Studies of the Resistance Vasculature

Thesis submitted for the degree of

Doctor of Philosophy

at the University of Leicester

by

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December 1997

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DEDICATION

I dedicate this work to my late father,

Mr J Kennedy Watt

BSc, ChM MB, FRCS (Lon), FRCSGLAS.

ACKNOWLEDGMENTS

I am greatly indebted to Professor Herbert Thurston for his continuing support over the years and without whose advice and faith I would not have completed this thesis.

I would like to thank the staff of the Department of Vascular Medicine for their assistance, the staff of Biomedical Services Unit for their care of the animals and the Department of Surgery for their help in obtaining samples from patients.

Finally, I would like to acknowledge the British Heart Foundation for financial support.

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CHAPTER 1 INTRODUCTION

1.1. HISTORICAL REVIEW

The recognition that a contracting heart created a pressure to drive blood through the arterial tree emerged with brilliant proof by William Harvey (1628) of the continuous circulation of blood within a closed system. A hundred years later, the Reverend Stephen Hales (1736) published his studies of blood pressure measurement in the horse and other animals. He cannulated the crural artery of the horse with a short brass tube connected to a long glass tube. On releasing the ligature, blood rose up the tube to a height of over nine feet. Hales also examined the dynamic of fluid flow in the mesenteric vascular bed showing that the resistance to flow increased the smaller the lumen of the vessel. Hales manometer was rapidly improved using mercury in the glass tube and sodium citrate in the arterial cannula to prevent clotting and thus a standard method for blood pressure measurement was established for use in animals. This technique, could not be applied to man and it was not until late in the 19th Century that the non-invasive sphygomanometer was developed for routine blood pressure measurements in man.

On the other hand, long before blood pressure could be measured and for that matter hypertension known to exist, the structural alteration of blood vessels and cardiac hypertrophy due to a raised blood pressure were identified by Richard Bright (1836). In his studies of albuminous urine, Bright noted at autopsy that many of his patients had evidence of cardiac hypertrophy with no evidence of valvular disease. Bright speculated that a change in resistance to blood flow in the small blood vessels might subject the heart to greater efforts to drive blood through the circulation. He

considered two possible changes, an alteration in the quality (i.e. viscosity) of the blood or narrowing of the small blood vessels. Johnson (1868) described thickening and narrowing of the intra renal vessels in patients dying of nephritis (Bright's disease) and Gull and Sutton (1872) extended this study, finding widespread changes - arteriocapillary sclerosis. However, whilst identifying the wide spread nature of structural change in hypertension they incorrectly concluded that this also was the cause of the renal disease. The concept of generalised arteriocapillary fibrosis stimulated the search for methods to measure blood pressure in man. The first successful studies by Mahomed (1874) lead to the realisation that hypertension could develop without renal disease.

Following Mahomed's untimely death from typhoid fever, Sir Clifford Allbutt (1890) distinguished the two main forms of hypertension, senile hyperpiesis (later termed essential hypertension) and hypertension associated with nephritis. The sphygmomanometer as we now know it was developed by Riva Rocci in 1896 and permitted safe, easily reproducible, measurements of systolic blood pressure. In 1905 Korotkoff described a technique for determining both systolic and diastolic blood pressure, which remains in wide clinical use. The use of this classic description of the five phases of the Korotkof sounds was proposed for use by Ettinger in 1907 and with the exception of adoption of a change from phase IV to phase V for the diastolic reading, blood pressure measurement has remained unchanged from that time.

Although, it was quickly appreciated that the majority of patients with hypertension were free from renal disease researchers remained preoccupied with the association between the kidney and blood pressure. This began with the work of Tigerstedt and Bergman (1898) who

demonstrated that injections of an extract of rabbit renal cortex but not medulla raised the blood pressure. They called this substance renin and demonstrated that the effect could be demonstrated in vivo but not in vitro on isolated vascular tissue. They suggested that renin, a heat labile substance, was secreted by the kidneys into the blood and by an indirect means raised blood pressure. However, this important study was ignored because of confusing reports of various pressor and vasodilator effects of kidney extracts and the lack of a reproducible experimental model of chronic renal hypertension. Many attempts were made to produce chronic hypertension by a variety of techniques which included partial nephrectomy, irradiation, renal vein banding and wrapping the kidneys in cellophane but these models failed to induce reproducible hypertension. After reviewing these experiments Goldblatt and his colleagues produced a reliable model of hypertension by constricting the renal arteries of a dog with silver clamps (Goldblatt et al. 1934). They showed that hypertension could be induced by bilateral renal artery clamping or unilateral clamping in combination with a contralateral nephrectomy. This pioneering work rekindled interest in renin and stimulated detailed biochemical studies which lead to the elucidation of the renin-angiotensin system.

In 1939 Braun-Mendez and colleagues demonstrated that renin reacted with a plasma substrate to form a heat stable, dialyzable short lived pressor substance which they called hypertensin. Almost simultaneously, Page (1939) demonstrated that renin only caused vasoconstriction of the rabbit ear artery when perfused with fresh blood not saline. Later Page and Helmer (1940) showed that incubating plasma with renin produced a vasoconstrictor substance which they called Angiotonin. It was rapidly realised that Angiotonin and Hypertensin were the same substance and a

common terminology Angiotensin was agreed for the pressor substance. Later the plasma substance on which renin acts was called angiotensinogen.

This classic series of studies lead to the idea that hypertension was caused by an increase in neurohumoral activity. Haemodynamic studies showed that all forms of hypertension were associated with a raised peripheral resistance. The majority of patients with hypertension had no obvious renal cause but the search for other evidence of neurohumoral activity continued. Pickering searched for a cause of the raised peripheral resistance showing that it was not due to an increase in blood viscosity and that the resistance to flow arose in the small pre-capillary vessels. He also noted that the peripheral resistance remained elevated in hypertensive patients even at maximum vasodilatation (Pickering 1936). Others confirmed these observations and it was agreed that the increased resistance was the result of a stable humoral factor.

Over the next ten years Pickering completed a series of studies of chronic renal hypertension in the rabbit. He concluded that there appeared to be a non-renal factor which maintained blood pressure in chronic hypertension and speculated that an alteration in vascular morphology may be the main mechanism for maintaining blood pressure (Pickering 1945). This hypothesis was taken up by Folkow and his coworkers who in 1958 showed that structural alteration of the vasculature could account for the raised total peripheral resistance in essential hypertension without the need to have an increase in smooth muscle activity by an increase in sensitivity or neurohumoral drive. Folkow showed that structural changes in the resistance vasculature with an increased wall thickness and reduced internal diameter would produce a greater resistance to flow than a structurally normal vessel. This hypothesis not only explained the enhanced reactivity

found in essential hypertension but also the raised resistance at maximal vasodilatation (Folkow 1958). Although, histological studies supported this hypothesis (Short 1966) its acceptance awaited morphological studies of living isolated small arteries.

The development of a small vessel bath system (myograph) has made possible direct studies of the structure and function of small vessels in vitro. This system has been used to study the development of structural and functional changes in mesenteric resistance vessels in genetic (spontaneously hypertensive rat) and experimental (Goldblatt two kidney one clip hypertensive rat) models of hypertension. This thesis will describe the development of vascular structure and the response to reversing hypertension. In addition, the effects of hypertension on the endothelium has been investigated with particular reference to the effect of high blood pressure on the recently described endothelial vasodilator EDRF.

1.2. PERIPHERAL VASCULAR RESISTANCE

Blood pressure depends on the cardiac output and the resistance to blood flow of the body tissues. There is general agreement that in the established phase of hypertension it is associated with raised total vascular resistance in the face of a normal cardiac output. Furthermore, since capillary pressure is normal in hypertension, the main increase in resistance must lie in the precapillary vessels.

1.2.1. What are resistance arteries?

For many years it was generally accepted that the peripheral resistance resides in the arterioles. However, recent blood pressure profile

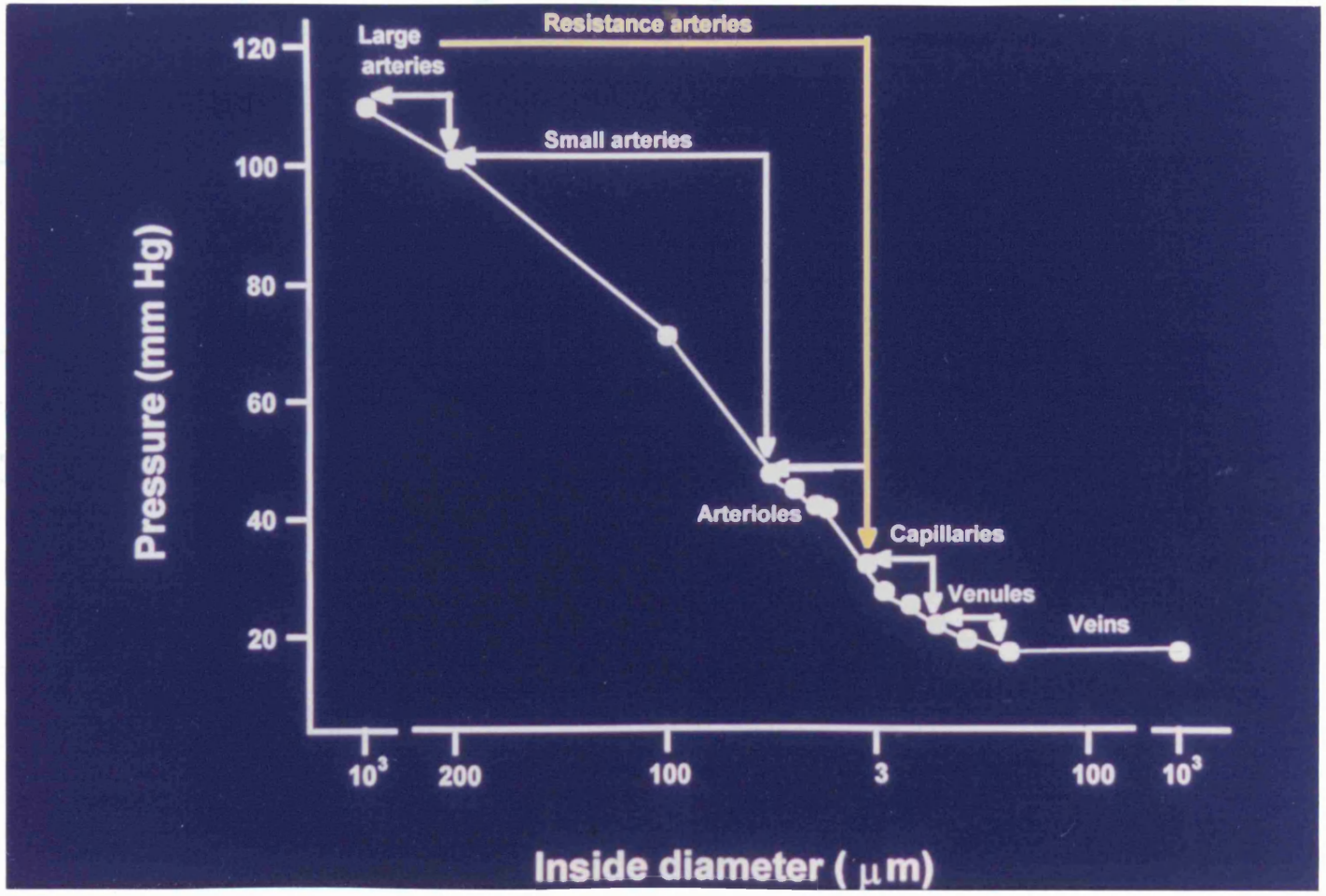

Figure 1. Vascular anatomy and hydrostatic pressure profile in the hamster cheek pouch. (Taken from Davis et al. 1986)

Tunica Media

This is generally the thickest layer and is the one which shows the greatest variation in structure and functional properties in different regions of the circulation. Because of these differences arteries are generally divided into elastic (larger) and muscular vessels but with the exception of the aorta there is no clear division between the two. The media comprises of smooth muscle cells bounded on the luminal side by a well defined internal elastic lamina and on the outer by the external elastic lamina which is less well developed in the smaller arteries.

In the large conduit arteries (i.e. aorta) the tunica media is made up of multiple concentric layers of elastic tissue separated by thin layers of connective tissue, collagen fibres and sparse smooth muscle cells which obliquely cross link successive elastic layers. Smooth muscle cells are long (25-50μm), thin (5μm) and contain contractile filaments of muscle which have the same basic form as in the muscle cells of the heart. The cells contract in response to depolarisation of the cell membrane and thus generate tension. In the smaller arteries the smooth muscle cells lie increasingly more circumferentially forming flat spirals.

Further away from the heart, the structure of the media changes and the vessels are known as the muscular arteries with the media consisting largely of spirally arranged smooth muscle cells. These are disposed in multiple layers with small amounts of connective tissue, collagen and elastic tissue. The number of layers diminish as the vessel radius diminishes. In the very small arteries and arterioles there may be only one or two such layers. Since there are few nerve fibres within the media it appears that the smooth muscle cells act as an electrical syncytium when stimulated by neuronal axons in the adventitia.

Tunica Adventitia

The adventitia is a fibrous supporting outer layer comprising of a loose connective tissue with a few elastin and collagen fibres, fibroblasts, macrophages and nerve fibres. It has an ill-defined boundary which merges into the surrounding tissues. It appears that nerves remain within the adventitia with little or no penetration into the smooth muscles of the media.

Large arteries (>1mm) have their own nutrient blood vessels, *vasa vasorum*. These originate from either the parent artery or a neighbouring one and they break into a capillary network which supplies the adventitia and in the larger vessels reaches as far as the inner layers of the tunica media. Nourishment of the tunica intima and the innermost layers of the tunica media depends predominantly on transport from the arterial lumen.

1.2.3. Haemodynamics of Blood Flow Through Resistance Arteries

Fluid flowing past the stationary wall of a tube (e.g. a vessel wall) always exerts a shear stress on the boundary because the fluid away from the wall is traveling faster than that at the boundary. Newtonian fluids, with a uniform viscosity e.g. water, glycerine and mercury, stress is directly proportional to the local rate of shear. Blood is a non-Newtonian fluid exhibiting a shear dependent viscosity. In the case of large arterial blood vessels this can be regarded as Newtonian and so considerable simplification is possible in describing the fluid dynamics. However, this is not the case in the microcirculation.

1.2.4. Poiseuilles formula

The flow of fluid through a tube can be expressed by Poiseuilles formula:

$$\text{flow per second} = \frac{(P_1 - P_2)\,\pi r^4}{8\eta L}$$

Where r is the radius of the tube, P1-P2 the pressure gradient, L the length of the tube and η the viscosity of the fluid. This formula can be re-arranged to define resistance to flow:

$$\text{Resistance (R)} = \frac{8\eta L}{r^4}$$

The important factor in this equation is the radius (r^4) and it follows that the resistance to blood flow in small arteries is inversely related to the internal diameter. Blood viscosity makes a contribution in vessels 20-100μm and length appears to be an important determinant of resistance in small arteries. However, vessel length and viscosity are not altered in hypertension and therefore these parameters do not significantly contribute to the elevated peripheral resistance.

It is important to bear in mind that the overall resistance of a vascular bed will be determined by the sum of the resistances and therefore the number of vessels. This vascular resistance in the whole tissue will be altered by variations in resistance artery lumen and/or reduction in the number of parallel vessels perfused (rarefaction).

The internal radius of resistance vessels depends on a number of factors, not just the degree of smooth muscle activity. Three other factors play an additional important role (1) vessel geometry (2) wall distensibility and (3) transmural pressure. These additional factors with muscle activity interact in vivo so that structure and function adapt to each other.

Cardiovascular structural design is governed by the law of Laplace which states:

$$T = \frac{P \times r}{w}$$

Where T is the tension per unit wall layer (wall stress), P is the transmural pressure and r the tube radius. Therefore as the pressure increases the wall tension will remain constant only if either the wall thickness increases or the radius decreases. Clearly the opposite applies with a fall in blood pressure.

Nature provides many examples of structural adaptation to increased pressures. An extreme example is found in the giraffe where there are enormous differences between the arterial blood pressure at the head and lower leg. At the heart beat the mean arterial blood pressure is high (250-300 mmHg) in the adult to provide adequate perfusion at the head which is over ten metres above the heart. The carotid artery at the jaw is exposed to a transmural pressure of 80-100 mmHg and has a normal wall to radius ratio. By contrast, because of the length of the leg the posterior tibial artery is grossly thickened with a pin hole lumen to withstand pressures exceeding 400-500 mmHg. Similarly, but less extreme examples, can be found in the venous circulation in man. The wall to radius ratio of veins are identical in the arms an legs of infants. However, as the child begins to walk the veins in the legs are exposed to a greater hydrostatic pressure and respond by developing an increased wall to radius ratio.

1.2.5. Control of the Systemic Circulation

The systemic circulation consists of numerous vascular beds connected in parallel. The relative resistance of each bed determines the proportion of cardiac output it will require. At rest the kidneys and intestinal organs receive ~20% of cardiac output, the brain and skeletal muscle 15% and

heart and skin 5%. During exercise flow to muscle increases greatly in association with a marked fall.

Resistance to flow depends on the internal diameter of the small arteries and arterioles. This may depend on increased smooth muscle activity or a structural alteration with a reduction in lumen diameter and an increased wall thickness. However, in both cases that state of smooth muscle cell tone will determine the resistance. The magnitude of intrinsic pre-capillary vessel tone varies considerably in different organs. Therefore, a given stimulus will not produce the same degree of dilatation or constriction in all beds.

1.2.6. Vascular Smooth Muscle Contraction

Vascular smooth muscle possess characteristics generally typical of excitable cells. The plasma membrane contains several specific ion channels and a number of active transport systems. These result in differences in ionic concentration and a potential difference across the membrane (50-70mV, inside negative). Changes in the conformation or charge distribution within channels form 'gating' mechanisms that regulate ion fluxes (Burnstock 1972; Somlyo & Somlyo 1968; Katz et al. 1982; Webb & Bohr 1981). The association of electrical membrane phenomena and mechanical activity has been demonstrated in several types of smooth muscle (Somlyo & Somlyo 1968).

Constrictor substances usually produce depolarisation and initiate or increase the frequency of action potentials in spike-generating tissue. Some tissues appear to respond to excitatory stimuli by graded depolarisation rather than spike-generation. Noradrenaline has been shown to produce depolarisation and contraction in rat caudal, cat basilar and rabbit

pulmonary arteries (Hermsmeyer et al. 1982; Harder et al. 1981; Hausler 1982). Papaverine and isoproterenol exert opposite effects (hyperpolarisation and relaxation) (Itoh et al, 1981).

Contractile mechanisms in vascular smooth muscle are similar to those in skeletal and cardiac muscle (Van Breeman et al. 1980; Webb & Bohr 1981; Adams & Schwartz 1980; Hartshorne 1980; Van Breemen et al. 1987; Barany & Barany 1981; Murphy 1982).

Vascular smooth muscle contains the major proteins myosin and actin but the arrangement of these two filaments is not as regular in smooth muscle, resulting in the absence of clear cross-striations. Myosin is composed of two "heavy" chains and two pairs of "light" chains. Extensions of the myosin molecule, containing the light chain, form cross-bridges toward the actin filament. The cross-bridges possess a magnesium dependent ATPase and an actin binding site.

Calcium binds to the protein calmodulin during initiation of the contractile process in vascular smooth muscle. The calcium-calmodulin complex then binds to the inactive catalytic subunit of myosin light-chain kinases, resulting in activation. This enzyme phosphorylates the myosin light-chain permitting the activation of the magnesium-dependent ATPase on the myosin cross-bridges by actin. Hydrolysis of ATP follows and results in tension development. Tension varies with the number of active cross-bridges and their cycling rate. Myosin light-chain phosphatase removes phosphate from the light-chain and restores the two filaments to their dormant state. An alternative mode of activating myosin ATPase by the protein lectonin has been proposed.

Calcium has a central role in the contractile process of all forms of muscle. During the resting state its concentration is approximately 10^{-7}M or less. Concentration related activation occurs at 10^{-7}M to 10^{-5}M. This calcium is primarily derived from the sacroplasmic reticulum in skeletal muscle. In cardiac muscle, influx of calcium occurs during the plateau of the action potential and is also released from the sacroplasmic reticulum. In smooth muscle calcium enters the cells through 'voltage'-activated channels that become operative on membrane depolarisation or through receptor-activated channels. Calcium can also be released from other intracellular binding sites as well as from the sacroplasmic reticulum. The contributions of these sources of calcium vary in different blood vessels and also depend on the mode of activation (Casteels 1980; Van Breeman 1980). Biologically active substances such as noradrenaline, angiotensin II and serotonin contract vascular smooth muscle by enhancing calcium influx through receptor-operated channels as well as release from intracellular stores. Agonist-induced depolarisation provides another mechanism for calcium entry through the voltage-sensitive channels.

Relaxation occurs on sequestration of calcium into the sacroplasmic reticulum or other stores or by efflux. Relevant substances can act by influencing one or more of these processes. cAMP promotes the uptake of calcium by the sacroplasmic reticulum and has also been shown to inhibit myosin light-chain kinase. Vasodilatation produced by nitroglycerin and nitroprusside correlates with increases in cGMP (Axelsson et al. 1979; Keith et al. 1982).

1.2.7. Local Mechanisms

Vascular smooth muscle cells readily respond to alterations in tissue PO_2, PCO_2 and pH. Hypoxia, hypercarbia and reduced pH result in

vasodilatation whereas changes in the opposite direction cause vasoconstriction. Overall there is a strong relationship between metabolic activity and blood flow or vascular resistance under normal conditions. The perfusion of various vascular beds with hypoxic blood results in vasodilatation only when PO_2 is greatly reduced i.e. to ~40mmHg (Haddy & Scott 1968; Sparks Jr 1980). Thus tissue O_2, CO_2 or hydrogen ion concentration cannot individually be responsible for resistance regulation. However, CO_2 tension is of critical importance in regulating blood flow in the cerebral circulation (Kuschinsky & Wahl 1978; Abboud 1981; Sokoloff 1981).

Other factors contributing to local regulation of vascular tone include nucleotide metabolites, such as adenosine, potassium ion and alterations in osmolarity (Haddy & Scott 1968; Sparks Jr 1980; Dobson Jr et al. 1971; Berne 1980). Metabolic vasodilatation in skeletal muscle also results in the opening of additional capillary channels. By contrast, post-capillary vessels appear to remain unaffected by local regulatory mechanisms. The pulmonary circulation displays a somewhat atypical response to hypoxia as the pulmonary arterial pressure increases. Where as, augmented blood flow may contribute to the pressure elevation. In addition, numerous other locally generated factors (prostaglandins, bradykinin, serotonin, histamine etc.) also influence vascular tone.

The power of local regulatory mechanisms is well illustrated by the phenomenon of autoregulation in blood flow. Changes in arterial transmural pressure or an increase in blood flow result in changes in smooth muscle tone and vessel resistance in the same direction and these in turn tend to maintain blood flow constant (Johnson 1980). Autoregulation normally operates in the pressure range 60-140mmHg in most organs.

