depends on sodium retention leading to an increase in plasma volume, with suppression of the renin-angiotensin system (Bennett & Thurston, 1996). Despite the beneficial effects of renin-angiotensin system blockade in the SHR, and 2K1C models of hypertension, renin-angiotensin system blockade does not prevent the development of hypertension in the 1K1C model of hypertension. Early studies using the AII antagonist saralasin, failed to lower the blood pressure in the 1K1C rat (Brunner, 1971). Similarly, later studies with the ACE inhibitor perindopril, and the AT_1 receptor antagonist losartan failed to prevent hypertension (Yu, *et al.*, 1993; O'Sullivan *et al.*, 1994; Lacy *et al.*, 1995)

Thus, this model was chosen because it allowed the effects of renin-angiotensin system inhibition on vascular structure to be studied, without the confounding effect of a fall in blood pressure. It was decided to investigate the effects of the angiotensin converting enzyme inhibitor, perindopril, angiotensin receptor antagonist, losartan and non-specific vasodilator, hydralazine, on the structure and function in mesenteric and femoral resistance arteries from 1K1C hypertensive rats. Treatment of sham operated controls was included so that drug effects could be assessed in the absence of a raised blood pressure. Functional studies were performed to determine whether treatment with antihypertensive agents would reverse the abnormal endothelial function, which develops with hypertension. Hydralazine treatment was included to allow us to determine if any observed benefits were related specifically to blockade of the renin-angiotensin system.

4.2 METHODS

Goldblatt 1K1C hypertension was induced in female Wistar rats (170-190g) bred from a colony at the University of Leicester, as described in Chapter 2. 1K1C rats and sham-operated controls were randomly allocated to one of eight groups, no treatment, treatment with Perindopril (1mgday-^{1}kg-^{1}), treatment with Losartan (10mgday-^{1}kg-^{1}) or treatment with hydralazine (50mgday-^{1}kg-^{1}). Perindopril and Losartan treatments were dissolved in drinking water, to which rats had free access. The drug concentrations in solution was adjusted according to the regularly monitored body weight and water intake, to maintain a constant dose per 100g body weight. Hydralazine was administered by oral gavage, because animals would not freely drink hydralazine in solution. The dosage was calculated according to the individuals body weight. Drugs were administered one week prior to clipping and were continued for the duration of the study. Systolic blood pressure was recorded at weekly intervals in all groups, using the photoplethysmograph technique in warmed (30°C), conscious restrained animals.

Rats were killed by stunning followed by cervical dislocation at either 4 or 8 weeks post-clipping. Heart weight and tibial lengths were recorded as indicators of cardiac hypertrophy. Femoral arteries and third order mesenteric arteries, approximately 200µm internal diameter, were dissected and mounted in the Mulvany myograph, as described in chapter 2. After making morphological observations, arteries were normalised and left to equilibrate for one hour. Following the equilibration period, arteries were subjected to a standard activation procedure, after which cumulative contraction response curves to noradrenaline were performed in the absence and presence of 10^{-6}M cocaine. This was followed by a series of cumulative dose-relaxation response curves. Arteries were maximally contracted with noradrenaline, and when the contraction reached a plateau relaxation response curves were performed to acetylcholine (10^{-10} to 10^{-4}mol/l), and

bradykinin (10^{-11} to 10^{-5}mol/l). The relaxation response curves were then repeated following a thirty-minute, pre-incubation with 10^{-5}M indomethacin. The arteries were then rinsed in PSS and allowed to rest at baseline for thirty minutes. Finally, the relaxation response to the endothelium-independent vasodilator sodium nitroprusside (10^{-9} to 10^{-4}mol/l) was observed.

4.3 STATISTICAL ANALYSIS

Differences in the physical characteristics, arterial morphology, and dose-responses between groups were determined using one way analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. A non-parametric ANOVA was performed using the Dunn's correction, a variation of the Bonferroni correction, in situations where the standard deviations of the populations were unequal. Comparisons of parameters between 4- and 8-week old animals were determined using the Students unpaired t-test. The Student's paired T-test was used to compare dose-responses in the presence and absence of cocaine or indomethacin. $P<0.05$ was considered significant. Numbers in brackets denote a reduced sample size.

4.4 PHYSICAL CHARATERISTICS

The systolic blood pressure (SBP) was elevated in the 1K1C rats immediately after clipping, and continued to rise throughout the four week study period (Figure 4.1). The SBP of the untreated eight week 1K1C rats rose up to five weeks post clipping, after which time, there was little further change in blood pressure (Figure 4.2). Treatment of the 1K1C rats with perindopril, hydralazine or losartan delayed the initial rise in SBP during the first week, but did not reduce the level of hypertension, which developed.

The systolic blood pressure was significantly raised in the untreated 1K1C rats at both 4 weeks (Table 4.1), and 8 weeks (Table 4.2), compared to the untreated sham rats ($P<0.001$). There was no further increase in SBP of the untreated 1K1C rats at 8 weeks, compared to the SBP of the untreated 1K1C rats at 4 weeks. This was likely due to an increase in the incidence of fatal cardiovascular complications, after the longer duration of hypertension. The mortality rate was increased in the untreated 1K1C rats from 20% in the 4 week study, to 33% in the 8 week study. Treatment with perindopril, losartan, or hydralazine did not affect the development of hypertension, at either 4-, or 8-weeks after surgery. Similarly, treatment did not alter the SBP of the sham-operated rats at either 4-, or 8-weeks.

The body weight was similar in all of the groups studied, at both 4 weeks (Table 4.1) and 8 weeks (Table 4.2), with the exception of a significant reduction in the body weight of the 8 week perindopril treated sham rats (264 ± 5g), compared to the untreated sham rats (287 ± 4g) ($P<0.05$).

The heart weight, heart weight to body weight ratio, and heart weight to tibial length ratios, were significantly increased in the 1K1C rats, at both 4 weeks (Table 4.1) and 8 weeks (Table 4.2), compared to the untreated sham rats. Cardiac hypertrophy was fully developed in the untreated 1K1C rats at 4 weeks, and there was no further change in

cardiac structure in the untreated 1K1C rats at 8 weeks. Treatment with perindopril, losartan, or hydralazine had no effect on the development of cardiac hypertrophy in this model of hypertension. However, cardiac hypertrophy was more marked in the hydralazine treated 1K1C rats at 4 and 8 weeks ($P<0.001$).

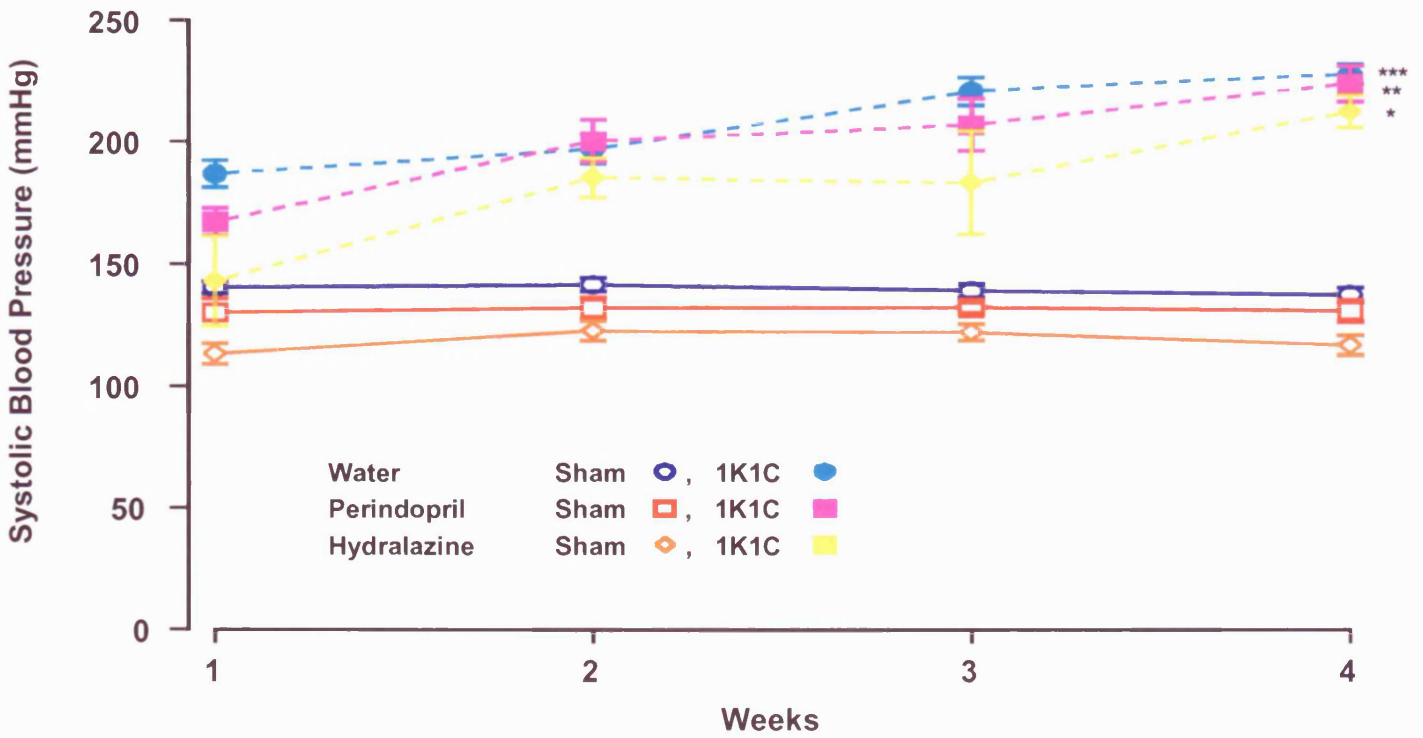


Figure 4.1. Systolic blood pressure profile of untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated sham-operated rats

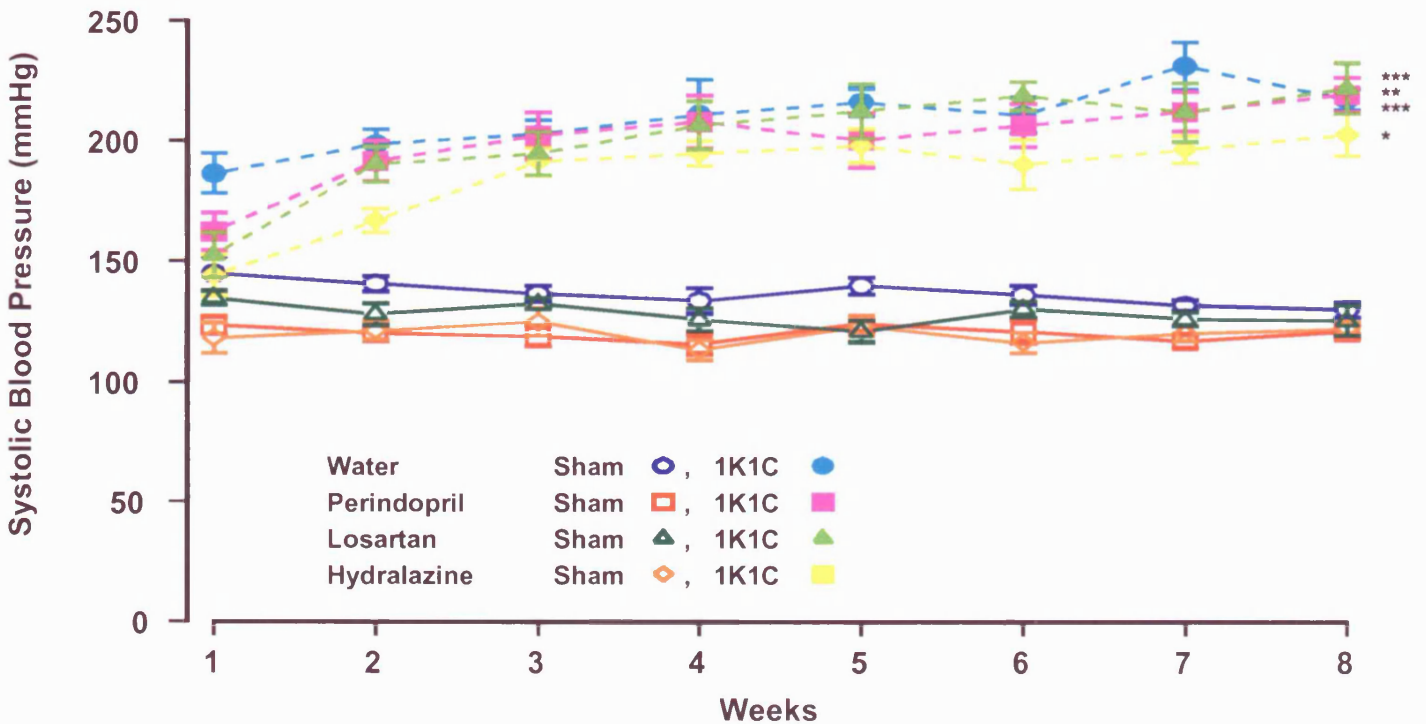


Figure 4.2. Systolic blood pressure profile of untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated sham-operated rats

Table 4.1. Physical characteristics of untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Systolic blood pressure (mmHg)	Body Weight (g)	Heart Weight(HW) (g)	HW / Weight (%)	HW / Tibia (g/mm)
SHAM	Water	14	139 ± 3	256 ± 5	0.72 ± 0.02	0.281 ± 0.005	1.993 ± 0.043
1K1C	Water	14	227 ± 6 ***	243 ± 3	0.96 ± 0.03 *	0.397 ± 0.012 **	2.698 ± 0.086 **
SHAM	Perindopril	12	130 ± 5	246 ± 5	0.65 ± 0.01	0.266 ± 0.005	1.831 ± 0.025
1K1C	Perindopril	12	223 ± 7 **	240 ± 5	1.03 ± 0.07 **	0.428 ± 0.026 ***	2.933 ± 0.213 **
SHAM	Hydralazine	12	116 ± 4	254 ± 4	0.78 ± 0.02	0.308 ± 0.003	2.169 ± 0.037
1K1C	Hydralazine	12	214 ± 7 *	255 ± 5	1.28 ± 0.04 ***	0.503 ± 0.017 ***	3.581 ± 0.137 ***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ ANOVA compared to untreated sham-operated rats

Table 4.2. Physical characteristics of untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Systolic blood pressure (mmHg)	Body Weight (g)	Heart Weight(HW) (g)	HW / Weight (%)	HW / Tibia (g/mm)
SHAM	Water	14	129 ± 3	287 ± 4	0.76 ± 0.01	0.268 ± 0.005	2.029 ± 0.026
1K1C	Water	14	217 ± 5 ***	284 ± 6	1.10 ± 0.06 **	0.388 ± 0.017 **	2.912 ± 0.142 *
SHAM	Perindopril	14	120 ± 2	264 ± 5 *	0.68 ± 0.01	0.257 ± 0.004	1.815 ± 0.028
1K1C	Perindopril	14	216 ± 6 **	275 ± 5	1.12 ± 0.07 *	0.410 ± 0.024 **	3.007 ± 0.170 *
SHAM	Losartan	13	128 ± 3	274 ± 6	0.68 ± 0.02	0.248 ± 0.006	1.807 ± 0.050
1K1C	Losartan	13	218 ± 7 ***	273 ± 5	1.10 ± 0.05 *	0.405 ± 0.020 **	2.960 ± 0.140 *
SHAM	Hydralazine	13	121 ± 3	283 ± 4	0.84 ± 0.01	0.297 ± 0.005	2.234 ± 0.031
1K1C	Hydralazine	15	201 ± 6 *	290 ± 5	1.35 ± 0.07 ***	0.465 ± 0.020 ***	3.590 ± 0.176 ***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons

4.5 MESENTERIC RESISTANCE ARTERY STUDIES

4.5.1 Morphological Characteristics

The normalised internal lumen diameter was similar in all mesenteric arteries at both 4 weeks (Table 4.3), and 8 weeks (Table 4.4). The media cross-sectional area (MCSA) and media thickness (MT) were not significantly increased in the 4 week untreated 1K1C mesenteric arteries, compared to the untreated sham rats, despite greater values. However, vascular hypertrophy did not appear fully developed in the untreated 1K1C mesenteries at 4 weeks, and there was a further significant increase in both MCSA (18,758 ± 687μm^2) ($P<0.01$), and MT (21.31 ± 0.96μm) ($P<0.05$) at 8 weeks (Table 4.4), compared to the mesenteric artery MCSA (15,716 ± 716μm^2) and MT (18.33 ± 0.68μm) in ths group at 4 weeks (Table 4.3). Consequently, at 8 weeks the MCSA, MT and M:L were significantly increased in the untreated 1K1C rats (8.33 ± 0.58%) compared to the untreated sham rats (6.00 ± 0.33%) ($P<0.05$) (Table 4.4).

Treatment with perindopril, losartan or hydralazine failed to prevent the development of vascular hypertrophy in the 1K1C mesenteric arteries at 4 weeks (Table 4.3). However, vascular hypertrophy was more marked in the hydralazine treated 1K1C mesenteric arteries 4 weeks, although this was not observed in either the hydralazine treated 1K1C mesenteries at 8 weeks, or in the sham rats at either 4-, or 8-weeks. At 8 weeks, treatment with perindopril, losartan or hydralazine did not prevent the increase in MT or M:L of the 1K1C rats. Although, perindopril, and losartan appeared to prevent some of the increase in MCSA of the 1K1C mesenteric arteries. Thus, these treatments may have a slight effect on the MSCA in this model of hypertension. Treatment with perindopril, losartan or hydralazine had little effect on the mesenteric artery structure of the sham rats.

Table 4.3. Mesenteric resistance artery morphology of untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
SHAM	Water	14	268 ± 9	13555 ± 606	14.97 ± 0.40	5.66 ± 0.24
1K1C	Water	14	250 ± 7	15716 ± 716	18.33 ± 0.68	7.52 ± 0.36
SHAM	Perindopril	12	253 ± 12	11081 ± 603	13.38 ± 0.71	5.53 ± 0.50
1K1C	Perindopril	12	260 ± 7	16593 ± 1166	18.88 ± 1.20	7.33 ± 0.52
SHAM	Hydralazine	12	267 ± 10	13583 ± 341	15.21 ± 0.45	5.74 ± 0.36
1K1C	Hydralazine	12	263 ± 8	21549 ± 1952 **	23.75 ± 1.82 ***	9.45 ± 0.81 ***

** P<0.01, *** P<0.001 ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.4. Mesenteric resistance artery morphology of untreated, perindopril treated, losartan treated or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
SHAM	Water	14	272 ± 9	14428 ± 564	16.02 ± 0.55	6.00 ± 0.33
1K1C	Water	14	261 ± 7	18758 ± 687 *	21.31 ± 0.96 **	8.33 ± 0.58 *
SHAM	Perindopril	14	256 ± 12	12752 ± 779	14.37 ± 0.52	5.61 ± 0.30
1K1C	Perindopril	14	247 ± 6	15408 ± 1145	18.96 ± 1.16	7.85 ± 0.60
SHAM	Losartan	12	263 ± 10	11941 ± 584	13.83 ± 0.48	5.36 ± 0.23
1K1C	Losartan	13	240 ± 5	15455 ± 826	18.92 ± 0.70	7.91 ± 0.26 *
SHAM	Hydralazine	13	248 ± 11	13340 ± 541	16.19 ± 0.61	6.73 ± 0.45
1K1C	Hydralazine	15	252 ± 10	17088 ± 1348	20.00 ± 1.29	8.17 ± 0.68

* $P<0.05$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

4.5.2 Mesenteric Resistance Artery Contractility

Activation Procedure

The contractile response to both high potassium, and high potassium containing 10^{-5}M noradrenaline (NAK), were similar in the untreated 1K1C and sham mesenteric arteries, at both 4 and 8 weeks, despite a slight increase in contractility of the untreated 1K1C mesenteric arteries. Treatment with perindopril or losartan did not alter the contractile response to high potassium, or NAK, in either the 1K1C, or sham mesenteric arteries. However, more marked contractions to both potassium, and NAK, were observed in the hydralazine treated 1K1C mesenteric arteries at 4 weeks (Table 4.5), and to NAK at 8 weeks (Table 4.6), when compared to the untreated sham rats.

When expressed as active media stress, to account for changes in wall thickness, the responses to both potassium and NAK were similar in all mesenteric arteries at both 4 weeks (Table 4.5), and 8 weeks (Table 4.6).

NA Contractility

The active tension generated in response to noradrenaline was similar in the untreated 1K1C and sham rats at 4 weeks, in both the absence (Table 4.7 & Figure 4.3), and presence of cocaine (Table 4.7 & Figure 4.4), despite a slight increase in the contractility of the untreated 1K1C rats. Again, at 8 weeks, the response was similar in the untreated 1K1C and sham rats in both the absence (Table 4.8 & Figure 4.5), and presence of cocaine (Table 4.8 & Figure 4.6). Treatment with perindopril, or losartan did not alter the active tension to noradrenaline of the 1K1C, or sham mesenteric arteries, at either 4 weeks (Table 4.7, Figure 4.3 & Figure 4.4), or 8 weeks (Table 4.8, Figure 4.5 & Figure 4.6). However, more marked contractions to both potassium, and NAK, were observed in the hydralazine treated 1K1C mesenteric arteries in both the absence (Table 4.7 & Figure

4.3), and presence of cocaine at 4 weeks (Table 4.7 & Figure 4.4), and at 8 weeks, in the presence of cocaine (Table 4.8), compared to the untreated sham rats.

The sensitivity to noradrenaline was similar in all mesenteric arteries, in the absence and presence of cocaine, at both 4 weeks (Table 4.7), and 8 weeks (Table 4.8). The addition of cocaine significantly increased the sensitivity to noradrenaline in all mesenteric arteries at both 4 weeks (Table 4.7), and 8 weeks (Table 4.8), with the exception of the perindopril treated sham-operated rats at 4 weeks.

When expressed as active media stress, the response to noradrenaline was similar in all mesenteric arteries at 4 weeks, in both the absence (Table 4.7 & Figure 4.7), and presence of cocaine (Table 4.7 & Figure 4.8). Similarly the active media stress was similar in all mesenteries at 8 weeks, in both the absence (Table 4.8 & Figure 4.9), and presence of cocaine (Table 4.8 & Figure 4.10).

Table 4.5. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline of mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
SHAM	Water	14	3.83 ± 0.21	4.66 ± 0.24	256 ± 11	312 ± 13
1K1C	Water	14	4.19 ± 0.27	5.43 ± 0.26	232 ± 15	300 ± 14
SHAM	Perindopril	12	3.32 ± 0.16	3.87 ± 0.15	252 ± 12	293 ± 12
1K1C	Perindopril	12	4.81 ± 0.36	5.96 ± 0.42	258 ± 17	318 ± 19
SHAM	Hydralazine	12	4.16 ± 0.09	5.17 ± 0.16	276 ± 10	342 ± 11
1K1C	Hydralazine	12	5.33 ± 0.20 **	6.94 ± 0.31 ***	234 ± 12	302 ± 14

** P<0.01, *** P<0.001 ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.6. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline of mesenteric resistance arteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
SHAM	Water	14	4.21 ± 0.25	5.14 ± 0.30	263 ± 15	322 ± 16
1K1C	Water	14	4.53 ± 0.31	5.76 ± 0.41	215 ± 16	274 ± 22
SHAM	Perindopril	14	3.80 ± 0.20	4.68 ± 0.22	265 ± 12 [13]	328 ± 15 [13]
1K1C	Perindopril	14	4.65 ± 0.35	6.07 ± 0.42	249 ± 16	325 ± 19
SHAM	Losartan	12	3.37 ± 0.24	4.25 ± 0.26	244 ± 15	308 ± 16
1K1C	Losartan	13	4.73 ± 0.34	6.01 ± 0.37	256 ± 22	324 ± 25
SHAM	Hydralazine	13	4.19 ± 0.35	5.51 ± 0.29	259 ± 21	344 ± 19
1K1C	Hydralazine	15	4.88 ± 0.33	6.45 ± 0.37 *	250 ± 17	330 ± 19

* $P<0.05$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.7. The maximum contractile response and EC_{50} to noradrenaline of mesenteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Max. NA (mN/mm)	NA EC_{50}	Max. NA active media stress (mN/mm^2)	Max. NA with cocaine (mN/mm)	NA EC_{50} with cocaine (μM)	Max. NA active media stress with cocaine (mN/mm^2)
SHAM	Water	14	4.59 ± 0.32	2.01 ± 0.25 (13)	307 ± 18	4.57 ± 0.26	1.27 ± 0.23	306 ± 16
1K1C	Water	14	5.47 ± 0.27	4.10 ± 0.72 (12)	301 ± 14	5.52 ± 0.29	2.15 ± 0.31	304 ± 15
SHAM	Perindopril	12	3.37 ± 0.21	1.88 ± 0.45 (9)	259 ± 19	3.61 ± 0.11	1.27 ± 0.21 (11)	276 ± 13
1K1C	Perindopril	12	6.08 ± 0.43	1.98 ± 0.23 (11)	324 ± 19	5.99 ± 0.40	1.91 ± 0.54 (10)	320 ± 18
SHAM	Hydralazine	12	5.14 ± 0.14	2.36 ± 0.21 (11)	340 ± 10	4.97 ± 0.15	1.36 ± 0.23	329 ± 11
1K1C	Hydralazine	12	7.20 ± 0.26 ***	3.28 ± 0.67	317 ± 19	7.17 ± 0.28 ***	1.92 ± 0.48	315 ± 18

*** $P<0.001$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.8. The maximum contractile response and EC_{50} to noradrenaline of mesenteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Max. NA (mN/mm)	NA EC_{50}	Max. NA active media stress (mN/mm^2)	Max. NA with cocaine (mN/mm)	NA EC_{50} with cocaine (μM)	Max. NA active media stress with cocaine (mN/mm^2)
SHAM	Water	14	5.23 ± 0.34	2.27 ± 0.33 (10)	328 ± 22	5.19 ± 0.35	1.33 ± 0.36 (12)	325 ± 22
1K1C	Water	14	6.02 ± 0.44	4.71 ± 0.72 (11)	285 ± 21	6.23 ± 0.44	2.16 ± 0.34	296 ± 22
SHAM	Perindopril	14	4.27 ± 0.21	2.59 ± 0.26 (8)	302 ± 14 (13)	4.21 ± 0.23	1.75 ± 0.39 (13)	296 ± 14 (13)
1K1C	Perindopril	14	6.15 ± 0.39	2.75 ± 0.29 (13)	330 ± 18	6.13 ± 0.41	1.59 ± 0.21 (13)	329 ± 18
SHAM	Losartan	12	4.04 ± 030	3.06 ± 0.61 (8)	294 ± 21	4.04 ± 0.31	1.70 ± 0.25 (10)	293 ± 20
1K1C	Losartan	13	5.97 ± 0.34	3.11 ± 0.47 (12)	322 ± 23	5.60 ± 0.52	1.86 ± 0.34 (12)	307 ± 32
SHAM	Hydralazine	13	5.37 ± 0.30	3.91 ± 0.85 (10)	337 ± 24	5.24 ± 0.34	2.05 ± 0.44	328 ± 25
1K1C	Hydralazine	15	6.48 ± 0.36	3.63 ± 0.47 (14)	332 ± 19	6.81 ± 0.36 *	1.62 ± 0.22	349 ± 19

* $P<0.05$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

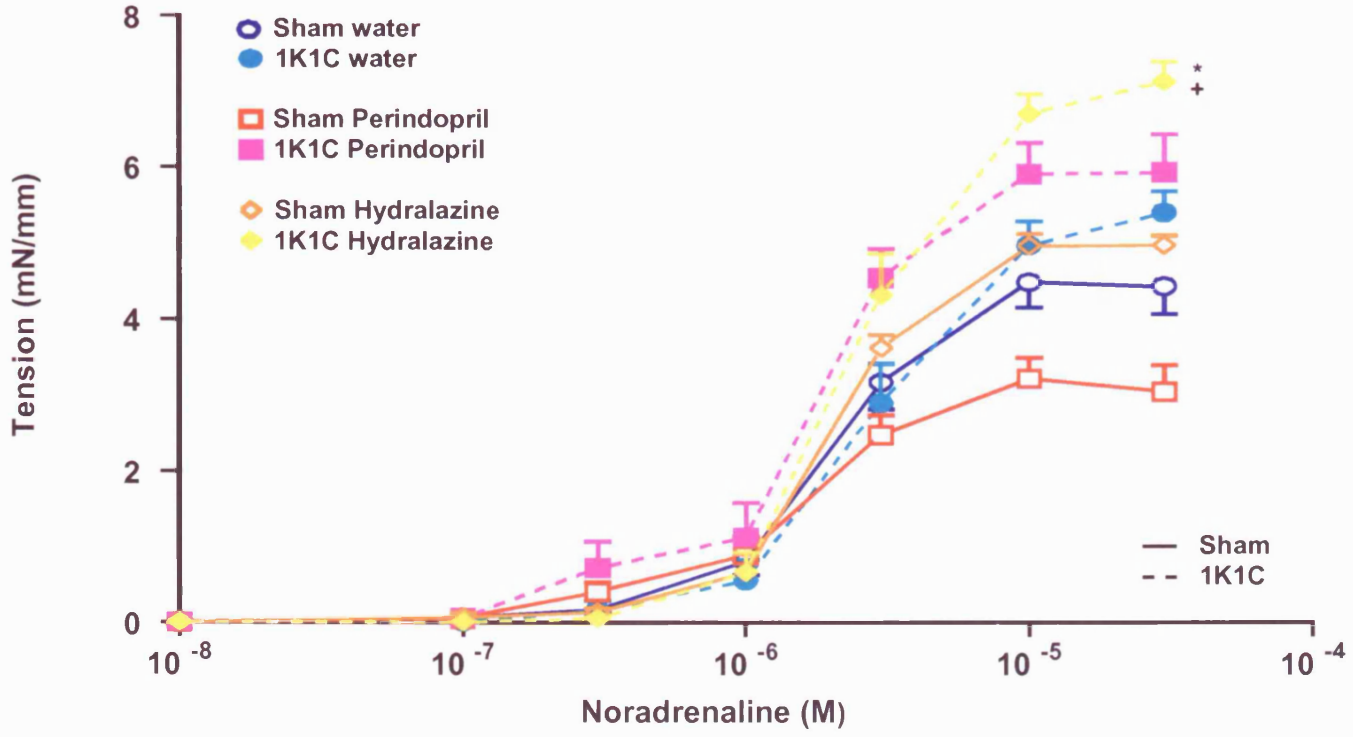


Figure 4.3. The contractile response to noradrenaline (mN/mm), of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

* $P<0.05$ ANOVA compared to untreated sham-operated rats

+ $P<0.05$ ANOVA treated 1K1C rats compared to untreated 1K1C rats

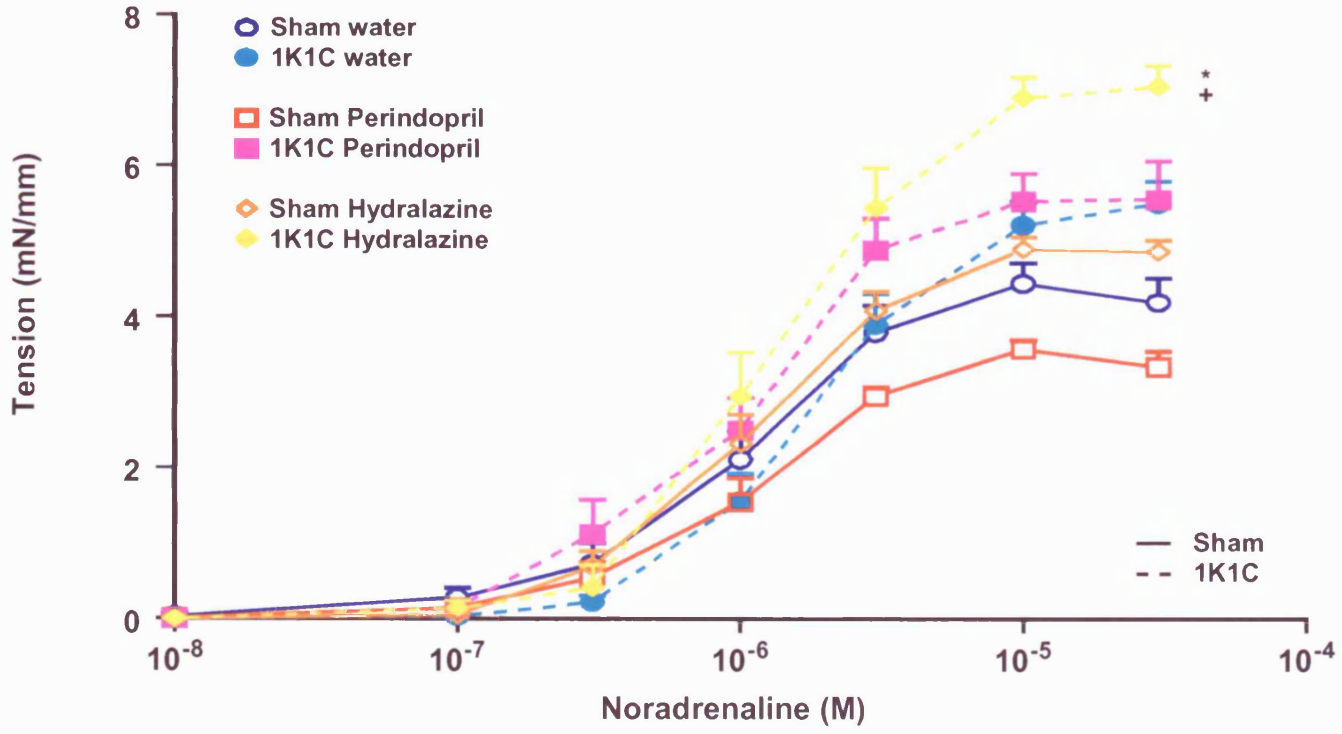


Figure 4.4. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

* $P<0.05$ ANOVA compared to untreated sham-operated rats

+ $P<0.05$ ANOVA treated 1K1C rats compared to untreated 1K1C rats

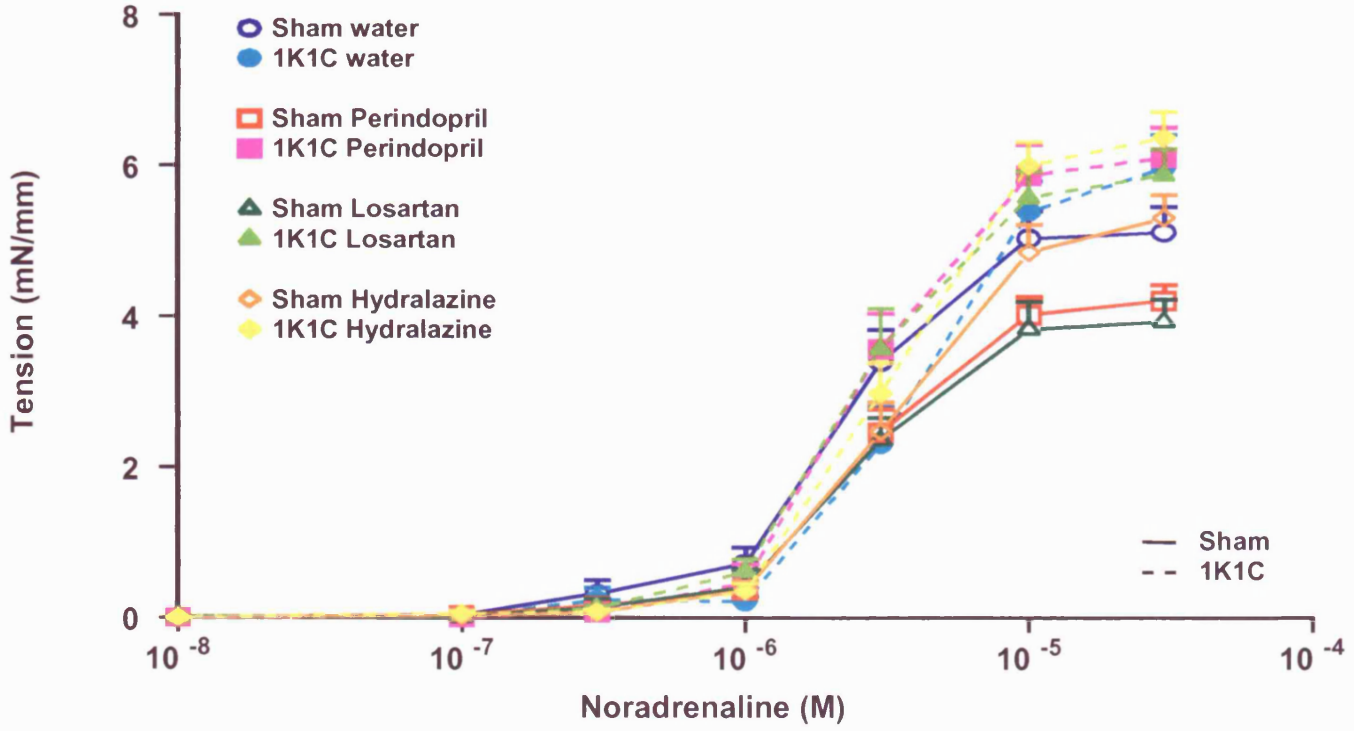


Figure 4.5. The contractile response to noradrenaline (mN/mm) of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

* P<0.05, ** P<0.01 ANOVA compared to treatment matched sham-operated rats

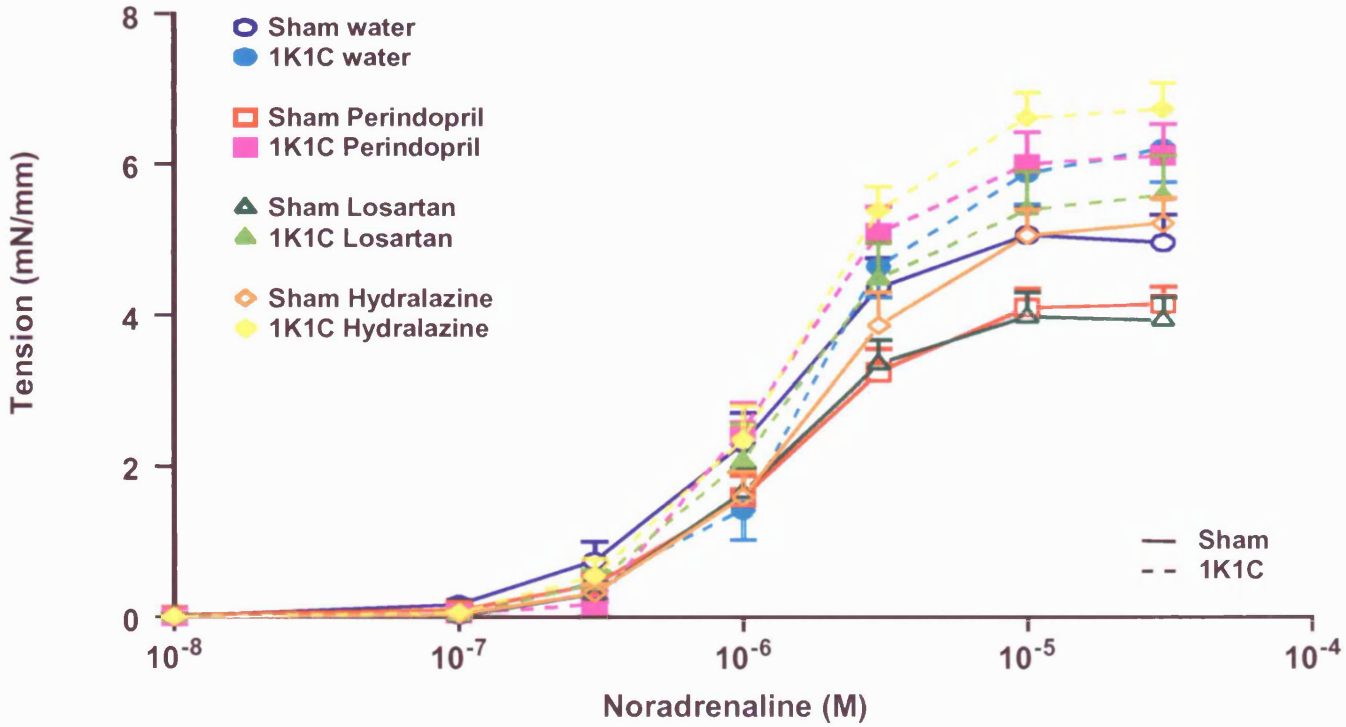


Figure 4.6. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

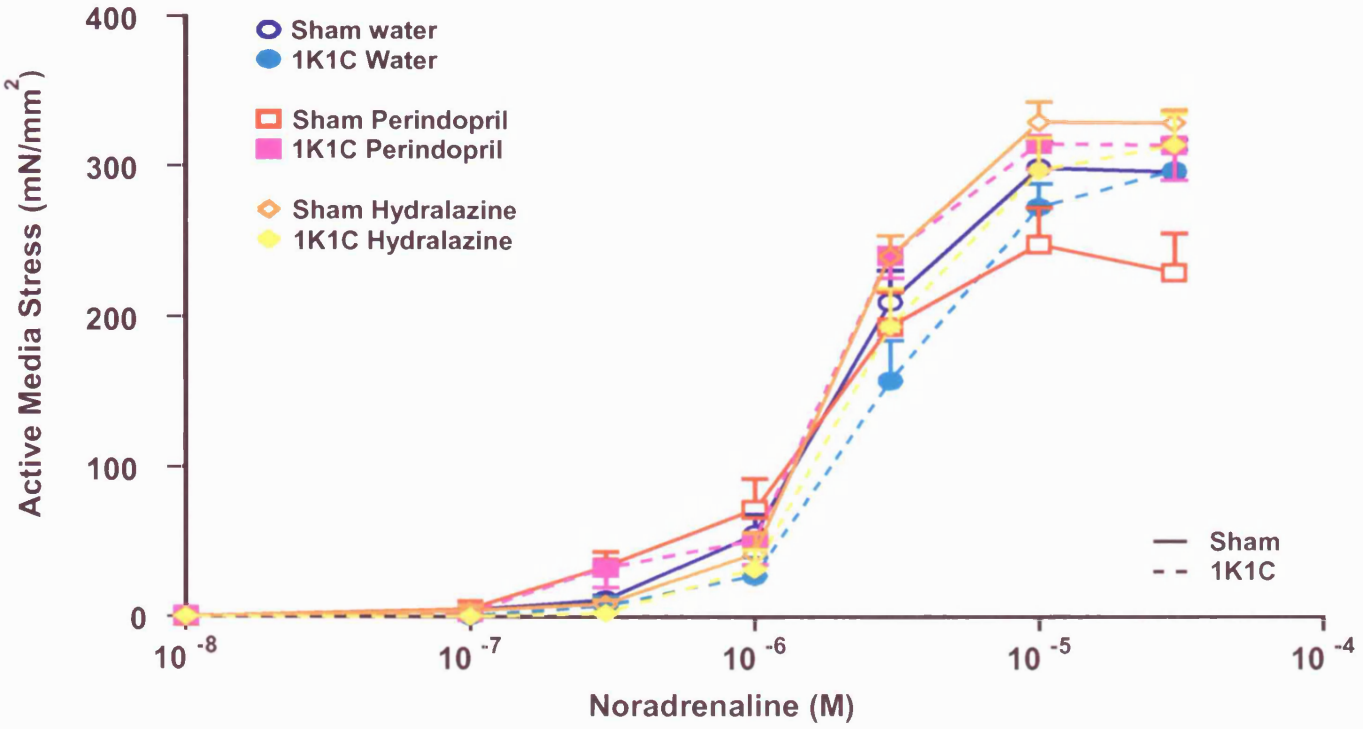


Figure 4.7. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2), of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

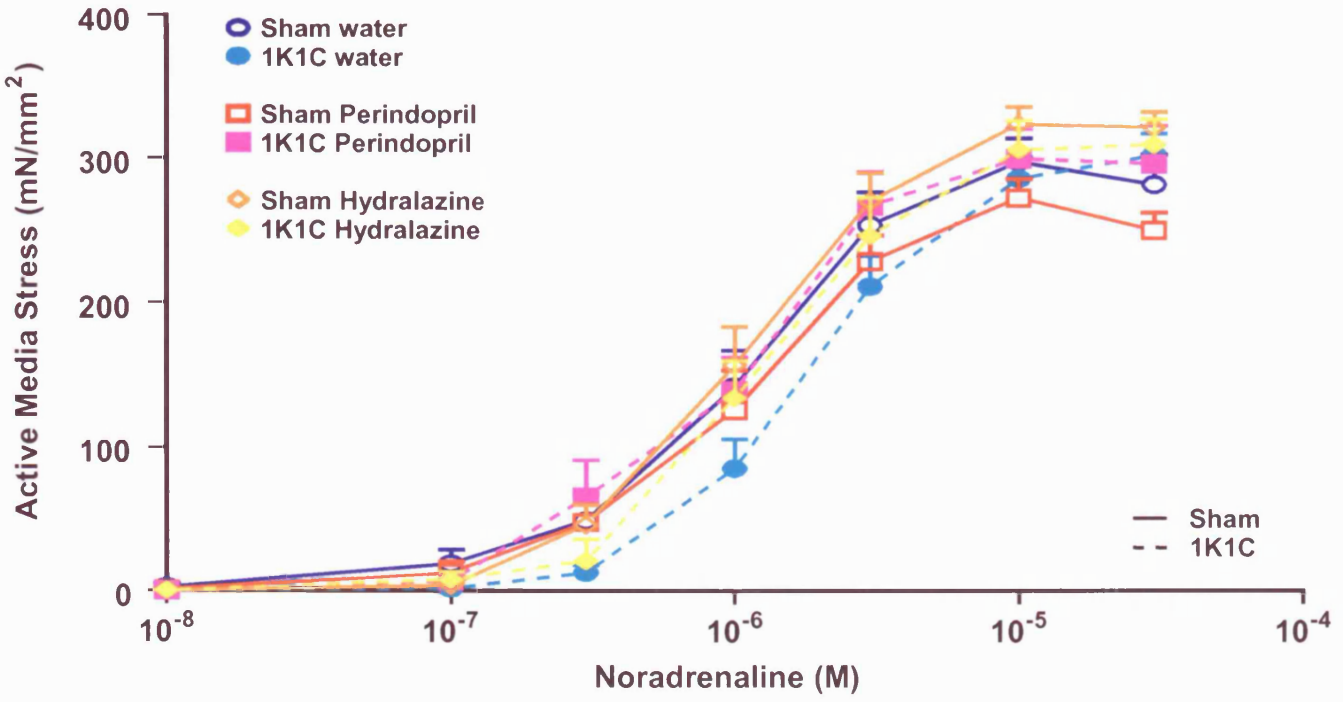


Figure 4.8. The contractile response to noradrenaline, expressed as active media stress (mN/mm2), in the presence of cocaine, of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

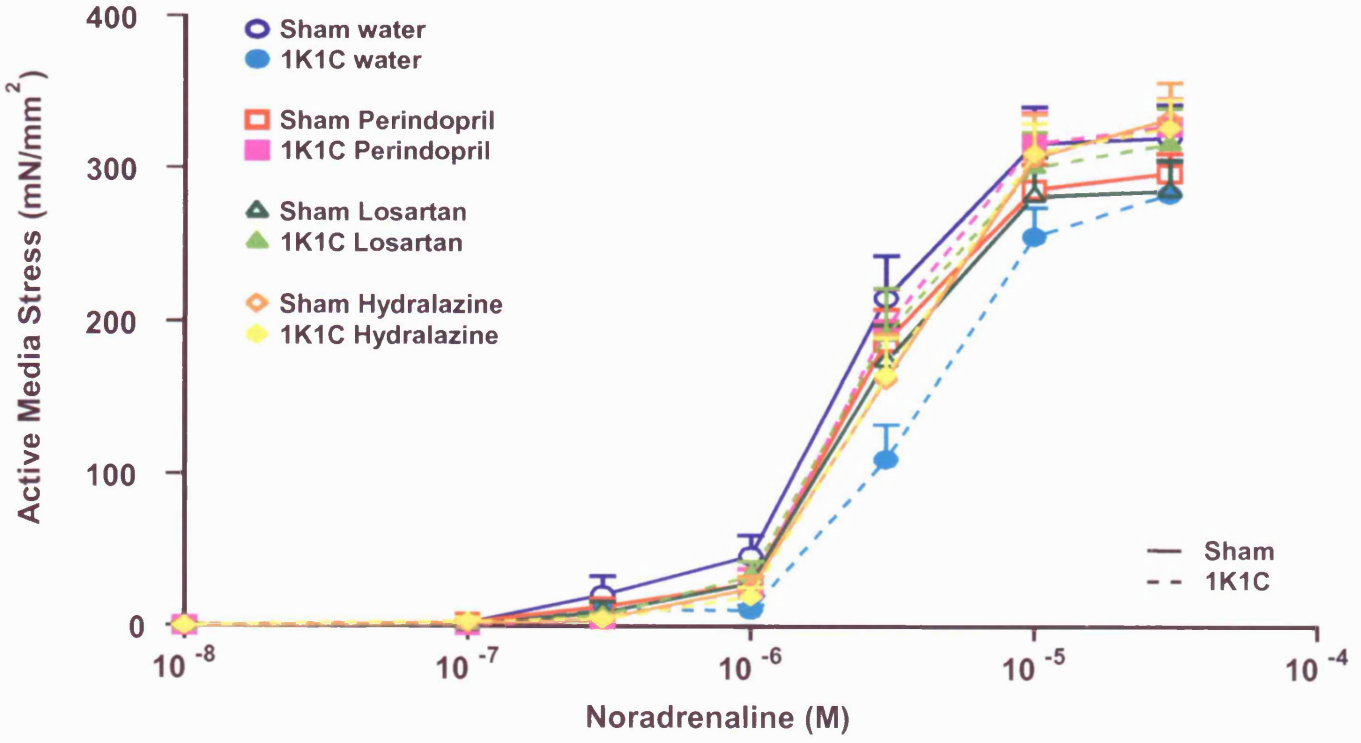


Figure 4.9. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2) of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

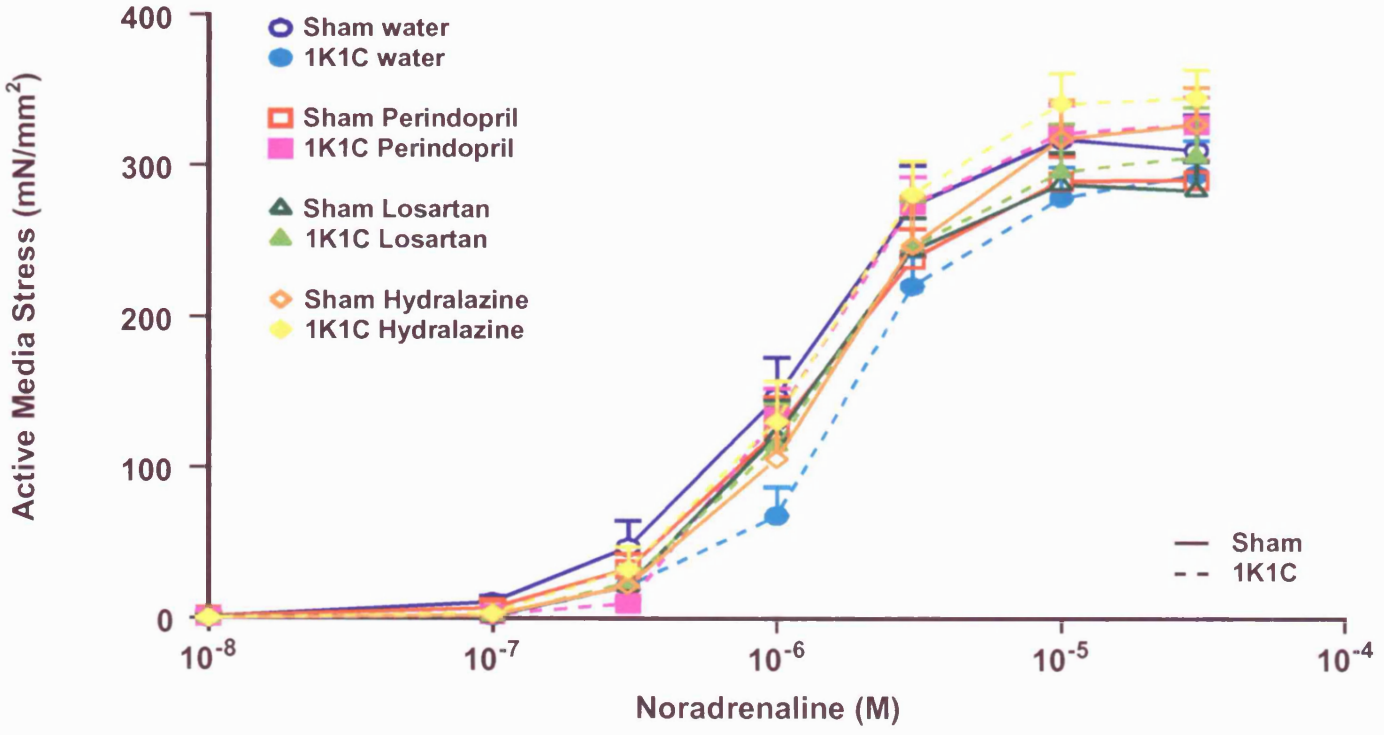


Figure 4.10. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2), in the presence of cocaine, of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

4.5.3 Endothelium-dependent relaxation

Acetylcholine

Low concentrations of acetylcholine (ACh) evoked a relaxation response in all mesenteric arteries, but higher concentrations caused the 1K1C arteries to re-contract. The relaxation response to ACh was impaired in the untreated 1K1C mesenteric arteries at 4 weeks (Figure 4.11), compared to the untreated sham rats, although the maximum relaxation was not significantly reduced in this group (Table 4.9). Treatment with perindopril or hydralazine did not prevent the impaired relaxation to ACh of the 4 week 1K1C mesenteric arteries (Table 4.9 & Figure 4.11).

There was no further impairment in the relaxation response to ACh in the 8 week untreated 1K1C mesenteries (Figure 4.12), compared to the relaxation response observed in this group at 4 weeks (Figure 4.11). However, higher concentrations of ACh evoked a slight re-contraction in the untreated and losartan treated sham rats, at 8 weeks. Consequently, the relaxation curve of the losartan treated 1K1C rats was not significantly different from that of the untreated sham rats at 8 weeks (Figure 4.12).

At 8 weeks, there was a significant reduction in the maximum relaxation ($P<0.05$) (Table 4.10), and relaxation response curve to ACh in the untreated 1K1C mesenteries (Figure 4.12), compared to the untreated sham rats. Treatment with perindopril, losartan, or hydralazine did not prevent the impaired relaxation response to ACh in the 8 week 1K1C mesenteries, although less re-contraction was observed in these arteries (Figure 4.12).

With the exception of a reduced sensitivity to ACh in the perindopril treated 1K1C mesenteries at 8 weeks (Table 4.10), compared to the untreated sham rats, the sensitivity to ACh was similar in all mesenteric arteries at both 4 weeks (Table 4.9), and 8 weeks (Table 4.10).