Regulatory mechanisms can be overcome at the extremes of blood pressure at which point blood flow will be proportional to the driving pressure. The ability of different organs to autoregulate varies being a powerful mechanism in the kidney and brain, somewhat less important in skeletal muscle and having an almost negligible effect in the skin. Passive mechanisms tend to counteract autoregulation to some extent because changes in distending pressure modify vessel radius and thereby resistance.

At least three major mechanisms have been proposed to account for the autoregulatory process. The tissue-pressure hypothesis suggests that increasing perfusion pressure leads to an increasing capillary filtration and an elevation of extravascular pressure and thus an increase in resistance. However, the majority of evidence support the metabolic and myogenic theories. The former is based on the hypothesis that a decrease in perfusion pressure will lower blood flow initially and reduce tissue PO_2 and cause an accumulation of metabolites. This will in turn lead to vasodilatation of the smaller arterial vessels, reduce resistance and raise blood flow towards original levels. The myogenic theory of autoregulation is an extension of observations by Bayliss and is based on the hypothesis that vascular smooth muscle responds to changes in distending pressure by contraction or relaxation. Thus, isolated strips of small mesenteric and cerebral arteries have been shown to contract when quickly stretched. Also, distension increases rhythmicity of isolated strips which normally display periodic contractions. Further elevation of distending pressure increases vasomotion in small arterial vessels.

The relative roles of metabolic and myogenic factors in autoregulation remain controversial and neither appear to account for the phenomenon

alone. Their respective contributions probably vary from organ to organ as well as from time to time as circumstances change. Normal vascular tone is a prerequisite for autoregulation because reactive hyperaemia or the administration of vasodilators or metabolic inhibitors obtunds the process.

The cerebral circulation is principally under the control of local factors with only minor contributions by systemic mechanisms, including the nervous system (Kuschinsky & Wahl 1978; Abboud 1981; Sokoloff 1981; Heistad et al. 1981). The highly selective permeability of the capillary endothelium, establishing a "blood-brain barrier" is a unique feature of the cerebral circulation.

1.3. SYSTEMIC MECHANISMS

Various neural and humoral factors influence vascular resistance. These include the autonomic nervous system, renin-angiotensin system, vasopressin, prostaglandins and the kallikinin kinin system.

1.3.1. Sympathetic Nervous System

The central nervous system plays a major role in the physiological regulation of blood pressure and heart rate in response to acute stimuli e.g. changes in posture, hypoxia and emotion.

Most blood vessels are richly innervated by sympathetic nerves. Nerve terminals form a network of anastamosing filaments that run along the periphery of vessels. Adrenergic innervation is usually limited to the junction of the adventitia and the media. However, neurons have been found to penetrate for a short distance into the media in some vessels

(e.g.. the proximal saphenous artery of the rabbit) (Fuxe & Sedvall 1965; Burnstock et al. 1970; Bevan & Purdy 1973; Bevan & Ljung 1974). Generally, arteries are more densely innervated than veins but the density of innervation of individual arteries and veins varies considerably. Terminal axons have also been shown to innervate pre-capillary sphincters.

The general finding that adrenergic fibres are limited to the adventitial-medial junction raises the question of the mode of activation of the more medial smooth muscle cells. This could be achieved by 1) diffusion of the transmitter substance 2) the inward electrical spread of the excitatory potential from innervated cells to the other smooth muscle cells or 3) conduction by smooth muscle cells along low-resistance pathways (Heistad et al. 1981). The relative importance of these mechanisms remains uncertain. It has been demonstrated that impulse propagation can occur in isolated vascular tissue but that vessels differ greatly in their ability to conduct action potentials (Bevan & Ljung 1974).

Stimulation of sympathetic nerves of the majority of organs, or intra-arterial administration of noradrenaline, results in vasoconstriction with an increase in vascular resistance which can be demonstrated by a reduction in blood flow or an increase in pressure in pump perfused systems. Sympathetic stimulation also causes venous constriction, leading to a reduction in venous capacitance and an increase in venous resistance (Mellander 1968, McCulloch et al 1982).

Sympathetic activation may affect vessels in different organs or even vessels within the same organ differently. For example blood is distributed away from the renal cortex during stimulation and increased sympathetic

activity produces a greater increase in resistance of the kidney than in the forelimb (Abboud 1972).

The ability to record pressures in small vessels has permitted more precise studies of vascular responses to sympathetic nerve stimulation. These studies have shown that the initial increment in total vascular resistance of the gastrocnemius muscle during sympathetic nerve activation depends on the constriction of the more distal arterial vessels. However, with continued stimulation the distal vessels tend to relax while the larger proximal segment progressively constrict (Folkow et al. 1971).

Responses to sympathetic stimulation in the perfused dog forelimb have also been studied in detail and revealed even greater complexity. Thus although, it was found that stimulation increased the overall vascular resistance, changes in the constituent muscle and cutaneous beds differed considerably. Vascular resistance of the large arterial segments increased in muscle and more particularly in skin. The small vessel resistance, comprising effects in small arteries, arterioles and smaller veins was little altered in skeletal muscle but fell markedly in skin. On the other hand the resistance of the venous segment composed of the larger veins increased greatly in the skin but with only a smaller change in the muscle. As a consequence, these responses resulted in a net distribution of blood flow from skin to skeletal muscle (Abboud 1972; Abboud & Eckstein 1966).

Arterial and venous constrictor responses to sympathetic stimulation and noradrenaline are mediated predominantly by activation of α_1-adrenoceptors but α_2-receptors may assume a greater role in large veins (Vanhoutte 1982). Arterial smooth muscle cells also possess β receptors (β_2). These probably are not innervated or do not respond greatly to

neuronally released noradrenaline but may be activated by circulatory epinephrine (Russell & Moran 1980).

An intravenous infusion of noradrenaline elevates total peripheral resistance whereas on the other hand, total resistance usually falls during epinephrine infusion, mainly because of β-mediated dilatation of vessels in skeletal muscle (Abel et al. 1980).

Sympathetic nerves supplying the skin and skeletal muscle also contain vasodilator fibres. The latter are part of a system which originates in the cerebral cortex, relayed in the hypothalamus and midbrain collicular region, but not in the medulla and emerges from the thoracolumbar cord. Activation of the system by hypothalamic stimulation results in vasodilatation in skeletal muscle but constriction of most other beds, as well as an increase in heart rate (Uvnas 1966; Folkow et al. 1968).

1.3.2. Adrenergic Receptors

Ahlquist concluded that two distinct adrenergic receptors, α and β, exist (Ahlquist 1948). Subsets have been identified by binding studies and the use of antagonists. Both α_1 and α_2-adrenoceptors cause arterial vasoconstriction. The observation that constrictor responses to sympathetic nerve stimulation are selectively attenuated by α_1 blockers, whereas parentally administered noradrenaline is most susceptible to α_2 blockers, has led to the hypothesis that neuronally released noradrenaline gains access primarily to α_1 receptors at post-junctional sites but that circulating noradrenaline activates both (Langer 1981; Drew & Whiting 1979).

Numerous biophysical and biochemical consequences of α-adrenoceptor activation have been described, including inhibition of adenylate cyclase in

some organs (e.g. platelets), elevation of cAMP in the brain and indirect linkage to guanylate cyclase (Langer 1981; Sabol & Nirenberg 1979). α_1 receptor activation has been shown to augment the breakdown of phosphatidyl inositol (Jacobs & Schultz 1982). Whereas, α_2 stimulation may specifically enhance calcium influx in vascular smooth muscle.

Activation of β_1-adrenoceptors causes an increase in cardiac rate and contractility and relaxes intestinal tone (Launds et al. 1967). Stimulation of β_2 receptors results in vasodilatation. Propanolol is non-selective and blocks both β_1 and β_2 receptors whereas the cardioselective betablocker, atenolol blocks β_1 receptors.

1.3.3. Cholinergic Receptors

Acetylcholine is an agonist for two major types of receptors that were historically called 'muscarinic' or 'nicotinic'. Smooth muscle cell receptors are termed 'muscarinic'. Activation of cholinergic receptors results in changes of cell membranes ultimately leading to various responses such as hyperpolarisation. Muscarinic receptors may be negatively coupled to adenylate cyclase and indirectly linked to guanylate cyclase, resulting in elevated concentrations of cyclic guanosine monophosphate (cGMP).

Table 1 Distribution and effect of receptor sites.

Blood vessels	*Adrenergic*	*Cholinergic*
skeletal muscle	constriction (α) dilatation (β)	dilatation
skin	constriction (α)	slight dilatation
kidney, intestine	constriction (α) slight dilatation (β)	dilatation

1.4. RENIN ANGIOTENSIN SYSTEM

The concept of a renal angiotensin system stemmed from the observations of Richard Bright (1836) which demonstrated the relationship between kidney disease and hypertension. The renal hormone renin was discovered by Tigerstedt & Bergman in 1898 but there followed a latent period with no further progress because of difficulties in reproducing the observations. Following the description of a stable model of renal hypertension by Goldblatt (1934) interest in renin was rekindled and it was quickly shown that renin was an enzyme which acted on a plasma substrate to produce a pressor substance (Braun-Mendez et al. 1940). Later experiments showed that renin cleaved the substrate to form angiotensin I, a non-pressor peptide. In the 1950's, Skeggs and his colleagues demonstrated that angiotensin I was converted by a chloride dependent enzyme (angiotensin converting enzyme) to form a potent vasoconstrictor substance, angiotensin II (Skeggs 1954). They went on to sequence angiotensin II and in 1957 angiotensin II was synthesised and large quantities were made available for experimental and clinical studies.

It rapidly became evident that angiotensin II was a potent vasoconstrictor peptide which also caused sodium retention. With the unfolding of the renin-angiotensin cascade and the discovery of the sodium retaining mineralocorticoid aldesterone it became clear that the system played an important role in both blood pressure and body fluid volume homeostasis. It is also linked with adrenergic vascular control mechanisms, to the prostaglandins and to the kallikrein-kinin system. The main effector peptide being angiotensin II, formed from renin substrate by a series of enzyme reactions.

1.4.1. Components of the Renin Angiotensin System

1.4.2. Renin

Renin is a proteolytic glycoprotein with a molecular weight of 38,000 with a high degree of substrate specificity to cleave the plasma substrate angiotensinogen at the Leu^{10}-Leu^{11} bond to form the decapeptide, angiotensin I. The kidney is the classic site for renin synthesis and most of the circulating renin is renal in origin, secreted by the juxtaglomerular apparatus. The importance of the myoepithelial cells of the juxtaglomerular apparatus were recognised by Goormaghtigh (1939) who proposed that this was the site of renin synthesis, storage and secretion. These cells are modified smooth muscle cells of the afferent arteriole.

The control of renin secretion is complex with intrarenal and extrarenal mechanisms. A major degree of control depends on the afferent arteriole baroreceptor mechanism. Juxtaglomerular cells act as pressor sensors detecting changes in perfusion pressure in the afferent arterioles. A fall in circulating blood volume and renal perfusion pressure leads to decreased afferent arteriole stretch and the juxtaglomerular apparatus cells to increasing renin release. A rise in perfusion pressure, with the associated increase in stretch, depresses renin release. The second intrarenal mechanism is located in the macula densa, a part of the distal convoluted tubule in close proximity to the juxtaglomerular apparatus. It is thought that these cells sense changes in renal tubular sodium concentration, feeding back on the juxtaglomerular apparatus cells to regulate renin release. Renin secretion is inversely related to the amount of sodium absorbed from the tubular fluid, the macula densa (Vander 1967, Freeman & Davis 1979, Kechin & Campbell 1980). Extrarenal mechanisms, which control renin release, include the sympathetic nervous system, circulating catecholamines

and a direct negative feedback by angiotensin II. Activation of the sympathetic nervous system increases renin release as does infusion of catecholamines (Dibona 1982). It is now known that this involves β_1 adrenoceptors stimulation and renin release can be inhibited by beta adrenergic blocking drugs.

1.4.3. Renin Substrate

Renin substrate is a glycoprotein with marked species variation. Human substrate can be split only by renin obtained from primates although human renin is capable of producing angiotensin I substrate from a variety of animals (Gordon & Sachin 1975). Renin substrate is synthesized by the liver and secreted into the circulation. The rate of secretion varies so that a constant level of plasma substrate is maintained despite alterations in plasma renin concentrations (Blair-West 1976).

1.4.4. Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE) is a non-specific dipeptidyl-carboxypeptidase which is membrane bound and widespread throughout the vascular tree. It converts angiotensin I to angiotensin II, an octapeptide, and also inactivates bradykinin to peptide fragments. Converting enzyme is present in plasma but the rate of conversion of angiotensin I to angiotensin II, in the plasma, is too slow to account for the immediate action of the decapeptide. The lungs are the most important site for the generation of angiotensin II with converting enzyme being located in the vascular endothelium of the pulmonary arteries which have been shown to contain very high concentrations of the converting enzyme (Aiken and Vane 1970). Converting enzyme has also been located in the brain, kidney and peripheral vascular tissue where angiotensin I may be converted to angiotensin II

locally (Aiken and Vane 1972). In fact angiotensin converting enzyme has been found in all blood vessels.

Inhibitors to ACE have been developed thus enabling scientists to block the conversion of angiotensin I to angiotensin II and the hydrolysis of bradykinin and substance P. ACE inhibitors are competitive inhibitors directed to the active site of the enzyme. Differences in binding characteristics is thought to explain their variation in potency, duration and action. ACE inhibitors reduce circulating angiotensin II levels in the blood which in turn reduces the effects of angiotensin II on vascular tone, aldesterone release and renal sodium handling. There are additional effects such as the potentiation of endothelium derived relaxing factor-mediated or prostaglandin-mediated vasodepressor effects of endogenous kinins.

At present there are three main classification for all the clinically important ACE inhibitors; 1) Sulphydryl-containing ligand (captopril), 2) Carboxyl-containing ligand (enalapril, ramipril, perindopril, quinapril), 3) Phosphorous-containing ligand (fosinopril) (Kostis 1989).

1.4.5. Angiotensin I

Angiotensin I is an inactive decapeptide product of the action of renin on renin substrate. Angiotensin I is subsequently cleaved by a converting enzyme to angiotensin II. It can also be hydrolysed, by aminopeptidases, to form a heptapeptide which is susceptible to conversion by converting enzyme and further degradation by aminopeptidases. The product that is formed is a heptapeptide, (des-Asp^{1})angiotensin II ('angiotensin III') which can modulate converting enzyme activity by product inhibition.

Renin secretion is inhibited by angiotensin II and (des-Asp^{1})angiotensin II and the later has been implicated in the steroidogenic actions of the renin angiotensin system (Freeman et al. 1977). Angiotensin I is an inactive precursor of angiotensin II but it does have some potential direct actions. It has been shown to act directly on the adrenal medulla to release catecholamines (Peach 1971), to facilitate the release of noradrenaline (Johnson et al. 1974) and to have direct actions on the central nervous system (Peach 1977). It has been proposed that angiotensin I may be important in the regulation of intrarenal blood flow distribution (Itskovitz and McGiff 1974). However, this could be the effect of tissue conversion of Angiotensin I to Angiotensin II as all have angiotensin converting enzyme present throughout the vascular endothelium. There appears to be no specific receptor site for angiotensin I but in high enough concentrations it can stimulate tissues that will normally respond to angiotensin II. However, it is probable that at physiological concentrations little or no action is exerted.

1.4.6. Angiotensin II

The octopeptide, angiotensin II, is still accepted as the main effector hormone of the renin angiotensin system. Angiotensin II is formed in tissue beds and in the circulation. It has a half life of 15-30 seconds and is a potent vasoconstrictor of vascular smooth muscle being 50 times more powerful on a molecular weight basis than noradrenaline (Peach 1977). The vasoconstrictor action is probably mediated by influx and intracellular release of calcium. Once formed, angiotensin II is rapidly broken down by plasma and tissue aminopeptidases and endopeptidases (Peach 1977). The heptapeptide fragment of angiotensin II may have some biological activity but the other fragments appear to be inactive.

1.4.7. Actions of Angiotensin II

Cardiovascular system

Angiotensin II increases cardiac contractility both by a direct action on the myocardium (Blumberg et al. 1975) and by potentiating the release of norepinephrine from the cardioaccelerator nerves (Stark et al. 1969). It should be noted that the direct positive intropic and chronotropic effects of angiotensin II are usually masked in vivo because the rise in systemic pressure elicits a baroreflex-mediated increase in cholinergic tone to the heart. By an action in the central nervous system angiotensin II elicits an increase in efferent nerve activity to the peripheral sympathetic nervous system that results in increases in cardiac output and total peripheral resistance, the two major determinants of blood pressure. Moreover, angiotensin II causes the release of epinephrine and norepinephrine from the adrenal medulla (Peach et al. 1966), facilitates the release of norepinephrine from the peripheral sympathetic neurons (Zimmerman & Gisslem 1968) and blocks the re-uptake of norepinephrine by peripheral sympathetic neurons (Khairallah 1972).

Vascular smooth muscle

Angiotensin II contracts the smooth muscle in blood vessels and is a determining factor in resistance vessel calibre. A smaller effect is seen in venous smooth muscle compared to arterial smooth muscle. Interaction between the sympathetic nervous system and angiotensin II may enhance the direct vasoconstrictor effect on blood vessels. In addition to causing an increase in vascular tone it has been suggested that angiotensin II can cause vascular hypertrophy by acting as a growth factor (Lever 1993).

Regional haemodynamics

Blood flow response to angiotensin II varies from one segment of a vascular bed to another and between different vascular regions (Peach 1977). The direct and indirect pressor effects of angiotensin II represent a major homeostatic mechanism involved in long term maintenance and control of blood pressure. Against this background, it is apparent that drug induced alterations of renin release are of great importance in experimental and clinical medicine (Keeton & Campbell 1984).

Vascular permeability

Giese (1973) demonstrated widening of the gaps between the endothelial cells of large arteries exposed to high concentrations of angiotensin II. It has been suggested that this effect may be mediated via the prostaglandin system or vascular permeability factor.

Vascular permeability factor has been described (Ferrara et al. 1992) as a potent mitogen for microvascular and macrovascular endothelial cells but it has not been shown to directly stimulate the proliferation of vascular smooth muscle cells. Angiotensin II is known to increase vascular permeability via pressor actions (Goldby & Beilin 1972). Vascular permeability factor (VPF) from vascular smooth muscle has also been called vascular endothelial cell growth factor (VEGF). VPF is a 34-42kDa heparin-binding, dimeric disulphide-bonded glycoprotein that binds to two high affinity receptors. These receptors each have tyrosine domains, predominantly located on the vascular endothelium (Ferrara et al. 1992).

VPF is among the most potent vascular permeability-enhancing factors and on a molar basis is 50,000 times as potent as histamine (Senger et al. 1990). This action makes it an alternative candidate as a mediator for normal and

pathological changes in vascular endothelial permeability. Williams and colleagues have suggested a role for VPF as a regulator of vascular function (Williams et al. 1995).

Nervous system

The direct injection of angiotensin II into the brain stem or infusion into the vertebral arteries causes peripheral vasoconstriction (Scroop & Lowe 1969). Also, the administration of angiotensin II into the central nervous system stimulates thirst and drinking. The mode of action may involve specific angiotensin receptors in the brain but it has been suggested that the drinking response may be mediated by dopaminergic pathways (Fitzsimons & Setter 1975). There is an interaction between angiotensin II and the sympathetic nervous system with a resultant facilitation of neurotransmission (Vanhoutte et al. 1981; Zimmerman 1981; Peach 1977). This appears to be due to enhanced noradrenaline synthesis (Roth 1972) and release (Hughes & Roth 1971) but in addition there is inhibition of neuronal reuptake of noradrenaline (Khairallah 1972). This interaction has been demonstrated in isolated artery preparations and it has been speculated that locally generated angiotensin II might modulate adrenergic neurotransmission.

Kidney

Studies have shown that angiotensin II can alter the distribution of blood flow in the kidney , reduce the glomerular filtration rate and directly enhance tubular sodium reabsorption (Peach 1977; Freeman & Davis 1979; Ploth & Navar 1979). Renal tubular sodium also is influenced indirectly by angiotensin II by regulation of aldosterone. In whole animal models intravenous infusion of angiotensin II produces diverse results in the kidney. The renal response to low doses (50pg/kg/min to 100ng/kg/min) is

a marked to moderate decrease in blood flow, a decrease in glomerular filtration with an increase in filtration fraction and decrease in urine flow and sodium excretion. There is a reduction in outer cortical blood flow with a variable effect on inner cortical and medullary blood flow. Larger doses of angiotensin II (>100ng/kg/min) cause a marked increase in mean arterial pressure with the development of natriuresis, chloriuresis and diuresis with the glomerular filtration returning to normal (Lever et al. 1992 and Healey et al. 1965). These effects have been observed after intra-arterial infusion and it is thought that this may be an intrarenal effect.

Normal circulating levels of angiotensin II are thought to play a direct renal role in the control of sodium, potassium and water homeostasis with angiotensin II exerting a direct stimulatory effect on sodium reabsorption independent of changes in glomerular filtration rates, renal plasma flow, filtration fraction or the intracortical distribution of blood flow (Johnson & Malvin 1977). Work, using tubular stop flow pressure techniques, has indicated that the presence of the renin angiotensin system is necessary for the full expression of the tubuloglomerular feed back mechanism. In addition, it has been suggested that angiotensin II may exert a modest but significant influence on renal haemodynamics (Ploth & Navar 1979). Mendelsohn (1979) has demonstrated local generation of angiotensin II in the rat kidney and postulates that there is an intrarenal renin angiotensin system with angiotensin II acting as a local regulatory hormone.

1.4.8. Extrarenal Renin Angiotensin System

Although the renin angiotensin system was considered originally to be a blood borne circulating system, there is considerable evidence for the existence of a tissue bound renin angiotensin system. Renin has been demonstrated in many organs, including blood vessels (Ganten et al. 1977;

Assad & Antonaccio 1982), the uterus and placenta (Ferris et al. 1972), brain (Ganten et al. 1976), adrenals and in the submaxillary glands of the white mouse (Boucher et al. 1974). However, only recently has it been appreciated that tissue bound renin angiotensin may contribute to blood pressure control and therefore play a role in hypertension (Dzau 1984, 1989).

When the renin angiotensin system was described it was assumed that the generation of angiotensin I and angiotensin II mainly occurred within the vascular compartment. Renin and angiotensin II interacted in the blood and the angiotensin converting enzyme was located on the endothelial surface, particularly in the pulmonary circulation. In 1964 Gould et al. demonstrated renin like activity in blood vessel walls and Daum et al. showed that some angiotensin II generation from renin took place outside the vascular compartment (Daum et al. 1966).