Incubation with indomethacin significantly improved the relaxation of the untreated, perindopril treated, losartan treated and hydralazine treated 1K1C mesenteries, at both 4 weeks (Table 4.11 & Figure 4.13,), and 8 weeks (Table 4.12 & Figure 4.14). In addition, indomethacin prevented the re-contraction to higher concentrations of ACh in the untreated and losartan treated sham mesenteries at 8 weeks (Table 4.12 & Figure 4.14).

Table 4.9. The maximum relaxation responses (E Max) and EC_{50} to acetylcholine (ACh) and sodium nitroprusside (SNP), of mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E Max (%)	ACh EC_{50} (μM)	BK E Max (%)	SNP E Max (%)	SNP EC_{50} (μM)
SHAM	Water	14	80 ± 6.2	0.05 ± 0.02 (8)	62 ± 7.3	79 ± 5.2	1.62 ± 0.54 (13)
1K1C	Water	14	69 ± 5.7	0.40 ± 0.23 (8)	34 ± 6.8 *	86 ± 3.6	0.86 ± 0.24 (12)
SHAM	Perindopril	12	89 ± 5.6	0.04 ± 0.01 (10)	69 ± 7.5	86 ± 3.2	0.40 ± 0.10
1K1C	Perindopril	12	59 ± 7.8	0.12 ± 0.04 (4)	43 ± 8.4 *	84 ± 3.8 (11)	0.68 ± 0.19 (11)
SHAM	Hydralazine	12	89 ± 4.7	0.04 ± 0.01 (11)	74 ± 4.7	78 ± 3.6	1.35 ± 0.71 (10)
1K1C	Hydralazine	12	70 ± 7.1	0.08 ± 0.04 (10)	26 ± 8.1 **	87 ± 2.8 (11)	0.68 ± 0.22 (10)

* $P<0.05$, ** $P<0.01$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.10. The maximum relaxation responses (E Max) and EC_{50} to acetylcholine (ACh) and sodium nitroprusside (SNP), of mesenteric resistance arteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E Max (%)	ACh EC_{50} (μM)	BK E Max (%)	SNP E Max (%)	SNP EC_{50} (μM)
SHAM	Water	14	85 ± 4.4	0.03 ± 0.01 (11)	62 ± 6.9	72 ± 3.2	1.63 ± 0.39 (13)
1K1C	Water	14	64 ± 5.0 *	0.12 ± 0.04 (5)	30 ± 6.2 **	80 ± 4.5	2.18 ± 0.68
SHAM	Perindopril	14	94 ± 2.1	0.04 ± 0.01 (13)	70 ± 6.0	83 ± 3.0 (13)	1.55 ± 0.35 (13)
1K1C	Perindopril	14	80 ± 4.5	0.14 ± 0.06 * (9)	45 ± 7.6	82 ± 3.8	0.97 ± 0.23 (13)
SHAM	Losartan	12	82 ± 5.7 (11)	0.09 ± 0.02 (9)	56 ± 7.6	85 ± 2.6 (10)	1.03 ± 0.41 (10)
1K1C	Losartan	13	74 ± 6.5	0.08 ± 0.02 (6)	38 ± 7.3	83 ± 3.3 (12)	0.82 ± 0.25 (11)
SHAM	Hydralazine	13	92 ± 2.0	0.04 ± 0.01 (11)	66 ± 6.6	72 ± 3.3	2.38 ± 0.59
1K1C	Hydralazine	15	74 ± 4.7	0.12 ± 0.02 (7)	35 ± 7.4 *	71 ± 4.8 (14)	2.17 ± 0.42 (14)

* $P<0.05$, ** $P<0.01$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

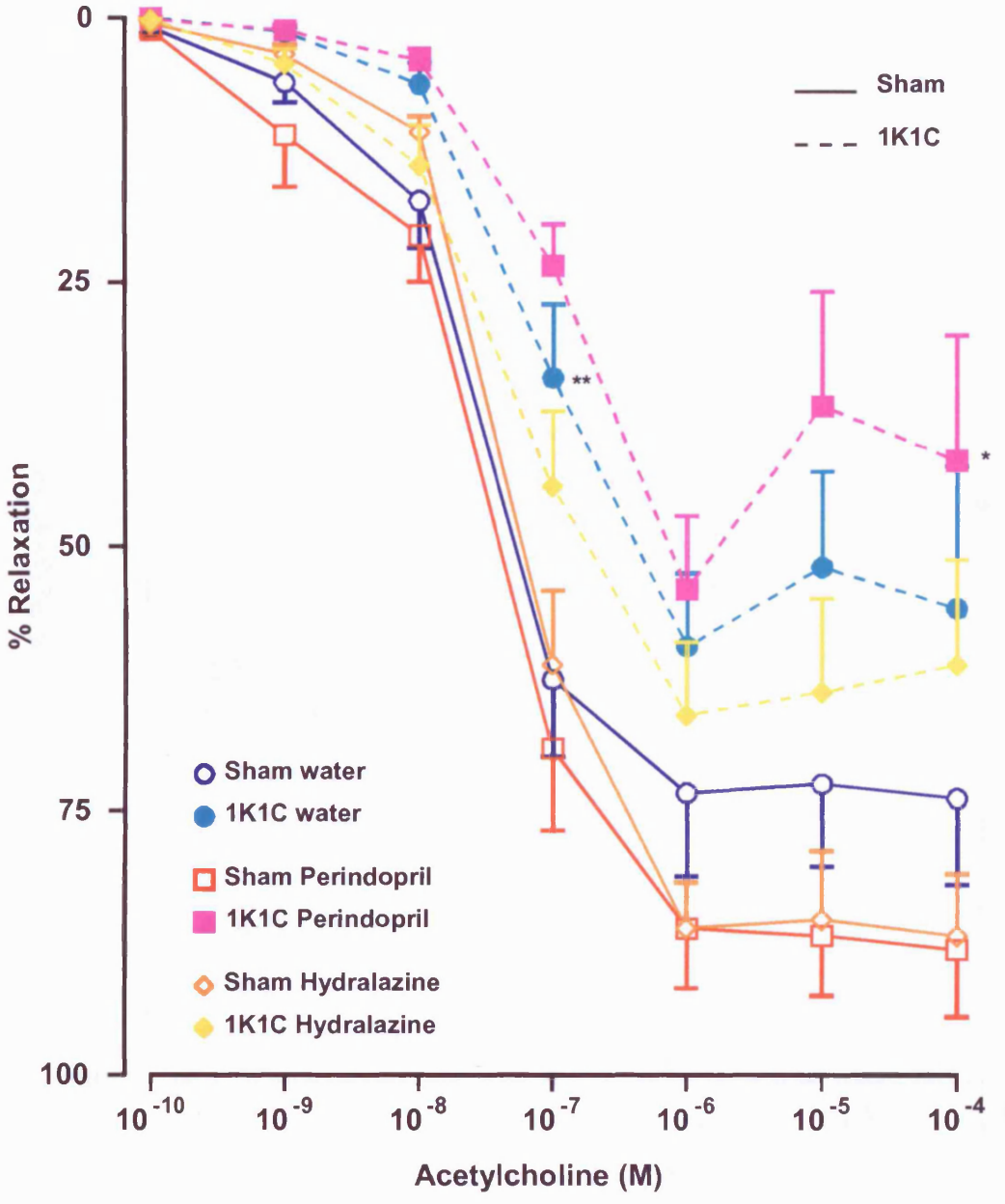


Figure 4.11. The relaxation response to acetylcholine of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

* P<0.05, ** P<0.01 ANOVA compared to untreated sham-operated rats

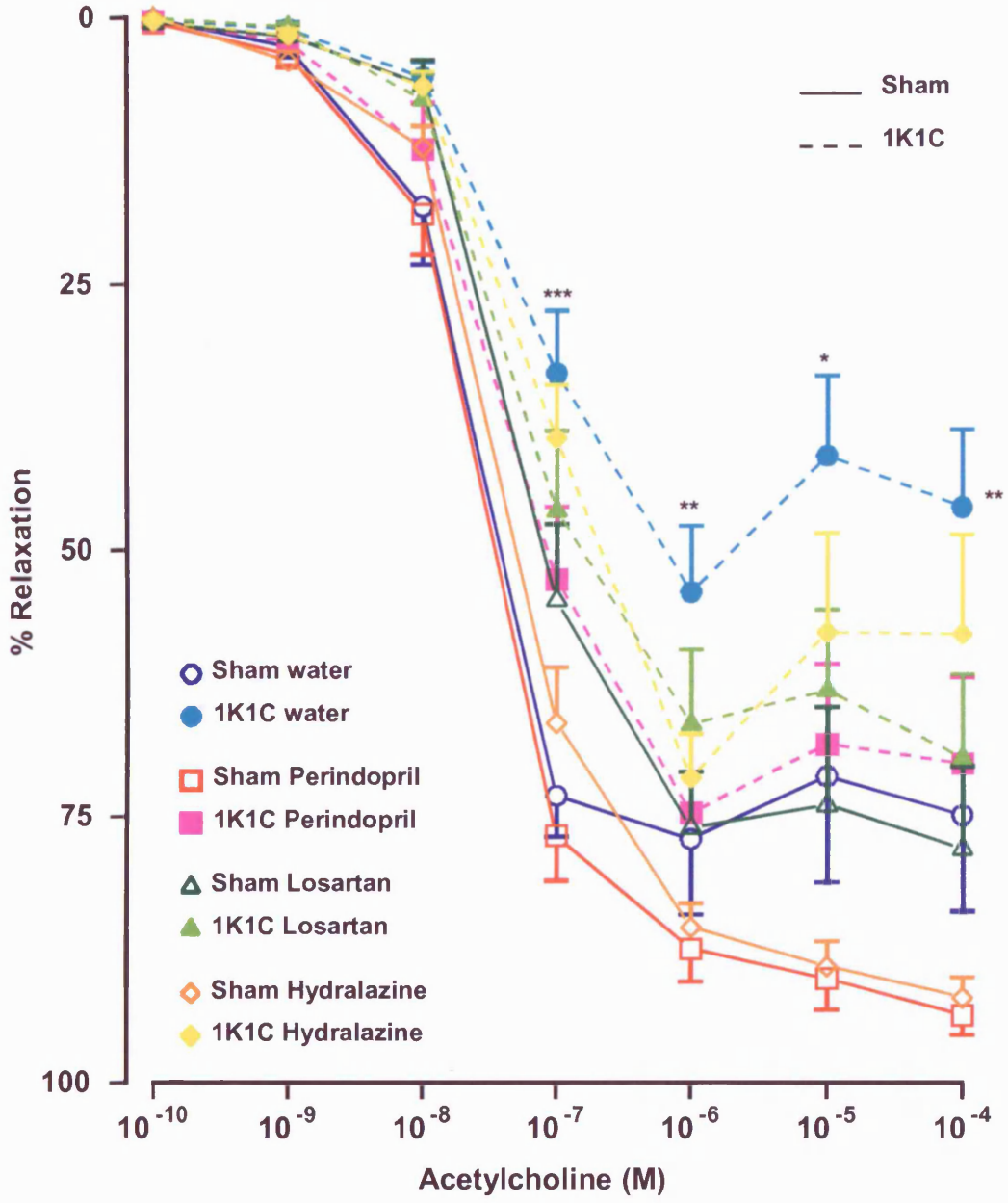


Figure 4.12. The relaxation response to acetylcholine of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

* P<0.05, ** P<0.01, *** P<0.001 ANOVA compared to untreated sham-operated rats

Table 4.11. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh), in mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E max (%)		ACh EC_{50}(μM)		ACh Mean R (%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
SHAM	Water	13	80 ± 6.3	88 ± 5.4	0.05 ± 0.02 (8)	0.08 ± 0.03 (9)	42 ± 5.6	49 ± 6.2
1K1C	Water	13	69 ± 6.2	92 ± 3.0 **	0.18 ± 0.08 (7)	0.10 ± 0.03 (9)	31 ± 6.0	48 ± 3.0 *
SHAM	Perindopril	12	89 ± 5.7	86 ± 5.3	0.04 ± 0.02 (10)	0.03 ± 0.01 (9)	52 ± 5.0	49 ± 5.2
1K1C	Perindopril	11	63 ± 7.0	89 ± 3.7 **	0.12 ± 0.04 (4)	0.05 ± 0.01 (9) *	25 ± 5.1	49 ± 5.1 **
SHAM	Hydralazine	12	89 ± 4.7	91 ± 3.0	0.04 ± 0.01 (11)	0.06 ± 0.01 (10)	48 ± 3.8	46 ± 3.6
1K1C	Hydralazine	11	72 ± 7.5	91 ± 2.8 *	0.09 ± 0.04 (9)	0.06 ± 0.01 (6)	37 ± 5.7	46 ± 5.9

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$ T-test of ACh relaxation compared to the control, in the absence of indomethacin.

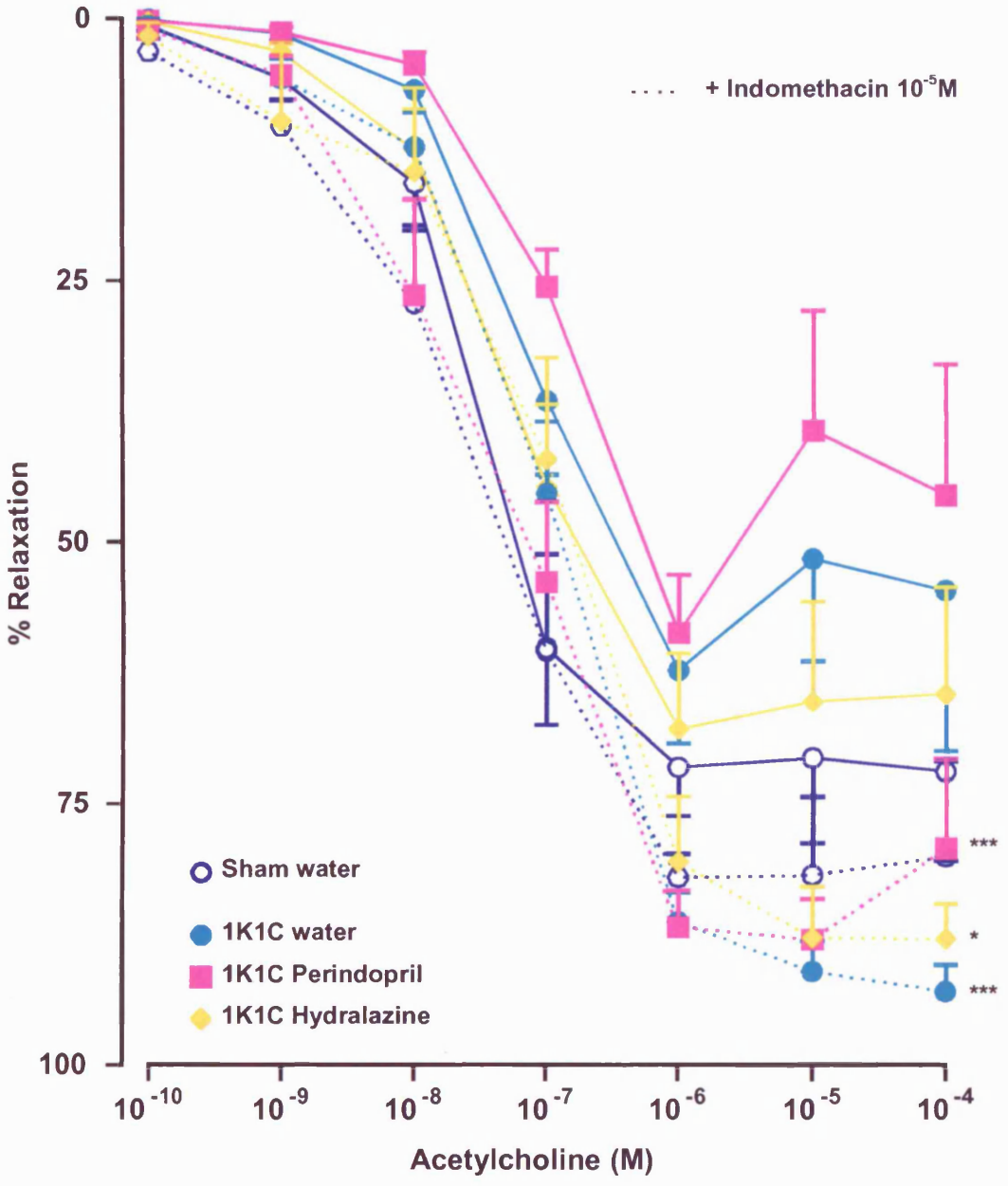


Figure 4.13. The relaxation response to acetylcholine of mesenteric resistance arteries, in the absence and presence of indomethacin 10^{-5}M, from 4 week 1K1C and sham-operated rats.

* P<0.05, *** P<0.001 T-test of dose response compared to that in the absence of indomethacin.

Table 4.12. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh), in mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E max (%)		ACh EC_{50}(μM)		ACh Mean R (%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
SHAM	Water	14	85 ± 4.4	90 ± 3.6	0.03 ± 0.01 (11)	0.04 ± 0.01 (11)	45 ± 5.2	52 ± 3.6
1K1C	Water	14	64 ± 5.0	86 ± 5.6 **	0.12 ± 0.04 (5)	0.04 ± 0.01 (11)	26 ± 4.2	48 ± 4.6 **
SHAM	Perindopril	14	94 ± 1.8	93 ± 2.1	0.04 ± 0.01 (13)	0.04 ± 0.01 (12)	53 ± 2.5	54 ± 2.7
1K1C	Perindopril	14	80 ± 4.5	95 ± 1.1 **	0.14 ± 0.06 (9)	0.11 ± 0.05 (10)	35 ± 3.9	50 ± 2.4 **
SHAM	Losartan	11	82 ± 5.7	90 ± 2.8	0.09 ± 0.02 (9)	0.07 ± 0.02 (9)	36 ± 4.3	49 ± 4.2 *
1K1C	Losartan	13	74 ± 6.5	86 ± 3.7	0.08 ± 0.02 (6)	0.06 ± 0.01 (10)	36 ± 4.7	43 ± 4.2
SHAM	Hydralazine	13	92 ± 2.0	92 ± 1.7	0.04 ± 0.01 (11)	0.06 ± 0.01 (11)	50 ± 2.1	54 ± 2.4
1K1C	Hydralazine	14	73 ± 4.8	92 ± 1.6 **	0.13 ± 0.02 (6)	0.14 ± 0.04 (12)	32 ± 4.3	54 ± 3.2 ***

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

** P<0.01, *** P<0.001 T-test of ACh relaxation compared to the control in the absence of indomethacin.

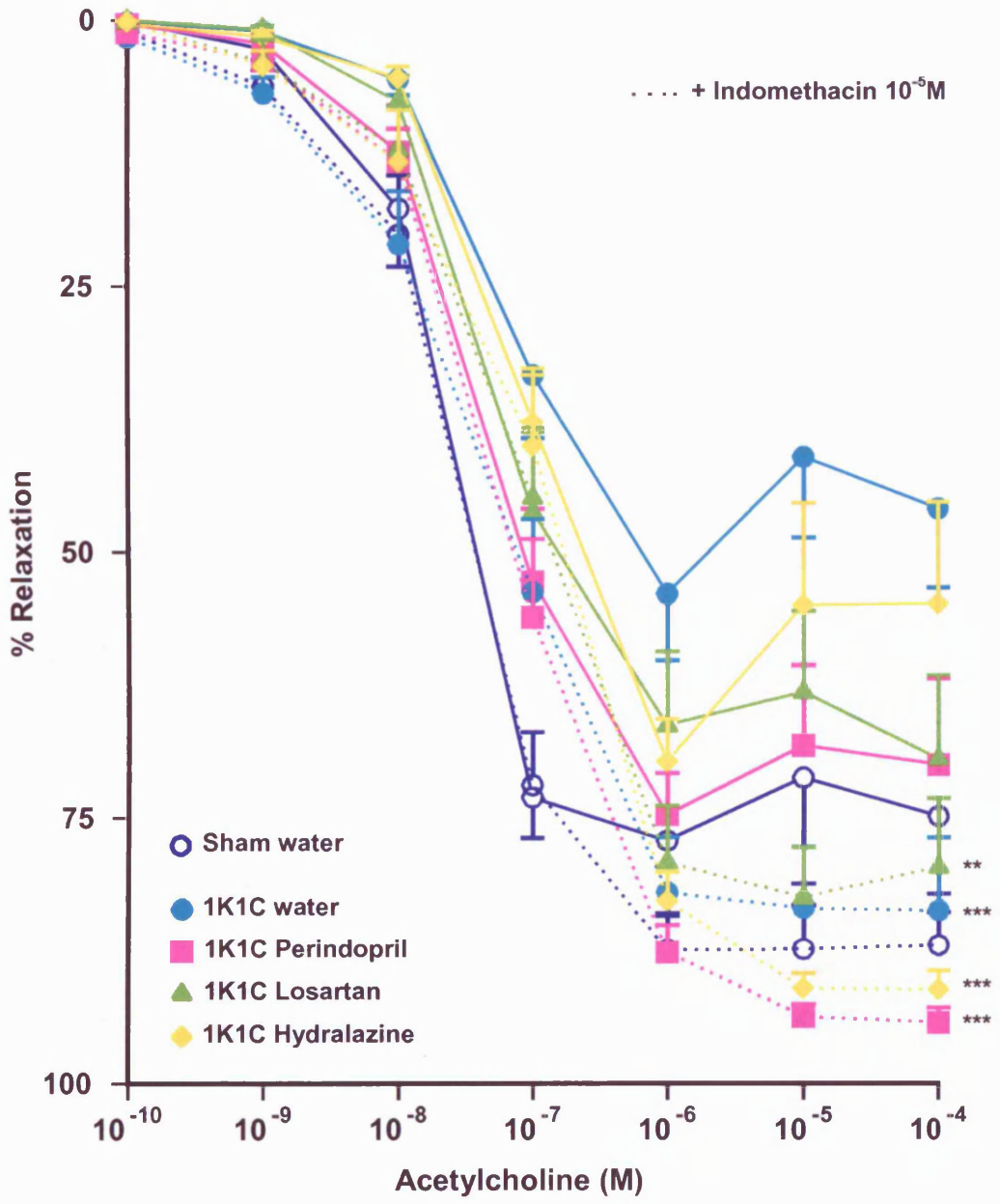


Figure 4.14. The relaxation response to acetylcholine, in the absence and presence of indomethacin 10^{-5}M, of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

** P<0.01, *** P<0.001 T-test of dose response compared to that in the absence of indomethacin.

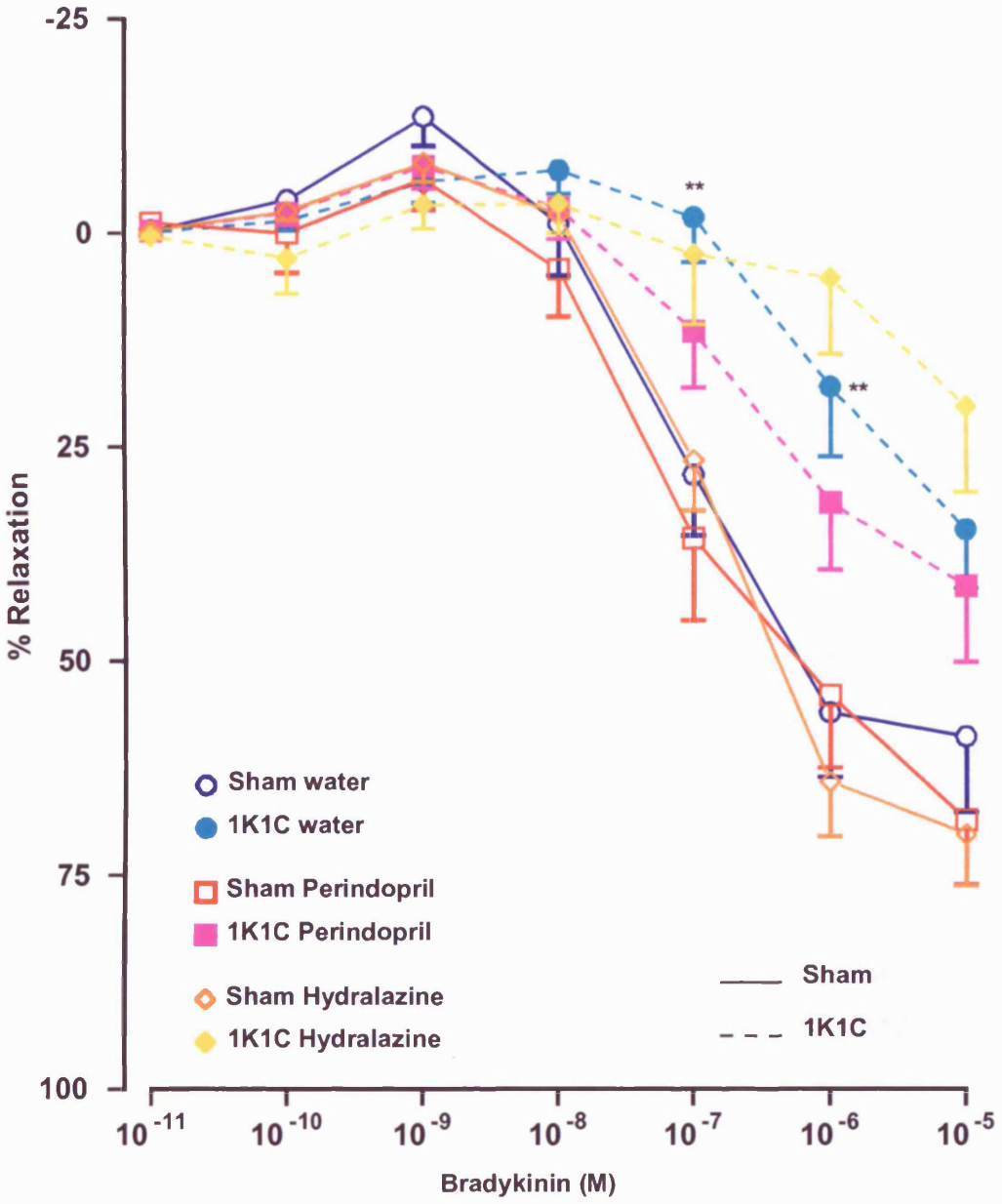


Figure 4.15. The relaxation response to bradykinin of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

** P<0.01 ANOVA compared to untreated sham-operated rats

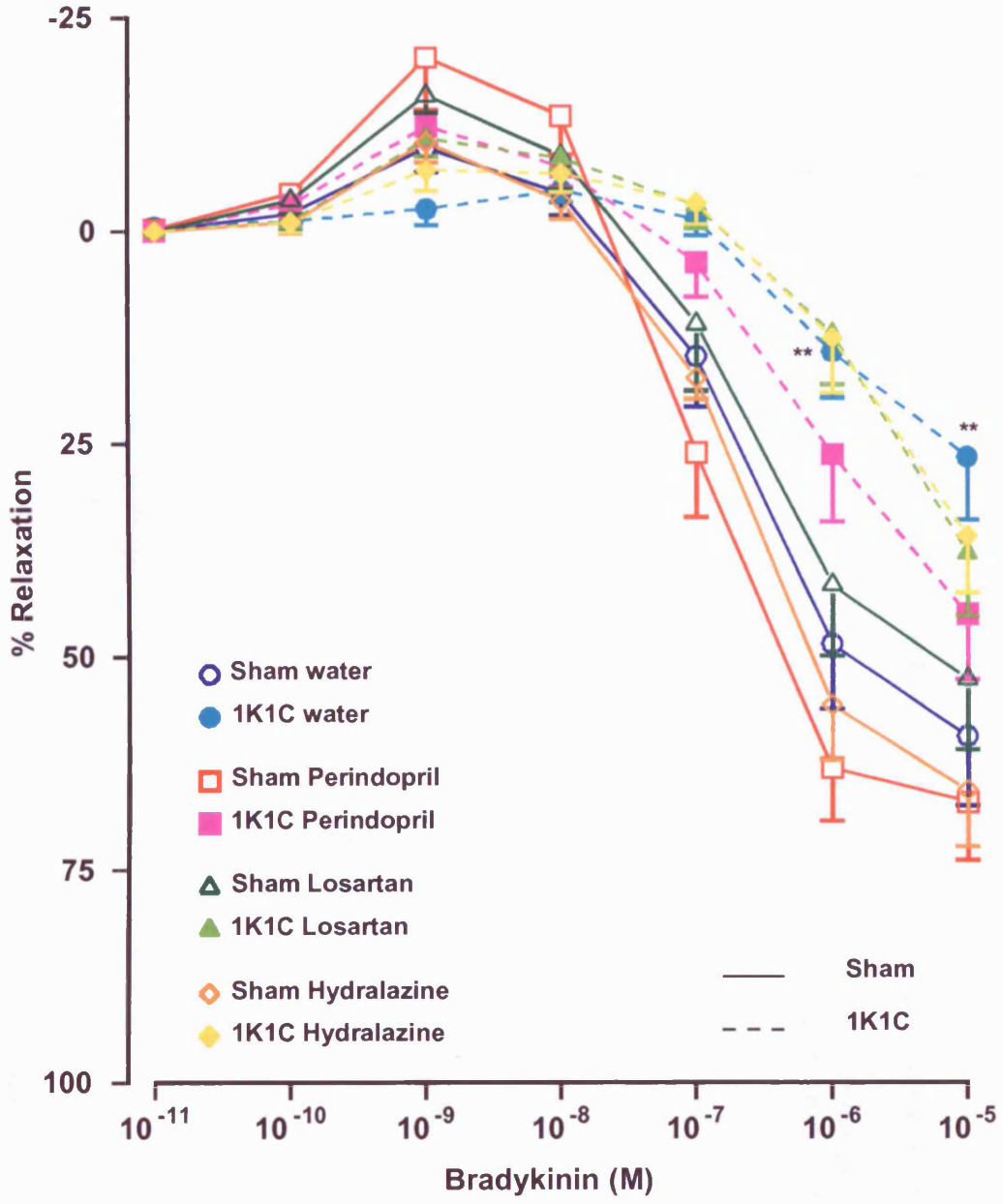


Figure 4.16. The relaxation response to bradykinin of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

** P<0.01 ANOVA compared to untreated sham-operated rats

Table 4.13. The effect of indomethacin 10^{-5}M on the relaxation to bradykinin (BK), in mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	BK E max (%)		BK Mean R (%)	
			Control	Indomethacin	Control	Indomethacin
SHAM	Water	13	60 ± 7.5	81 ± 4.3 *	17 ± 5.1	31 ± 3.9 *
1K1C	Water	13	36 ± 7.1	76 ± 6.3 ***	6 ± 3.9	21 ± 4.1 *
SHAM	Perindopril	12	69 ± 7.5	75 ± 6.9	22 ± 5.7	29 ± 4.4
1K1C	Perindopril	11	45 ± 8.7	75 ± 5.7 *	11 ± 4.6	29 ± 6.5 *
SHAM	Hydralazine	12	74 ± 4.7	86 ± 3.4	21 ± 3.4	32 ± 3.3 *
1K1C	Hydralazine	11	29 ± 8.5	61 ± 8.2 *	5 ± 5.7	20 ± 3.5 *

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ T-test of BK relaxation compared to that in the absence of indomethacin.