Other studies have shown that angiotensin I can be generated throughout the vascular tree (Mizuno et al. 1988, Hilgers et al. 1989, Hilgers et al. 1991, Campbell et al 1990 and Higashimori et al 1991). The rat hindlimb has been used to demonstrate that there can be spontaneous and induced release of immunoreactive angiotensin I and angiotensin II. However, Hilgers et al. showed that spontaneous production of angiotensin peptides was abolished by prior bilateral nephrectomy thus, suggesting that part of the activity was due to renal renin (Hilgers et al. 1989)

Further direct evidence that the generation of angiotensin I from endocrine renin angiotensin system actually occurs outside the plasma compartment comes from studies that analyse the metabolism and production of angiotensin I across tissue beds in vivo. These studies show that turnover

of angiotensin I occurs in many beds with local generation replacing that taken up from the circulation and metabolised (Admiraal et al. 1990, Danser et al. 1992).

1.4.9. Vasopressin

The peptide vasopressin (antidiuretic hormone, ADH) is formed in the hypothalamus and stored and released from the posterior pituitary (Bie 1980). Its primary physiological role is concerned with renal water reabsorption by controlling the permeability of collecting ducts to water. Although vasopressin has potent vasoconstrictor activity its role in circulatory control remains unclear largely because much higher doses are required to raise blood pressure than to produce antidiuresis (Johnston et al. 1981). However, this difference may be attributed to buffering by the baroreceptors. Vasopressin probably contributes to circulatory regulation in stressful states such as hemorrhage and may provide a backup mechanism to the renin angiotensin system (Johnston et al. 1981; Cowley et al. 1980; Gavras 1982). Development of hypertension in models using sodium overload has been shown to involve vasopressin (DiPette et al. 1982).

1.4.10 Kallikrein Kinin System

Kinins are potent vasodilator peptides formed from globulins (kininogens) by kinases (e.g. kallikrein). Salivary glands, pancreas, kidney and plasma contain high concentrations of kallikrein (Regoli & Barabe 1981; Carretero & Scicli 1981). Also, kallikrein has been shown to activate renin (Seally et al. 1978).

Glandular kallikrein forms kallidin (lysyl-bradykinin) which in turn is converted to the peptide bradykinin by an aminopeptidase. Bradykinin is inactivated by kinase II, now known to be angiotensin converting enzyme.

Kinins affect blood clotting mechanisms, fibrinolysis and capillary permeability, in addition to participating in local control of blood flow. In the kidney, kinins influence water and electrolyte secretion. Angiotensin II and prostaglandins can release bradykinin in organs such as the kidney. Bradykinin in turn is capable of releasing prostaglandins.

Functional coupling of the kallikrein-kinin system with prostaglandins amplifies the vasodilator and diuretic actions of kinins and may be essential to some of the effects of these peptide hormones on blood vessel and renal function (McGiff et al. 1975). Together, prostaglandins and kinins constitute a major blood pressure regulating system which opposes the effects of circulating hormones such as angiotensins, ADH, noradrenaline and mineralcorticoids as well as excitation of the adrenergic nervous system (McGiff and Nasjletti 1973).

1.5. PROSTAGLANDINS

The role of arachidonic acid derivatives has been investigated as early as 1936 (Von Euler 1936). The term "prostaglandin" was coined by Von Euler and along with Goldblatt, Von Euler demonstrated a smooth muscle stimulating acidic lipid, in human seminal plasma.

Two families of highly active substances are derived from arachidonic acid: prostaglandins and leukotrienes (Moncada & Vane 1979; Moncada 1982; Sirois & Borgeat 1980). Prostaglandins are biosynthesised from arachidonic acid and dihomo-y-linolenic acid, both of which are derived from the essential unsaturated long-chain dietary fatty acids. Prostaglandins are a group of unsaturated acidic lipids containing a 20-carbon skeleton.

Arachidonic acid is released from membrane phospholipids by phospholipases. In the prostaglandin pathway, cyclo-oxygenase forms the intermediate endoperoxides PGG_2 and PGH_2 from arachidonic acid. These in turn, depending on the tissue, are transformed into thromboxane A_2 (TXA_2), prostacyclin (PGI_2) or into PGE_2 or $PGF_{2\alpha}$.

In the lipoxygenase pathway, arachidonic acid is converted into hydroxyperoxy eicosatetraenoic acid (HPETE) and then into a variety of leukotrienes. Leukotrienes are found in leukocytes, mast cells and in lung tissue. These substances comprise the "slow-reacting substance of anaphylaxis" (SRS-A). Their cardiovascular role remains to be elucidated but they have been shown to constrict coronary arteries (Michelassi et al. 1982).

Several relatively stable prostaglandins were described ($PGF_{2\alpha}$, PGE_1, PGE_2, PGD_2 etc.) before it was discovered that their synthesis could be inhibited by anti-inflammatory agents such as indomethacin and aspirin (Vane 1971). Following this discovery, a series of short-lived derivatives of arachidonic acid metabolism were identified (eg. PGG_2, PGH_2, TXB_2) (Samuelsson et al. 1978 [for review]).

In 1976 Vane and his colleagues discovered a physiological antagonist to thromboxane A_2. They showed that microsomes from arterial walls enzymatically transformed PGG_2 and PGH_2 to an unstable product which relaxed arterial strips and prevented platelet aggregation. This substance became known as prostacyclin (PGI_2) (Moncada et al. 1976; Higgs & Moncada 1983; Vane et al. 1987; Moncada & Vane 1978 [for review]).

Prostacyclin synthesis, the principal metabolite of arachidonic acid in blood vessels, begins with the liberation of arachidonic acid by phospholipase A_2 from the endothelial phospholipids and is stimulated by a number of factors. These include stress exerted on the cell membrane and by bradykinin, thrombin, platelet-derived growth factor and adenine nucleotides (Moncada & Vane 1979; Moncada 1982; Feigen 1981). Prostacyclin has a short half life in the blood stream (1-2 minutes). The platelet anti-aggregatory and vasodilator actions of prostacyclin are mediated by increasing intracellular cyclic AMP formation. Therefore, locally produced prostaglandins may participate in the regulation of blood flow since they play a role in modulating sympathetic transmission and renin release

Prostaglandins and arachidonic acid derivatives have been shown to play a role in control of cerebral blood flow and cerebral metabolism (Pickard 1981). Additionally, in some segments of the gut and circulation intrinsic tone has been related to the endogenous production of prostaglandins (Eckenfels & Vane 1972). A vast number of pharmacological effects have been ascribed to the prostaglandins but it is dangerous to generalise between different tissues and between species. Some arachidonate derivatives are peripheral vasodilators, whilst others are vasoconstrictors (Bergstrom et al. 1968). Some prostaglandins even function as feedback modulators of noradrenaline release at sympathetic nerve terminals (Hedqvist 1970).

Arachidonate metabolites are released with tissue trauma and there is abundant evidence for their participation in the inflammatory process, oedema formation (involving both cyclo-oxygenase and lipoxygenase pathways), platelet aggregation (involving generation of TXA_2 by the platelets) and the ability of the vascular endothelium to resist platelet-

deposition (by producing the anti-aggregatory and vasodilator prostaglandin) (Moncada & Vane 1978; Moncada & Vane 1980; Williams 1979).

Table 2 Source and major biological actions of arachidonic metabolites in the cardiovascular system.

Metabolite	**Source**	**Target**	**Action**
PGI_2	endothelium	platelets	inhibition
	vasculature	vascular smooth muscle	relaxation
		macrophages	inhibition
TXA_2	platelet	platelet	stimulation
		vascular smooth muscle	contraction
PGD_2	mast cells	platelets	inhibition
	platelets	vasculature	differing
PGE_2	vasculature	vasculature	relaxation
	endothelium	leukocytes	inhibition
	other cells		
$PGF_{2\alpha}$	vasculature	vasculature	contraction
	other cells		
12-H(P)ETE	platelet	leukocytes	stimulation
	endothelium		
	vasculature		
	myocytes	vascular smooth muscle	contraction
LTB_4	granulocyte	granulocyte	stimulation
		vascular permeability	increase
LTC_4 LTD_4	leukocyte	vascular permeability	increase
		vascular smooth muscle	contraction

1.6. THE ENDOTHELIUM

The endothelium is the inner most lining of the vascular tree and consists of a single uniform layer of flattened, polygonal cells which are approximately 25-30μm long and 10-15μm wide (Simionescu 1977). Endothelial cells are orientated with their long axis in the direction of blood flow. The endothelial surface varies in different vascular beds. In large i.e. conduit arteries, the surface is smooth with longitudinal folds (Simionescu 1977; Hirsch 1977; Clark 1976; Christensen 1972). However, in regions where a high surface area is important, such as in the pulmonary artery, the lumen surface is compared to finger like projections known as microvilli (Smith 1971). This anatomical variation enhances the capacity of the pulmonary endothelium for angiotensin I to angiotensin II conversion.

For many years the vascular endothelium was regarded as a semi-permeable layer permitting the passage of nutrient and waste products to and from the underlying smooth muscle but serving mainly as a barrier to the diffusion of macromolecules. However, it has become clear that the endothelium serves many important functions and that these cells are metabolically versatile. The endothelium provides a non-stick surface preventing blood cell adherence and produces factors involved in homeostasis including heparin sulphate and tissue plasminogen activator. It also synthesises connective tissue components such as laminin, fibrinonectin and a variety of smooth muscle growth factors. The endothelium is an important source of vasoactive substances such as prostacyclin, endothelium derived relaxing factor (EDRF, nitric oxide) and a putative hyperpolarising factor. These contribute to vasodilation and inhibit platelet function. Other endothelial derived factors are vasoconstrictors such as endothelin-1, thromboxane A_2 and prostaglandin H_2. In addition, the endothelium also plays an important

role in the renin angiotensin system. Angiotensin converting enzyme (ACE) is a membrane bound enzyme found in all vascular endothelium and the high concentration in the pulmonary circulation acts as the major site for the conversion of angiotensin I to the potent vasoconstrictor angiotensin II. As well as being responsible for the inactivation of bradykinin endothelial cells play an important role in inactivating other vasoactive substances (e.g. mono aminoxidase) and degrading catecholamines (e.g. serotonin).

1.6.1. Endothelium Derived Relaxing Factor

One of the most powerful substances released by the endothelium is endothelium-derived relaxing factor (EDRF). EDRF was discovered accidentally in 1980 by Furchgott and Zawadzki (Furchgott & Zawadzki 1980). Furchgott reported that his laboratory had for many years used the helical strip of the rabbit thoracic aorta as a standard isolated vascular preparation for studying the pharmacology of vasoactive drugs (Furchgott 1955). However, while being able to relax the strips with a variety of vasodilators they never managed to induce relaxation using acetylcholine or other muscarinic agonists such as carbachol and methacholine. Usually they observed a slight to moderate contraction with these agents when used at a high concentration. However, when they changed their technique to arterial ring preparations a technician accidentally added carbachol to a noradrenaline pre-contracted ring and to his surprise the vessel partially relaxed. They then undertook a series of experiments comparing the responses of pre-contracted helical strips and rings with muscaranic drugs. The strips contracted in response to the addition of acetylcholine and carbachol whereas the rings relaxed with low concentrations of carbachol but higher concentrations evoked a contraction similar to that observed in the aortic strips. Within a short period of time, Furchgott demonstrated that the lack of relaxation response in the helical strip was the result of

unintentional removal of the endothelium and that if great care was taken to avoid damaging the intimal surface a contracted ring or helical strip preparation would relax in response to exposure to carbachol.

Since rubbing the intimal surface but not the adventitial surface was found to eliminate the relaxation response to acetylcholine it was suggested that endothelial cells were required for the relaxation response. By contrast, removal of the endothelium did not interfere with the relaxation response to other common non-muscarinic vasodilators (Furchgott 1981).

Acetylcholine induced endothelium dependent relaxation has been observed in a wide variety of arteries from different mammals (Furchgott 1984; Peach et al. 1985 and Vanhoutte et al. 1986). Endothelium dependent responses have also been obtained in isolated blood vessels from lower vertebrates, including amphibians and bony fish (Miller & Vanhoutte 1986). The presence of endothelium dependent responses in blood vessels which are not innervated by adrenergic nerves suggest that the ability of the endothelium to modulate the response of the underlying vascular smooth muscle is an ancestral phenomenon (Vanhoutte 1987).

Acetylcholine induced endothelium dependent relaxations to acetylcholine have been demonstrated in a wide variety of blood vessels from laboratory animals and more recently in human blood vessels (Aalkjaer et al. 1987; Luscher et al. 1987 and Greenberg et al. 1987). Many other vasodilators, such as bradykinin, histamine, Ca^{2+} ionophore A23187, ATP, ADP and substance P were also found to cause endothelium dependent relaxation. Therefore, the endothelium has the capacity to release endothelium derived relaxing factor in response to constrictor substances and this can be regarded as a protective or modulating function (Quilley & McGiff 1994).

In the search to identify this endothelial derived relaxing factor it has been established that the relaxation of animal and human blood vessels does not depend on the synthesis and release of prostacyclin because the acetylcholine induced endothelium dependent vasodilator responses can not be blocked by treatment with the cyclo-oxygenase inhibitor indomethacin (Furchgott 1987). Other studies have demonstrated that EDRF is a highly labile substance, with an extremely short half life (6-30 seconds), which is readily degraded by superoxide anions and by a number of antioxidant substances (Griffith et al. 1984; Rubanyi et al. 1985; Gryglewski et al. 1986; Rubanyi & Vanhoutte 1986).

Some early studies suggested that EDRF was a lipoxygenase product but in 1987 evidence was presented to show that EDRF was nitric oxide or a similar molecule and that it acted through the same second messenger as organic nitrates i.e. cyclic GMP. Moreover, nitrovasodilators such as glycerol trinitrate and sodium nitroprusside act as nitric oxide donors (Palmer et al. 1987). Palmer and his colleagues demonstrated the release of nitric oxide (NO) from endothelial cells in culture and that this was indistinguishable from EDRF in biological activity, stability and susceptibility to inhibitory agents (Palmer et al. 1987). However, controversy continues about whether EDRF is NO per se or another NO derivative such as S-nitrosocysteine. Myers and colleagues in 1990 suggested that EDRF was a compound that comprised NO within its structure and also that it was a more potent vasodilator than NO. They concluded that EDRF was more likely to be a nitrosylated compound such as a nitrothiol rather than authentic NO. (Myers et al. 1990). Never the less, it is now generally accepted that endothelial cells can synthesize NO from the terminal guanidino nitrogen of L-arginine (Palmer et al. 1988)

The physical characteristics of EDRF were studied by Rubanyi and his colleagues in 1985 (Rubanyi et al. 1985) using a superperfusion system of two femoral arteries where one of the femoral arteries had the endothelium removed. The recipient coronary artery was mounted as a ring preparation and was denuded of endothelium. This system allowed them to show that there was a basal release of EDRF in response to stimulation by acetylcholine. They also suggested that either noradrenaline inactivated the EDRF or caused the release of two or more relaxing substances from the endothelium. They postulated that one is responsible for the initial, rapid and transient relaxation and the other maintained the inhibitory response or in some cases produced a secondary relaxation.

EDRF(NO) is formed from L-arginine by oxidation of the guanidine-nitrogen terminal of L-arginine (Palmer et al. 1988). EDRF(NO) activates soluble guanylyl cyclase in vascular smooth muscle and platelets which causes an increase in cyclic 3'5'-guanosine monophosphate (cGMP) resulting in vascular smooth muscle relaxation and platelet inhibition. EDRF(NO) production can be inhibited by analogues of L-arginine such as L-N^G-monomethylarginine (L-NMMA), L-nitroargininemethylester (L-NAME), and N_w-nitro-L-arginine (L-NOARG) which can be restored by L-arginine but not D-arginine (Bennett et al 1992). In 1989 Rees and colleagues demonstrated that the vasculature in rabbits was in a constant state of vasodilation. They infused L-NMMA, which induced a long-standing rise in blood pressure, which could then be reversed by L-arginine (Rees et al. 1989).

Nitric oxide may appear to be only one of several endothelium derived relaxing factors because studies using canine blood vessels indicate that another vasodilator factor may be released simultaneously with EDRF (NO)

(Vanhoutte 1987). This other factor causes relaxation of vascular smooth muscle by hyperpolarising the smooth muscle cell membrane, by activating the sodium potassium pump and has been referred to as endothelium derived hyperpolarising factor (EDHF) (Feletou & Vanhoutte 1987; Garland & McPherson 1992).

This discovery of an endothelial derived relaxing factor provided a satisfactory explanation for the apparent paradox of the divergent in vivo and in vitro responses to vasoactive agents.

Physiological importance of EDRF

Although the discovery and early evaluation of the role of EDRF in regulating basal tone utilised the agonist acetylcholine it is important to consider that this may not represent a physiological stimulus. On one hand studies of large blood vessels indicate that acetylcholine released from cholinergic nerves (which is the only source for the cholinergic transmitter) is unable to diffuse through the blood vessel wall to the muscarinic receptors on the endothelium and release EDRF (Cohen et al. 1984 and Vanhoutte & Cohen 1984). While on the other hand there is considerable evidence that other factors such as shear stress, catecholamines, vasopressin, and the process associated with platelet aggregation and blood coagulation stimulate EDRF release (Rubanyi et al. 1986). Increase in shear stress with a raised blood flow leads to flow mediated dilation of large arteries and it has been shown that shear stress and acetylcholine induced relaxation share the same G protein. Stimulation of α_2 receptors by noradrenaline and physiological concentrations of AVP also stimulate EDRF release (Katusic et al 1984). Interestingly, incubating isolated small arteries with the nitric oxide synthetase antagonist, L-NAME, enhances the

noradrenaline contractile response by inhibiting basal and stimulated EDRF release.

The media of blood vessels does not normally produce nitric oxide (NO) but they can be induced to do so under certain conditions (Bernhard et al. 1991). It has been demonstrated that at least two enzymes exist for the production of NO, a constitutive endothelial enzyme producing picomoles of NO and the inducible form producing nanomoles of NO.

Recently, several isoforms of NO synthase have been identified (Nathan 1992; Lowenstein et al. 1994). There is the constitutive NO synthase (cNOS) and the inducible NO synthase (iNOS). cNOS has been shown to be calcium dependent, released in small pulsatile quantities and involved in regulation. iNOS has been shown to be calcium independent, continually released in much larger quantities and it is postulated that it plays a role in the host's defense. When NOS cells are given an appropriate stimulus (eg. endothelial cells stimulated with acetylcholine) receptor activation leads to an increase in cytosolic calcium which activates the NOS to give off a short burst of NO. The activators of NOS are substances released from nerves (eg. acetylcholine) and those released from platelets (eg. serotonin). iNOS differs from cNOS in that it can continuously release large amounts of NO. It has been postulated that the role of NO in activated immune cells is to act as a killer molecule (Anggard 1994). Studies of patients with septic shock who have been successfully treated with the nitric oxide synthetase antagonist, L-NMMA (L-arginine analogue) when they have become resistant to catecholamines suggesting that NO may play a crucial role in some physiological conditions (Petros et al. 1991; Wright et al. 1992).

1.6.2. Endothelial Derived Contracting Factors

In 'normal' endothelium the response to vasoactive agents, such as acetylcholine, is vasodilatation but under certain conditions the same vasoactive agents can produce vasoconstriction. It has been suggested that the endothelium releases both relaxing and contracting factors and that when the contracting factor(s) predominate when there is endothelial cell dysfunction. Several studies have been undertaken to identify these contracting factor(s).

One endothelial derived contracting factor is triggered by anoxia and the metabolism of arachidonic acid does not play a role in its genesis (De Mey & Vanhoutte 1983). Another contracting factor appears to be a prostanoid, possibly thromboxane A_2 or PGH_2. This substance has been demonstrated in isolated veins (De Mey & Vanhoutte 1982; Miller & Vanhoutte 1985) and in cerebral arteries in the dog (Shirahase et al. 1987). This contracting factor appears to be produced when the isolated vessels are exposed to exogenous arachidonic acid (Miller & Vanhoutte 1985) or by acute stretching (Katusic et al. 1987). Acute stretching of the endothelium results in production of an endothelial derived contracting factor which acts on the underlying smooth muscle and the contractile response may contribute to the phenomenon of autoregulation.

When the endothelium releases excess amounts of a contracting factor this can override the effects of EDRF causing vasoconstriction. Therefore, pathophysiological conditions which results in the over production of contracting factor(s) will increase vascular tone and contribute to a rise in peripheral resistance.

Endothelin

Recently, a Japanese group isolated and characterised a new peptide which is an endothelial-derived contracting factor (Yanagisawa et al. 1988). This peptide proved to be a potent vasoconstrictor and is the most potent vasoconstrictor identified to date. They named the peptide endothelin and its true physiological role has yet to be established. Circulating levels of endothelin-1 are very low suggesting that in health the peptide is produced in small quantities (Luscher et al 1992, Wagner et al. 1992, Stewart et al. 1990). So far scientists have found three ways in which to inhibit the production of endothelin. These involve cGMP and cAMP dependent pathways and the ability of the vascular smooth muscle to produce an inhibitory factor. Endothelin has also been shown to release prostacyclin from endothelial cells which may suggest a role in a positive feedback mechanism (Warner et al. 1989).

Two distinct endothelin receptors have been identified, ET_A and ET_B (Arai et al. 1990, Vane 1990). Endothelial cells have ET_B receptors linking them to the formation of EDRF(NO) and prostacyclin while in vascular smooth muscle ET_A receptors predominate and appear to mediate contraction and proliferation. Receptor agonists to endothelin have recently been developed (Bazil et al. 1992). These antagonists have been shown to decrease blood pressure suggesting that endothelin may contribute to blood pressure autoregulation (Nishikibe et al. 1992, Luscher et al. 1993).

1.7. HYPERTENSION

Hypertension in man can be defined as an increased blood pressure which exceeds an arbitrary figure. Blood pressure distribution in man is

represented by a unimodal bell shaped curve which is skewed to the right. A major problem concerns how to decide who should be classified as hypertensive and who should not. For practical reasons a cut off point for systolic and diastolic pressure has been adopted but it must be recognised that this is arbitrary. The most widely used criteria is that set by the World Health Organisation (WHO) currently sets the upper systolic limit as 160mmHg with the diastolic limit at 95mmHg (Korotkoff phase V) but many countries accept that a diastolic of 90mmHg is a more reasonable upper limit. Twenty five percent of the population have a diastolic pressure exceeding 90mmHg with 14.5% having a diastolic pressure exceeding 95mmHg. There is a grey area around this cut off point where treatment may not prove as beneficial in a minority of cases.