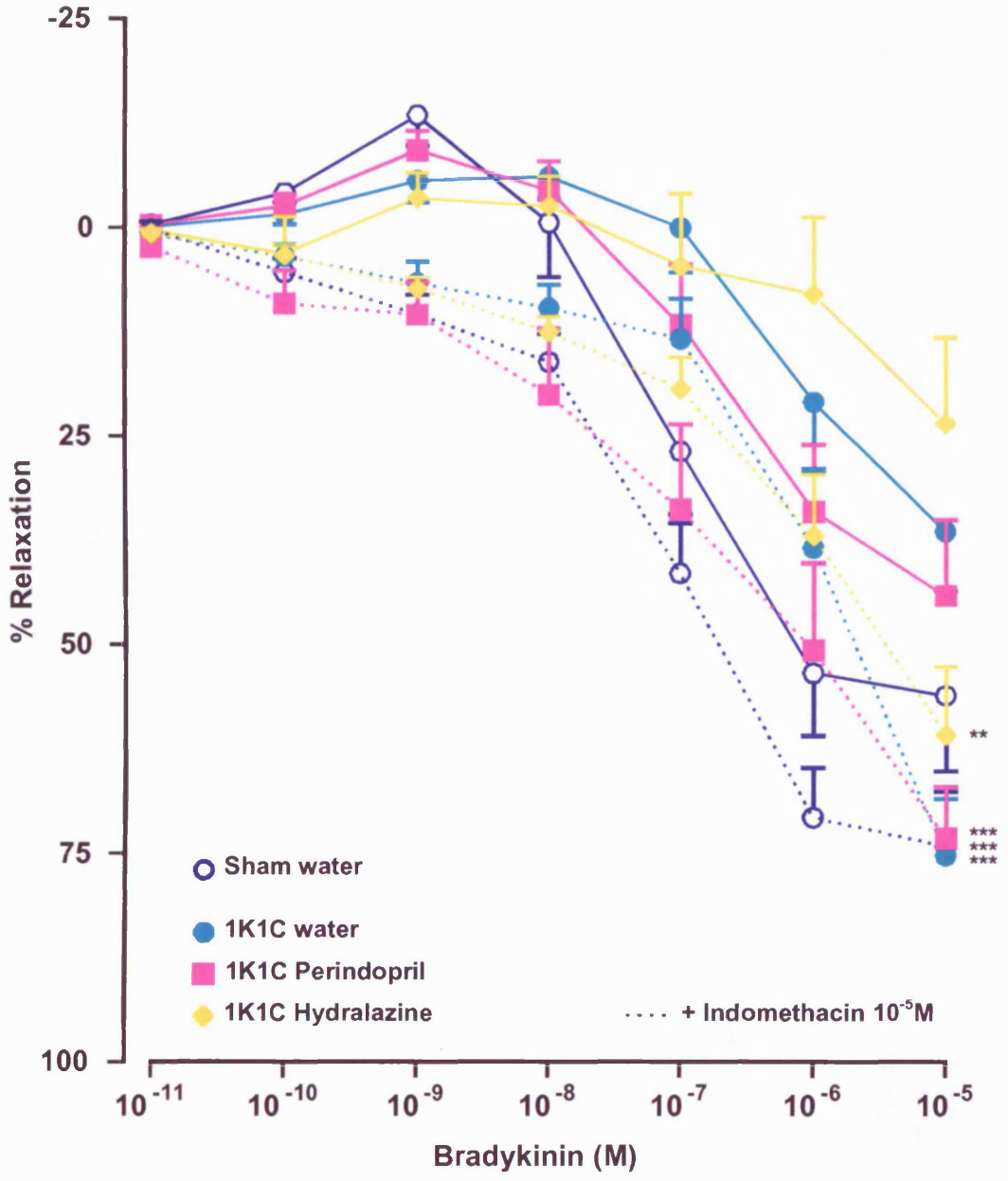


Figure 4.17. The relaxation response to bradykinin of mesenteric resistance arteries, in the absence and presence of indomethacin 10^{-5}M, from 4 week 1K1C and sham-operated rats.

** P<0.01, *** P<0.001 T-test of dose response compared to that in the absence of indomethacin.

Table 4.14. The effect of indomethacin 10^{-5}M on the relaxation to bradykinin (BK), in mesenteric resistance arteries from untreated, perindopril treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	BK E max (%)		BK Mean R (%)	
			Control	Indomethacin	Control	Indomethacin
SHAM	Water	14	62 ± 7.0	81 ± 4.8 *	15 ± 4.0	29 ± 4.2 *
1K1C	Water	14	30 ± 6.2	70 ± 5.0 ***	4 ± 2.8	21 ± 3.4 ***
SHAM	Perindopril	14	70 ± 6.0	83 ± 3.9	17 ± 4.7	36 ± 3.7 **
1K1C	Perindopril	14	45 ± 7.6	80 ± 3.7 ***	7 ± 3.6	24 ± 3.3 **
SHAM	Losartan	11	56 ± 7.6	90 ± 5.9 *	11 ± 4.6	31 ± 4.5 **
1K1C	Losartan	13	38 ± 7.3	74 ± 4.5 ***	4 ± 2.9	24 ± 3.5 ***
SHAM	Hydralazine	13	66 ± 6.6	66 ± 7.1	18 ± 3.0	21 ± 2.8
1K1C	Hydralazine	13	34 ± 7.5	67 ± 6.9 **	4 ± 2.4	18 ± 3.2 **

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ T-test of BK relaxation compared to that in the absence of indomethacin.

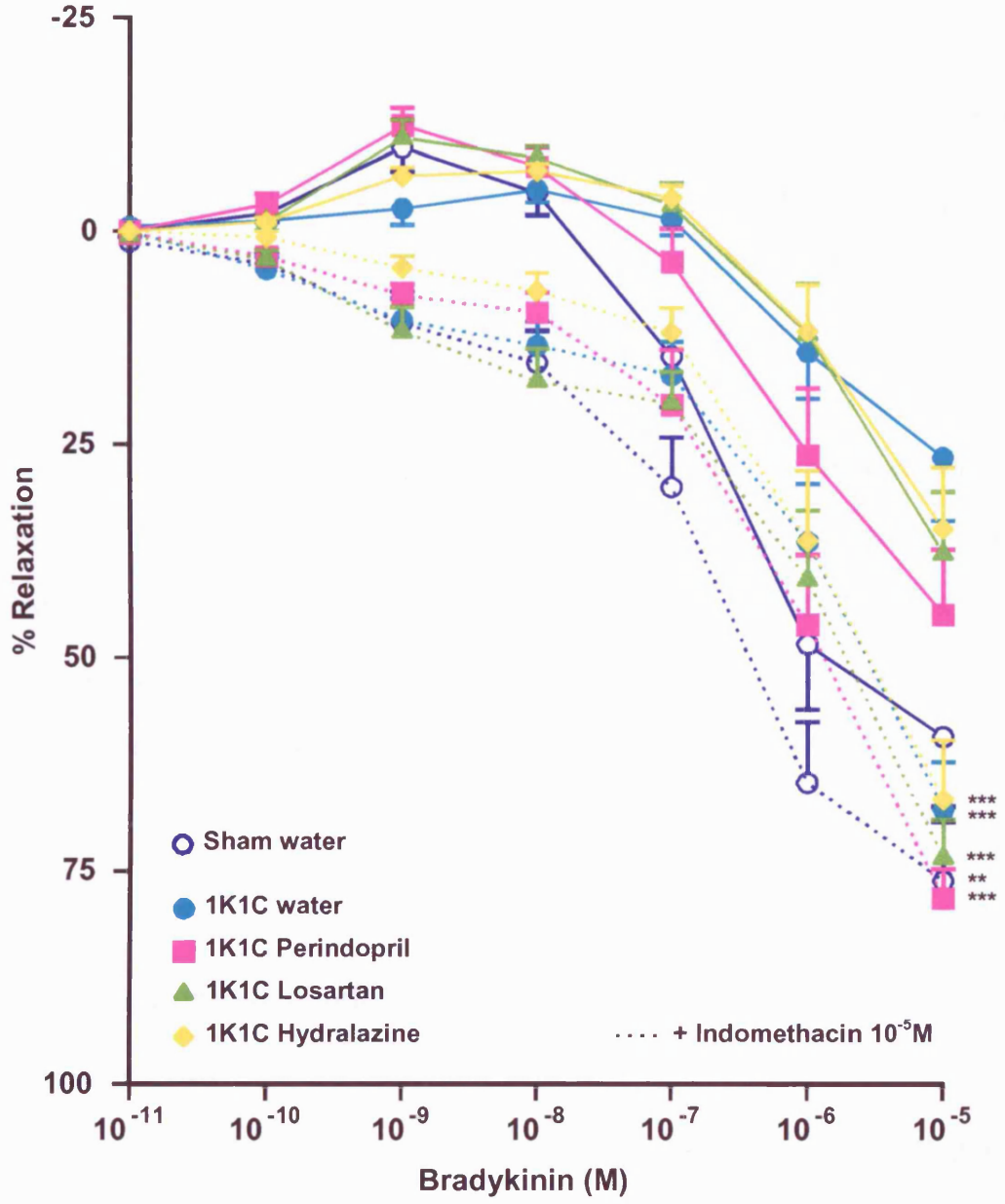


Figure 4.18. The relaxation response to bradykinin, in the absence and presence of indomethacin 10^{-5}M, of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

** P<0.01, *** P<0.001 T-test of dose response compared to that in the absence of indomethacin.

Bradykinin

The mesenteric arteries relaxed to higher concentrations of bradykinin, but lower concentrations caused the mesenteric arteries to contract. There was a significant reduction in the relaxation response to bradykinin in the untreated 1K1C mesenteric arteries at both 4 weeks (Table 4.9 & Figure 4.15), and 8 weeks (Table 4.10 & Figure 4.16). Treatment with perindopril, losartan or hydralazine did not prevent the impaired relaxation response in the 4 week (Figure 4.15), or 8 week (Figure 4.16) 1K1C mesenteric arteries.

Incubation with indomethacin significantly improved the relaxation response to bradykinin in the untreated, perindopril treated, losartan treated, or hydralazine treated 1K1C and sham mesenteries, at both 4 weeks (Table 4.13 & Figure 4.17), and 8 weeks (Table 4.14 & Figure 4.18), although only the relaxation response of the untreated sham rats is shown. Incubation with indomethacin also prevented the contractile response to lower concentrations of bradykinin in the mesenteric arteries, thus suggesting the involvement of a vasoconstrictor prostanoid in this response.

4.5.4 Endothelium-independent relaxation

Sodium Nitroprusside

The maximum relaxation of the mesenteric arteries, and sensitivity to SNP were similar in all mesenteric arteries at both 4 weeks (Table 4.9), and 8 weeks (Table 4.10). The relaxation response to SNP was also similar in all of the mesenteric arteries at both 4 weeks (Figure 4.19), and 8 weeks (Figure 4.20).

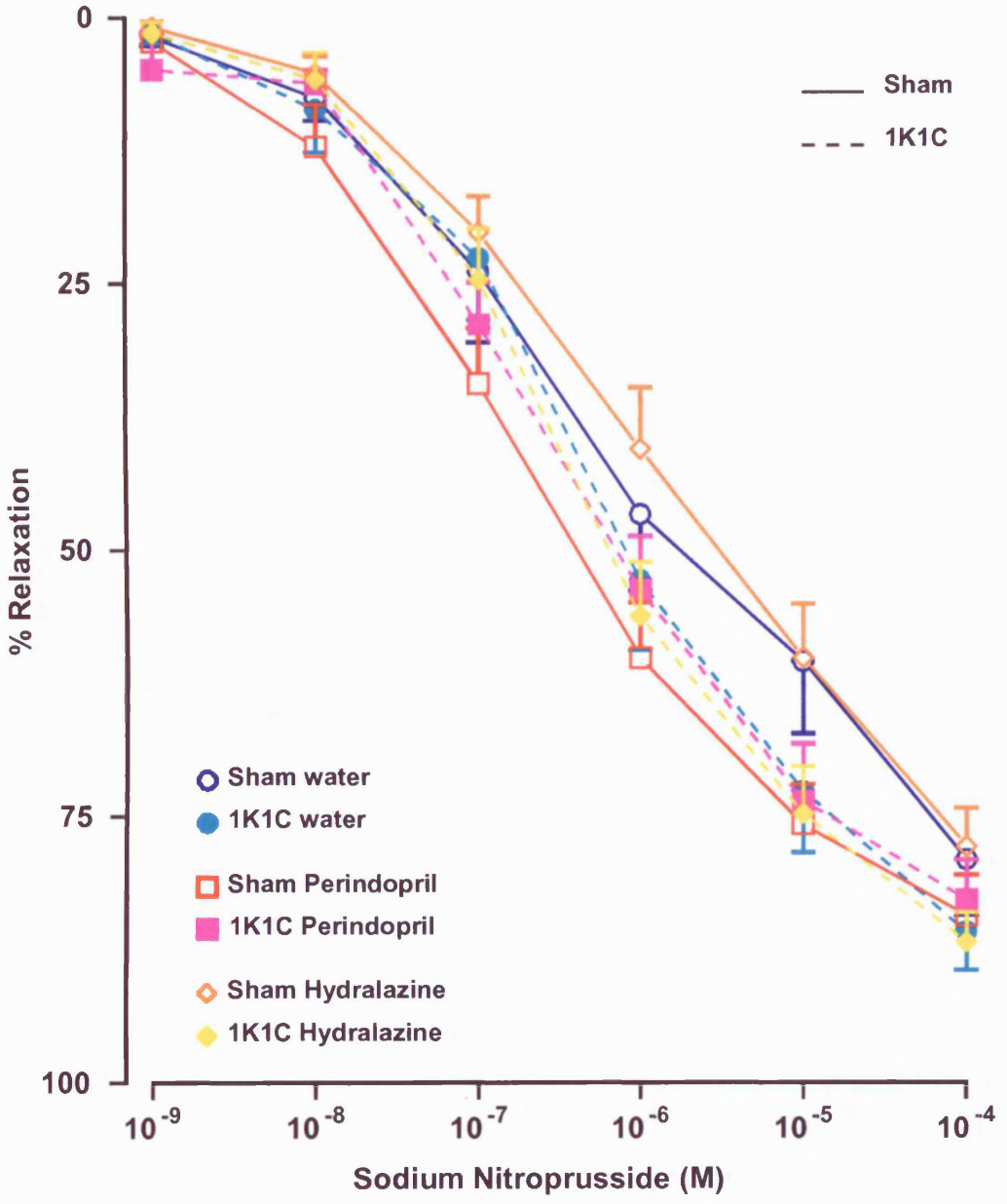


Figure 4.19. The relaxation response to sodium nitroprusside of mesenteric resistance arteries from 4 week 1K1C and sham-operated rats.

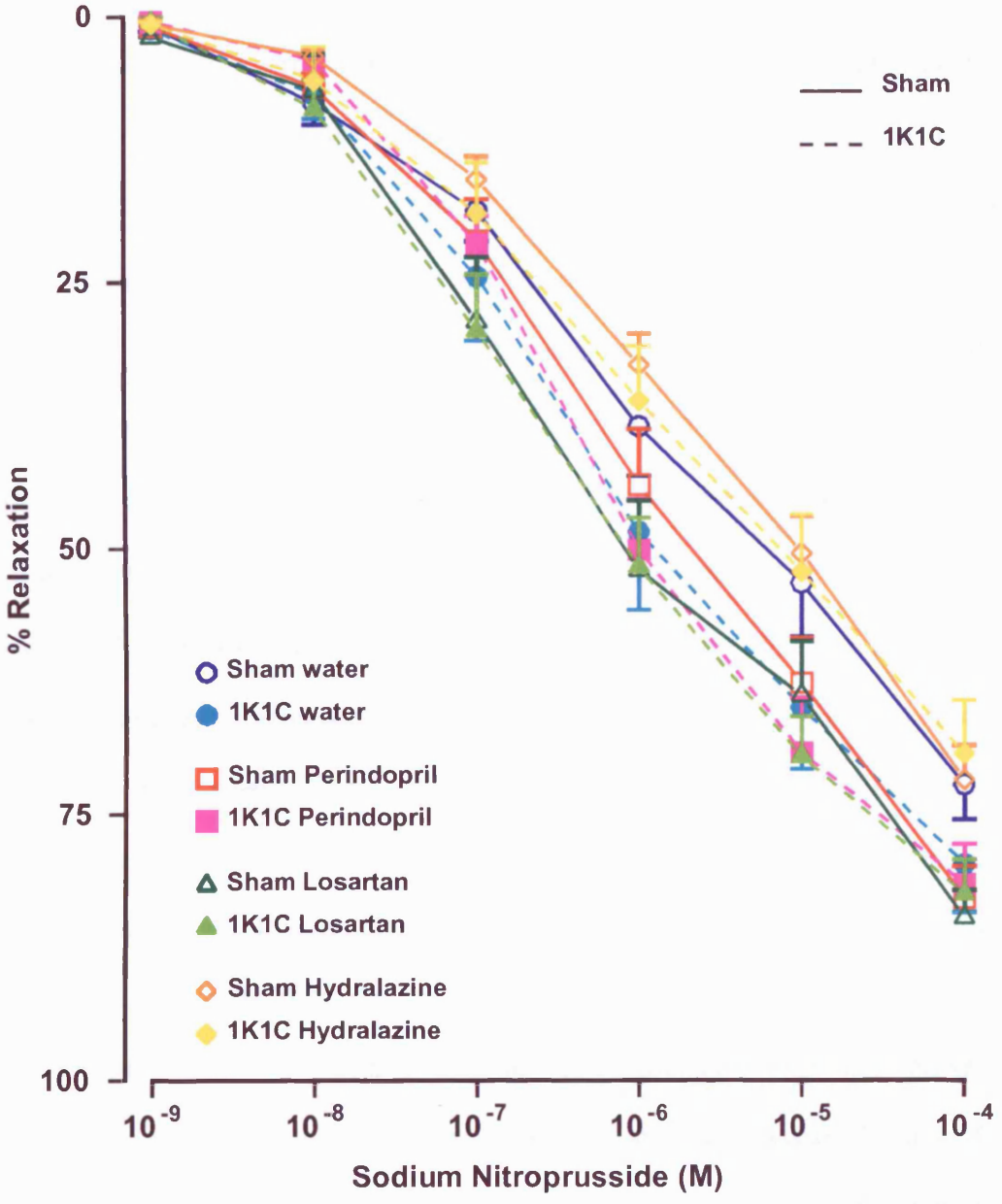


Figure 4.20. The relaxation response to sodium nitroprusside of mesenteric resistance arteries from 8 week 1K1C and sham-operated rats.

4.6 FEMORAL RESISTANCE ARTERY STUDIES

4.6.1 Morphological Characteristics

The normalised internal lumen diameter was similar in all femoral arteries at both 4 weeks (Table 4.15), and 8 weeks (Table 4.16). The medial cross-sectional area (MCSA) was significantly increased in the untreated 1K1C femoral arteries at 4 weeks (21,458 ± 923μm^2), compared to the untreated sham femoral arteries (18328 ± 615μm^2) ($P<0.05$) (Table 4.15). There was a non-significant increase in the MCSA of the untreated sham femoral arteries at 8 weeks (20398 ± 850μm^2), compared to the MCSA in this group at 4 weeks (18328 ± 615μm^2). This increase in MCSA at 8 weeks resulted in a lack of significance between the MCSA of the untreated 1K1C femoral (23203 ± 733μm^2), compared to the untreated sham rats (20398 ± 850μm^2) (Table 4.16). The media thickness (MT) was significantly increased in the untreated 1K1C femoral arteries at both 4 weeks (Table 4.15), and 8 weeks (Table 4.16), compared to the untreated age-matched sham femoral arteries ($P<0.01$). However, the media to lumen ratio (M:L) was similar in all femoral arteries at both 4 weeks (Table 4.15), and 8 weeks (Table 4.16), despite a slight increase in the M:L of the 1K1C femoral arteries.

Treatment with perindopril, losartan or hydralazine did not prevent the development of structure in the 1K1C femoral arteries, at either 4 weeks (Table 4.15), or 8 weeks (Table 4.16). Although, there was a slight reduction in the MCSA of the perindopril treated 1K1C femoral arteries. In addition, vascular hypertrophy was slightly more marked in the hydralazine treated 1K1C femoral arteries. Treatment with perindopril, losartan or hydralazine had no significant effect on the MCSA or MT of the sham femoral arteries.

Table 4.15. Femoral resistance artery morphology of untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Lumen Diameter (μm)	MCSA (μm^2)	Media Thickness (μm)	Media : Lumen (%)
SHAM	Water	14	246 ± 9	18328 ± 615	22.13 ± 0.71	9.35 ± 0.56
1K1C	Water	14	232 ± 11	21458 ± 923 *	26.85 ± 1.11 **	12.17 ± 0.95
SHAM	Perindopril	12	235 ± 10	19388 ± 514	24.23 ± 1.27	10.83 ± 1.14
1K1C	Perindopril	12	215 ± 9	19282 ± 749	25.56 ± 0.76	12.23 ± 0.87
SHAM	Hydralazine	12	243 ± 10	20454 ± 866	24.58 ± 0.98	10.46 ± 0.81
1K1C	Hydralazine	12	231 ± 11	22641 ± 643 ***	27.99 ± 0.49 ***	12.56 ± 1.14

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.16. Femoral resistance artery morphology of untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Lumen Diameter (µm)	MCSA (μm^2)	Media Thickness (µm)	Media : Lumen (%)
SHAM	Water	14	251 ± 8	20398 ± 850	23.76 ± 0.93	9.69 ± 0.62
1K1C	Water	14	235 ± 7	23203 ± 733	28.52 ± 1.02 **	12.40 ± 0.79
SHAM	Perindopril	14	243 ± 7	19757 ± 662	23.60 ± 0.62	9.85 ± 0.45
1K1C	Perindopril	14	229 ± 7	21769 ± 797	27.16 ± 0.91	12.09 ± 0.64
SHAM	Losartan	13	241 ± 9	19403 ± 605	23.60 ± 0.89	10.11 ± 0.75
1K1C	Losartan	13	236 ± 9	22041 ± 580	27.03 ± 1.12	11.88 ± 0.96
SHAM	Hydralazine	13	236 ± 13	19596 ± 697	24.31 ± 0.79	10.94 ± 0.99
1K1C	Hydralazine	15	241 ± 9	23953 ± 1071 ***++	28.74 ± 1.49 **++	12.38 ± 0.98

** P<0.01 ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

4.6.2 Femoral Resistance Artery Contractility

Activation Procedure

The contractile responses generated to high potassium, and to high potassium with 10^{-5}M noradrenaline (NAK), were similar in all femoral arteries at both 4 weeks (Table 4.17), and 8 weeks (Table 4.18). However, when expressed as active media stress, to take account of alterations in wall thickness, there was a non-significant reduction in contractility of the 1K1C femoral arteries, compared to the untreated sham femoral arteries.

Noradrenaline (NA) Contractility

The maximum active wall tension to noradrenaline was similar in all femoral arteries, in the absence and presence of cocaine, at both 4 weeks (Table 4.19), and 8 weeks (Table 4.20). The active wall tension response curves to noradrenaline was also similar at 4 weeks, in the absence (Figure 4.21), and presence of cocaine (Figure 4.22), and at 8 weeks in the absence (Figure 4.23), and presence of cocaine (Figure 4.24). The sensitivity to noradrenaline was similar in all femoral arteries at both 4 weeks (Table 4.19), and 8 weeks (Table 4.20). However, at 8 weeks the sensitivity to noradrenaline was further increased in the untreated sham femoral arteries, in the presence of cocaine, (2.11 $\pm$0.32μM) (Table 4.20), compared to the sensitivity observed in this group at 4 weeks (3.23 $\pm$ 0.42) ($P<0.05$) (Table 4.19).

Expressing the contractile responses as active media stress revealed a significant reduction in the contractile response of the 4 week hydralazine treated 1K1C femoral arteries, in both the absence (Figure 4.25 & Table 4.19), and presence of cocaine (Figure 4.26 & Table 4.19). At 8 weeks, there was a significant reduction in active media stress response in the untreated and hydralazine treated 1K1C femoral arteries, in the absence of cocaine (Figure 4.27), compared to the untreated sham rats. Similarly, there was a

significant reduction in the active media stress of the untreated, losartan treated and hydralazine treated 1K1C femoral arteries at 8 weeks, in the presence of cocaine (Table 4.20 & Figure 4.27).