The recognised associated risks of hypertension are cerebrovascular, coronary, peripheral vascular and renal disease. The Framingham study (1970) showed that the risks of cerebrovascular and coronary disease were strongly correlated with hypertension. A blood pressure of 160/95mmHg resulted in a 5.3 fold increase in the incidence of stroke dependent on age and sex whereas there was only a weak correlation between blood pressure and ischaemic heart disease. Overall the results showed that hypertensives were 7 times more likely to have a stroke, 4 times more likely to have congestive heart failure, 3 times more likely to have coronary heart disease and twice as likely to suffer from peripheral arterial disease. Controlling the blood pressure led to a reduction in the risk of renal failure. Other factors, which can be broadly classified as genetic and environmental influences, contribute as risk factors associated with hypertension. These include smoking, obesity, physical inactivity, pregnancy, oral contraception in women, race high dietary salt intake, familial history, stress and increasing age.

1.7.1. Total Peripheral Resistance and Vascular Changes in Hypertension

Hypertension, which is probably the most common risk factor for cardiovascular disease, is recognised as a major health problem. Hypertension is an asymptomatic disorder prior to the onset of cardiovascular complications. The mean arterial pressure is determined by the cardiac output and the resistance to blood flow in the systemic circulation. This is known as total peripheral resistance. It is generally agreed that in human essential and experimental animal models of hypertension that the increased blood pressure depends on an elevated total peripheral resistance in the face of normal cardiac output. Therefore, it could be said that hypertension is a disease of regulation.

All blood vessels offer resistance to blood flow in the circulatory system. In hypertension an impairment of the function of one or more of the controlling systems that normally regulate vascular resistance may contribute to the raised peripheral vascular resistance. These systems include 1) the autonomic nervous system, which expresses the activity of reflex neurogenic arc and of the central nervous system; 2) the kidney, which functions in salt and water metabolism and in the production of regulatory hormones with either pressor or depressor actions; 3) the adrenal cortex, which produces steroid hormones that help regulate sodium stores in the body and 4) the blood vessel wall, which regulates its activity through the production of local hormones such as prostaglandins and kinins.

In hypertension, alteration in these neurogenic, humoral and local factors affect the small blood vessels in such a way that total peripheral vascular resistance is increased. In the early stages of essential hypertension

sympathetic nervous system activity is increased, cardiac output is increased and the total peripheral resistance remains normal. However, in the established phase of hypertension total peripheral resistance is raised with cardiac output remaining at normal levels.

Pickering said that hypertension is a disease of the blood vessels. However, it is difficult to determine the primary event that initiates the overall process leading to an elevated arterial pressure. This is particularly true of the most frequent types of the disease; clinical essential hypertension and experimental, spontaneous hypertension in the rat. Even when the initiating factor is known, such as renal ischaemia or mineralocorticoid excess, the sequence of changes leading from the primary event to increased total peripheral resistance remains unknown.

It is apparent that despite the level of cardiac output, hypertension would not occur if the mechanisms controlling blood vessel resistance were not abnormal. The characterisation of structural and functional changes in the vasculature may provide an aspect of understanding the hypertensive process.

Morphological studies have demonstrated that the vasculature undergoes dramatic modifications when exposed to elevated arterial pressure. Early on, morphological changes were recognised but, while blood pressure could be measured, the relationship between the two was not recognised (Rojo-Ortega & Hatt 1977). It is now recognised that many of the morphological changes characterise the malignant phase of hypertension but it is unlikely that they are contributing to the elevated vascular resistance during the developmental phase of the disease. In malignant or established forms of essential hypertension, large renal arteries show

atherosclerotic changes consisting of intimal thickening with splitting of the internal elastic lamina (Hepinstall 1974). However, these larger arteries do not show histological changes in the early stages. Similarly, focal lesions in small arteries and arterioles are probably the consequence of elevated blood pressure rather than a vascular change that contributes to increased peripheral vascular resistance.

Two types of structural change that they alter the background on which vasoconstrictor influences operate occurs in hypertension and can have haemodynamic consequences. 1) increased vascular wall thickness and 2) rarefaction of resistance vessels. The importance of these structural changes to elevated blood pressure is demonstrated by the observation that vascular beds in hypertensive animals show higher flow resistance under conditions of maximal vasodilation induced either pharmacologically or by reactive hyperaemia (Borecek & Bohr 1977; Conway 1963; Sivertsson 1970; Weiss 1974; Angus et al. 1976; Folkow et al. 1977; Harthing et al. 1978; Amery et al. 1969). In studies where active wall tension generated by vascular smooth muscle is presumed to be removed, the difference in resistance between hypertensive and normotensive animals depends upon structural changes in vascular beds.

Following on from Short's early studies (Short 1966), Folkow and associates have provided clinical and experimental evidence to suggest that increased vascular wall thickness may account for the increased peripheral vascular resistance and enhanced vascular reactivity seen in hypertension (Folkow 1982; Folkow et al. 1977; Folkow 1978; Lundgren et al. 1974; Folkow et al 1972; Folkow et al. 1958; Folkow et al. 1970). They have hypothesized that media hypertrophy of resistance vessels increases the bulk of wall tissue so that it encroaches on the lumen even when the blood

vessel is relaxed. This increase in wall to lumen ratio also results in a steeper resistance curve when the smooth muscle contracts in response to vasoactive stimuli. They observed that resting vascular tone was normal in the forearm and hand vessels in hypertensive patients but that vascular resistance was increased at maximum vasodilation (Folkow et al. 1972; Folkow et al. 1958). Sensitivity to noradrenaline was not altered in hypertensive patients but the change in vascular resistance produced by increasing doses resulted in a steeper resistance curve than that in normotensive subjects. They postulated that a change in the media to lumen ratio of the blood vessels would account for this increased vascular resistance.

A change in media to lumen ratio also characterises the vasculature of the spontaneously hypertensive rat (SHR) (Folkow et al. 1977; Folkow 1978; Folkow et al. 1970). Dose response curves for vasoconstrictor agents were studied in isolated hindquarters of SHR and normotensive control rats, perfused at a constant flow. The findings showed that 1) vascular resistance was higher in the SHR under conditions of maximal vasodilation 2) sensitivity to vasoconstrictor agents were similar in SHR and controls but 3) dose response curves of vascular resistance to noradrenaline were steeper and the maximum response attained was greater in SHR than in control rats.

Folkow and associates showed that these observations could mimic a mathematical model in which an increase throughout the media of 30% would produce the same results. Folkow put forward the hypothesis that the structural change in the vasculature could be sufficient to maintain the elevated vascular resistance in hypertension even in the presence of normal vascular smooth muscle tone.

Structural changes of the vasculature leading to an increased media thickness of resistance vessels in hypertension could include: 1) smooth muscle cell hypertrophy 2) smooth muscle cell hyperplasia and/or 3) increased amount of paracellular matrix.

Smooth muscle cell hypertrophy

Smooth muscle cell hypetrophy has been suggested by many scientists as the cause of morphological changes in hypertension. Mallov observed that the aortae in DOCA-hypertensive rats and Goldblatt two kidney one clip rats contained greater quantities of smooth muscle per unit of wet tissue compared to those of normotensive rats (Mallov 1965). Wolinsky and associates reported an increased media thickness with no change in cellular DNA content in aortae from Goldblatt two kidney one clip rats (Wolinsky et al. 1974). Also, smooth muscle cell hypertrophy has been reported in blood vessels from SHR (Guanberg et al. 1981).

Smooth muscle cell hyperplasia

In several experimental models of hypertension the uptake of tritiated thymidine by vascular tissue in hypertensive animals is greater than that in control animals suggesting cellular proliferation. The models studied include DOCA rats (Crane & Ingle 1964), Goldblatt one kidney one clip renal hypertensive rats (Rorive et al. 1980) and rats and rabbits made hypertensive by coarctation (Bevan 1976; Bevan et al. 1980).

Mulvany and associates measured the morphological properties of resistance vessels in mesenteric vascular beds of the SHR and normotensive rats. They observed that relaxed blood vessels in SHR had a 16% smaller lumen diameter and a 49% thicker media at a given transmural pressure than did those of normotensive rats (Mulvany et al. 1978). Using a three

dimensional dissector technique they went on to demonstrate that medial hypertrophy of SHR resistance vessels was due to smooth muscle cell hyperplasia. These findings have been confirmed by morphological, biochemical and immunological studies of isolated smooth muscle cells (Owens et al. 1988). In addition, scanning electron microscopy has revealed that cell size is normal in the SHR (Miller et al. 1987)

Increased amount of paracellular matrix

There is a considerable volume of evidence to indicate an increase in the paracellular matrix in blood vessels from hypertensive patients and animals (Rojo-Ortega & Hall 1977). Tobian and associates have presented extensive evidence supporting the concept that extra water, which accumulates in blood vessels in hypertensive animals, increases vascular wall stiffness (Tobian et al. 1969; Tobian & White 1956, Tobian & Binion 1952; Tobian et al. 1961). This greater wall stiffness reduces the average calibre of the arterioles and thus increases resistance to blood flow.

Rarefaction of resistance vessels

A reduction in the number of resistance vessels also could contribute to increased vascular resistance in hypertension. Hutchins and Darnell (1974) observed a 50% reduction in the number of resistance vessels (12-25μm) in cremaster muscle in the SHR compared with normotensive rats. Also, arteriolar rarefaction has been reported in the mesenteric (Henrich et al. 1978), cutaneous (Haack et al. 1979) and skeletal muscular vasculatures (Henrich & Hertel 1979) in SHR and in the conjunctiva in patients with essential hypertension (Harper et al. 1979).

1.7.2. Importance of Transmural Pressure

Regardless of the 'type' of structural alteration that occurs in the vasculature in hypertensive animals the change in morphology is not a primary but an adaptive change in response to the elevation in arterial pressure (Folkow 1978). Indirect evidence by Lundgren and associates, during perfusion studies, suggested that increased media thickness occurred in the hindlimb vasculature after development of a sustained increase in arterial blood pressure in Goldblatt two kidney one clip rats. Reversibility of the structural alteration required 2-3 weeks following removal of the clip from the renal artery, whereas arterial blood pressure returned to normal 1 day after the clip was removed (Lundgren et al. 1974). Moreover, Folkow et al. (1971) observed that aortic obstruction lowered the blood pressure in hind quarter vascular beds in the spontaneously hypertensive rat. This haemodynamic change (reduction in arterial pressure) was immediate, whereas reversibility of structural changes required 2-3 weeks. Thus, it appears an increase in transmural pressure is the dominant stimulus for structural changes in the vasculature in hypertensive animals.

1.7.3. Cardiac Effects of Hypertension

Systemic arterial hypertension leads to an increase in cardiac work and left ventricular hypertrophy. Left ventricular hypertrophy is associated with a reduction in left ventricular compliance which leads to a reduction the stroke volume in hypertensives.

1.7.4. Additional Regulatory Factors

Guyton and associates have critically analysed overall circulatory control and evaluated the relative roles of the baroreceptors, the chemoreceptors, the central and autonomic nervous systems, the renin-angiotensin-

aldosterone system, capillary fluid shifts and renal excretory function (Guyton 1980).

Nervous control mechanisms are ideally suited to short-term circulatory control because they respond very quickly and can profoundly affect vascular function. They are directed predominantly toward maintenance of systemic blood pressure rather than cardiac output. For example, blood pressure will tend to remain relatively constant in the face of induced changes in cardiac output or during infusion of fluids as a consequence of reflexly mediated changes in vascular resistance. After baroreceptor denervation or destruction of the central nervous system, these interventions result in wide variations in pressure. Thus, the primary function of nervous mechanisms probably is to mediate the rapid circulatory adjustments to changing situations rather than the long-term setting of blood pressure levels.

However, the renin-angiotensin-aldesterone system participates in short- as well as longer-term blood pressure control by influencing renal sodium reabsorption and by direct and possibly centrally mediated effects on blood vessels. According to Guyton and associates, the kidney is the primary regulator of blood pressure in the long term. They demonstrated that a direct relationship exists between systemic blood pressure and sodium and urine output. Thus, any variation in pressure initiates a compensatory change in urinary volume and in turn plasma volume which will tend to return pressure to normal levels. However, these adaptations imply adequate renal function and disease states that compromise renal function may severely strain this homoeostatic process.

There are numerous other examples of cardiac and vascular adaptation to a changing internal environment and to disease. Cardiac dilatation and hypertrophy, in response to chronically increased volume and pressure, and in the blood vessels hypertrophy response, in the presence of persistently elevated pressure, are some of these adaptations (Folkow 1982).

1.7.5. Importance of Neural and Humoral Influences

Although most studies suggest that structural changes are secondary to the increase in blood pressure a number of publications have indicated that other factors may contribute to morphological alteration of the vasculature in hypertensive animals. It has been observed that the increased wall thickness in cerebral arteries in stroke prone spontaneously hypertensive rat requires an intact sympathetic nerve supply for full development (Hart et al. 1980). Similarly, Bell and Overbeck suggested that neural or humoral influences may play an important role in determining structural changes in the vasculature in aortic coarctation hypertensive rats (Bell and Overbeck et al. 1979). They observed an elevated resistance in vascular beds below the aortic obstruction which were not exposed to elevated blood pressure levels. They suggested that this depended on neural activity because nerve section dramatically reduced hindlimb resistance in these rats.

1.7.6. Functional Changes in the Vasculature

Since structural change which could increase total peripheral resistance in hypertension is primarily an adaptive phenomenon other mechanisms must be considered. Functional changes in the blood vessel will contribute to the altered state of the vasculature when 1) there is an increased sensitivity to vasoactive stimuli in isolated vascular preparations from hypertensive animals 2) the magnitude of the change in sensitivity are qualitatively and

quantitatively different for each agonist 3) functional vascular changes often precede or accompany increased blood pressure.

Studies have attempted to characterise mechanisms for functional vascular changes but have been complicated by the marked individualities of these changes observed under different conditions. Several factors contribute to the variations in experimental results 1) the primary cause of hypertension 2) the time course of hypertension 3) animal species 4) age and sex of animal 5) the technique used to evaluate a functional change and 6) the anatomical location of the vascular bed.

Increased sensitivity to vasoconstrictor stimuli

Many studies have shown that isolated vascular preparations from hypertensive animals show increased sensitivity to vasoactive stimuli (Webb et al. 1981; Mulvany et al. 1980; Webb & Vanhoutte 1982; Collis & Vanhoutte 1978).

This functional change is usually measured as a reduction in the ED_{50} i.e. the dose required to elicit a half maximal response. The increased vascular sensitivity to some vasoactive stimuli may be masked in the hypertensive animal by compensatory mechanisms. For example, increased activity of the neuronal uptake mechanism may partially mask the increased sensitivity to noradrenaline in vascular preparations isolated from spontaneously hypertensive rats (Collis & Vanhoutte 1977). The observations of many investigators indicate that the sensitivity of vascular smooth muscle in hypertension is variable. The changes vary in different strains of hypertensive rats and vascular beds. Therefore, it is certain that not all vascular smooth muscle in all types of hypertension is abnormal in its sensitivity.

Altered maximum response to vasoconstrictor stimuli

McGregor and Smirk observed that isolated perfused mesenteric vascular beds of SHR and renal hypertensive rats constricted to a greater degree than did those from normotensive rats when using noradrenaline or serotonin (McGregor & Smirk 1970). From these observations they suggested that altered pressor responsiveness could not be due to a structural change and that a differential augmentation of responsiveness by the two agonists demonstrates a functional alteration in smooth muscle of resistance vessels in hypertensive animals. Collis and Vanhoutte observed that in the renal vasculature of the spontaneously hypertensive rats, responses to serotonin were potentiated to a greater degree than responses to noradrenaline, angiotensin II or barium (Collis & Vanhoutte 1977).

It has also been shown that increased vascular sensitivity to noradrenaline in hypertension is greater than that to potassium (Holloway & Bohr 1973). Comparison of vascular response to noradrenaline and to calcium has yielded differential sensitivities in hypertensive preparations. The response to calcium of potassium-depolarised vascular smooth muscle is not different, whereas smooth muscle sensitivity to calcium is greater in hypertensive animals when noradrenaline is used a the activating agent.

Diminished vasodilation

Aortic-strip preparations from the spontaneous hypertensive and renal hypertensive rats do not relax to a variety of vasodilators when compared to normotensive controls (Table 3). These observations have been supported by several investigators but many studies have shown no difference in the ability of the vascular preparations from the hypertensive animals to relax. In fact some vascular preparations from hypertensive

animals are more sensitive to isoproterenol and nitroprusside than their normotensive controls. A review of these findings is shown in Table 3.

Table 3 Review of vasodilator responses in animal hypertension models.

Reference	Preparation	Agonist	Results (compared to control)
Shibata & Cheng	SHR aortic strip	isoproterenol, Ach, Mg^{2+}, MN^{2+}	decrease % relaxation
	renal hypertensive aortic strip	isoproterenol	decrease % relaxation
Cohen & Berkowitz	SHR + renal hypertensive aortic strip	cAMP, cGMP, isoproterenol, nitroglycerin, adenosine	decrease % relaxation
Antonaccio et al	SHR aortic strip	nitroglycerin	decrease % relaxation
Triner et al	SHR aortic strip	isoproterenol	decrease % relaxation
Hutchins et al	SHR cremaster microvessels	isoproterenol	decrease max % diameter increase
Bell at al	Blood perfused hindlimb (aortic coarctation)	nitroprusside	impaired max vasodilation
Shibata & Cheng	SHR aortic strip	nitroglycerin, papaverine isoproterenol, Ach	No difference in % relaxation
	renal hypertensive aortic strip	Mg^{2+}, Mn^{2+}, nitroglycerin papaverine, isoproterenol	No difference in % relaxation
Folkow et al	Forearm resistance vessels (human essential hypertension)	vasodilator metabolites	no difference in ability to relax
Brody et al	Blood perfused hindlimb (1K1C dog)	histamine, nitroglycerin	no difference in vasodilation
Cohen et al	Blood perfused renal vasculature (DOCA hypertensive rat)	nitroprusside	increased sensitivity

1.7.7. Blood Pressure Maintenance

Hypertension is associated with structural changes in the vasculature with an increased wall to lumen ratio. These changes may act as a vascular amplifier and exposure to vasoconstrictor agents leads to an increase in resistance and an alteration in the slope of dose response curves. Therefore, the structure of the blood vessel can maintain vascular tone in the presence of normal humoral activity. Changes in blood vessel structure are slow to be reversed. An MRC trial showed a slow rise in blood pressure after withdrawal of long term treatment with bendrofluazide or propanolol. A major factor which is thought to increase the blood vessel wall to lumen ratio is exposure to high blood pressure. However, in the spontaneously hypertensive rat the animals may have inherited a predisposition to vascular hypertrophy. Genetic predisposition could therefore elevate blood pressure via structural changes in two ways: 1) Increase in transient pressor responses to stress as a result of sympathetic over activity could cause hypertrophy through exposure of the resistance vasculature to increased pressure and 2) The vessels could be genetically susceptible to pressure load.

Rarely is it possible to point to a single cause of hypertension. Therefore, essential hypertension is multifactorial. There are however some similar discrepancies in hypertensive subjects when they are compared to normotensive controls.

- modest over activity of sympathetic nervous system, possibly causal.
- over active vascular second messenger systems
- structural changes maintain blood pressure increases at later stages. result of pressure surges caused by autonomic overacting?

- resetting of baroreceptors and alterations in baroreceptors are secondary to blood pressure increases
- genetic predisposition, neurogenic drive and perhaps some humoral systems are all important in predisposing these structural changes in certain individuals
- genetic and environmental influences interact with these processes at several levels to create a sustained blood pressure rise.

1.8 AIMS OF THE EXPERIMENTAL STUDIES

The following studies described in this thesis are an attempt to evaluate the alteration in vascular structure and function in hypertension.

The preliminary experiments were designed to investigate at what age structural and functional abnormalities become apparent in the spontaneously hypertensive rat. This led to a further series of experiments to evaluate the role thromboxane A_2 may play in the endothelial abnormality found in the SHR.

The second part of this thesis was designed to study the development of structural and functional changes during acute and chronic phases of renovascular hypertension. This led to a second series of experiments to investigate the changes before and after reversing renovascular hypertension.

The final part of this thesis was designed to investigate the effects of endothelin-1 in isolated human resistance vessels.

CHAPTER 2 MATERIALS and METHODS

2.1. ANIMAL MODELS OF HYPERTENSION

2.1.1. Spontaneously Hypertensive Rat

The spontaneously hypertensive rat (SHR) was derived, by selective inbreeding, from outbred wistar rats, taking offspring with high blood pressures and inbreeding for 20 generations. Approximately ten years later a similar breeding program selecting animals with low blood pressures lead to the production of the Wistar Kyoto (WKY) normotensive strain (Okamoto & Aoki 1963).

The spontaneously hypertensive rat is widely used as an animal model of essential hypertension using the Wistar-Kyoto (WKY) as the normotensive control. Although the blood pressure of the SHR is only slightly greater than the WKY at birth (Gray 1984; Morton et al. 1990), hypertension develops as the animal matures peaking between 12 to 18 weeks of age.

The female SHR and WKY rats used in these studies were obtained from the Biomedical Services Unit inbred colony at the University of Leicester. All animals had free access to food and water and were maintained on a 12 hour light/dark cycle. An SHR was considered to be hypertensive if the anaesthetised systolic blood pressure was 140mmHg or above at 11 weeks of age and a WKY rat was considered to be normotensive if the systolic blood pressure was 130mmHg or less.

2.1.2. Goldblatt Two Kidney One Clip Hypertension

Nine week old white female wistar rats, weighing 170-190g, were used throughout. Goldblatt two kidney one clip hypertension was induced by

placing a constricting silver clip around the left renal artery under ether anaesthesia, the contralateral kidney being left undisturbed (Byrom 1969). A loin incision was made in the abdomen, the left kidney exposed and lifted clear of the body wall. The kidney, renal artery and vein were carefully cleaned of fat and connective tissue with cotton buds. The artery was separated from the vein using a small pair of forceps and a silver clip with an internal diameter of 0.2mm was placed round the main renal artery. The clips were made from annealed silver ribbon which was cut into 15mm lengths and bent around a steel feeler gauge to obtain a precise internal diameter. In order to facilitate the clipping process, clips were arranged to have one short and one long arm. After clipping the kidney was replaced taking care not to occlude the artery. The fat was replaced, the wound sutured in layers and the animal allowed to recover. The contralateral kidney remained untouched. A control group of rats underwent a sham operation where the surgical procedure involved placing a non-constricting strip of silver was placed between the renal artery and vein.

The rats were maintained on standard laboratory rat chow with water ad libitum. Indirect anaesthetised systolic blood pressure was measured at intervals after surgery. When a clipped animal attained a systolic blood pressure greater than or equal to 150mmHg it was considered to be hypertensive. Sham animals were used as controls and their systolic blood pressure did not exceed 130mmHg.

2.2. INDIRECT MEASUREMENT OF SYSTOLIC BLOOD PRESSURE

The indirect systolic blood pressure was measured using the light plethysmographic technique described by Swales and Tange (1970). This

method uses a photoelectric sensor to detect changes in light translucency in the tail of the rat. The signal from the photosensor was relayed to an amplifier containing a frequency filter and displayed using an oscilloscope. The animals were maintained under light ether anaesthesia on a warming pad. An inflatable cuff, connected to a mercury sphygmomanometer, was placed around the tail with the photoelectric sensor immediately behind the cuff. The sphygmomanometer cuff was inflated to occlude blood flow and then deflated slowly until a pulse wave reappeared on the oscilloscope. The mercury sphygmomanometer reading at this point was taken to be the systolic blood pressure. This indirect measurement of blood pressure correlates well with the conscious direct mean arterial pressure (Swales & Tange 1970) and is a reliable method for differentiating between hypertensive and non-hypertensive rats.

All animals were weighed using a digital balance at the time of blood pressure measurement and on the day of study.

2.3. ASSESSMENT OF CARDIAC HYPERTROPHY

All animals were killed by stunning followed by cervical dislocation. The heart was removed after sacrifice, cleaned of fat and blood clots removed before being blotted dry and weighed using a four figure balance. The heart to body weight ratio (%) was calculated (heart weight (g)/ body weight (g) x 100) to assess the degree of cardiac hypertrophy.

2.4. PREPARATION OF TISSUES

All animals were killed by stunning followed by cervical dislocation. The mesenteric bed was dissected free and placed in cold (4^{o}C) physiological salt solution (Appendix A). Arterial resistance vessels (approximately 3mm in length) were taken from the mesenteric bed which supplies the jejunum at a point 8-10cm from the pylorus, cleaned of connective tissue and fat for study in a myograph. The third generation branch of the mesenteric artery was used in all except the 3 week old SHR and WKY animals from which the second generation branch was taken.

2.5. TECHNIQUES FOR STUDYING RESISTANCE ARTERIES

Various approaches have been developed to study resistance artery structure and function. These have involved in vivo studies of exteriorised vascular beds in anaesthetised animals (Duhling 1972; Gray 1973) and by observing vessels through intracranial windows (Johansson et al. 1982; Wahl et al. 1974; McCulloch et al. 1982).

In vitro studies of isolated blood vessels remain the most common technique in use but for a long time only relatively large conduit arteries could be studied. However, the development of the small artery myograph has permitted studies of resistance size (200-300μm internal diameter) arteries. The myograph was developed by Bevan and Osher in 1972 and later modified by Mulvany and Halpern in 1977 to produce the instrument now used in vascular laboratories throughout the world. It is possible to study the vessels in the myograph under normalised conditions, that is to say at a diameter defined for each individual vessel that corresponds to a

given transmural pressure. This has been established as 90% of the diameter the vessel would have been in situ under a transmural pressure of 100mmHg (Mulvany & Halpern 1977). This 'normalised' setting for the internal diameter is such that the vessel gives a maximum isometric response.

The Mulvany / Halpern myograph consists of an 12ml organ bath in which there are two pairs of stainless steel mounting heads. One mounting head from each pair is attached to a micrometer screw gauge that permits movement longitudinally while the other mounting head is connected to a force transducer. The signal from the transducer is amplified and recorded by a two channel flat bed recorder. Vessels are attached between the two mounting heads by means of fine stainless steel wires secured at each end to screws in the mounting heads (Figure 2).

Other small vessel organ bath techniques have been developed where only one end of each of the mounting wires is secured. The mounting of the vessels is technically easier but the disadvantage is that the wires need to be much thicker (usually >100μm in diameter) compared to the Mulvany/Halpern myograph which uses wires of 30 or 40μm in diameter. This increase in wire size, to prevent bending, restricts studies to larger more proximal small arteries (Hogestatt et al. 1983; Nielsen-Kudsk et al. 1986).

The main disadvantage in the myograph methodology is that when the vessel is stretched to a 'normalised' diameter it is no longer cylindrical in shape and the transmural pressure is not evenly distributed throughout the internal circumference but concentrated between the two wires. The

myograph also precludes the investigation of the responses to raised intraluminal pressure and the intraluminal application of vasoactive agents.

2.5.1. Mulvany / Halpern Myograph

Resistance arteries, (i.e. <300μm), two per myograph, were mounted on two 40μm stainless steel wires in a myograph. As previously described, one wire was attached to a strain gauge force transducer and the other was connected to a micrometer to accurately measure the distance between the two wires (Figure 2). The artery was initially threaded onto one wire which was attached to one jaw at the top end with a screw. The artery was then carefully pulled along the wire until the bottom end of the vessel was clear of the bottom of the jaw. The wire was then pulled taught and anchored under the bottom screw. An incision was made in the top part of the artery, at the location of the top of the jaw. This made a hole in the artery so as the second wire could be passed down and also it effectively cut away the portion of artery that had been handled with forceps and therefore damaged. The second wire was passed down the vessel, using the first wire as a guide, being careful not to cause damage to the endothelium or smooth muscle. The second wire was then secured to the jaw at the top and bottom with screws ensuring that there was no slack in the wire. The jaws were positioned so that when the two mounting heads were moved together the two wires were parallel and touched, thus giving a micrometer reading for the minimum diameter, i.e. two times the internal diameter of the wires. Additionally, the artery was trimmed at the top end if it protruded past the jaw to prevent problems during the normalisation procedure. The exact length of each vessel exposed between the jaws was measured using a filar micrometer eyepiece.

The arteries were mounted in normal physiological salt solution, heated to 37°C and gassed with 5%CO_2/95%O_2 to achieve a pH of 7.4 (Mulvany and Halpern 1977).

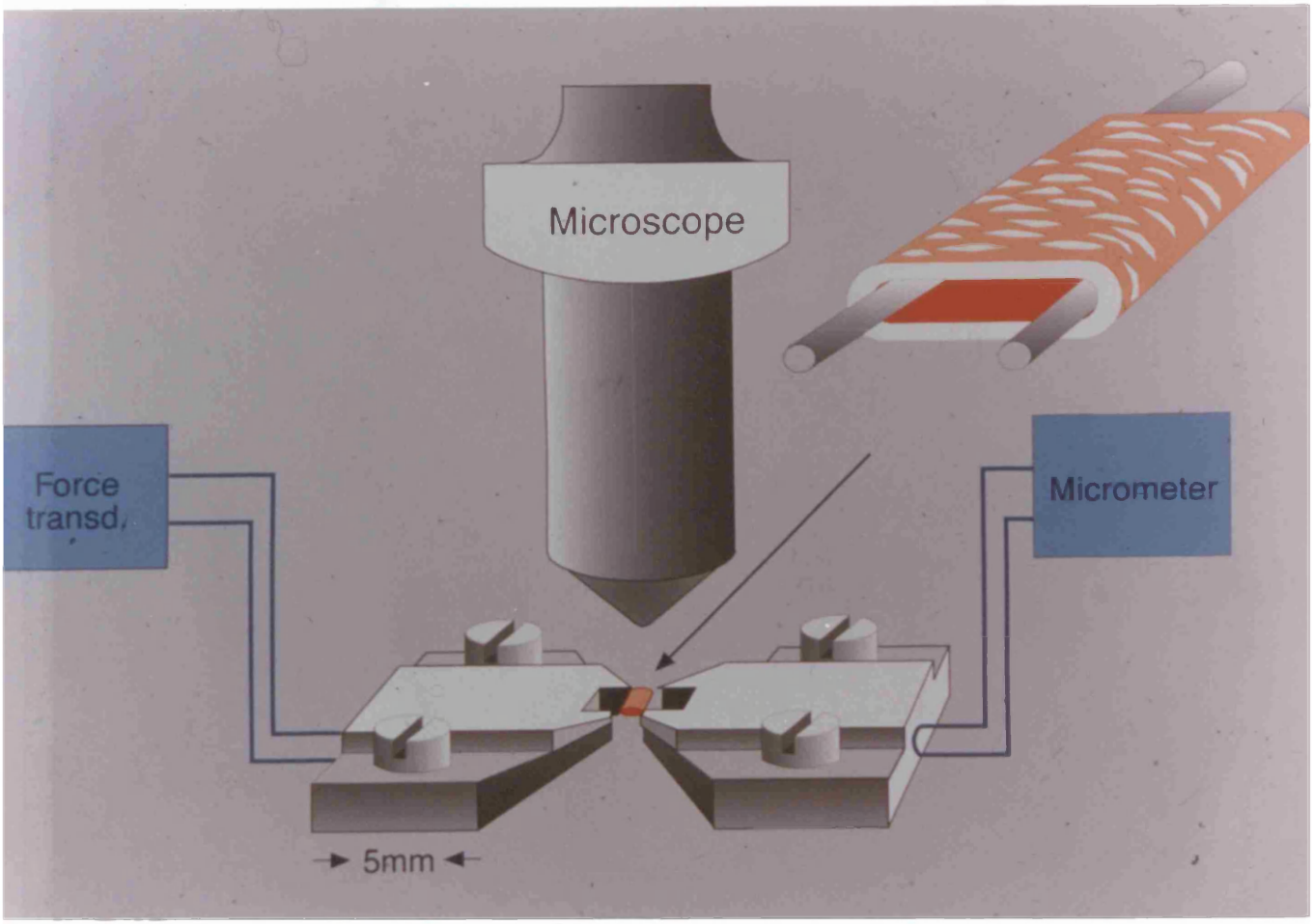

Figure 2. Diagrammatic representation of a vessel on two wires in a myograph.

2.5.2. Vessel Morphology

The Mulvany / Halpern myograph is equipped with a window in the floor of the bath to permit morphological measurements by light microscopy. Arteries were allowed to equilibrate for 60 minutes before morphological measurements were made. The mounting wires were moved apart until the transducer registered a minimal force (0.2mN). The myograph was then placed on an Olympus microscope with a x25 Leitz objective immersion lens and a Zeiss x8 filar micrometer eyepiece (total magnification x200). The filar micrometer eyepiece reading was calibrated using a graticule placed on the microscope stage. Measurements, were then taken over the vessel from the outside of the media, inside of the media, outer and inner edge of the wire, outer and inner edge of the second wire, inside of the media and finally the outside of the media. This process was repeated at three locations along the length of the vessel. These readings were used to obtain the media thickness (m), distance between the wires (f), wire thickness (d) and the segment length (a) using the micrometer calibration factor (μm/unit).

The vessel dimensions were then calculated as follows:

Internal circumference (IC) = $(2 + \pi)\, d + 2f$
(μm)

Internal diameter (l_1) = IC / π
(μm)

Media cross-sectional area = $\pi\, (m^2 + m\, l_1)$
(μm^2)

Wall cross-sectional area = $\pi\, ((m+i)^2 + (m + i)l_1)$
(μm^2)

where *i* is the intimal thickness

The vessels were then normalised as described below and the wall dimensions of the normalised vessel re-calculated for this internal diameter.

The calculation assumes that the wall cross-sectional area remains constant and that the length of the vessel does not change following normalisation.

Using this system the effective internal diameter (L), wall (W) and media thickness (M) can be obtained. The segment volume (VS) and the media volume (MV) can then be calculated as follows:

$$VS = A (\pi W^2 + LW)$$

$$MV = A (\pi M^2 + LM)$$

2.5.3. Determination of Normalised Lumen Diameter

In the past many pharmacological studies used isolated rings or strips of artery that were set up with a fixed tension, irrespective of the vessel size. This resulted in considerable variability because the smaller arteries were stretched to a greater intra-mural tension than the larger arteries. The active force generated by a blood vessel increases as the internal circumference is increased until you reach the optimum circumference for that blood vessel after which if you continue to increase the internal circumference the active force generated by the vessel starts to decrease. At this point there is an increase in the passive tension generated by the vessel (Figure 3).

Several methods have been employed to ensure blood vessels are studied under standard conditions which will minimise the variability of the in vivo behaviour of the vessel. One approach has been to perform an active tension / internal diameter (or circumference relationship) and then stretch the vessel to the internal diameter at which optimum tension is developed. The most commonly used method, for standardising studies of resistance size arteries, is to stretch the vessel, measure wall tension and determine the

effective pressure from Laplace's equation. This relates wall tension (T) to effective transmural pressure and radius (r) of the hollow vessel.

$$\text{Transmural Pressure (kPa)} = T / r$$

The effective transmural pressure can be calculated from any given internal diameter or circumference and wall tension. This calculation can be used to plot a length tension curve of an individual blood vessel and obtains the circumference or internal diameter corresponding to an effective internal pressure of 100mmHg (13.3kPa). Normalisation is performed by progressively stretching a blood vessel, by moving the mounting heads in the myograph apart, by use of the micrometer. The passive tension developed with each stretch is recorded and the internal circumference calculated from the micrometer reading. In this way, vessels can be passively stretched and changes in tension and circumference used to calculate the effective pressure. The procedure is continued until an effective pressure equal or greater than 13.3kPa (100mmHg) is achieved. A computer program fits an exponential curve to the data to obtain L_{100} (the internal diameter corresponding to a transmural pressure of 100mmHg) and the vessel set to 90% of the internal diameter that the artery would have had in-vivo, under a transmural pressure of 100mmHg (13.3 kPa) (Figure 4). Experience has shown that this setting for the wall tension will produce a maximal active force when pharmacological agents are applied (Mulvany & Halpern 1977).

Following normalisation the arteries were allowed to equilibrate for a further 60 minutes with the physiological salt solution being changed at 20 minute intervals. At the end of this period the vessels were stimulated three times with a high potassium physiological salt solution.

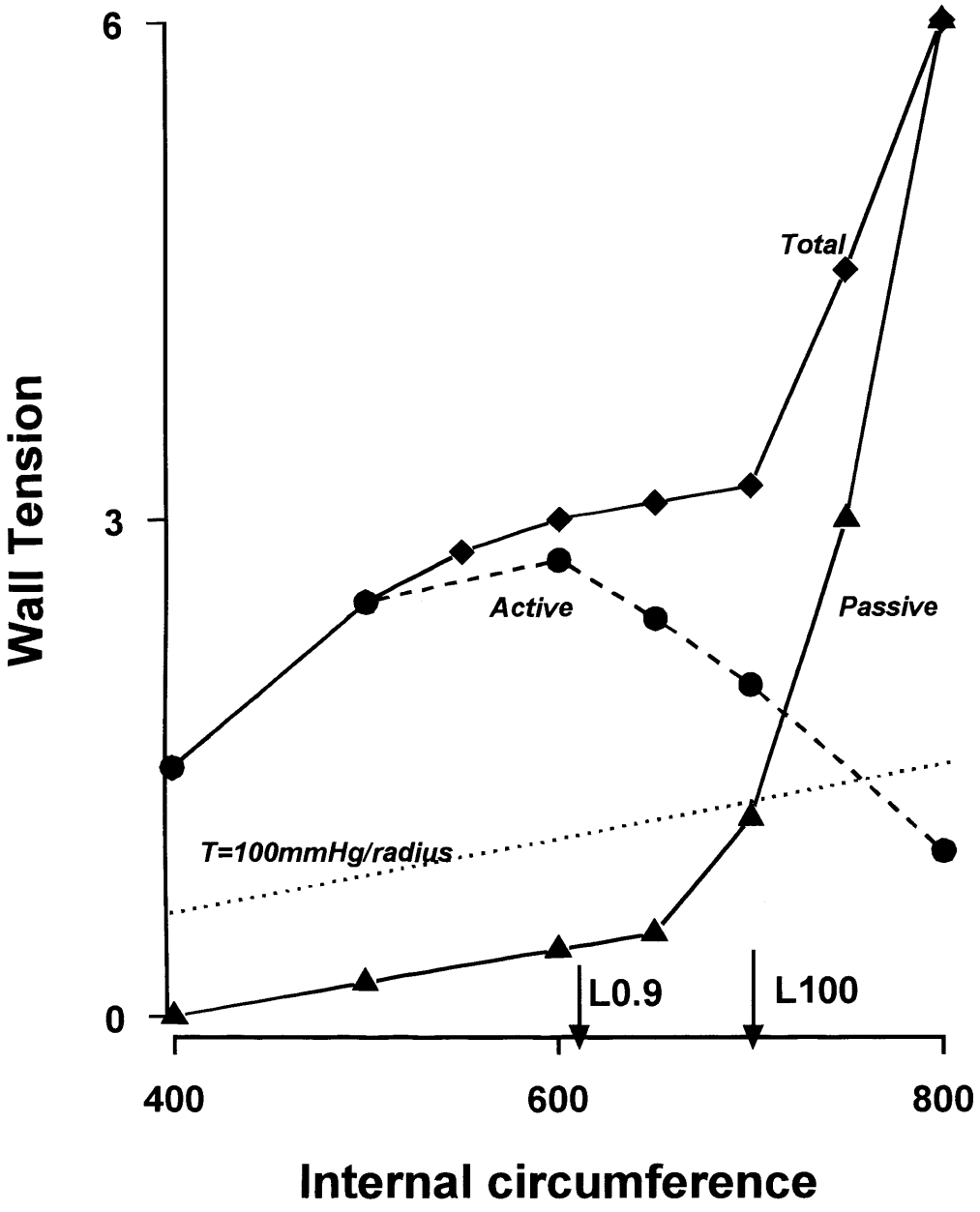

Figure 3. Graphical representation of the relationship between total, passive and active wall tension with blood pressure and the internal circumference of the blood vessel.

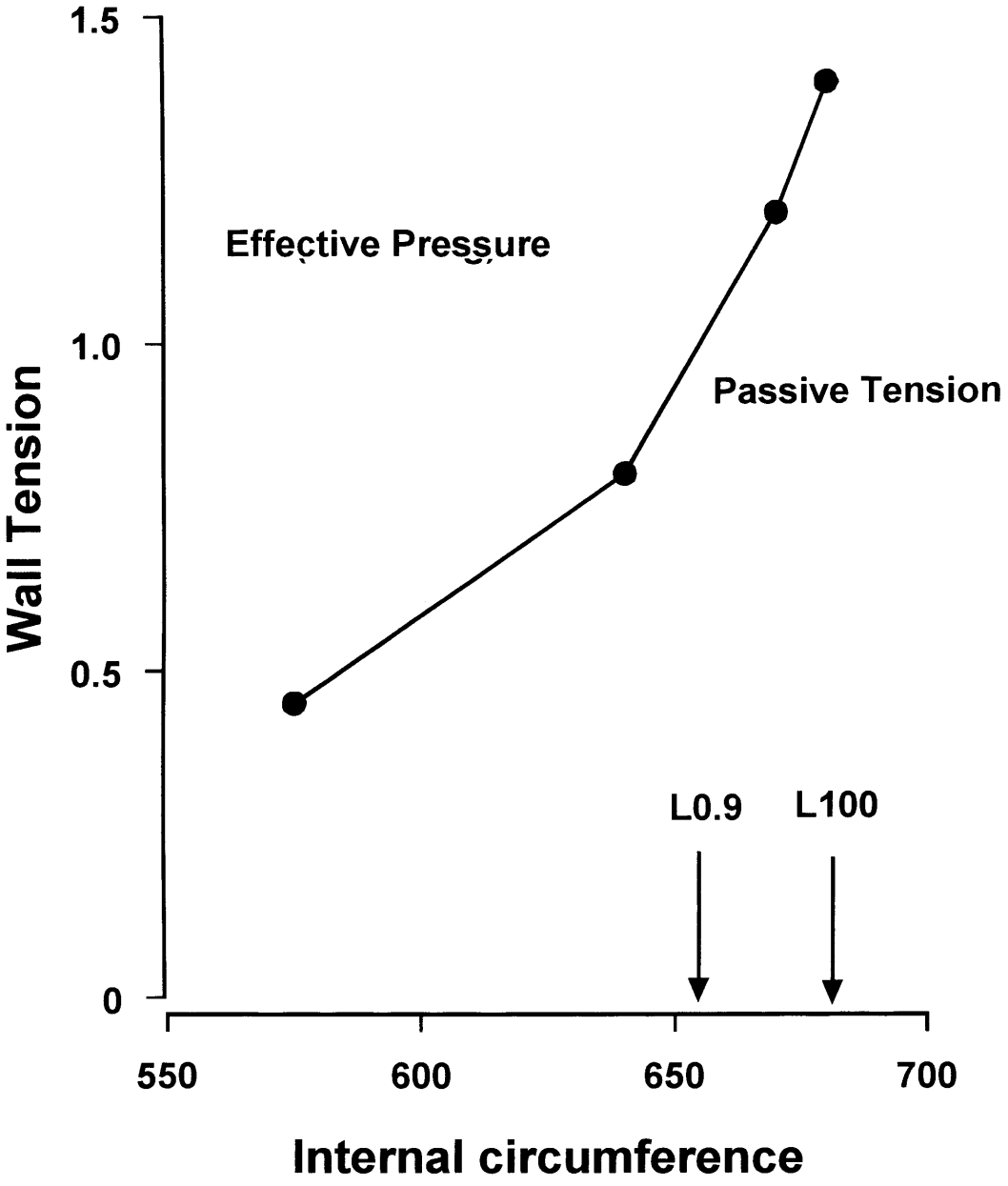

Figure 4. Shows the exponential curve of active tension/circumference relationship in relation to transmural pressure of 100mmHg (13.3kPa). L100 is the circumference the vessel would have had in situ under a transmural pressure of 100mmHg. L0.9 is 90% of L100

2.6. ASSESSMENT OF VASCULAR CONTRACTILITY

Contractile responses of isolated small arteries were assessed using a standard protocol. All studies were preceded by three stimulations with a high potassium solution and once with high potassium solution containing noradrenaline before a cumulative dose contraction curve to noradrenaline was performed.

The high potassium physiological salt solution (123mM) was prepared by substituting potassium chloride for sodium chloride in equimolar quantities (Appendix A). The myograph was drained of physiological salt solution and replaced with high potassium physiological salt solution that had been heated to 37°C and gassed with $5\%CO_2/95\%O_2$. The vessels were allowed to achieve a maximum contraction before being rinsed at least 3 times with fresh physiological salt solution. When the vessels had returned to baseline this procedure was repeated two more times. Finally high potassium physiological salt solution containing noradrenaline (± arterenol hydrochloride, 10^{-5}M) was added to the myograph and the contraction recorded.

A cumulative dose contraction curve to noradrenaline (10^{-8}M to 10^{-4}M) was performed in the presence of cocaine (10^{-6}M) to prevent noradrenaline re-uptake by the sympathetic nerves. Cocaine was added to the bath twenty minutes before application of noradrenaline. Each concentration of noradrenaline was added when the response to the previous dose had either plateaued or after 2 minutes if no response was recorded. Then the vessels were rinsed and allowed to return to baseline.

2.7. ASSESSMENT OF ENDOTHELIAL FUNCTION

Assessment of endothelial function was performed by recording the relaxation response to acetylcholine and sodium nitroprusside of maximally contracted vessels. The relaxation response to acetylcholine depends on the presence of an intact endothelium whereas sodium nitroprusside is a nitric oxide donor and causes relaxation independent of the endothelium.

The vessels were maximally contracted with noradrenaline (10^{-5}M) and when a steady plateau was reached (2-3 minutes) cumulative concentrations of acetylcholine (10^{-9}M to 10^{-4}M) were added to the bath and the relaxation recorded. After this the vessels were rinsed in physiological salt solution and allowed to return to baseline.