The addition of cocaine, to prevent noradrenaline re-uptake, significantly increased the sensitivity to noradrenaline in all of the femoral arteries studied, with the exception of the 4 week untreated and hydralazine treated 1K1C femoral arteries.

Table 4.17. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline of femoral resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
SHAM	Water	14	5.32 ± 0.32	6.65 ± 0.36	244 ± 17	304 ± 19
1K1C	Water	14	5.21 ± 0.33	6.34 ± 0.40	198 ± 16	243 ± 20
SHAM	Perindopril	12	5.31 ± 0.23	6.41± 0.29	227 ± 17	276 ± 21
1K1C	Perindopril	12	5.08 ± 0.25	6.25 ± 0.35	201 ± 12	248 ± 16
SHAM	Hydralazine	12	5.60 ± 0.30	6.89 ± 0.37	233 ± 15	287 ± 19
1K1C	Hydralazine	12	5.76 ± 0.22	6.80 ± 0.26	211 ± 13	249 ± 16

Table 4.18. The maximum contractile responses to high potassium and high potassium with 10^{-5}M noradrenaline of femoral resistance arteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Potassium (mN/mm)	NAK (mN/mm)	Potassium Active media stress (mN/mm^2)	NAK Active media stress (mN/mm^2)
SHAM	Water	14	5.76 ± 0.24	7.07 ± 0.31	247 ± 13	302 ± 16
1K1C	Water	14	5.39 ± 0.35 [13]	6.51 ± 0.35	193 ± 14 [13]	231 ± 15
SHAM	Perindopril	14	5.12 ± 0.31	6.56 ± 0.38	218 ± 15	280 ± 17
1K1C	Perindopril	14	5.04 ± 0.22	6.60 ± 0.24	188 ± 9	247 ± 13
SHAM	Losartan	13	5.05 ± 0.37	7.12 ± 0.47	215 ± 14	303 ± 17
1K1C	Losartan	13	5.03 ± 0.34	6.23 ± 0.37	191 ± 15	234 ± 16
SHAM	Hydralazine	13	5.34 ± 0.46	6.61 ± 0.51	225 ± 23	278 ± 25
1K1C	Hydralazine	15	5.28 ± 0.30	6.66 ± 0.34	195 ± 19	244 ± 21

Table 4.19. The maximum contractile response and sensitivity (EC_{50}) to noradrenaline of femoral resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	Max. NA (mN/mm)	NA EC_{50} (μm)	Max. NA active media stress (mN/mm^2)	Max. NA with cocaine (mN/mm)	NA EC_{50} with cocaine (μM)	Max. NA active media stress with cocaine (mN/mm^2)
SHAM	Water	14	6.08 ± 0.33	5.14 ± 0.75 [13]	278 ± 17	6.22 ± 0.31	3.23 ± 0.42 [13]	284 ± 16
1K1C	Water	14	5.78 ± 0.39	4.23 ± 0.45	221 ± 18	6.10 ± 0.32	3.20 ± 0.42 [13]	232 ± 16
SHAM	Perindopril	12	5.99 ± 0.30	5.24 ± 1.22 [11]	257 ± 20	6.06 ± 0.27	2.91 ± 0.61	259 ± 19
1K1C	Perindopril	12	5.67 ± 0.30	4.65 ± 0.76	224 ± 13	5.73 ± 0.33	3.45 ± 0.59	226 ± 15
SHAM	Hydralazine	12	5.75 ± 0.30	6.08 ± 0.57 [8]	242 ± 20	6.09 ± 0.32	3.57 ± 0.78	254 ± 18
1K1C	Hydralazine	12	4.84 ± 0.27	6.99 ± 0.68	177 ± 14 ***	5.29 ± 0.21	4.98 ± 0.86	193 ± 12 ***

*** $P<0.001$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.20. The maximum contractile response and sensitivity (EC_{50}) to noradrenaline of femoral resistance arteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	Max. NA (mN/mm)	NA EC_{50} (μm)	Max. NA active media stress (mN/mm^2)	Max. NA with cocaine (mN/mm)	NA EC_{50} with cocaine (μM)	Max. NA active media stress with cocaine (mN/mm^2)
SHAM	Water	14	6.90 ± 0.30	4.37 ± 1.11 [13]	293 ± 22	6.99 ± 0.30	2.11 ± 0.32	298 ± 14
1K1C	Water	14	6.29 ± 0.35	6.92 ± 0.96	224 ± 15	6.41 ± 0.37	3.63 ± 0.84	229 ± 16 *
SHAM	Perindopril	14	6.39 ± 0.36	3.18 ± 0.49 [13]	273 ± 16	6.23 ± 0.35	1.86 ± 0.20	266 ± 17
1K1C	Perindopril	14	6.13 ± 0.24	5.55 ± 0.80 [13]	230 ± 14	6.39 ± 0.25	3.14 ± 0.44	240 ± 13
SHAM	Losartan	13	6.89 ± 0.42	5.45 ± 1.20	295 ± 17	6.94 ± 0.37	2.24 ± 0.46	298 ± 17
1K1C	Losartan	13	6.07 ± 0.30	4.04 ± 0.77	230 ± 16	6.00 ± 0.25	2.88 ± 0.43	227 ± 14 *
SHAM	Hydralazine	13	5.98 ± 0.50	6.05 ± 1.14	252 ± 25	6.23 ± 0.49	3.39 ± 0.72	262 ± 24
1K1C	Hydralazine	15	5.98 ± 0.39	6.39 ± 0.65	222 ± 23	6.27 ± 0.35	3.01 ± 0.41	231 ± 22 +

* $P<0.05$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

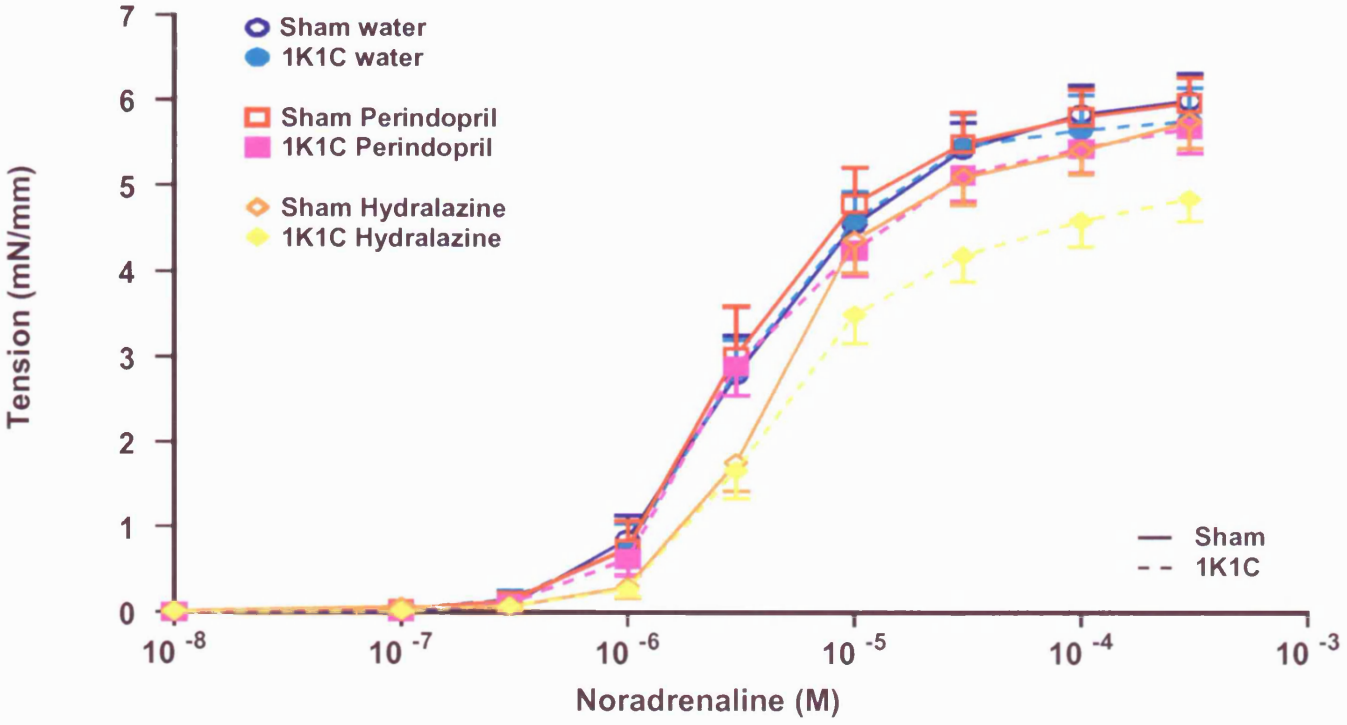


Figure 4.21. The contractile response to noradrenaline (mN/mm) of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

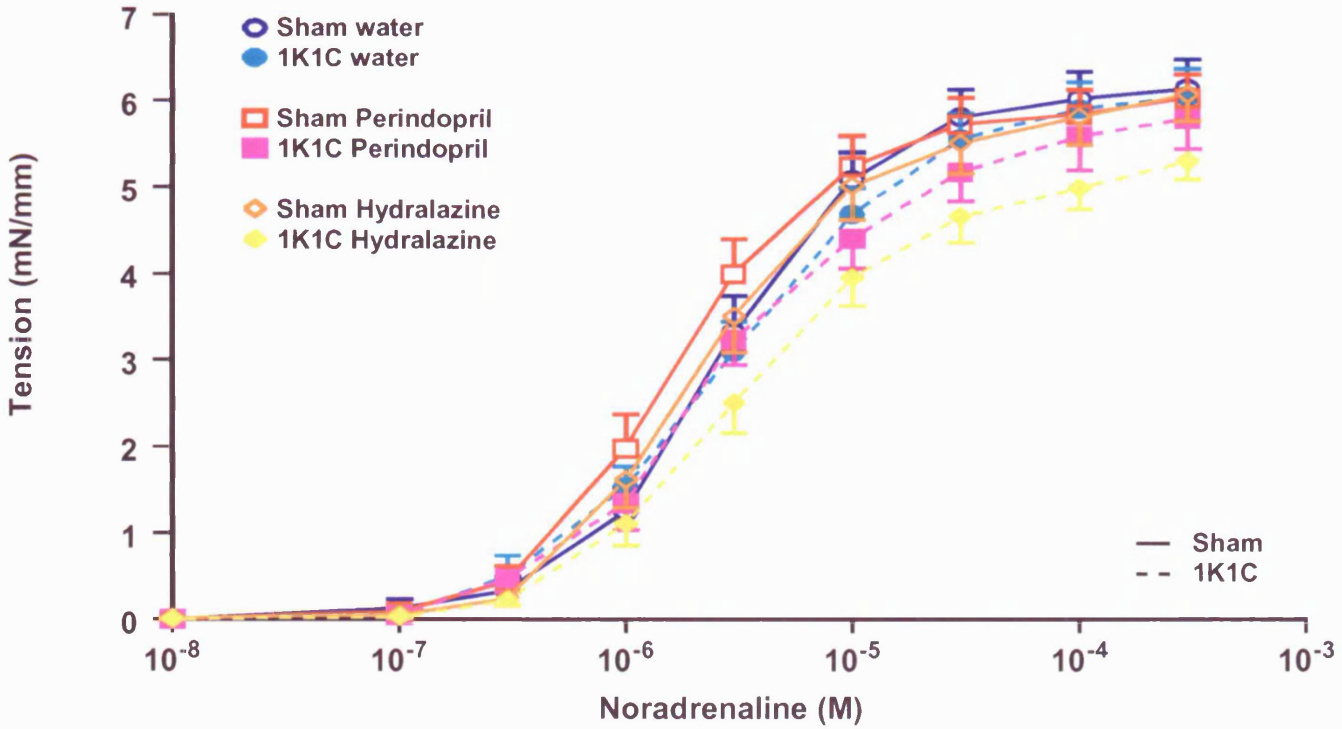


Figure 4.22. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

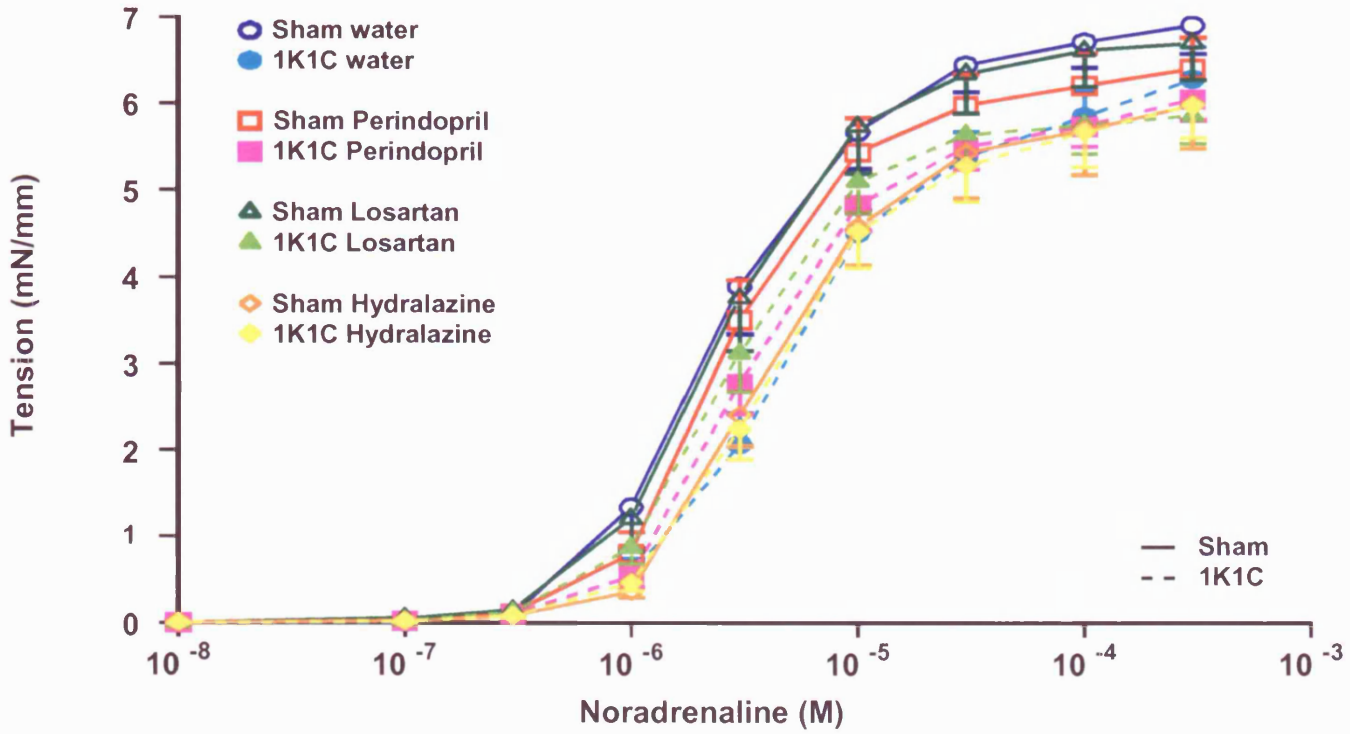


Figure 4.23. The contractile response to noradrenaline (mN/mm) of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

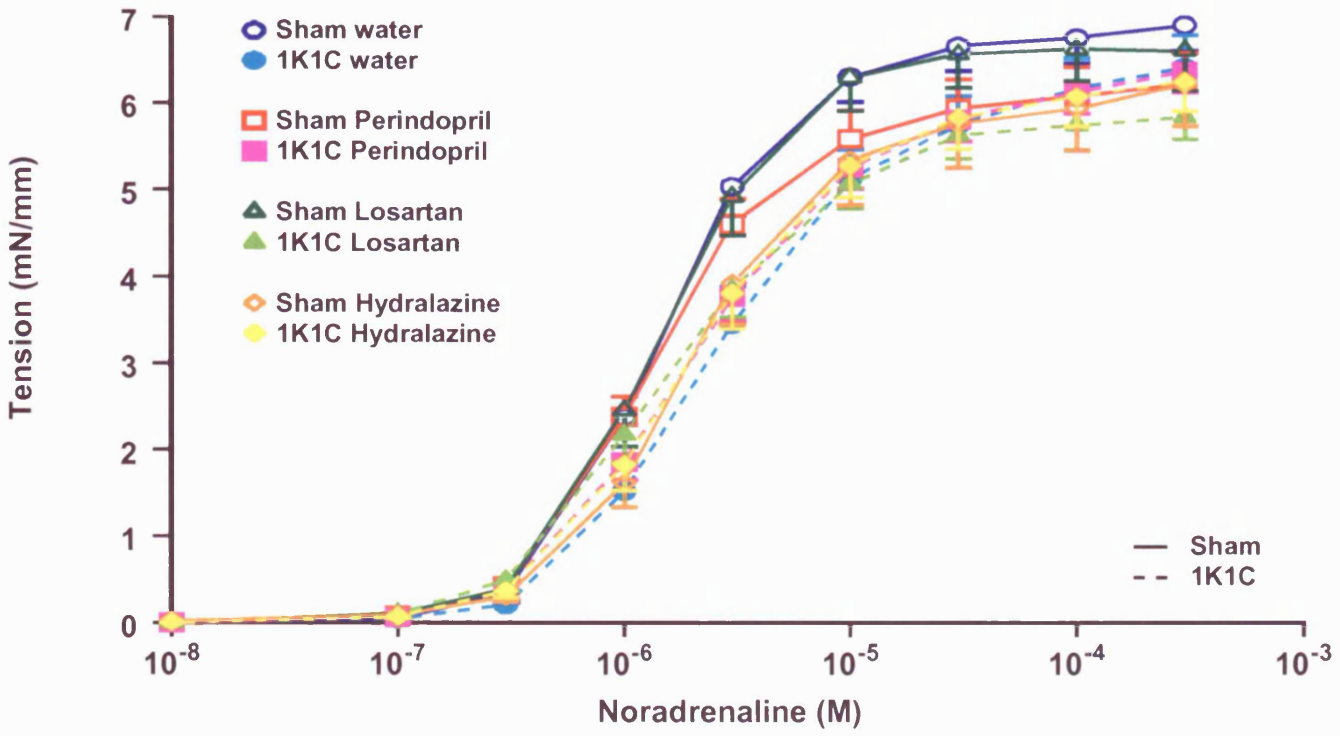


Figure 4.24. The contractile response to noradrenaline (mN/mm), in the presence of cocaine, of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

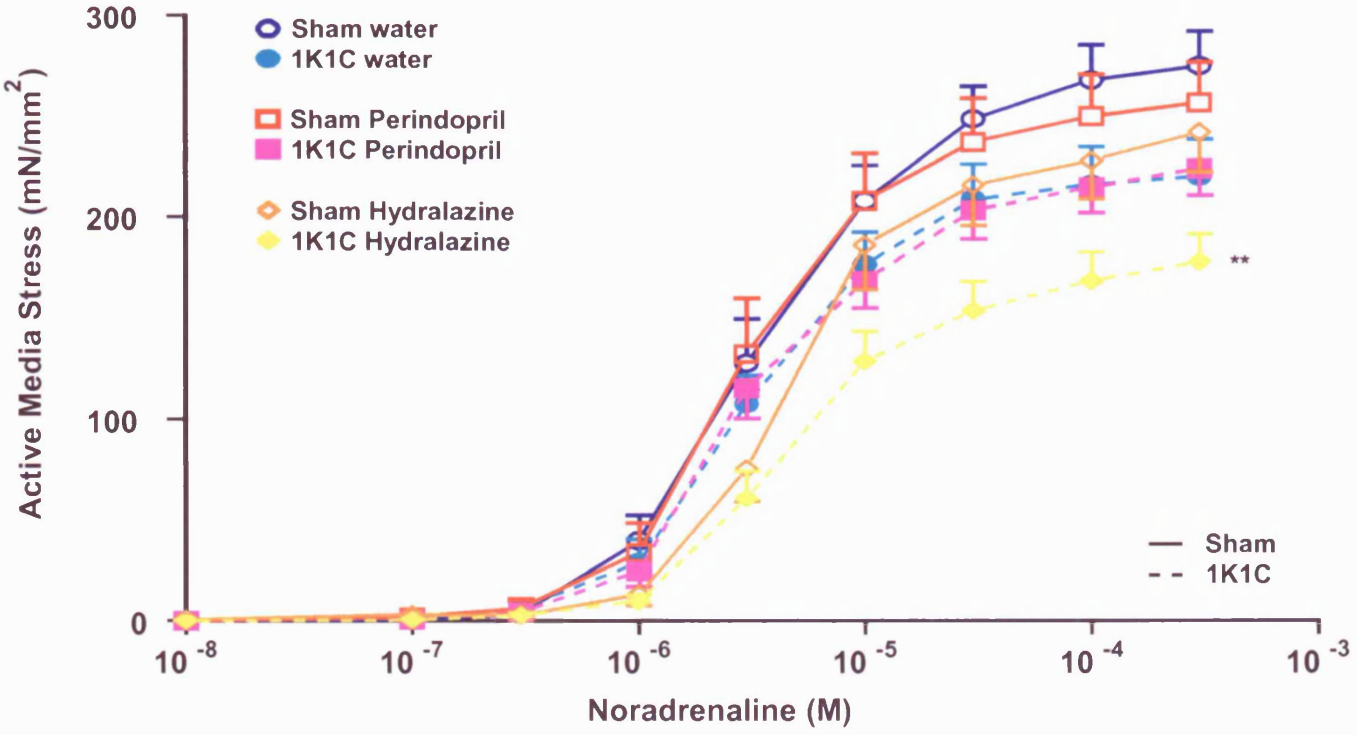


Figure 4.25. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2) of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

** $P<0.01$ ANOVA compared to untreated sham-operated rats

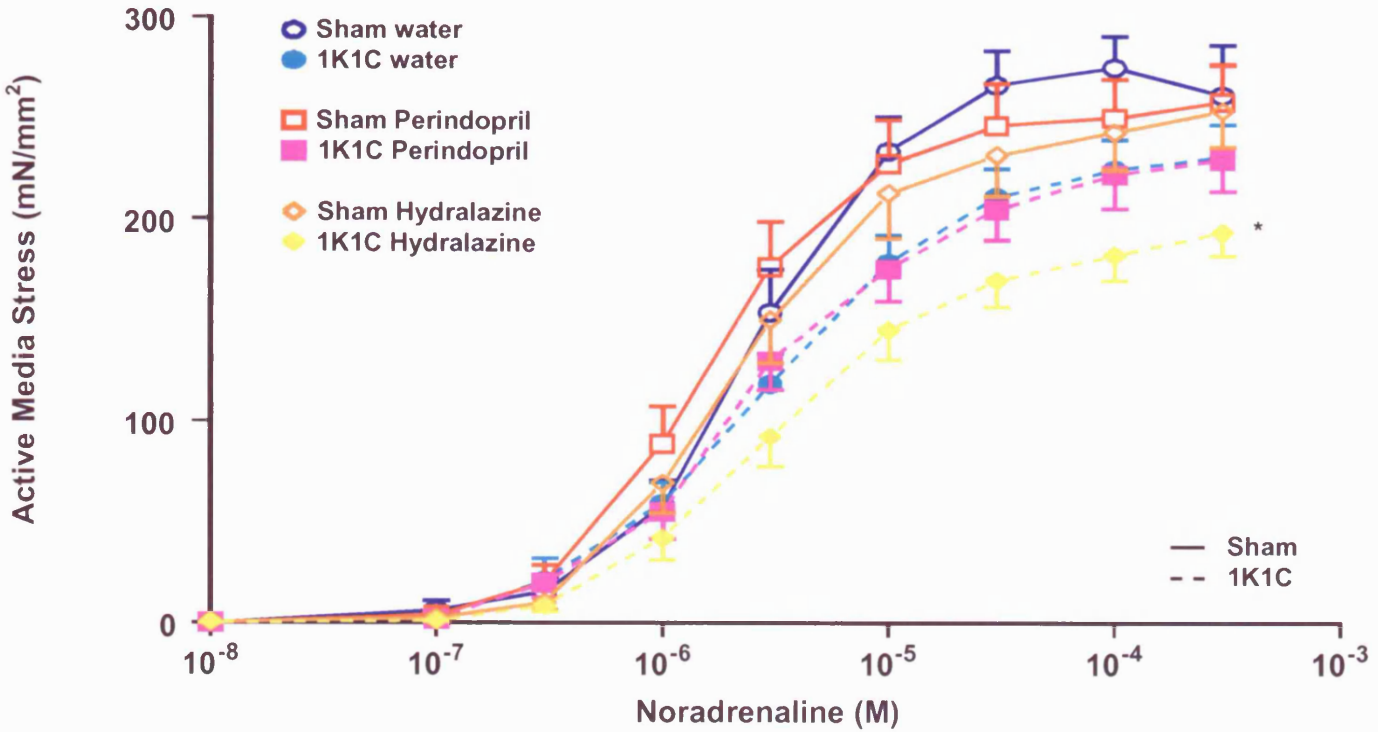


Figure 4.26. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2), in the presence of cocaine, of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

* $P<0.05$ ANOVA compared to untreated sham-operated rats

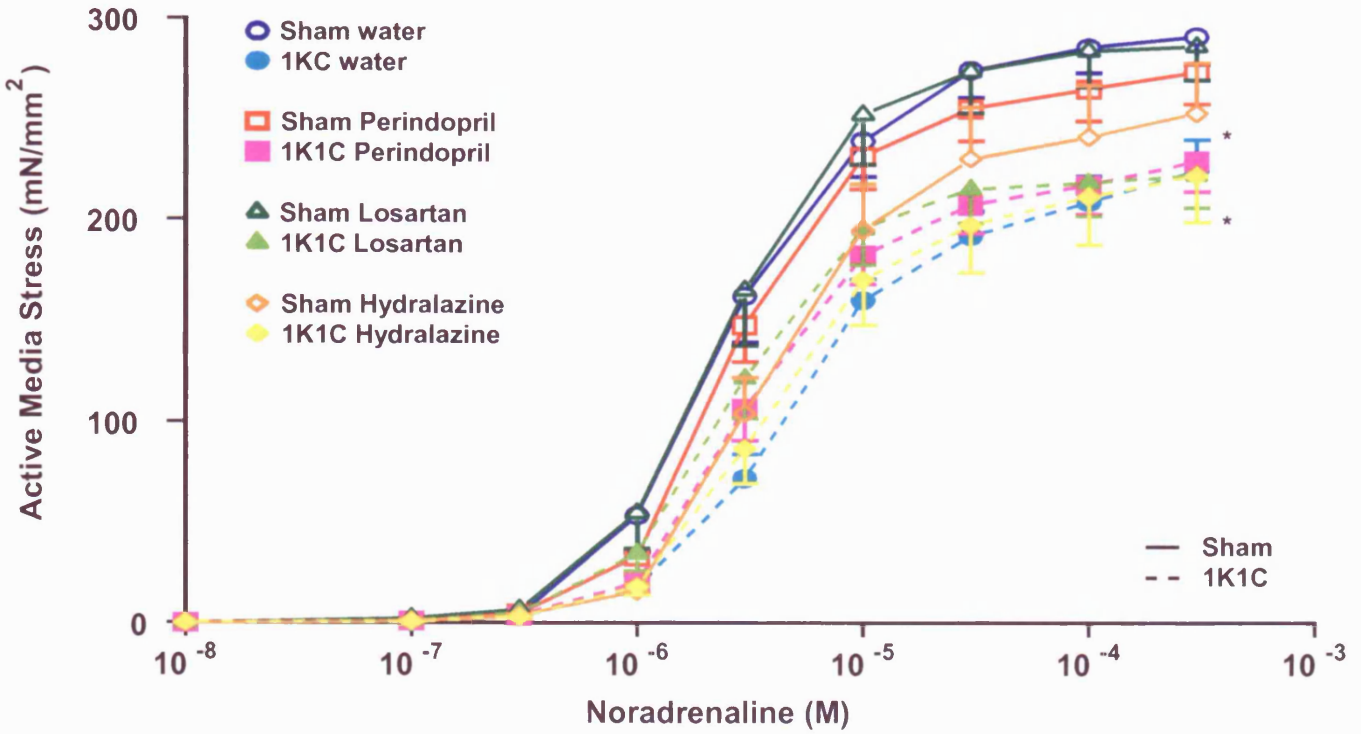


Figure 4.27. The contractile response to noradrenaline, expressed as active media stress (mN/mm^2) to noradrenaline of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