After a 20 minute period the vessels were maximally contracted again with noradrenaline (10^{-5}M) and when a steady plateau was reached (2-3 minutes) cumulative concentrations of sodium nitroprusside (10^{-9}M to 10^{-4}M) were added to the bath and the relaxation recorded.

2.8. SOLUTIONS AND DRUGS

The recipe for physiological salt solution, high potassium physiological salt solution and high potassium physiological salt solution containing noradrenaline are detailed in Appendix A. Noradrenaline (± arterenol hydrochloride), cocaine hydrochloride, acetylcholine chloride, nicardipine hydrochloride, and H7 (1-(5-isoquinolinylsulfonyl)-2-methylpiperazine) were obtained from the Sigma Chemical Company and dissolved in distilled water. Caffeine was purchased from the Sigma Chemical Company and dissolved in physiological salt solution. Indomethacin was obtained from

Sigma and dissolved in absolute alcohol. Thromboxane A_2 (U-46,619; 9,11-dideoxy-11$_\alpha$,9$_\alpha$-epoxymethano-Prostaglandin $F_{2\alpha}$) was donated by The Upjohn Company and was dissolved in methyl acetate. Endothelin-1 was donated by Pfizer UK Ltd and was dissolved in distilled water. SQ29548 ([1S-[1$_\alpha$,2$_\beta$(5Z),3$_\beta$,4$_\alpha$]]-7-[3-[[2-[(Phenylamino)-carbonyl]hydrazino] methyl]-7-oxabicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid) was donated by Squibb and was dissolved in absolute alcohol. Dazmegrel (UK 38,485; 3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid) was donated by Pfizer UK Ltd and dissolved in absolute alcohol. All drugs were diluted with physiological salt solution and expressed as the final concentration in the bath.

2.9. EXPRESSION OF RESULTS AND STATISTICAL ANALYSIS

The results are expressed as the mean ± standard error of the mean (SEM). Where two mesenteric arteries were taken from one animal the responses were averaged. Similarly, when two or more vessels were taken from one human biopsy the responses were averaged. Contractile responses are expressed as active tension (milli Newtons/mm) which is calculated from the measured force (mN) divided by twice the vessel length (mm). Results also are expressed as active media stress (mN/mm^2) which takes account of the effects of vascular structure on the contractile force and is calculated by dividing the active tension (mN/mm) by the media thickness (μm^2). Sensitivity to vasoconstrictor agents was expressed in terms of the ED_{50} which is the concentration required to produce a half maximal response. The relaxation response to acetylcholine and sodium nitroprusside are expressed as the percentage decrease from the maximal noradrenaline induced contraction (%).

Statistical analysis was performed using two-tailed Student's unpaired or paired t-test or for multiple comparisons and dose responses by two-way analysis of variance with a Scheffé test where appropriate. A value of $p<0.05$ was considered to be significant.

CHAPTER 3 RESISTANCE ARTERY FUNCTION IN GENETIC HYPERTENSION: ALTERATIONS IN ENDOTHELIAL DEPENDENT RELAXATION

3.1. INTRODUCTION

Elevated peripheral resistance is a consistent feature of experimental (Ferrario & Page 1978) and essential (Lund-Johansen 1980) hypertension. This partly depends on increased neurohumoral activity, but there is also evidence of a significant contribution by changes in vascular responsiveness. Increased contractions in response to vasoconstrictors and impaired relaxation in response to vasodilators have been attributed to structural changes in the arterial wall (Folkow 1978). However, functional alterations may have an important role. In particular, the endothelium must be considered since many vasodilators exert their effects by causing endothelial cells to release a substance (endothelium derived relaxing factor) that relaxes vascular smooth muscle (Furchgott & Zawadzki 1980; Peach et al. 1985).

Endothelial damage, leading to intimal proliferation, occurs in hypertension (Huttner & Gabbiani 1983) and decreased endothelium dependent relaxation with acetylcholine has been reported in aortic rings in spontaneously hypertensive (Konishi & Su 1983) and New Zealand genetic hypertensive rats (Winquist et al. 1984). Similar impaired responses have been observed in DOCA-salt and renal hypertensive rats. Moreover,

reversal of hypertension restored endothelium dependent relaxation to normal (Lockette et al. 1986).

The few studies of endothelium dependent relaxation in resistance vessels indicate that the response to acetylcholine is more powerful than that found in larger vessels. Thus, in normal rats maximally contracted mesenteric resistance vessels were completely relaxed when exposed to acetylcholine (DeMey & Gray 1985). Similar responses have been observed using vessels from normotensive rabbits (Owen & Bevan 1985) and man (Aalkjaer et al. 1987b). In addition, endothelium dependent relaxation was shown to increase as the internal diameter of the vessels decreased (Aalkjaer et al. 1987b). However, there is relatively little information about the effects of hypertension on endothelium dependent relaxation in resistance vessels. Accordingly, acetylcholine induced relaxation was studied in mesenteric resistance vessels during the development of hypertension in the spontaneously hypertensive rat and compared to Wistar-Kyoto controls.

3.2. METHODS

Female spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were studied at 3, 6, 12 and 18 weeks of age. Systolic blood pressure was measured under light ether anaesthesia in all animals except those of 3 weeks of age. Therefore, studies carried out on young SHR assumed that those animals would have developed hypertension with the inverse holding true for the WKY.

Animals were killed by stunning and cervical dislocation. The heart was removed, cleaned of blood clots and fat before being weighed. Arterial

resistance vessels were taken from the superior mesenteric bed which supplies the jejunum at a point 8-10cm from the pylorus. Third generation branches were used in all animals except the 3 week old animals where the second generation branch was used. Arteries were cleaned of fat and surrounding tissue before being mounted in a myograph. Two vessels from each animal were studied and the results averaged.

3.3. PROTOCOL

The vessels were maintained in physiological salt solution at 37°C and gassed with $5\%CO_2/95\%O_2$ to achieve a pH of 7.4. After equilibrating for 60 minutes, the vessel length and morphology was measured using water immersion light microscopy (see Chapter 2). Media cross sectional area (equivalent to media volume per unit length) was calculated from the media thickness and the internal diameter. The vessels were normalised as described in Chapter 2 and the internal diameter was set to $L_{0.9}$ which is 90% of the internal diameter the vessel would have had in-vivo and under a transmural pressure of 100mmHg (13.3 kPa).

After the vessels were normalised a further 60 minutes was allowed to elapse before they were stimulated three times with a high potassium physiological salt solution (KPSS) followed by KPSS containing noradrenaline (10^{-5}M). The bathing medium was replaced after each stimulus and the contraction allowed to return to baseline.

A cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline was added the bathing medium was replaced several times

with fresh physiological salt solution and the vessels allowed to return to baseline. The vessels were then maximally contracted with noradrenaline (10^{-5}M) and cumulative concentrations of acetylcholine (10^{-9}M to $3x10^{-5}$M) were added and the relaxation observed. Finally, the vessels were maximally contracted with noradrenaline and cumulative concentrations of sodium nitroprusside (10^{-9}M to 10^{-4}M) were added and the relaxation observed. The relaxation response was then expressed as the percentage decrease from noradrenaline maximal contraction.

3.4. RESULTS

3.4.1. Physical Characteristics

The blood pressure, body weight, heart weight and heart to body weight ratio are shown in Table 4. With the exception of the 3 week old animals, in which measurements were not made, blood pressure was higher in the SHR compared to the WKY, and the greatest difference was observed in 12 week old animals ($p<0.01$). There was no significant difference in body weight, heart weight and heart to body weight ratio between SHR and WKY until 18 weeks of age. Eighteen week old SHRs were significantly heavier and had an increased heart weight and heart to body weight ratio compared to WKY ($p<0.01$).

3.4.2. Vessel Morphology

Mesenteric vessel morphology is shown in Table 5. The internal diameter of the vessels in the SHR at each age group was similar to those of the WKY rats. The media thickness was increased in the SHR compared to the WKY at all ages and the greatest differences were found at 6 ($p<0.05$) and 18 ($p<0.01$) weeks of age. The media volume was increased in the SHR

compared to the WKY at all ages but was only significant at 18 weeks of age ($p<0.05$). The media to lumen ratio was significantly increased in the SHR compared to the WKY at 6 ($p<0.05$) and 18 ($p<0.01$) weeks of age.

3.4.3. Contraction Studies

3.4.3.1. High potassium and noradrenaline potassium contraction

123mM Potassium (KPSS) contraction

At all ages, except 3 weeks, the maximal contractile (mN/mm) response to potassium was significantly greater in the SHR compared to the age matched WKY (Table 6). When this contractile response was expressed as media stress (mN/mm^2), with the exception of rats at 3 weeks of age, the responses remained significantly different in the SHR compared to WKY ($p<0.01$) (Table 6). In 6 ($p<0.05$) and 12 ($p<0.05$) week old animals the force generated was significantly increased in the SHR whereas in 18 week old rats ($p<0.01$) there was a significant reduction in the SHR compared to age matched WKY.

Noradrenaline potassium contraction

The maximal contractile response to 123mM potassium containing noradrenaline (10^{-5}M) was similar in the SHR compared to WKY at 3, 12 and 18 weeks of age. However, at 6 weeks of age the maximal contractile response was greater in the SHR compared to the WKY animals ($p<0.05$) (Table 6). When the contractile responses were expressed as media stress, the responses in the SHR at 3, 6, 12 and 18 weeks of age were comparable to the WKY animals (Table 6).

3.4.3.2. Noradrenaline contraction response

Noradrenaline produced a concentration dependent contraction of mesenteric resistance vessel segments. With the exception of the 3 week old rats the absolute response (mN/mm) to noradrenaline was greater in the SHR than the WKY vessels ($p<0.001$) (Table 7, Figures 5 to 8). When the contractile responses were expressed as media stress (mN/mm^2), the contraction per unit volume of smooth muscle, the noradrenaline responses in the 3 week old SHR was significantly reduced ($p<0.05$) compared to the age matched WKY rats. At 6 weeks and 18 weeks of age the SHR response remained unchanged from WKY and at 12 weeks of age the SHR response was significantly greater compared to the 12 week old WKY rats ($p<0.05$) (Table 7, Figures 9 to 12). The sensitivity, as expressed by the ED_{50}, showed that there was no significant difference to noradrenaline between the WKY and SHR animals at any age (Table 7).

3.4.4. Relaxation Studies

3.4.4.1. Acetylcholine relaxation response

Acetylcholine produced a concentration dependent relaxation of maximally contracted (noradrenaline $10^{-5}M$) mesenteric resistance vessel segments. However, after the endothelium was mechanically disrupted by drawing a 40μm stainless steel wire back and forth in the lumen several times, this response was markedly attenuated but there was no change in the maximal contractile response to noradrenaline.

Acetylcholine induced relaxation was greater in the 3 week old SHR compared to the WKY rats ($p<0.001$) but when high concentrations of acetylcholine ($>10^{-7}M$) were used the SHR vessels showed a tendency to contract again (Table 7, Figure 13). Relaxation was significantly impaired in

the 6, 12 and 18 week old SHR compared to the age matched WKY rats ($p<0.001$) (Table 7).

The pattern of relaxation responses resemble that observed in young SHRs. Thus, at low concentrations of acetylcholine ($<10^{-7}$M) similar degrees of relaxation occurred in the SHR and WKY vessels, but higher concentrations of acetylcholine caused the SHR vessels to re-contract so that little or no relaxation was observed at the maximum concentration of acetylcholine. By contrast, the WKY vessels demonstrated a continuous concentration dependent relaxation response to acetylcholine (Figures 14 to 16).

3.4.4.2. Sodium Nitroprusside relaxation response

Sodium nitroprusside produced a concentration dependent relaxation of maximally contracted (noradrenaline 10^{-5}M) mesenteric resistance vessel segments. The sodium nitroprusside induced relaxation was similar in the SHR (85 ± 4%) compared to the WKY (84 ± 3%) animals with no alteration in the sensitivity (SHR: 0.83 ± 0.02μM; WKY: 1.33 ± 0.30μM) (Figure 17).

Table 4. Physical characteristics of spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY)

	N	Blood Pressure (mmHg)	Body Weight (grams)	Heart Weight (grams)	Heart:Body Weight Ratio (%)
3 weeks					
WKY	10		41 ± 3	0.2391 ± 0.018	0.588 ± 0.03
SHR	10		39 ± 4	0.2265 ± 0.062	0.582 ± 0.04
6 weeks					
WKY	10	92 ± 4	90 ± 10	0.4628 ± 0.013	0.440 ± 0.01
SHR	10	115 ± 11	112 ± 3	0.4949 ± 0.018	0.444 ± 0.01
12 weeks					
WKY	8	120 ± 5	204 ± 5	0.7854 ± 0.031	0.385 ± 0.01
SHR	7	156 ± 7**	202 ± 5	0.8339 ± 0.039	0.409 ± 0.01
18 weeks					
WKY	6	124 ± 2	224 ± 9	0.8196 ± 0.033	0.367 ± 0.01
SHR	6	168 ± 11**	249 ± 4*	1.0286 ± 0.022**	0.417 ± 0.002**

* $p<0.05$, ** $p<0.01$ SHR compared to age matched WKY control

Table 5. Mesenteric vessel morphology of spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY).

	No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
3 weeks					
WKY	19	117 ± 7	11.71 ± 0.47	4982 ± 293	9.85 ± 0.64
SHR	20	135 ± 8	13.33 ± 0.97	6358 ± 674	10.18 ± 0.89
6 weeks					
WKY	20	184 ± 11	10.50 ± 0.62	6289 ± 351	5.73 ± 0.44
SHR	20	170 ± 9	12.59 ± 0.04*	7115 ± 468	7.78 ± 0.02*
12 weeks					
WKY	13	201 ± 11	11.05 ± 0.60	7543 ± 501	6.26 ± 0.54
SHR	12	204 ± 12	13.08 ± 1.00	8993 ± 905	6.53 ± 0.59
18 weeks					
WKY	10	248 ± 403	8.66 ± 0.70	7043 ± 403	3.55 ± 0.49
SHR	11	224 ± 16	13.04 ± 0.53**	9722 ± 2047*	5.99 ± 0.42**

* $p<0.05$, ** $p<0.01$ SHR compared to age matched WKY control

Table 6. Tension and media stress responses to high potassium and potassium containing noradrenaline (NAK) in mesenteric vessels from spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats.

	123mM Potassium		123mM Potassium + Noradrenaline (10^{-5}M)	
	Active Tension (mN/mm)	Media Stress (mN/mm^2)	Active Tension (mN/mm)	Media Stress (mN/mm^2)
3 weeks				
WKY	1.18 ± 0.10	104 ± 9	1.93 ± 0.19	169 ± 8
SHR	1.41 ± 0.06	118 ± 8	2.22 ± 0.15	181 ± 21
6 weeks				
WKY	1.72 ± 0.11	178 ± 11	2.55 ± 0.25	269 ± 26
SHR	2.57 ± 0.13***	218 ± 16*	3.68 ± 0.33*	315 ± 42
12 weeks				
WKY	2.44 ± 0.08	228 ± 10	3.16 ± 0.20	310 ± 34
SHR	3.31 ± 0.18***	265 ± 17*	4.02 ± 0.45	330 ± 47
18 weeks				
WKY	2.68 ± 0.10	348 ± 28	3.37 ± 0.22	407 ± 50
SHR	3.36 ± 0.19**	257 ± 12**	4.39 ± 0.40	336 ± 26

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ SHR compared to age matched WKY control

Table 7. Tension, media stress responses and sensitivity to noradrenaline and acetylcholine in mesenteric vessels from spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats.

	Noradrenaline Maximum Active Tension (mN/mm)	Noradrenaline Maximum Media Stress (mN/mm^2)	Noradrenaline sensitivity (ED_{50}) (μM)	Maximum Acetylcholine Relaxation (%)
3 weeks				
WKY	1.64 ± 0.17	146 ± 16	3.455 ± 0.51	56 ± 9
SHR	1.47 ± 0.08	123 ± 11*	3.091 ± 0.33	66 ± 7***
6 weeks				
WKY	2.36 ± 0.19	245 ± 23	1.929 ± 0.36	44 ± 9
SHR	3.11 ± 0.22***	275 ± 30	2.079 ± 0.31	42 ± 7***
12 weeks				
WKY	3.00 ± 0.25	281 ± 17	2.283 ± 0.65	62 ± 7
SHR	4.17 ± 0.41***	335 ± 38*	1.857 ± 2.10	43 ± 9***
18 weeks				
WKY	3.25 ± 0.32	398 ± 64	1.179 ± 0.25	67 ± 8
SHR	4.44 ± 0.33***	344 ± 29	1.486 ± 0.45	34 ± 7***

* $p<0.05$, *** $p<0.001$ SHR compared to age matched WKY control

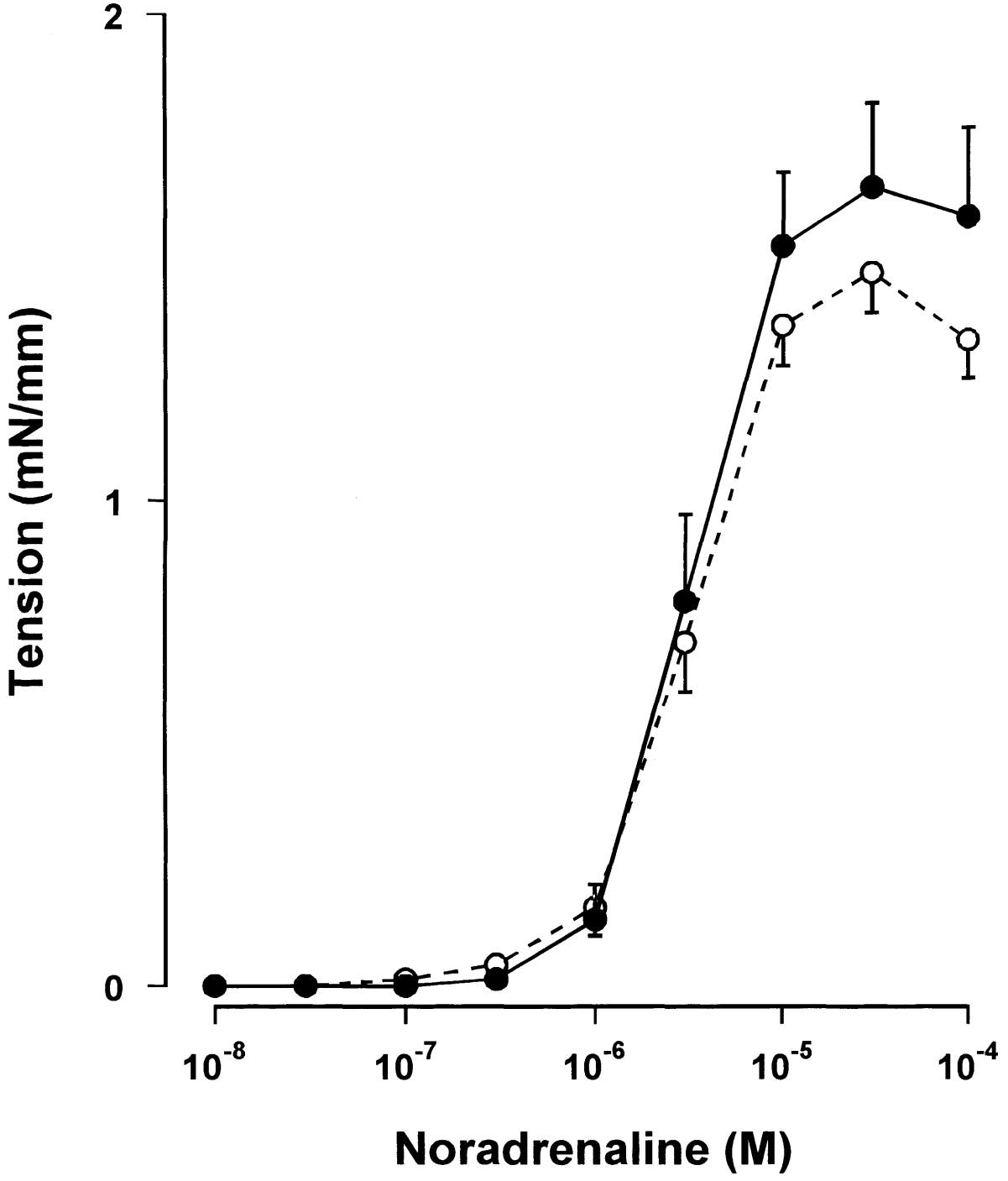

Figure 5 The noradrenaline contractile response of mesenteric vessels from 3 week old SHR (O) and WKY (●) rats

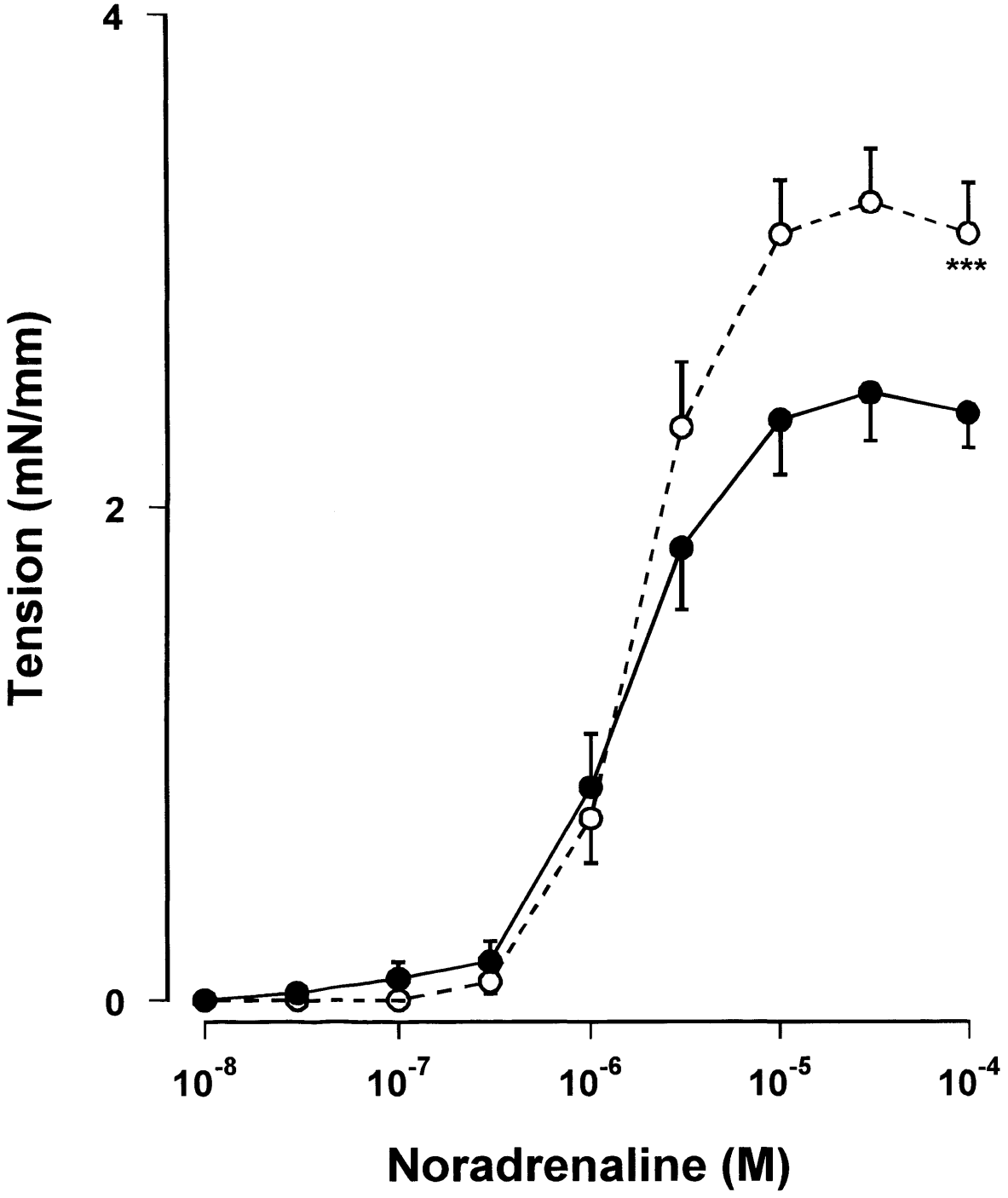

Figure 6 The noradrenaline contractile response of mesenteric vessels from 6 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