* $P<0.05$ ANOVA compared to untreated sham-operated rats

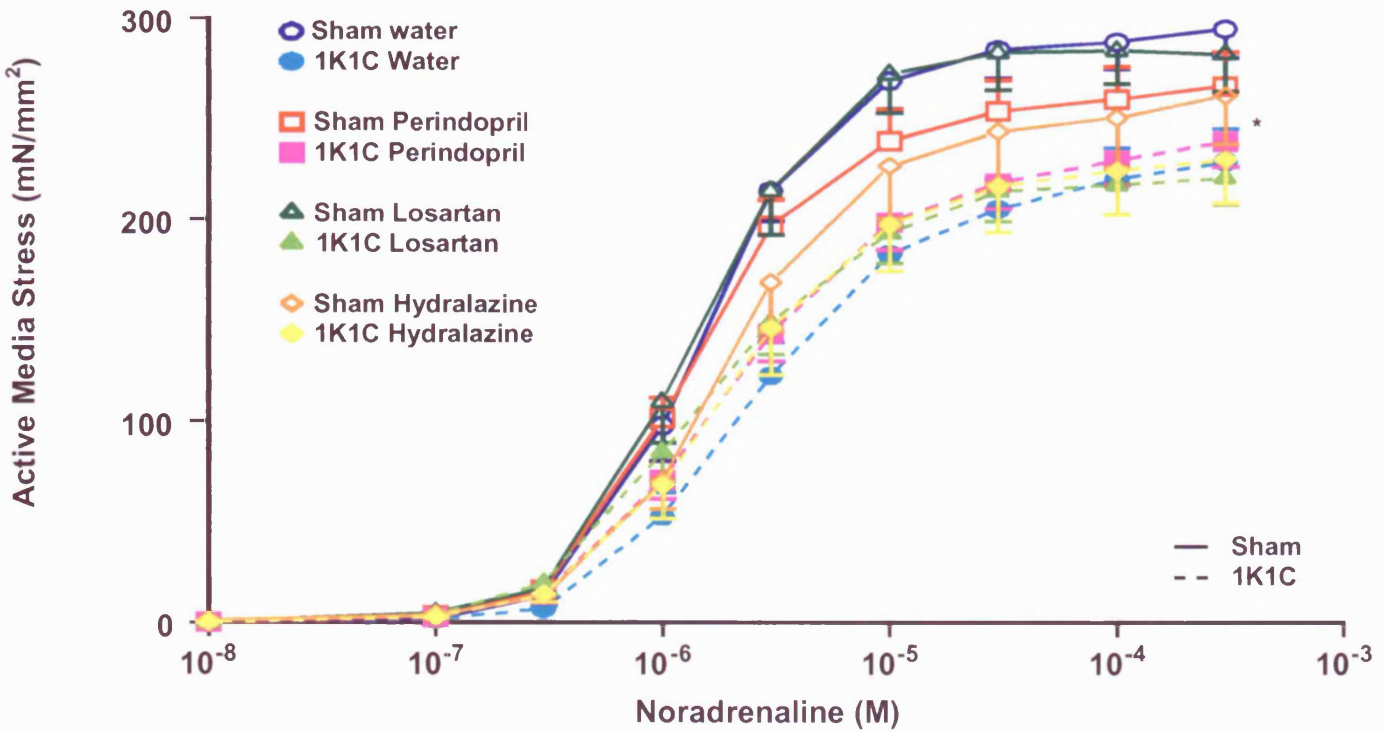


Figure 4.28. The contractile response to noradrenaline, expressed as active media stress (mN/mm2), in the presence of cocaine, of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

* $P<0.05$ ANOVA of untreated 1K1C compared to untreated sham-operated rats

4.6.3 Endothelium-dependent relaxation

Acetylcholine (ACh)

The maximum relaxation to ACh was similar in all femoral arteries at both 4 weeks (Table 4.21), and 8 weeks (Table 4.22). At both 4- and 8-weeks, low concentrations of acetylcholine evoked a relaxation response in these arteries, but higher concentrations caused the arteries to re-contract. The dose-response curves to ACh were also similar in all femoral arteries at both 4 weeks (Figure 4.29), and 8 weeks (Figure 4.30), although a significant increase in relaxation was observed in the untreated 4 week 1K1C femoral arteries, at 10^{-6}M ACh, compared to the response to 10^{-6}M ACh in the untreated sham femoral artery ($P<0.05$) (Figure 4.29). Treatment with perindopril, losartan or hydralazine did not alter the relaxation response of the femoral arteries to ACh.

Incubation with indomethacin significantly improved the relaxation of arteries from the untreated, perindopril treated, losartan treated, and hydralazine treated 1K1C and sham femoral arteries, at both 4 weeks (Table 4.23 & Figure 4.31), and 8 weeks (Table 4.24 & Figure 4.32), although only the relaxation response of the untreated sham rats is shown. Thus, suggesting the involvement of a vasoconstrictor prostanoid in the contractile response to higher concentrations of acetylcholine.

Bradykinin (BK)

Femoral arteries did not relax to low concentrations of bradykinin and higher concentrations caused arterial contraction (Figures 4.33 & 4.34). There was a slight reduction in the contractile response to bradykinin in the 1K1C femoral arteries, compared to the sham femoral arteries, although the overall bradykinin response curve followed a similar pattern in all femoral arteries studied.

Indomethacin failed to alter the contractile response to bradykinin at 4 weeks in the untreated, perindopril treated, and hydralazine treated 1K1C and sham femoral arteries

(Table 4.23 & Figure 4.35). Similarly, there was little change in the response to bradykinin at 8 weeks, but there was less re-contraction in the 8 week untreated sham femorals in the presence of indomethacin (Table 4.24 & Figure 4.36).

4.6.4 Endothelium-independent relaxation

Sodium Nitroprusside (SNP)

The maximum relaxation and sensitivity to SNP were similar in all femoral arteries at both 4 weeks (Table 4.21), and 8 weeks (Table 4.22), with the exception of a significant reduction in the maximum relaxation to SNP in the perindopril treated sham femoral arteries, at 4 weeks ($P<0.05$) (Table 4.17). Similarly, there was a reduction in the sensitivity of the perindopril treated 1K1C femoral arteries at 8 weeks, compared to the untreated sham femoral arteries ($P<0.05$) (Table 4.22). The relaxation responses were similar in all femoral arteries studied at both 4 weeks (Figure 4.37), and 8 weeks (Figure 4.38).

At 8 weeks, there was a significant reduction in the relaxation response to SNP in the 8 week untreated 1K1C femoral arteries, compared to the relaxation response of this group at 4 weeks ($P<0.05$). However, this decrease in sensitivity of the untreated 8 week 1K1C rats failed to achieve significance upon comparison of the EC_{50} values.

Table 4.21. The maximum relaxation response (E Max) to acetylcholine (ACh), and maximum relaxation (E Max) and sensitivity (EC_{50}) to sodium nitroprusside (SNP), of femoral resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E Max (%)	SNP E Max (%)	SNP EC_{50} (μM)
SHAM	Water	14	41 ± 4.8	81 ± 4.1 (13)	0.37 ± 0.10 (13)
1K1C	Water	14	57 ± 4.4	81 ± 2.4	0.53 ± 0.12 (12)
SHAM	Perindopril	12	36 ± 5.7	66 ± 5.0 **	0.41 ± 0.14
1K1C	Perindopril	12	42 ± 5.7	73 ± 3.2	0.88 ± 0.29 (11)
SHAM	Hydralazine	12	55 ± 4.5	77 ± 2.1	0.22 ± 0.05 (11)
1K1C	Hydralazine	12	57 ± 7.0	78 ± 3.1	0.60 ± 0.17 (11)

** $P<0.01$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

Table 4.22. The maximum relaxation response (E Max) to acetylcholine (ACh), and maximum relaxation (E Max) and sensitivity (EC_{50}) to sodium nitroprusside (SNP), of femoral resistance arteries from untreated, perindopril treated, losartan treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E Max (%)	SNP E Max (%)	SNP EC_{50} (μM)
SHAM	Water	14	39 ± 4.1	71 ± 3.3	0.36 ± 0.11
1K1C	Water	14	41 ± 4.4	70 ± 2.4	1.58 ± 0.44
SHAM	Perindopril	14	35 ± 3.9	69 ± 3.0	0.56 ± 0.16 [13]
1K1C	Perindopril	14	50 ± 6.0	67 ± 3.9	1.05 ± 0.15 *
SHAM	Losartan	13	41 ± 3.4	69 ± 3.8 [12]	0.30 ± 0.06 [12]
1K1C	Losartan	13	51 ± 5.0	77 ± 3.5 [12]	0.49 ± 0.17 [12]
SHAM	Hydralazine	13	51 ± 5.2	65 ± 4.2	0.07 ± 0.33 [12]
1K1C	Hydralazine	15	44 ± 5.4	67 ± 2.9 [14]	1.15 ± 0.35 [13]

* $P<0.05$ ANOVA compared to untreated sham-operated rats with Bonferroni correction for multiple comparisons.

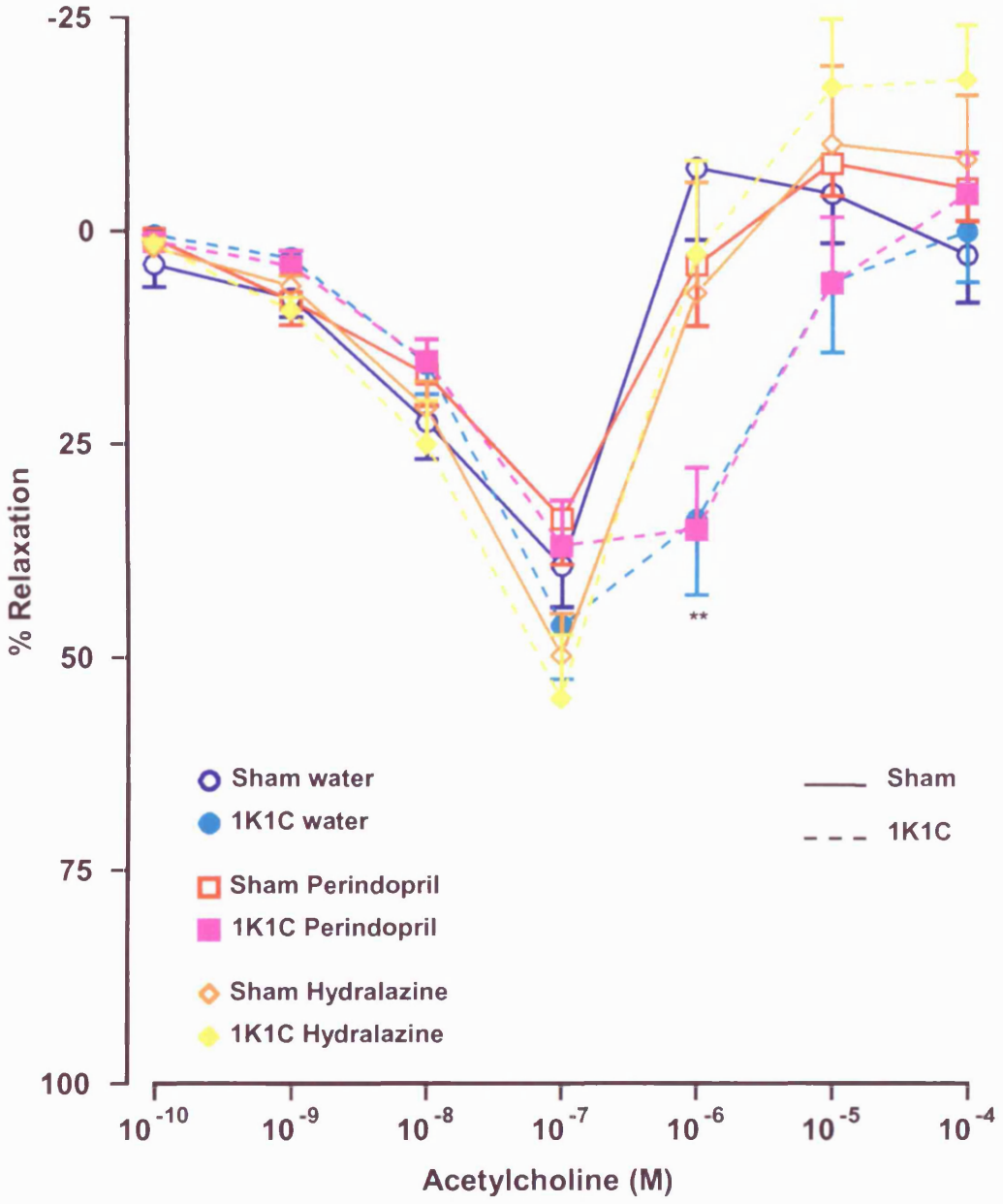


Figure 4.29. The relaxation response to acetylcholine of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

** P<0.01 ANOVA compared to untreated sham-operated rats

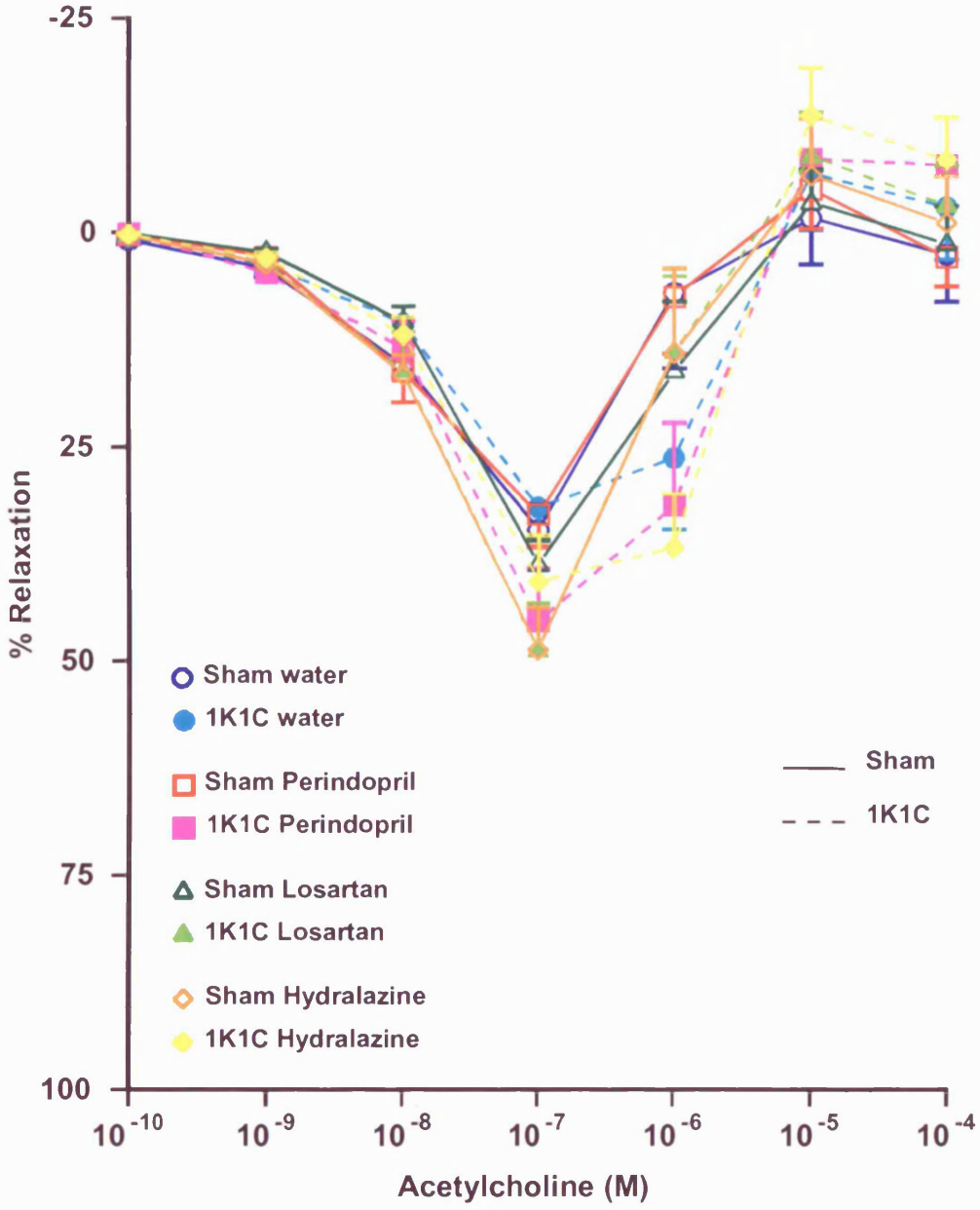


Figure 4.30. The relaxation response to acetylcholine of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

Table 4.23. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh) or bradykinin (BK), in femoral resistance arteries from untreated, perindopril treated, or hydralazine treated 4 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)		BK Mean R (%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
SHAM	Water	14	41 ± 4.8	59 ± 5.4 *	9 ± 4.8	30 ± 4.2 **	-2 ± 3.3 (13)	6 ± 4.7 (13)
1K1C	Water	14	57 ± 4.4	64 ± 3.8	15 ± 4.9	35 ± 4.3 **	6 ± 3.4	3 ± 3.8
SHAM	Perindopril	12	36 ± 5.7	42 ± 4.6	7 ± 3.9	22 ± 3.9 *	-5 ± 2.4	-1 ± 4.5
1K1C	Perindopril	12	42 ± 5.8	60 ± 6.1 *	13 ± 4.4	31 ± 5.2 *	3 ± 2.9	6 ± 4.9
SHAM	Hydralazine	12	55 ± 4.5	61 ± 3.7	10 ± 5.7	32 ± 2.8 **	-3 ± 4.1	5 ± 3.8
1K1C	Hydralazine	12	57 ± 7.0	68 ± 5.0	8 ± 5.8	37 ± 4.1 ***	4 ± 4.6	1 ± 4.7

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ T-test of ACh or BK relaxation compared to the control in the absence of indomethacin.

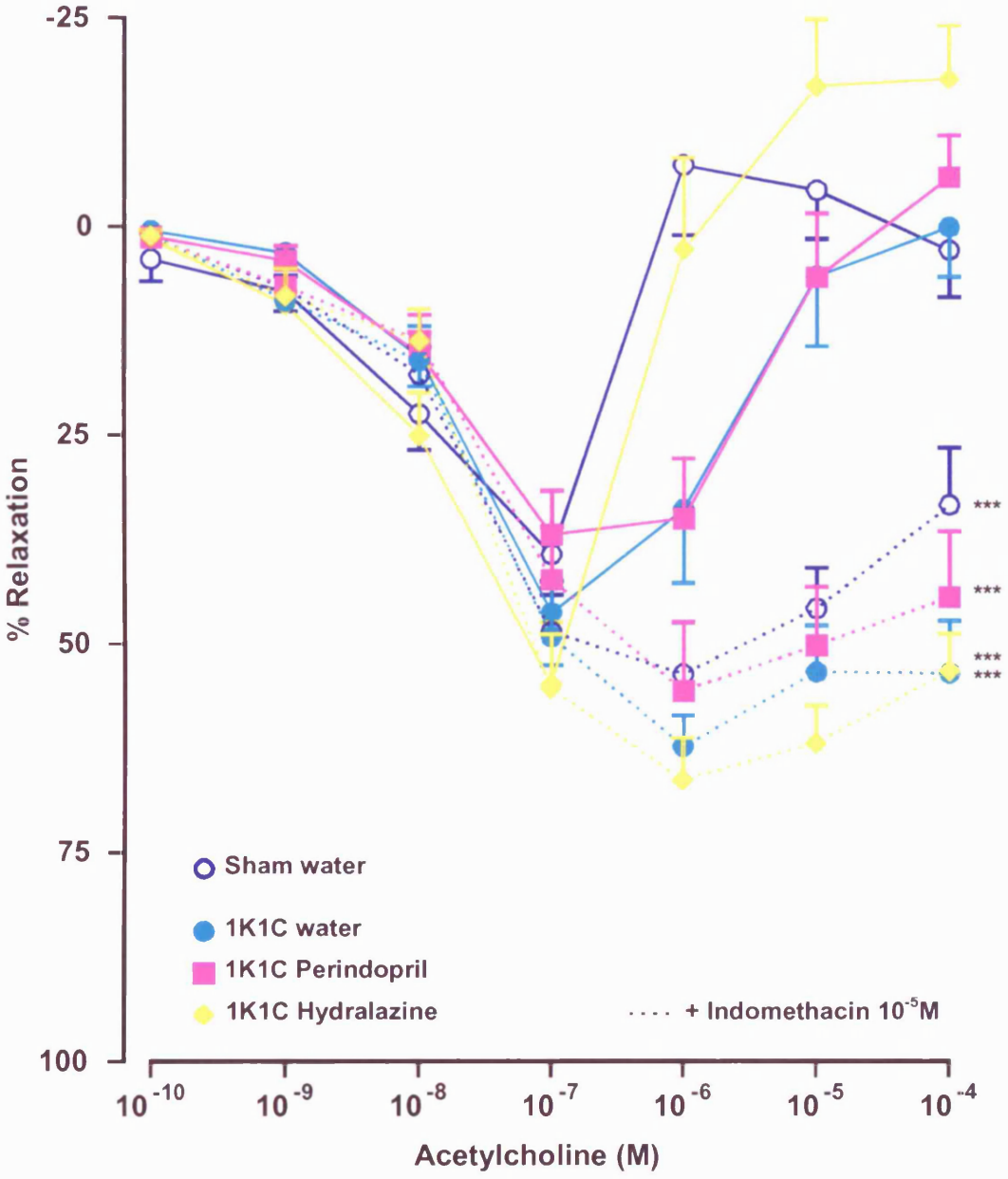


Figure 4.31. The relaxation response to acetylcholine, in the absence and presence of indomethacin 10^{-5}M, of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

••• P<0.001 T-test of dose response compared to that in the absence of indomethacin.

Table 4.24. The effect of indomethacin 10^{-5}M on the relaxation to acetylcholine (ACh) or bradykinin (BK), in femoral resistance arteries from untreated, perindopril treated, or hydralazine treated 8 week 1K1C and sham-operated rats.

Rats	Treatment	N	ACh E max (%)		ACh Mean R (%)		BK Mean R(%)	
			Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
SHAM	Water	14	39 ± 4.1	52 ± 4.7 *	10 ± 3.6	26 ± 3.9 **	-4 ± 1.4	6 ± 2.8 **
1K1C	Water	14	41 ± 4.4	63 ± 4.4 **	9 ± 4.1	30 ± 3.5 ***	-1 ± 5.9	1 ± 6.0
SHAM	Perindopril	14	35 ± 3.9	47 ± 4.2 *	8 ± 3.2	26 ± 2.9 ***	-8 ± 2.5	1 ± 1.6 **
1K1C	Perindopril	14	50 ± 6.0	58 ± 6.2	15 ± 4.3	27 ± 4.1 ***	-3 ± 2.0	-1 ± 3.0
SHAM	Losartan	13	41 ± 3.4	51 ± 5.1	11 ± 2.9	26 ± 3.6 **	-10 ± 4.1	-3 ± 1.2
1K1C	Losartan	13	51 ± 5.0	66 ± 4.8 *	10 ± 4.3	40 ± 4.2 ***	1 ± 2.2	-2 ± 3.6
SHAM	Hydralazine	13	51 ± 5.2	56 ± 5.1	11 ± 4.4	34 ± 3.8 ***	-7 ± 2.9	-1 ± 2.2
1K1C	Hydralazine	14	42 ± 5.6	58 ± 2.5	10 ± 3.6	34 ± 3.9 ***	-2 ± 1.6	-2 ± 1.9

E max represents the maximal relaxation, EC_{50} represents the concentration of each drug required to achieve 50% of E Max, Mean R represents the mean of the points on each curve.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ T-test of ACh or BK relaxation compared to the control in the absence of indomethacin.

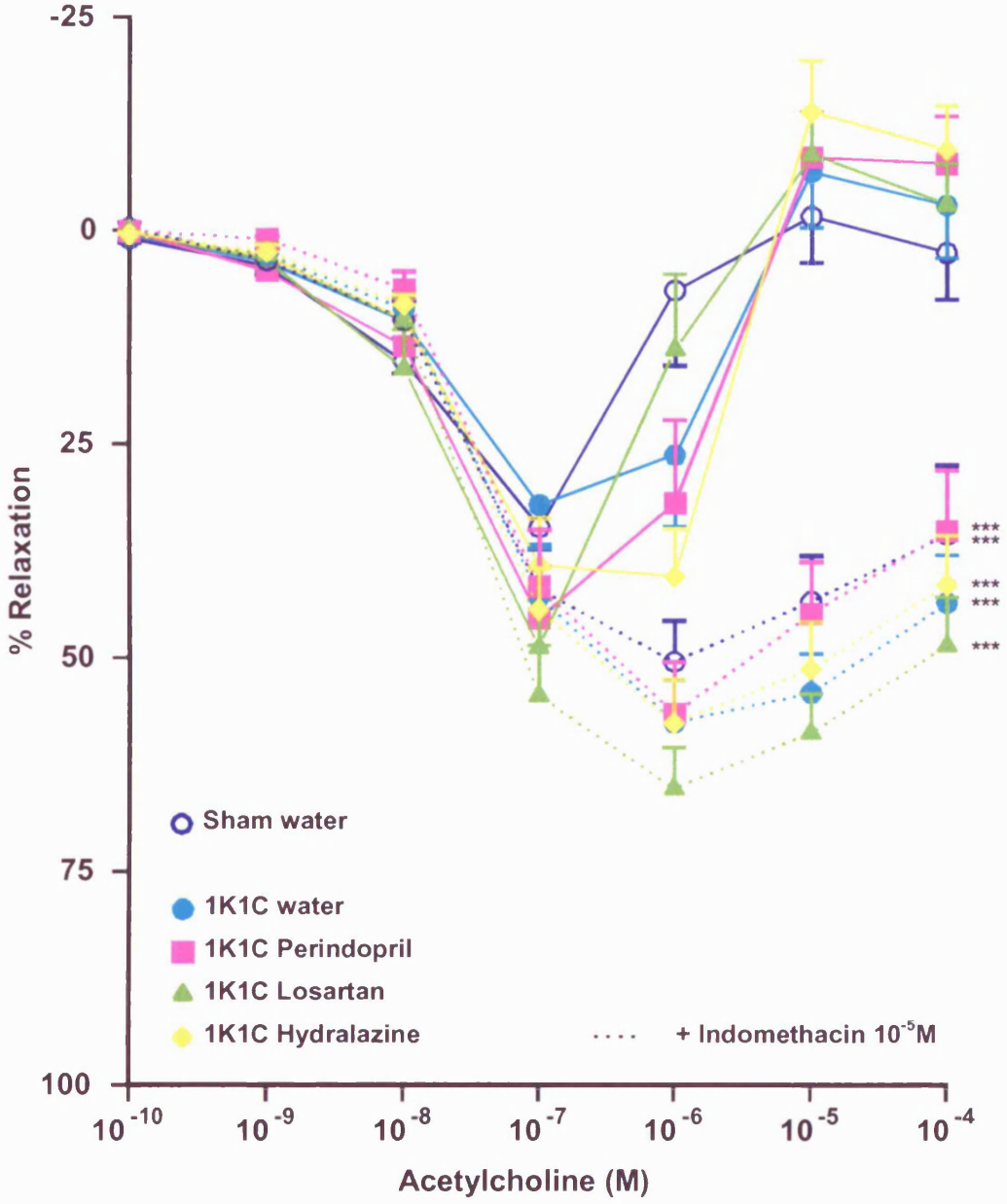


Figure 4.32. The relaxation response to acetylcholine, both in the absence and presence of indomethacin 10^{-5}M, of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

••• P<0.001 T-test of dose response compared to that in the absence of indomethacin.