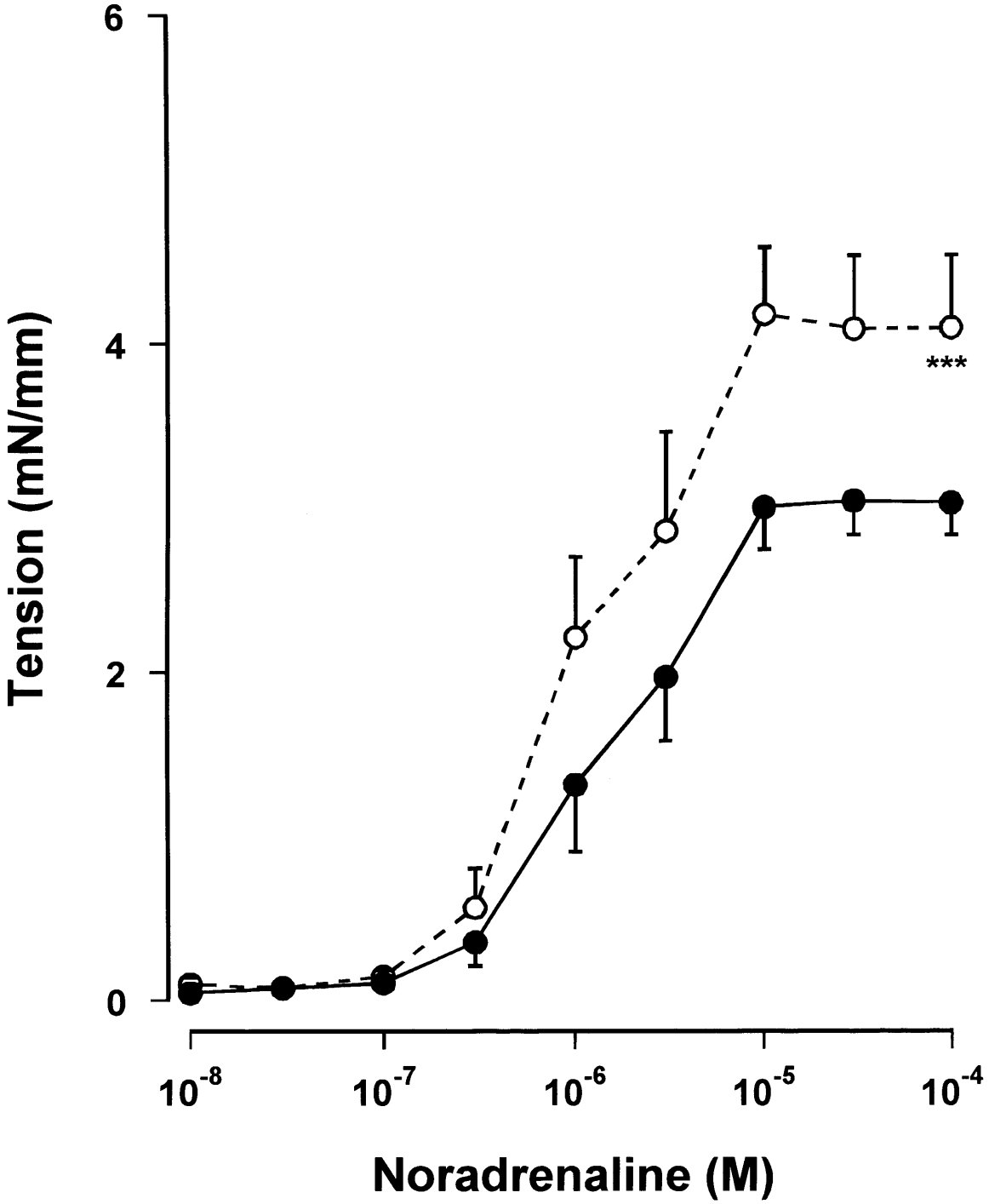

Figure 7 The noradrenaline contractile response of mesenteric vessels from 12 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

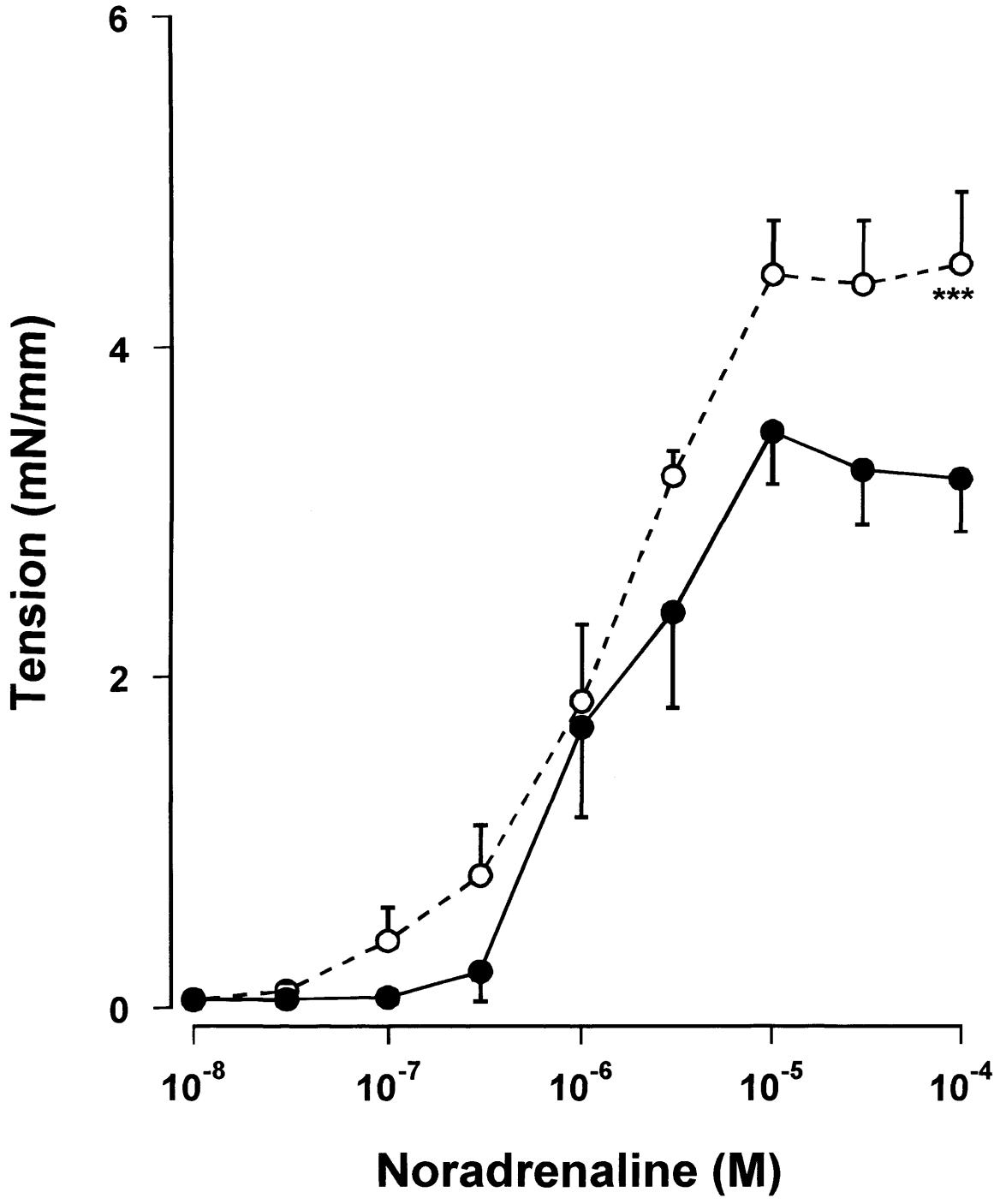

Figure 8 The noradrenaline contractile response of mesenteric vessels from 18 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

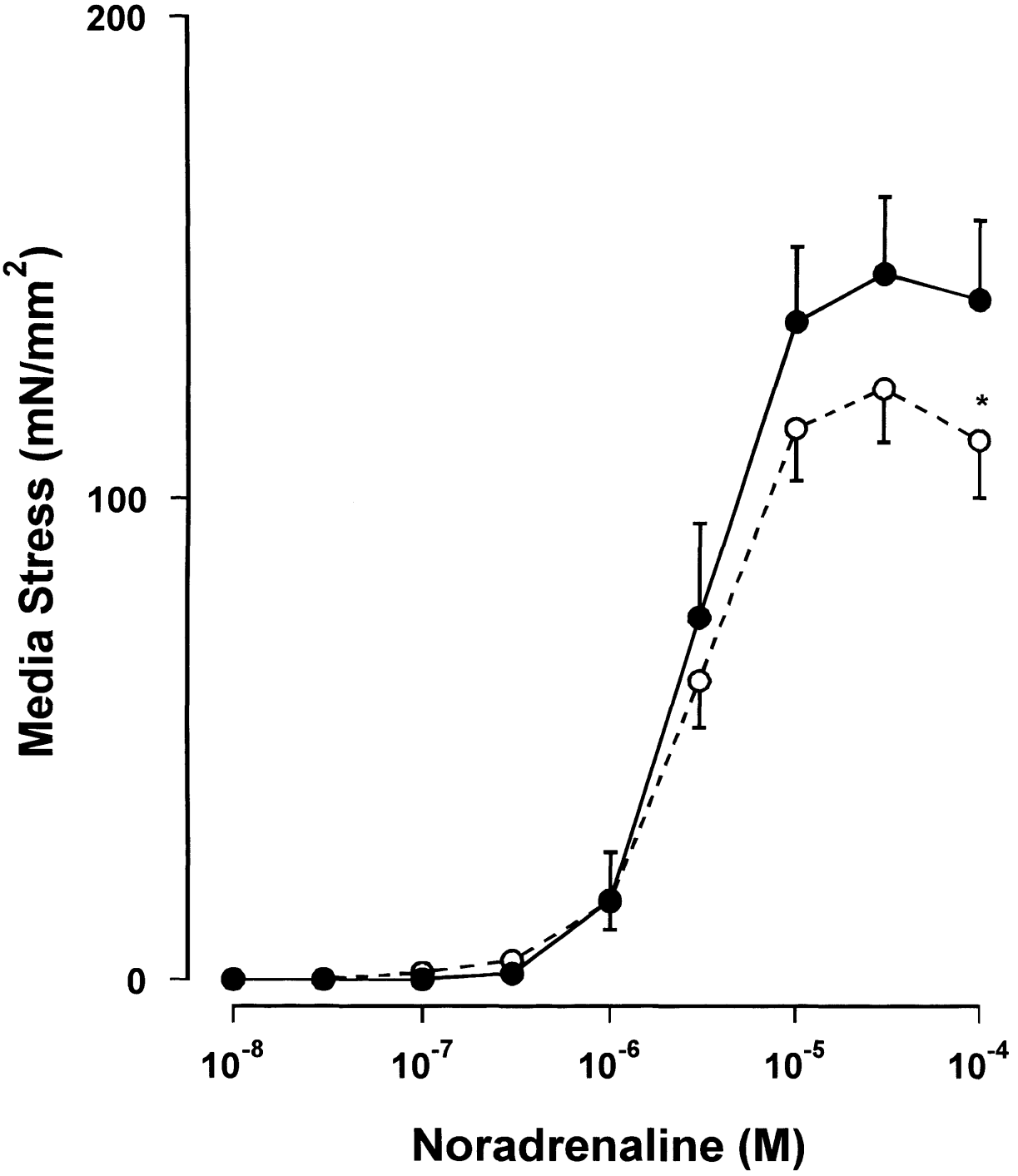

Figure 9 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from 3 week old SHR (O) and WKY (●) rats
***p<0.05 denotes comparison between the two lines**

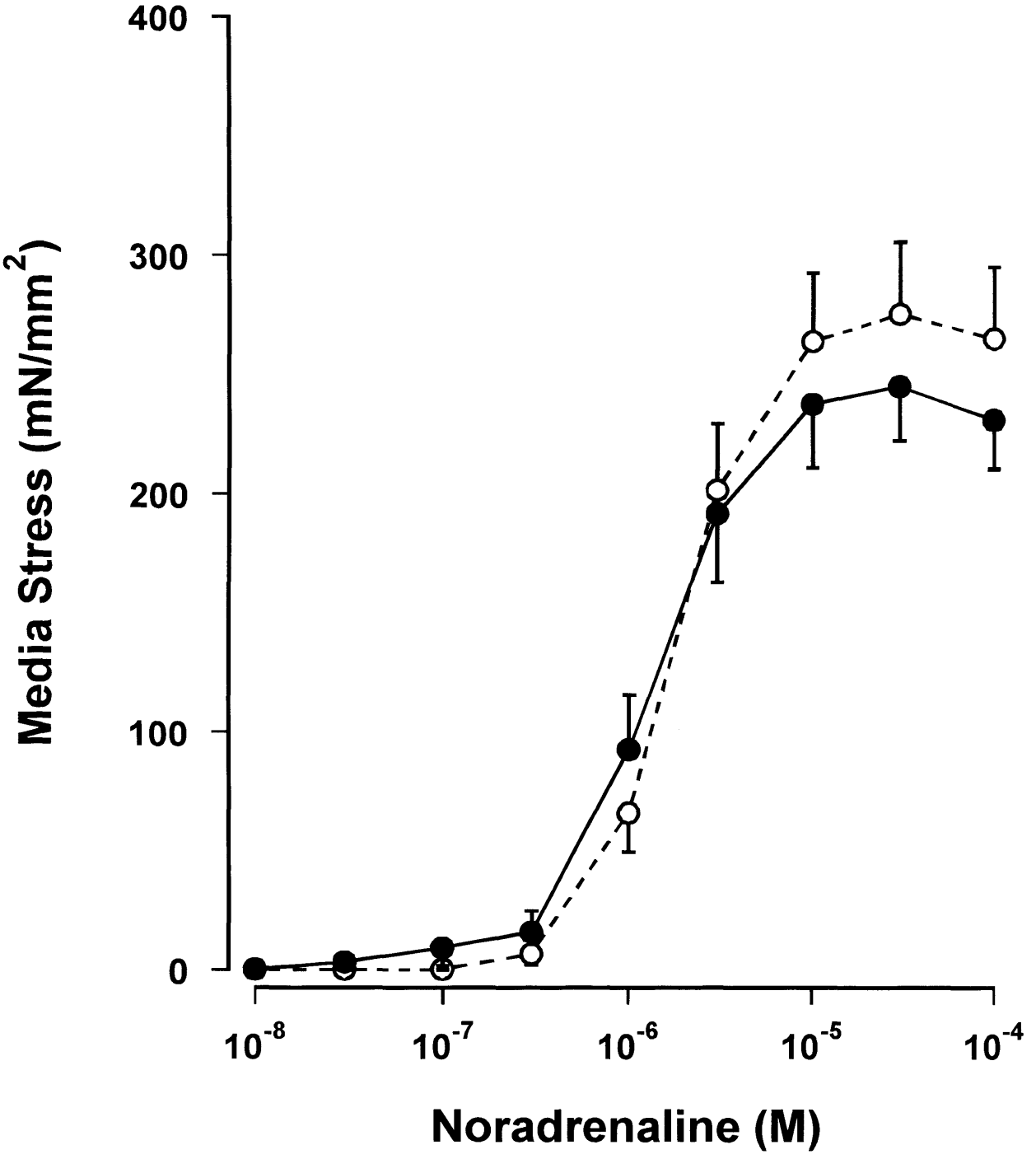

Figure 10 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from 6 week old SHR (O) and WKY (●) rats

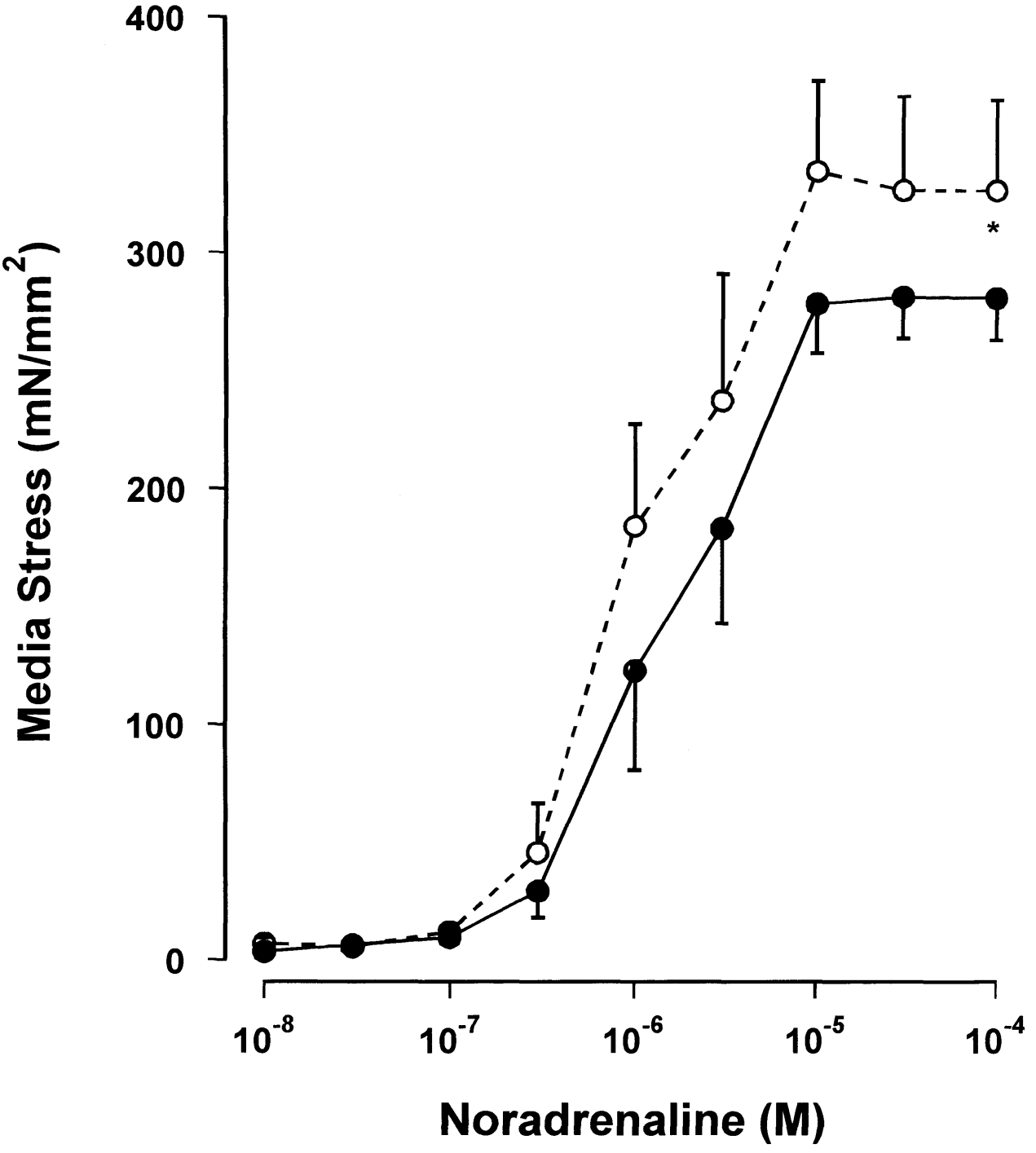

Figure 11 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from 12 week old SHR (O) and WKY (●) rats
***p<0.05 denotes comparison between the two lines**