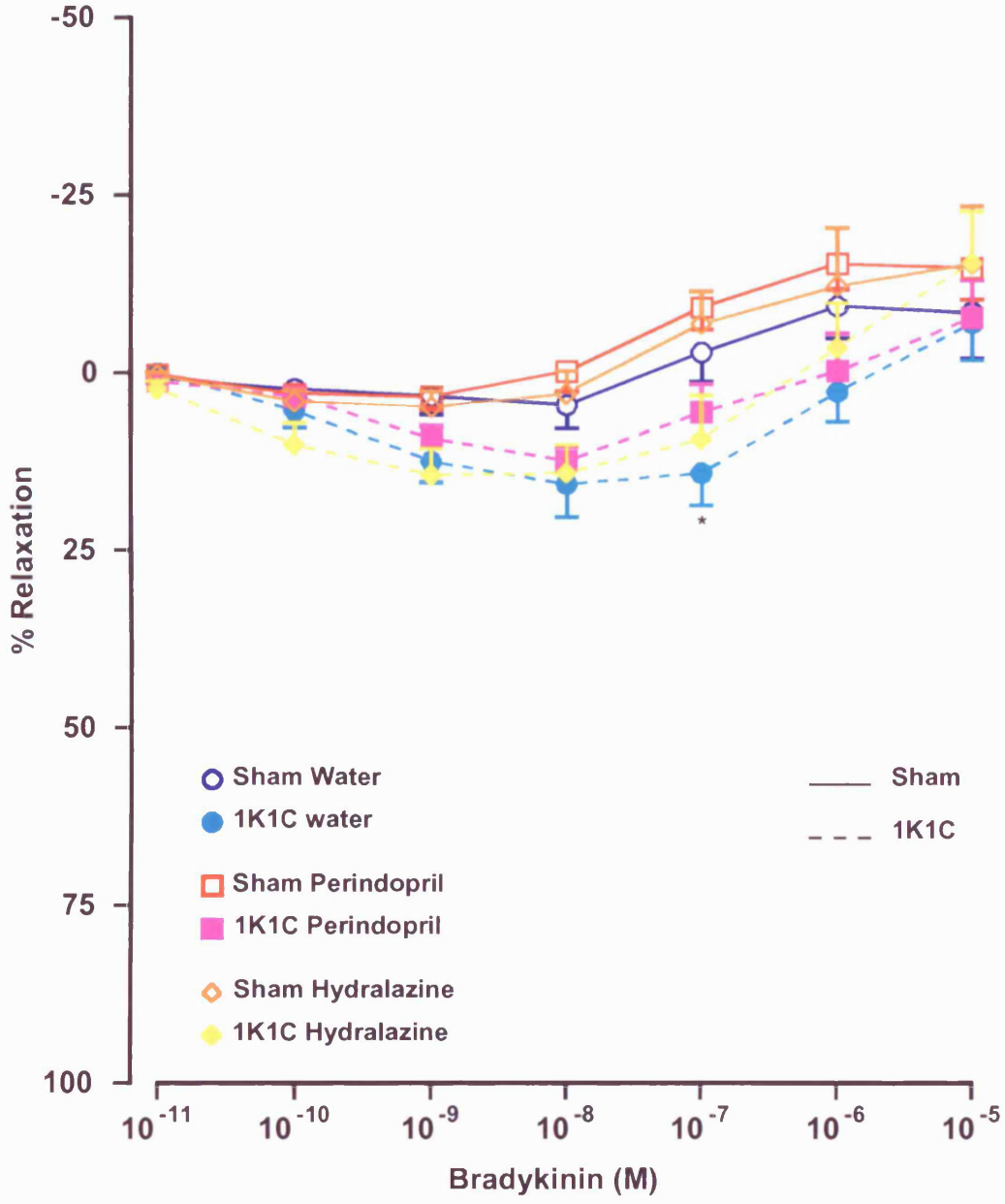


Figure 4.33. The relaxation response to bradykinin of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

* P<0.05 ANOVA compared to untreated sham-operated rats

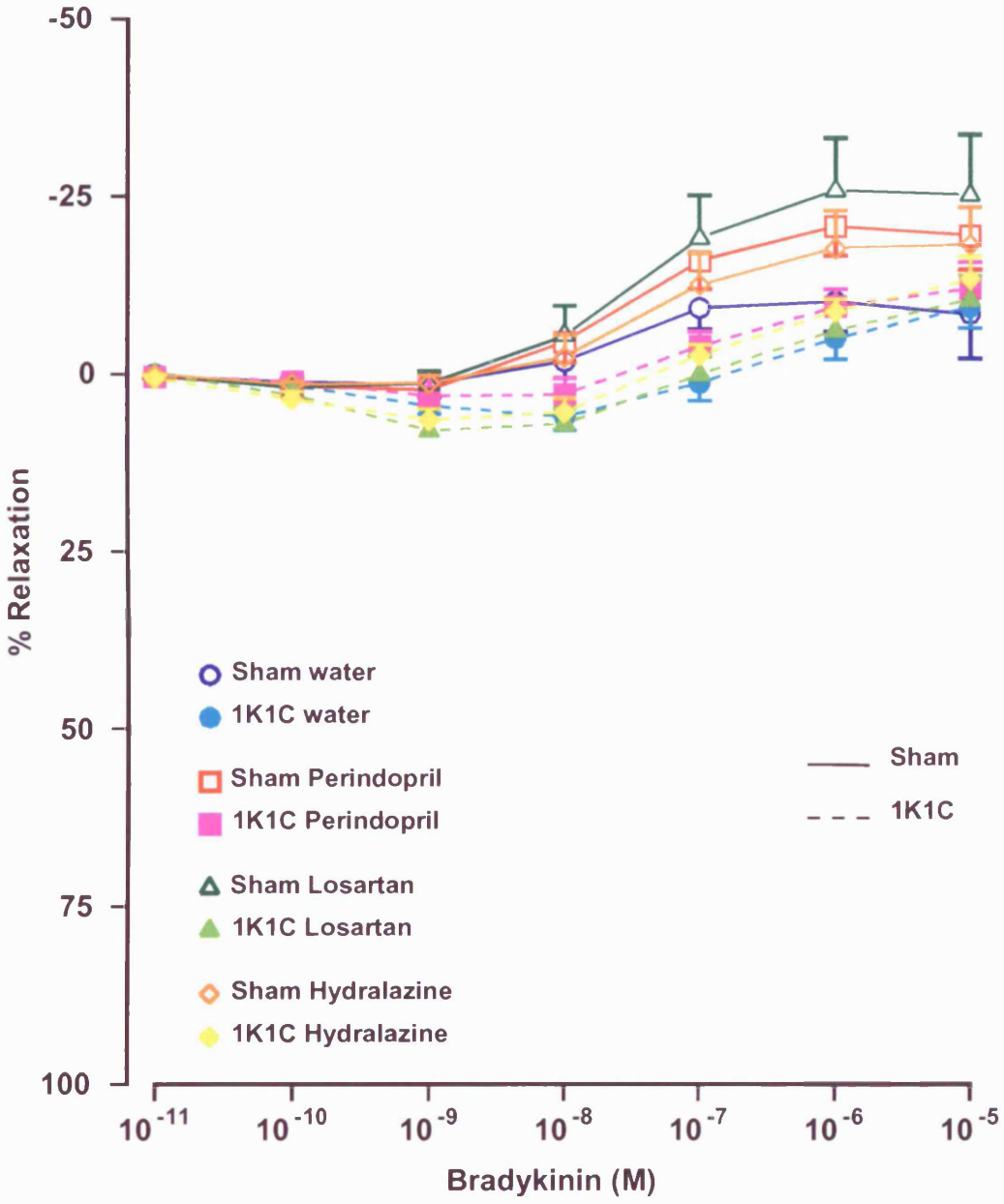


Figure 4.34. The relaxation response to bradykinin of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

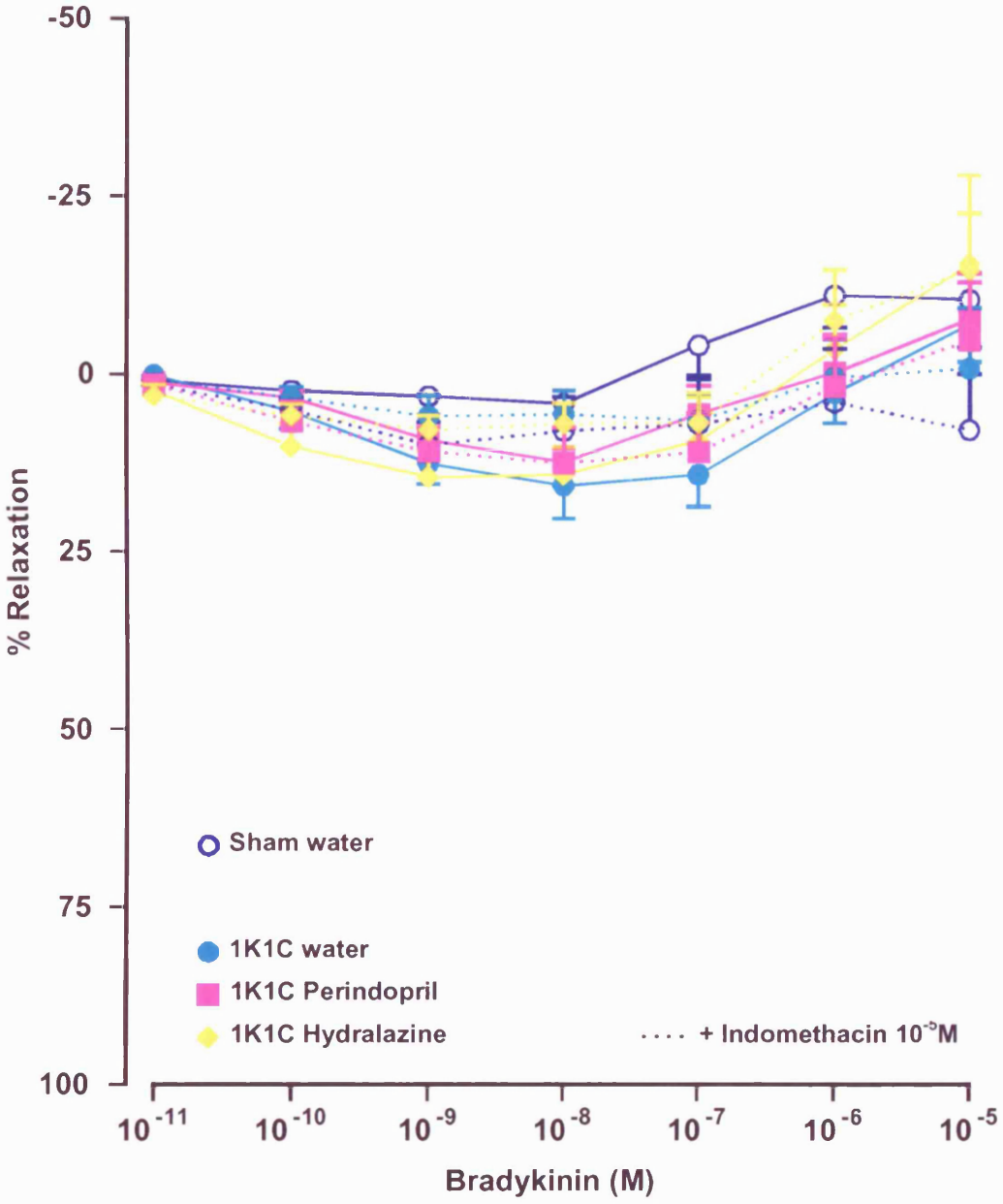


Figure 4.35. The relaxation response to bradykinin of femoral resistance arteries, in both the absence and presence of indomethacin 10^{-5}M, from 4 week 1K1C and sham-operated rats.

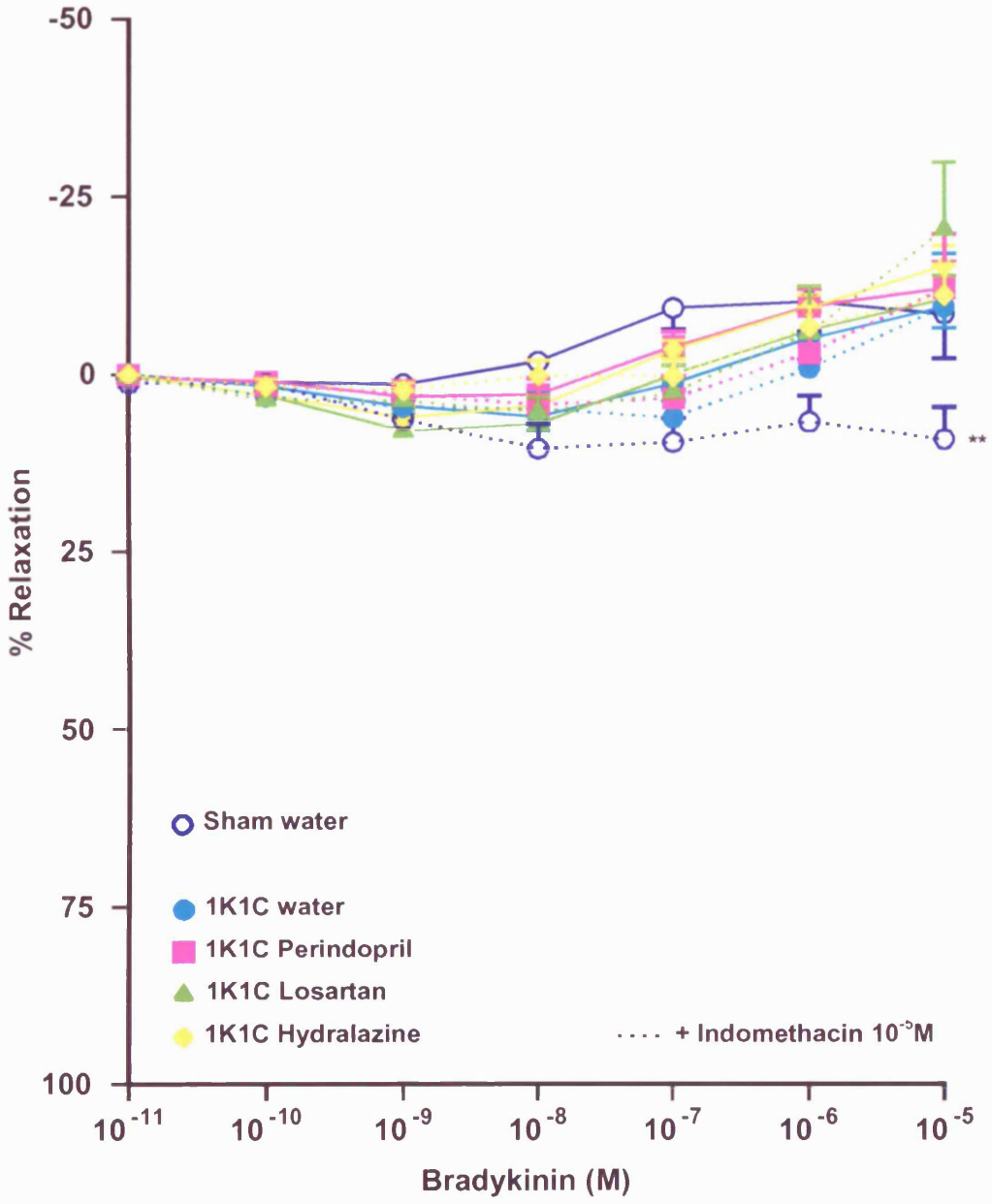


Figure 4.36. The relaxation response to acetylcholine, in both the absence and presence of indomethacin 10^{-5}M, of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

** $P<0.01$ T-test of dose response compared to the that in the absence of indomethacin.

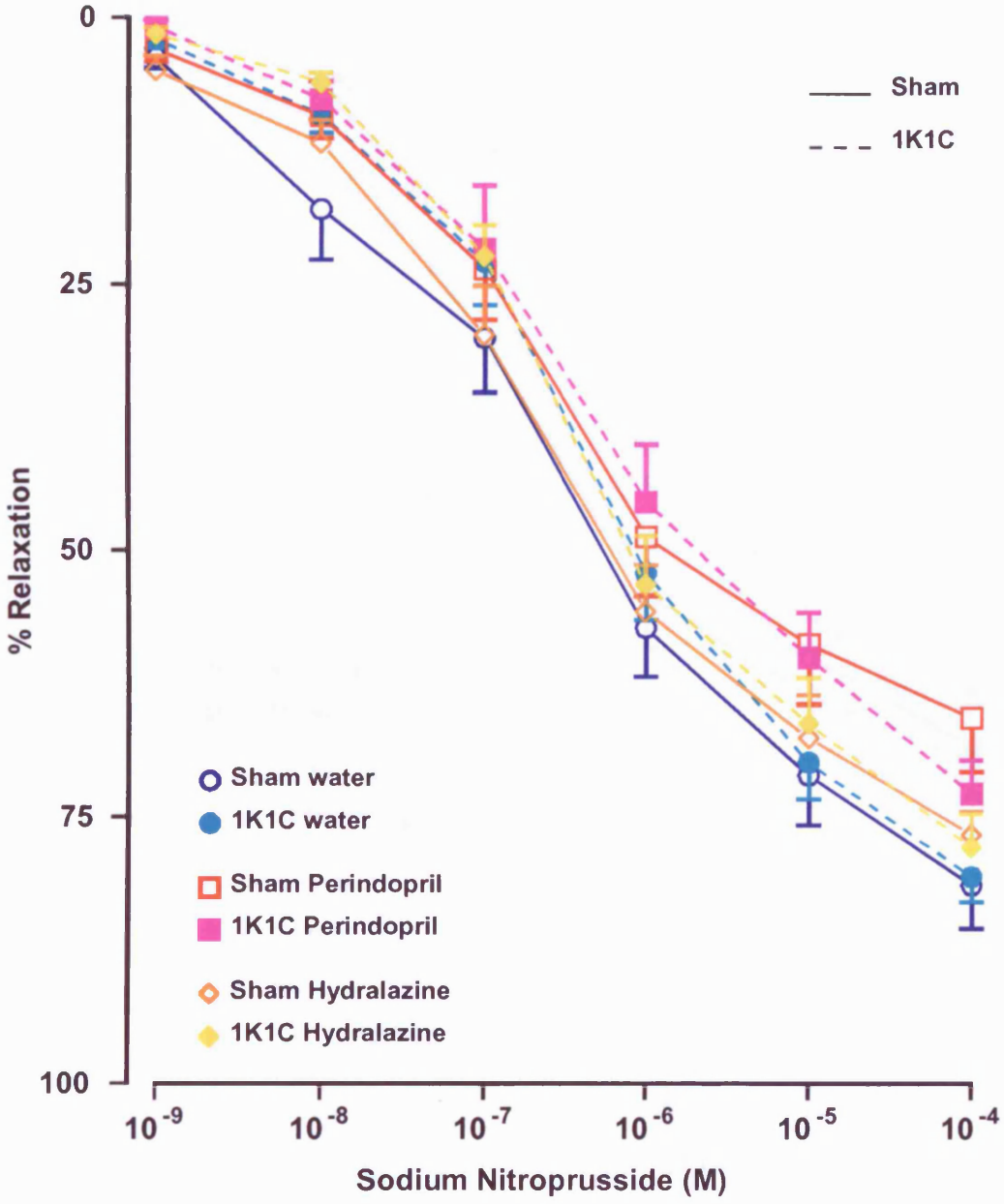


Figure 4.37. The relaxation response to sodium nitroprusside of femoral resistance arteries from 4 week 1K1C and sham-operated rats.

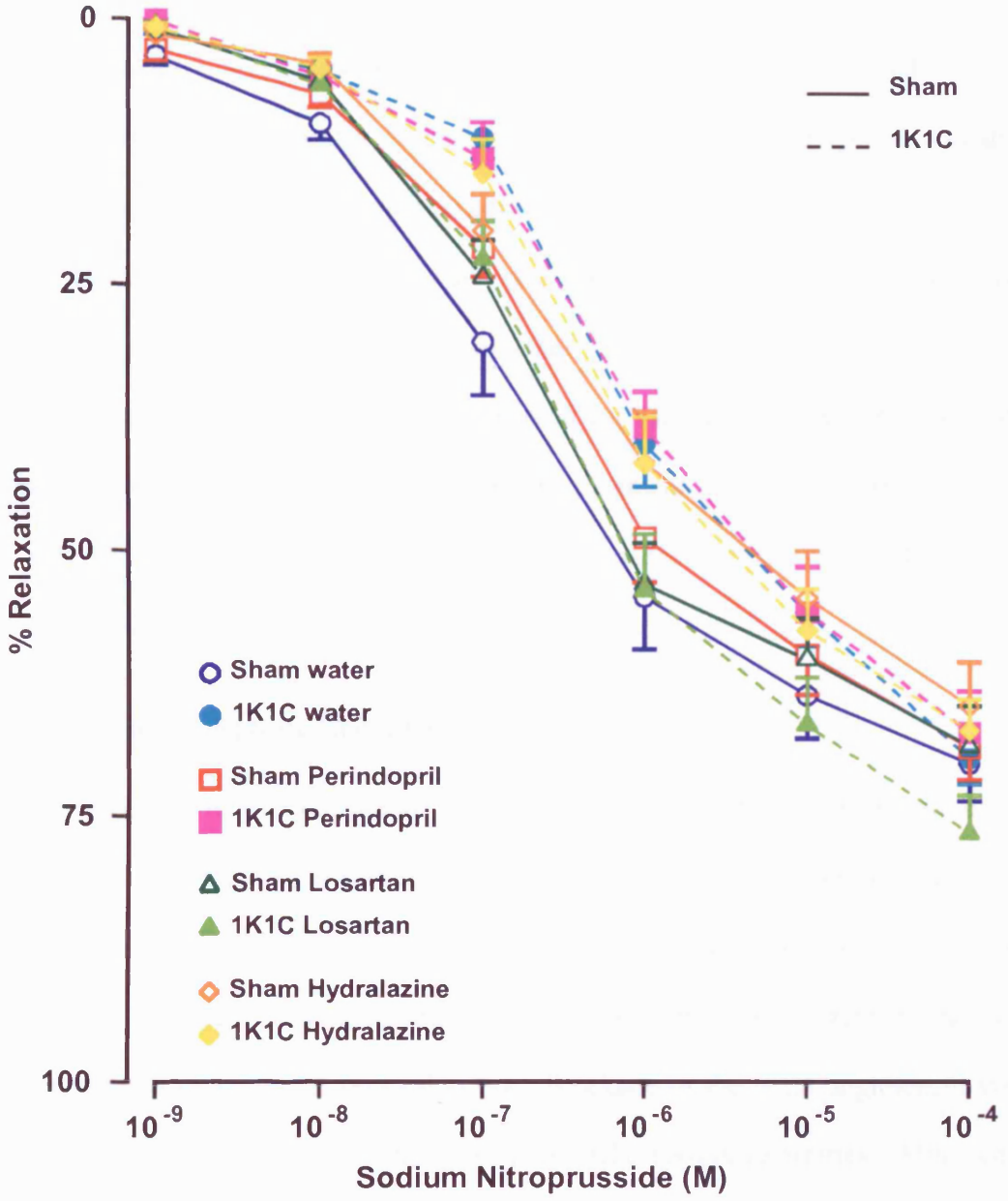


Figure 4.38. The relaxation response to sodium nitroprusside of femoral resistance arteries from 8 week 1K1C and sham-operated rats.

4.7. SUMMARY OF 1K1C RESULTS

4.7.1. Physical Characteristics

The systolic blood pressure was significantly elevated in the untreated 1K1C rats. Blockade of the renin-angiotensin system, and treatment with the non-specific vasodilator hydralazine failed to prevent the development of hypertension in this model.

The body weight of the rats in this study were unaltered by hypertension, or by treatment with perindopril, losartan or hydralazine.

The untreated 1K1C rats displayed marked cardiac hypertrophy. Blockade of the renin-angiotensin system failed to prevent the development of cardiac hypertrophy in the 1K1C rats. In addition, hydralazine treatment increased cardiac hypertrophy over that observed in the untreated 1K1C rats.

4.7.2. Resistance Artery Structure

The mesenteric artery structure was increased in the untreated 1K1C rat, although this increase only reached significance at 8 weeks. Also, there was evidence of an increase in structure in the femoral arteries at 4 weeks, although at 8 weeks only the media thickness was increased. This may suggest the involvement of a different mechanism in the structural alterations of the femoral artery. Blockade of the renin-angiotensin system did not prevent the development of structure in the 1K1C resistance arteries. Although, the structure was not as marked in the perindopril treated 1K1C resistance arteries, or in the losartan treated 1K1C mesenteric arteries. Thus, suggesting these treatments may have a slight effect on the structure of the 1K1C resistance arteries.

4.7.3. Resistance Artery Contractility

The active tension generated in response to contractile agents was increased in the untreated 1K1C mesenteric arteries. However, this increase was shown to be entirely due

to the structural alterations present in these arteries. In contrast, there was a slight reduction in the contractility of the untreated 1K1C femoral arteries, which was unrelated to structural alterations. Thus, suggesting a reduction in the femoral artery smooth muscle cells ability to contract because of hypertension.

4.7.4. Resistance Artery Relaxation

The endothelial-dependent relaxation to acetylcholine, and bradykinin, was impaired in the untreated 1K1C mesenteric arteries. There was also a slight impairment in the relaxation to acetylcholine in the 8 week untreated and losartan treated sham rats. Blockade of the renin-angiotensin system did not prevent the impaired relaxation to acetylcholine and bradykinin in the hypertensive mesenteric arteries, although there was a slight improvement in the response at 8 weeks to ACh, after treatment with perindopril or hydralazine. The response to the endothelium-independent vasodilator SNP was unaltered by hypertension, suggesting that the ability of the smooth muscle cell, to respond to nitric oxide was normal.

In contrast to the mesenteric arteries, the relaxation response of the femoral arteries to acetylcholine and bradykinin, were unaltered by hypertension. The femoral arteries all displayed re-contraction to higher concentrations of acetylcholine, and contracted in response to bradykinin. The relaxation response of the femoral arteries to SNP was also unaltered by hypertension.

Incubation of the arteries with indomethacin improved the relaxation response to acetylcholine in the 1K1C mesenteric arteries, and the relaxation to bradykinin in all mesenteric arteries. Thus, suggesting that vasoconstrictor prostanoids are involved in the abnormal relaxation response to both acetylcholine, and bradykinin, in the mesenteric arteries. Similarly, indomethacin improved the relaxation response of all femoral arteries to acetylcholine, but had little effect on the contractile response to bradykinin. Thus,

suggesting that vasoconstrictor prostanoids are involved in the re-contraction observed in all femoral arteries, and that re-contraction is a normal femoral artery response and not a consequence of hypertension.

4.8. DISCUSSION

The 1K1C hypertensive rat was chosen for these studies because the renin-angiotensin system can be inhibited, without affecting the development of hypertension (O'Sullivan, *et al.*, 1994; Garcia *et al.*, 1996). Thus a possible contribution of AII could be assessed, whilst the effects of elevated blood pressure on vascular remodelling remained intact. In the present study, as expected, treatment with perindopril, or losartan did not prevent the rise in blood pressure, despite being administered for one week prior to renal artery constriction. The lack of blood pressure lowering effect with perindopril could be attributed to incomplete renin-angiotensin system blockade because angiotensin II generation has been reported to occur by ACE independent pathways (Okunishi *et al*, 1984). However, other studies have cast doubt on the importance of non ACE pathways in the rat heart and small arteries where treatment with an ACE inhibitor has been shown to block angiotensin II generation completely (Okunishi, *et al.*, 1993; Nishimura, *et al.*, 1996). The inclusion of the losartan treatment arm overcomes these difficulties since the angiotensin II receptor antagonist inhibits the physiological actions of angiotensin II, irrespective of the nature or site of formation (Siegl, *et al.*, 1995), including a mitogenic response demonstrated in culture cells (Timmermans, *et al.*, 1993). Therefore, renin-angiotensin system blockade using perindopril, or losartan permits the separation of changes in vascular structure and function attributable to raised blood pressure, from that due to a non-pressor angiotensin mediated trophic effect.

Although inhibition of the renin-angiotensin system had little or no effect on final degrees of hypertension, which developed, the early rise of blood pressure after renal artery constriction was delayed. Others have reported this effect, which probably relates to inhibition of the initial high renin phase of 1K1C hypertension present during the first week after surgery (Wang & Prewitt, 1990; Lacy, *et al*, 1995). Thus, angiotensin II

inhibition only appears to delay but cannot prevent the development of hypertension in this model. Hydralazine treatment also had no effect on the blood pressure development in 1K1C hypertensive rats, a disappointing result making it impossible to observe a response of vascular structure to blood pressure lowering in the presence of an intact and possibly activated renin-angiotensin system.

Cardiac hypertrophy appeared to be established completely by four weeks after renal artery constriction in the untreated 1K1C rat, and there was little change after this time. Treatment with perindopril, losartan or hydralazine all failed to prevent the development of cardiac hypertrophy in the 1K1C hypertensive rat. Thus, the development of cardiac hypertrophy appeared to be largely pressure dependent with little or no contribution by angiotensin II. This view is supported by a previous study, which reported that renin-angiotensin system blockade, had no effect on cardiac or thoracic aortic structural change in this model (O'Sullivan, *et al.*, 1994). By contrast, hydralazine treatment brought about a greater degree of cardiac hypertrophy than observed in the untreated 1K1C hypertensive rat, without a significant change in blood pressure. This suggested a specific drug effect possibly as a consequence of the increased cardiac output and cardiac work associated with treatment with vasodilator treatment (Swales, 1994). Also, hydralazine treatment activates both the sympathetic nervous system and the renin-angiotensin system, both of which have been implicated as stimulators of cardiovascular growth. A similar, but less marked increase of cardiac mass was observed in the sham animals treated with hydralazine. This suggests that the growth stimulation by hydralazine may amplify blood pressure induced changes in the 1K1C hypertensive rat. Pressure-independent trophic effects of hydralazine were previously suggested in a study by Jespersen and colleagues (Jespersen, *et al.*, 1985).

The growth pattern of both the sham and 1K1C hypertensive rats was normal over the time course of this study and weight gain was not adversely affected by eight weeks losartan, or hydralazine treatment. However, long-term perindopril treatment caused significant growth retardation in the eight week sham controls. Again, others have made similar observations with perindopril treatment in the WKY rat (Black, *et al.*, 1997) and in SHR and WKY rats treated from weaning to 24 weeks (Chapter 3).

The induction of 1K1C hypertension led to marked structural alteration of both the mesenteric and femoral small arteries. This was not associated with a significant change in the lumen diameter of the mesenteric artery, which is in contrast to reports of a reduced lumen diameter in both human essential hypertension (Aalkjaer *et al.*, 1987a) and the spontaneously hypertensive rat (Mulvany & Halpern, 1977). On the other hand, although the change failed to achieve significance, the lumen diameter of the femoral was smaller in the 1K1C hypertensive rat compared to the sham control. Media cross sectional area, media thickness and media to lumen ratio were significantly increased (compared to the sham operated controls students "t" test) in mesenteric arteries taken from both four and eight week 1K1C hypertensive rats. However, analysis of variance only revealed significant differences at eight weeks after renal artery constriction. Less marked changes in the media cross sectional were observed in the femoral arteries but nonetheless, media thickness was significantly greater in both the four, and eight week hypertensive rats. Thus, the two vascular beds respond differently to the induction of 1K1C hypertension. The morphological changes observed in the mesenteric artery denote smooth muscle growth, whereas structural alteration of the femoral artery largely appears to have resulted from remodelling with only a small contribution by growth. Studies of the nuclear incorporation of [^{3}H] thymidine in the first week after the induction of 1K1C hypertension support this view with a marked increase in DNA synthesis in the mesenteric, compared to

a non significant change in femoral arteries (Lacy, *et al*., 1995) In the present study, no attempt was made to examine the cellular basis of structural change, although a previous histological study using the stereo dissector technique suggested that mesenteric growth was the result of vascular smooth muscle cell hypertrophy (Korsgaard & Mulvany, 1988). This is markedly different from both the spontaneously hypertensive rat where growth is reported to be the result of hyperplasia (Heagerty, *et al.*, 1993a, 1993b; Mulvany, *et al.*, 1985; Owens, *et al.*, 1988; Yang, *et al.*, 1989) and subcutaneous arteries from patients with essential hypertension, where remodelling occurs without growth (Heagerty, *et al.*, 1993a; Korsgaard, *et al.*, 1993; Lee *et al.*, 1995).

It was recently reported that vascular hypertrophy of the femoral and thoracic aorta in 1K1C hypertension could be related to increased synthesis of PDGF. This increase in PDGF-A expression positively correlated to both the rise in blood pressure, and vascular structure (Dobrian *et al.*, 1999). Similarly, a recent report showed increased PDGF-A expression in the 1K1C small mesenteric artery wall, in spite of renin-angiotensin blockade with Losartan (Parker *et al.*, 2000). Thus, suggesting that vascular hypertrophy is a result of alterations in growth factor expression, as a consequence of the increased pressure. Many studies involving the AII infusion model of hypertension also support the finding that increased expression of PDGF-A accompanies vascular hypertrophy (Berk & Rao, 1993; Parker, *et al.*, 1998)

Treatment with perindopril appeared to have little effect on the development of mesenteric structural change in four week 1K1C hypertensive rats (Fish *et al.*, 1996). On the other hand, there was an increase in mesenteric artery structure with hydralazine treatment. Similarly, ACE inhibition failed to alter significantly four week femoral artery structure. However, the results were unclear because there was little difference between the perindopril sham and 1K1C hypertensive rats at this time point. Consequently, the

values of these groups lay between those of the four week untreated sham and 1K1C hypertensive rats. Again, hydralazine administration appeared to increase femoral artery structural development in the 1K1C rats, which is in keeping with the augmented mesenteric artery and cardiac hypertrophy. Thus, the rise in blood pressure following renal artery constriction was unaffected by hydralazine treatment, and structural change appeared more marked at four weeks, than at eight weeks of hypertension. This discrepancy may be related the great mortality at eight weeks versus four weeks and certainly, there was a trend towards lower blood pressures in the eight week hydralazine treated 1K1C hypertensive rats.

Blockade of the renin-angiotensin system produced different responses in the mesenteric, compared to the femoral arteries in the eight week study. In the mesenteric artery, there was a reduction in media cross sectional area with slight decreases in media thickness and media to lumen ratio, in both hypertensive and normotensive rats. However, normalisation of vascular structure was in no way achieved, as is reported to occur in both the 2K1C and SHR models of hypertension after renin-angiotensin system blockade (Korsgaard *et al.*, 1991; Bennett & Thurston, 1996; Thybo, *et al.*, 1994). Interestingly, in the present study, there was also a noticeable reduction in mesenteric structure in the sham rats at both ages, after blockade of the renin-angiotensin system. Thus, resetting of vascular structure was observed as a result of renin-angiotensin blockade in both the normotensive and hypertensive rats. Therefore, it was not possible to completely rule out some effects of renin-angiotensin system blockade, on vascular structure in the present study. By contrast, there was little change in femoral artery structure of the eight week 1K1C hypertensive rats treated with either perindopril or losartan. This perhaps reflects the limitation of optical morphological analysis where large changes in arterial dimension are absent. Wang and colleagues reported that treatment with the ACE inhibitor lead to a

reduction in wall cross sectional area of the aorta and cremaster arterioles in both four week 1K1C and sham operated rats, in the absence of a change in blood pressure (Wang & Prewitt, 1990). They suggested that this indicated a trophic effect of AII either as a direct action on the arterial wall or by an indirect action via the sympathetic nervous system. However, they could not exclude antiproliferative effect due to bradykinin potentiation during ACE inhibitor treatment. This discrepancy was addressed by a second study using both the ACE inhibitor delapril, and the AT_1 receptor antagonist TCV-116, and confirmed a specific beneficial effect of angiotensin II blockade on aortic hypertrophy in the model (Yu, *et al.*, 1993). Conversely, a more recent study by Prewitt and his colleagues failed to confirm a beneficial effect of AT_1 receptor blockade with losartan in the 1K1C hypertensive rat suggesting that angiotensin was not necessary for aortic or mesenteric hypertrophy in the 1K1C model (Parker, *et al.*, 2000). Also, studies of the response of vascular DNA synthesis to renin angiotensin blockade using both perindopril and losartan showed no effect of treatment in the 1K1C aorta, mesenteric or femoral arteries (P.Lacy, personal communication).

Studies of vascular growth in the angiotensin II infusion model of hypertension have also failed to produce consistent evidence for a non pressor AII trophic effect. A number of studies suggest a dissociation between blood pressure elevation and the development of structural change (Lever, 1993; Simon *et al.*, 1998). However, a recent study has provided strong evidence that the vascular wall structure was mediated solely by the raised blood pressure in this model. Wall hypertrophy was not observed in the AII infusion rat in this study, unless blood pressure was also raised (Parker, *et al.*, 1998).

The finding that AII can act as a bifunctional growth factor also may complicate studies implicating direct effects of AII on growth. The action of AII appears to be dependent upon the conditions under which it is studied (Gibbons, *et al.*, 1992; Simon &

Altman, 1992; Itoh, *et al.*, 1993; Griendling & Alexander, 1994). Its actions are complex and may be dependent on the balance of many factors including the presence, and/or absence of receptor subtypes, the presence of endothelial-derived substances, and the presence of other growth factors. Alterations in the levels of any of these factors, during different experimental conditions may explain the differing reports, both in favour of, and against direct effects of AII on growth. Therefore, many studies may not reflect the fine balance between the influence of growth stimulatory and inhibitory factors *in vivo*.

It is also important to consider the lack of influence of the endothelial cells in some culture studies, which suggested direct trophic effects of AII, as many early investigations were carried out in vascular smooth muscle cells alone. Endothelial cells are known to release growth inhibitory substances, such as EDRF and heparin sulphates. Thus, in the absence of this cell type there may be a reduction in the availability of growth inhibitory substances (De Mey, *et al.*, 1991a; Schiffers, *et al.*, 1992; Luscher *et al.*, 1993c; Scott-Burden & Vanhoutte, 1993). Additionally, it is reported that endothelial cells may disappear from multi-passaged cell lines (Angus & Lew, 1992b). Schiffers and colleagues confirmed the complex interplay of growth stimulatory and inhibitory actions of substances within the vasculature using organoid tissue culture techniques. Segments of carotid and renal arteries were cultured in both the presence, and absence of endothelium. They concluded that both stimulatory and inhibitory factors were present in the vascular wall, which could influence DNA synthesis and cellular proliferation. They also confirmed a role for the endothelium in the suppression of DNA synthesis (Schiffers, *et al.*, 1994b). Interestingly, they were unable to demonstrate an effect of AII on DNA synthesis in these arterial segments from the WKY, in the absence of endothelium.

It has also been reported that nitric oxide can negatively modulate renin-angiotensin system activity by inhibition of ACE activity and via down-regulation of AT_1

receptors (Guan *et al.*, 1996). More recently, it was reported that AII may exert an antimitogenic action via the AT_2 receptors, present in abundance on endothelial cells (Stoll *et al.*, 1995; Pueyo & Michel, 1997; Horiuchi *et al.*, 1999), This receptor subtype was suggested to be the antagonistic receptor to the AT_1 subtype. However, only small numbers of the AT_2 receptor subtypes are reported on the vascular smooth muscle cells (Unger, *et al.*, 1996) and their stimulation may lead to trophic effects, by contrast to stimulation of those on the endothelial cells (Levy, 1998). In addition, reports suggest that cultured vascular smooth muscle cells fail to express the AT_2 receptor subtype (Stoll, *et al.*, 1995). Thus, in the absence of the endothelial AT_2 receptor and growth inhibitory endothelial-derived substances, the vascular smooth muscle cell may be more greatly influenced by growth factors. Therefore, the organoid culture technique (Schiffers, *et al.*, 1994b), may prove to be a more useful tool in clarifying the role of AII, and other potential growth factors, in structural changes which occur during hypertension. Further studies on the expression of both early growth genes, and mitogen activated protein kinase in complete arteries may provide more insight into the mechanisms involved in vascular hypertrophy.

The potassium, NAK and noradrenaline contractile responses were slightly increased in both the four and eight week untreated 1K1C mesenteric arteries. This appeared largely to be a consequence of altered structure in these arteries, since the difference disappeared upon expression of the results as active media stress. Blockade of the renin-angiotensin system either by perindopril or losartan had no effect on the active tension response in the 1K1C mesenteric arteries supporting the view that structural change had not been altered by treatment. On the other hand, contractile responses were reduced in both the perindopril treated and losartan treated sham rats at both time points. There are several mechanisms by which ACE inhibition may influence smooth muscle contractility.

These include the enhancement of release of endothelial-derived relaxing factors (Collis & Keddie, 1981; Vanhoutte, *et al.*, 1989b; Atkinson, 1995) and a reduction in sympathetic nervous system activity (Collis & Keddie, 1981; Zimmerman, 1981). However, these specific mechanisms are unlikely to be involved as there was no reduction in contractility in the sham mesenteric arteries when the results were expressed in terms of active media stress response. This is in keeping with the observed reduction in arterial structure in the sham following treatment with either the ACE inhibitor or AT_1 receptor antagonist. Thus, the absence of a treatment effect on contractility of the 1K1C mesenteric arteries and the marked effect in the sham reflects the failure of renin-angiotensin system blockade to prevent structural change from developing in the hypertensive rat. In contrast to the mesenteric artery, the contractile response (active tension) of the femoral artery to potassium, NAK and noradrenaline was unaffected by the induction of 1K1C hypertension. However, when the contractile responses were expressed in terms of media stress, a significant reduction was observed in femoral arteries from the untreated, losartan treated and hydralazine treated 1K1C rats. This indicates a reduction in the contractile ability of the femoral artery smooth muscle cells, which could not be prevented by either renin-angiotensin system blockade or by treatment with hydralazine. The reduced active media stress may be a consequence of alterations in the cell to cell interactions or alternatively, to a decreased average active force per cell cross sectional area (Korsgaard & Mulvany, 1988). The observed impairment was more marked in the eight week 1K1C rats, suggesting that it is a consequence of the progression of hypertensive disease. The difference in active media stress between femoral and mesenteric arteries further supports the view that structural alteration in the femoral artery is a consequence of remodelling, whilst the change in mesenteric artery reflects growth.

A leftwards shift in the contractile response to noradrenaline was observed in both the femoral and mesenteric arteries, in the presence of cocaine. This is related to inhibition of the neuronal amine pump, by cocaine, leading to a lack of re-uptake of NA into the nerve terminal. Therefore, for increasing concentrations of NA, an increased level of NA is seen within the synapse, resulting in increased contractility in the presence of cocaine (Mulvany, 1989).

There was a significant reduction in the acetylcholine induced endothelial-dependent relaxation response in mesenteric arteries from both four and eight week 1K1C hypertensive rats, compared to sham operated rats, with a re-contraction to high concentrations of acetylcholine (Fish, *et al.*, 1996). Arteries from sham operated animals showed a full relaxation response, but there was a slight tendency to re-contraction at higher concentrations of acetylcholine in the eight week sham rats. Impaired endothelium-dependent relaxation has been previously reported in mesenteric arteries from both genetic and experimental models of hypertension (Bennett, *et al.*, 1993; Bennett, *et al.*, 1996; Lockette, *et al.*, 1986). This could be the result of functional changes in either the endothelium or the vascular smooth muscle cells. Alternatively, it could be related to morphological changes in the wall of the artery, resulting in a decreased diffusion pathway for vasoactive substances to the vascular smooth muscle. A change in the ability of mesenteric smooth muscle cells to react to nitric oxide is unlikely to be involved because responses to endothelium-independent nitric oxide donor SNP were unaffected by hypertension and were similar to that of the sham operated rats. Incubation with indomethacin abolished the re-contraction response indicating the involvement of cyclo-oxygenase-derived vasoconstrictor prostanoids. This is in agreement with previous studies of endothelial dysfunction in the (Watt & Thurston, 1989), 2K1C and 1K1C models of

hypertension (Bennett, *et al.*, 1993). Re-contraction could result from greater sensitivity in the hypertensive arteries, increased release of vasoconstrictor prostanoids or alternatively, reduced release of another endothelium-dependent relaxing factor unmasking the effects of vasoconstrictor prostanoids.

Recent studies of the SHR and SHR-SP models of hypertension (including my study reported in Chapter 3) have demonstrated a decrease or absence of the EDHF component of endothelial-dependent relaxation in hypertension (Mantelli, *et al.*, 1995; Sunano, *et al.*, 1999), rather than a reduction in EDNO. At the present time, there is less information about alterations in the components of endothelium-dependent relaxation in the 1K1C model of hypertension. However, recent work from our laboratories has revealed evidence of a decrease in the potassium sensitive component of acetylcholine relaxation in the 1K1C rats compared to the sham rats indicating reduced EDHF release. However, the results were preliminary and there was little difference in the ODQ/indomethacin insensitive component of relaxation between the hypertensive and normotensive (Hanvesakul, 1998). Thus, further work clearly needs to be carried out to elucidate changes of the components of endothelial dependent relaxation in the 1K1C hypertensive rat.

A similar reduction in endothelium-dependent relaxation of mesenteric arteries from the untreated 1K1C rats was observed in response to bradykinin. Again, the specific mechanism involved remains unclear but incubation with indomethacin significantly improved the relaxation response of both the 1K1C rats and sham rats to bradykinin, suggesting the involvement of cyclo-oxygenase-derived vasoconstrictor prostanoids.

There were marked differences in the relaxation response of femoral arteries compared to mesenteric arteries in the sham operated rats. High concentrations of acetylcholine were associated with re-contraction of femoral arteries from both

hypertensive and normotensive rats, suggesting that re-contraction was not a consequence of hypertension. Moreover, the addition of the cyclo-oxygenase inhibitor indomethacin improved the relaxation response to ACh to the same extent in all femoral arteries. This again indicates the involvement of vasoconstrictor prostanoids and suggests that there was little effect of hypertension on this response.

Treatment with perindopril or hydralazine failed to prevent the development of impaired endothelium-dependent relaxation in mesenteric arteries from either the four, or eight week 1K1C hypertensive rats. Additionally, losartan also was ineffective in the eight week 1K1C hypertensive rat in this respect. However, these treatments resulted in smaller re-contraction in response to higher concentrations of acetylcholine in the eight week hypertensive rats. Similarly, the slight re-contraction observed in the mesenteric arteries from the eight week sham rats also was abolished by treatment with perindopril, or hydralazine. Additionally, there was a slight improvement in the maximal relaxation response of the four week shams with these agents. Blockade of the renin angiotensin or treatment with hydralazine also failed to improve the relaxation of femoral arteries, but with the exception of hydralazine brought about a reduction in the re-contraction response to high concentrations of acetylcholine. Thus, overall neither blockade of the renin-angiotensin system, nor hydralazine treatment brought about significant improvements in the maximal relaxation responses to either acetylcholine, or bradykinin in the mesenteric or femoral arteries from 1K1C hypertensive rats. However, drug treatments appeared to have some effect by reducing the vasoconstrictor prostanoids component of relaxation.

Previous studies have reported that treatment with an ACE inhibitor improved acetylcholine induced relaxation of the SHR (Clozel, 1991; Rubanyi *et al.*, 1993b; Bennett, *et al.*, 1996), and 2K1C hypertensive rat (Bennett & Thurston, 1996). However, it was not possible to separate an effect due to blood pressure reduction from a specific pressure

independent AII trophic response. There are many reports of specific ACE inhibitor effects on vascular function, which include the bradykinin potentiation leading to an increase in PGI_2 and EDRF/nitric oxide release. Others suggest that AT_1 receptor antagonists also may be able to stimulate the production of EDRF/nitric oxide, and prostaglandins in various tissues (Cachefeiro *et al.*, 1996; Cachofeiro *et al.*, 1997; Maeso *et al.*, 1998) and thus improve endothelium-dependent relaxation. In addition, losartan has been reported to block vascular thromboxane A_2 and prostaglandin F_2 receptors (Timmermans, *et al.*, 1993; Rodrigo, *et al.*, 1997). Thus, ACE inhibitor or AII receptor antagonist treatment could result in the inhibition of prostaglandin vasoconstrictor activity involved in the re-contraction response observed in hypertensive mesenteric or femoral arteries in this study. A further possible explanation for the reduced re-contraction to higher concentrations of ACh in mesenteric arteries, following ACE inhibition, or the AT_1 receptor antagonist might result from potentiation of EDHF activities. This might shift the balance from re-contraction at high concentrations of acetylcholine, to relaxation. More recently, both perindopril and losartan have been reported to stimulate EDHF production in SHR mesenteric arteries (Onaka, *et al.*, 1998; Kahonen, *et al.*, 1999). Nonetheless, treatment with perindopril, losartan or hydralazine produced only a minor improvement of endothelial formation in the 1K1C hypertensive model. Significant impairment remained suggesting that endothelial dysfunction is largely the result of continual exposure to the elevated blood pressure.

In conclusion, this study suggests that blood pressure per se is an important determinant of cardiovascular structure, since renin-angiotensin system blockade appeared to have only small effects on vascular structure in the absence of a fall blood pressure. It is therefore unlikely that angiotensin II plays a major role in the facilitation of vascular

structural change of the mesenteric and femoral arteries via a non pressor action on either arterial vascular smooth muscle cells or matrix components.

The development of endothelial dysfunction in the 1K1C was largely unaffected by blockade of the renin-angiotensin system or by hydralazine treatment, although slight effects of treatment on the prevention of arterial re-contraction were observed. Again, this indicates that endothelial dysfunction in hypertension is largely related to the elevated blood pressure in hypertension.

CHAPTER FIVE

CONCLUSIONS

CONCLUSIONS

1. Prolonged perindopril administration prevented the rise in blood pressure, and the development of structural alteration of mesenteric and femoral small arteries in the spontaneously hypertensive rat

2. The antihypertensive effect of perindopril was attenuated by the administration of a high salt diet. Rats receiving combination treatment achieved similar blood pressure levels and vascular structural alteration as was observed in the untreated spontaneously hypertensive rat.

3. Perindopril, or losartan administration failed to prevent the rise in blood pressure, or normalisation of vascular structural alterations in the Goldblatt one-kidney, one-clip hypertensive rat. However, it was not possible to rule out some effects of renin-angiotensin blockade on vascular structure in this study.

4. These studies suggest that blood pressure elevation is an important determinant of structural vascular change in genetic or experimental hypertension. There was little evidence of a major direct angiotensin II mediated trophic effect, although slight effects of the renin-angiotensin system could not be ruled out.

5. Mesenteric and femoral small arteries showed marked differences in their responses to endothelium-dependent vasodilator agents. Thus, acetylcholine produced concentration-dependent relaxation of mesenteric arteries, whereas femoral arteries relaxed only in response to low concentrations of acetylcholine, with high

concentrations causing re-contraction. Bradykinin also produced concentration-dependent relaxation in mesenteric arteries, but caused little or no relaxation of femoral small arteries.

6. The acetylcholine and bradykinin responses of mesenteric arteries were impaired in both the spontaneously hypertensive rat, and Goldblatt one-kidney, one-clip hypertensive rats. In the SHR, this appears to depend upon cyclo-oxygenase-derived vasoconstrictor prostanoids, with some evidence of a compensatory increase in EDNO activity. There was little change in the response of femoral small arteries to acetylcholine, or bradykinin in either model of hypertension. However, there was a slight reduction in the ability of the femoral artery smooth muscle cells to respond to nitric oxide in the 1K1C rats.

7. Perindopril administration prevented the development of endothelial dysfunction in the spontaneously hypertensive rat, but this benefit was largely attenuated when blood pressure elevation was maintained by salt loading. However, there was a small contribution of specific drug effects in this model, which appeared unrelated to blood pressure. Similarly, treatment with either perindopril, or losartan had a slight effect on the development of endothelial dysfunction in the Goldblatt one-kidney, one-clip hypertensive rat. These results suggest that endothelial dysfunction is closely related to the blood pressure elevations observed during hypertension, although some improvements in endothelial function were observed as a result of drug treatment.

APPENDIX A EQUIPMENT AND SUPPLIERS

United Kingdom suppliers unless otherwise stated

Myograph (models 410A and 500A)	Myograph Trading Ltd, Aarhus, Denmark
Kistler Morse transducers	Myograph Trading Ltd, Aarhus, Denmark
Stainless steel wire	Myograph Trading Ltd, Aarhus, Denmark
Technival 2 stereomicroscope	Carl Zeiss Jena Ltd
Olympus stage light microscope	Olympus Ltd
Filar micrometer eyepiece	Carl Zeiss Jena Ltd
Water immersion objective lens	Leitz Ltd
Flat bed 2 channel recorder	Fisons Ltd
Heidolph T50 water heater/circulator	Scientific Industries
Large water bath	Grant instruments Ltd
Suction pump	BDH Ltd
$5CO_2/95O_2$	BOC Ltd
Silver wire for clips	Thessco Ltd
Sutures	Ethicon Ltd
Blood Pressure Equipment	IITC INC/Life Science Instruments,Woodland Hills, California
Sphygmomanometer	Richardsons Ltd
Trabecular scissors	Altomed Ltd
Watchmakers forceps (size 5)	Richardsons
Assorted dissecting instruments	Richardsons
Assorted surgical instruments	Richardsons

Chemicals

Noradrenaline, acetylcholine cocaine hydrochloride, hydralazine, indomethacin, L-NOARG and SNP were obtained from Sigma chemical company, Poole, Dorset. Bradykinin was obtained from ICN, Basingstoke, Hampshire. Perindopril and losartan were gifts received from Pfizer, Sandwich, Kent and Merck, Enfield, Middlesex respectively. Other general chemicals were obtained from the laboratory suppliers BDH chemicals Ltd, Poole, Dorset and Fisons Scientific equipment, Loughborough.

APPENDIX B SOLUTIONS

Physiological salt solution (PSS)

Compound	**g/L**	**Molarity (mM)**
NaCl	6.90	118
$NaHCO_3$	2.10	25
Glucose	1.08	5.4
KCl	0.34	4.6
$CaCl_2$	0.37	2.5
$MgSO_4.7H_2O$	0.25	1.0
KH_2PO_4	0.14	1.0

High Potassium Physiological salt solution

Compound	**g/L**	**Molarity (mM)**
KCl	9.23	123.7
$NaHCO_3$	2.10	25
Glucose	1.08	5.4
$CaCl_2$	0.37	2.5
$MgSO_4.7H_2O$	0.29	1.17
KH_2PO_4	0.16	1.18

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