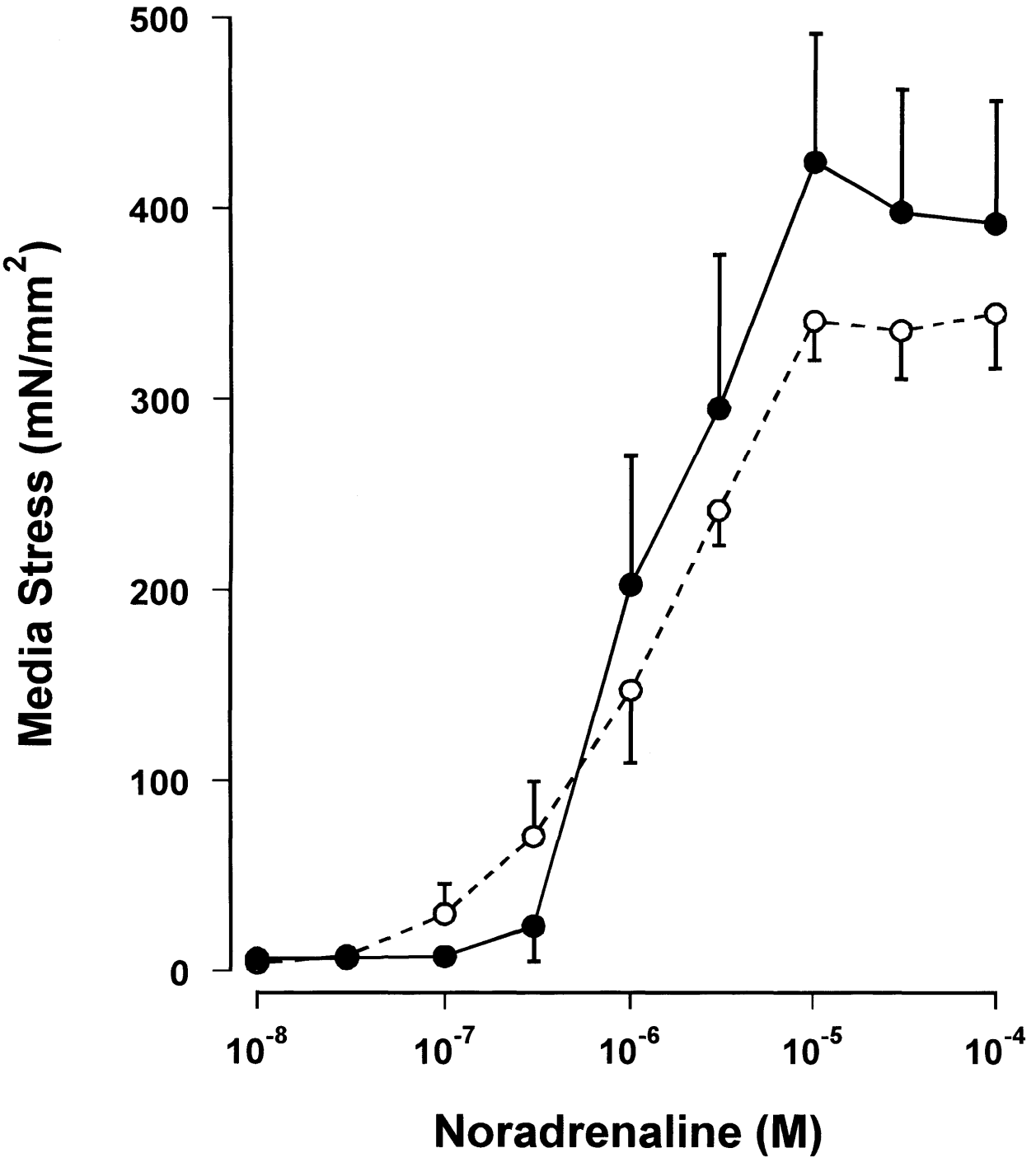

Figure 12 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from 18 week old SHR (O) and WKY (●) rats

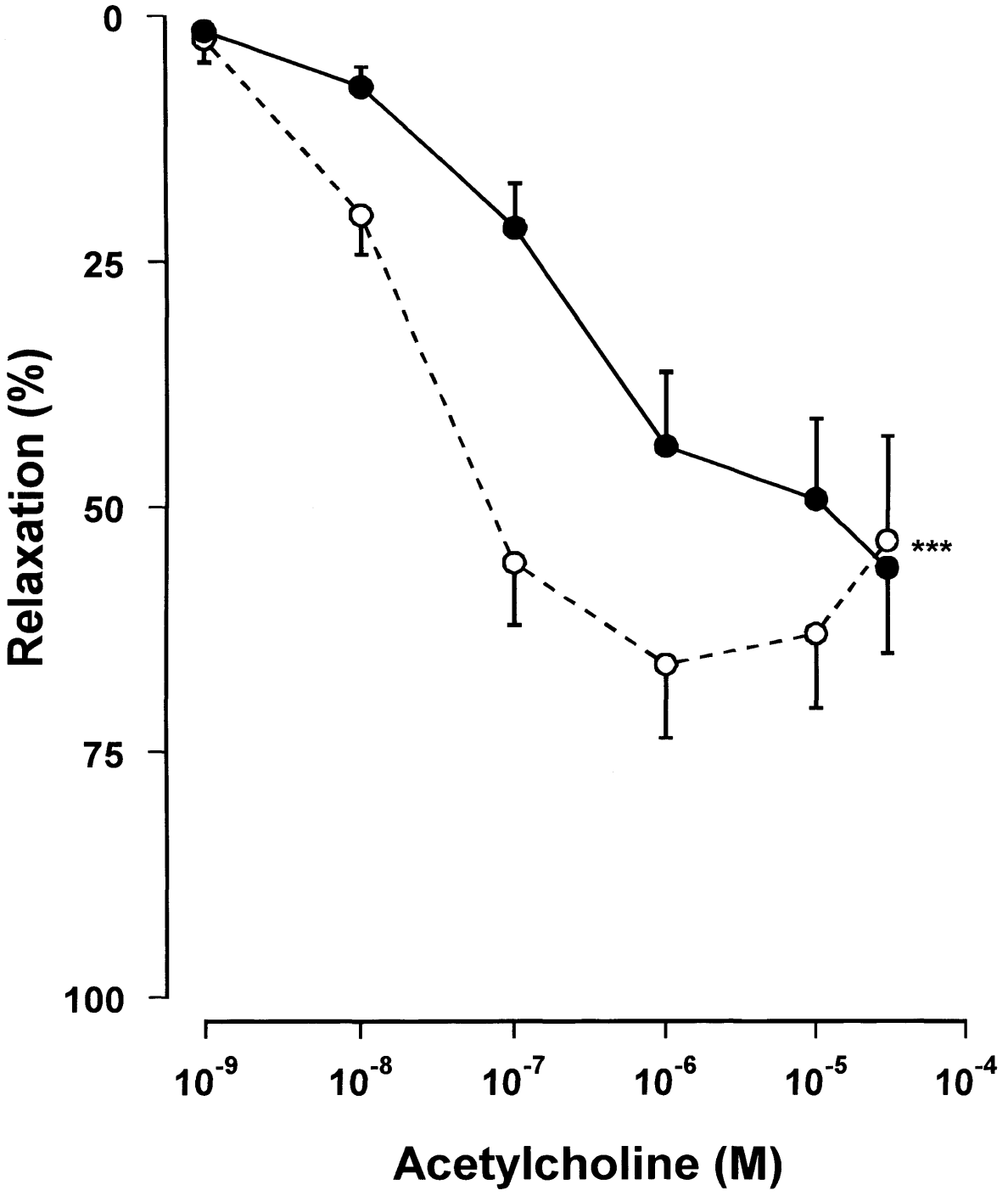

Figure 13 The acetylcholine relaxation response of mesenteric vessels from 3 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

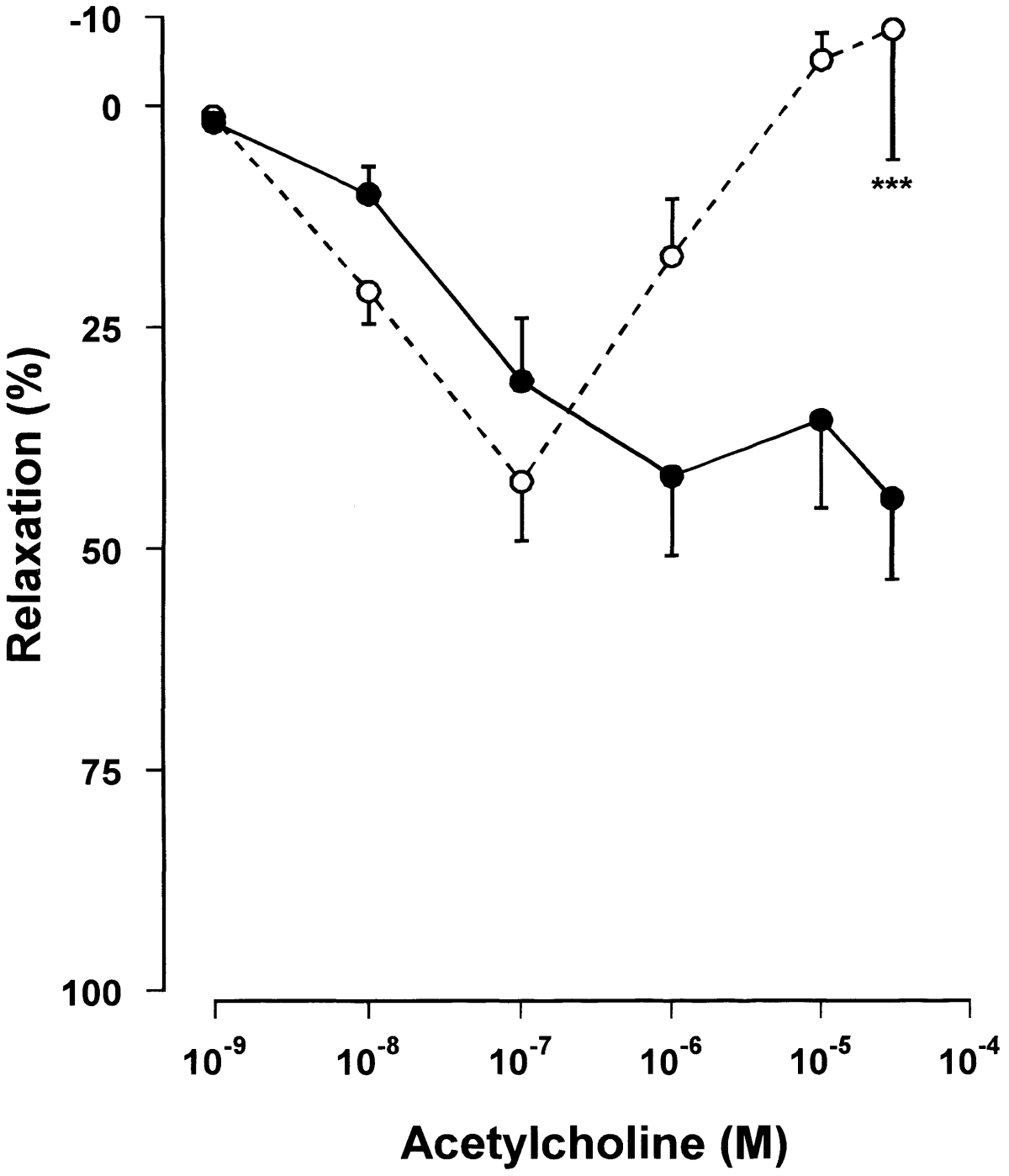

Figure 14 The acetylcholine relaxation response of mesenteric vessels from 6 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

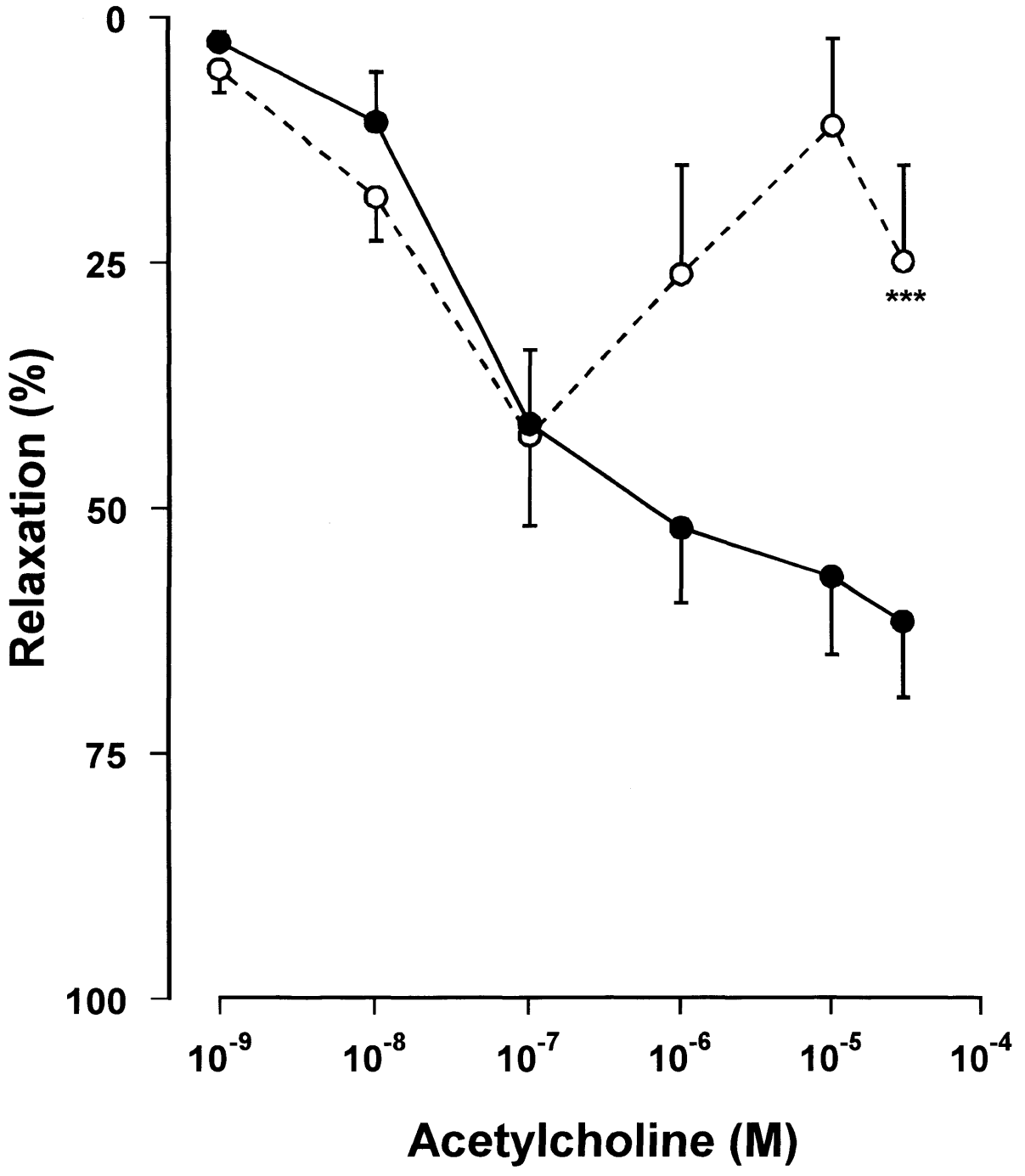

Figure 15 The acetylcholine relaxation response of mesenteric vessels from 12 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

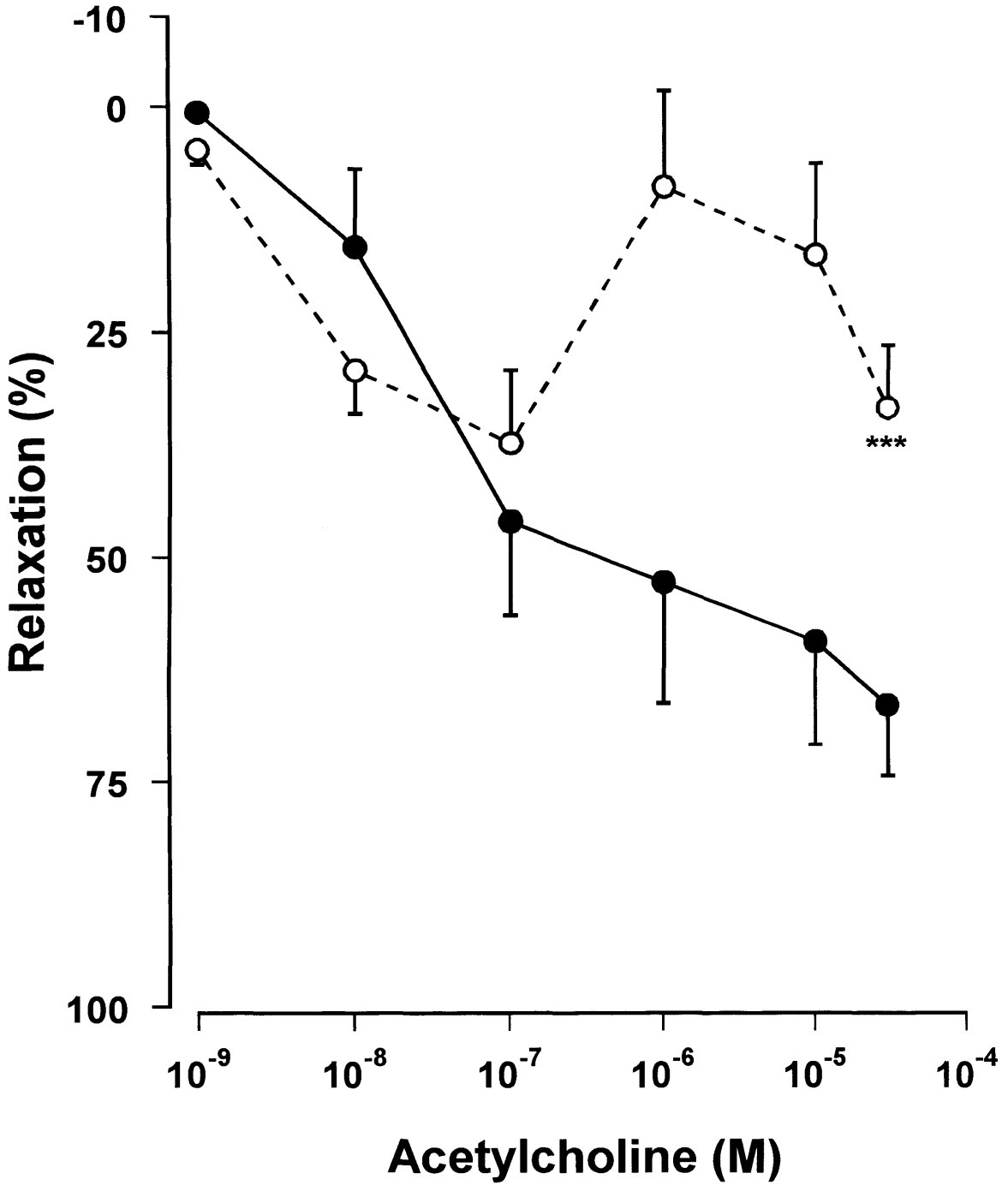

Figure 16 The acetylcholine relaxation response of mesenteric vessels from 18 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

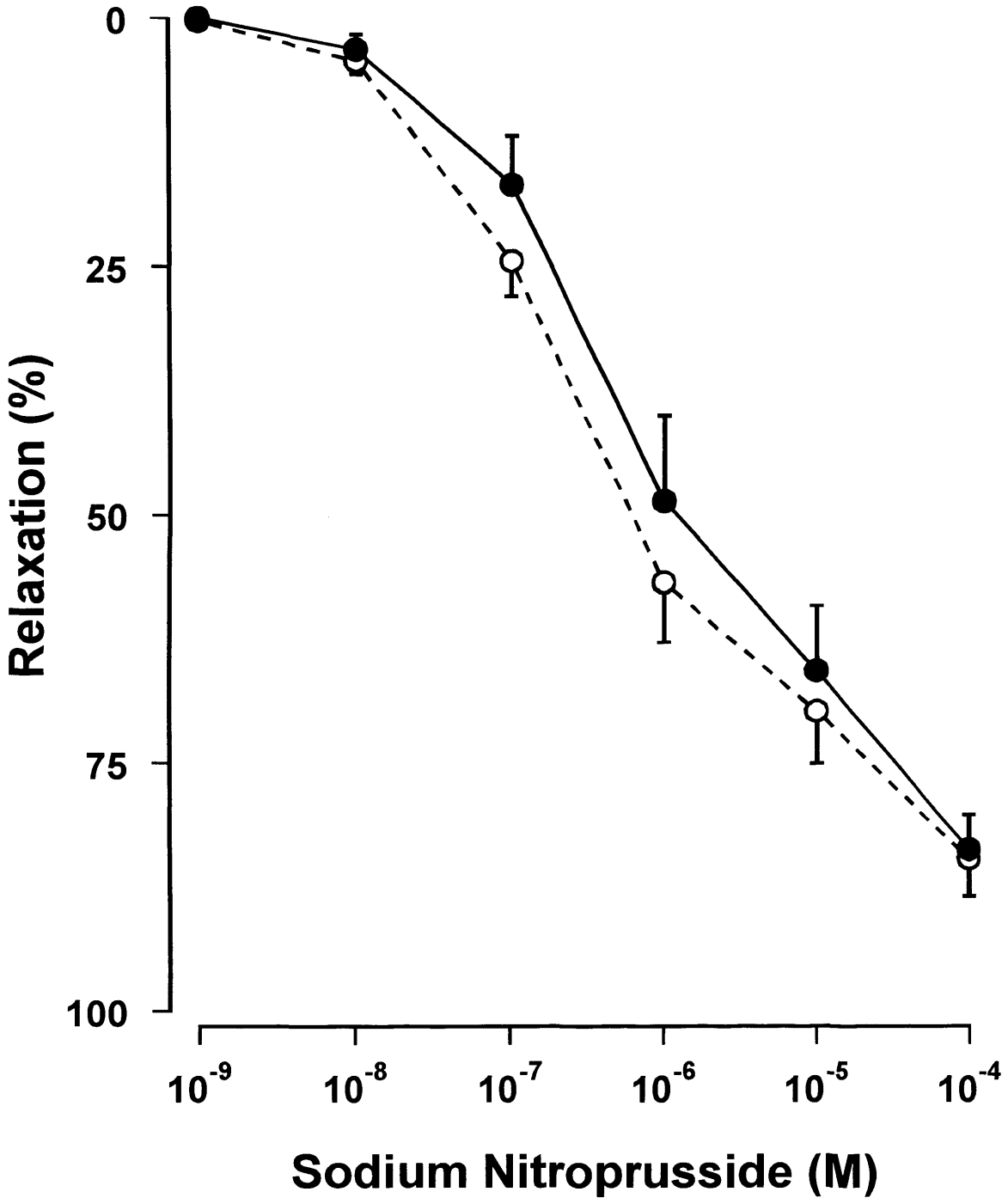

Figure 17 The sodium nitroprusside relaxation response of mesenteric vessels from 12 week old SHR (O) and WKY (●) rats

3.5. DISCUSSION

Blood pressure in the SHR, from 6 weeks of age, was always higher than that measured in the WKY although the differences were not significant until 12 weeks of age. In the young SHR (3 weeks) the heart weight was similar to the WKY animals. However, from 6 weeks of age the heart weight in the SHR was progressively greater than that of the WKY and by 18 weeks of age there was evidence of cardiac hypertrophy as shown by the heart to body weight ratio. Similar findings have also been reported by Mulvany and colleagues (Mulvany et al. 1980b) when they studied SHR and WKY animals at 6, 12 and 24 weeks of age.

The internal diameter of the vessels was similar in the SHR and WKY animals at all ages although other investigators have suggested a 15-20% reduction in the internal diameter of SHR (Mulvany et al 1978 and 1980b). Structurally, the mesenteric arteries in the SHR had a thicker media at all ages. By 6 weeks of age the media thickness of the SHR had increased by 20% and at 18 weeks of age the media thickness in the SHR was 51% greater than that of the WKY animals. These findings are directly comparable with those of Mulvany and co-workers (Mulvany et al. 1980b). The cross sectional area (media volume) was similar in the SHR and WKY except in the 18 week old animals where the media volume of the SHR was 30% greater than that of the WKY. However, the media to lumen ratio results were less clear cut in that at 6 and 18 weeks of age the SHR had a significant 36% and 69% increase (respectively) compared to the WKY whereas at 3 and 12 weeks there was no significant difference.

The present study involved the use of vessels small enough to be involved in the regulation of peripheral resistance. The increased media thickness and media to lumen ratio observed is in keeping with Folkow's hypothesis that structural alteration in the form of media hypertrophy could contribute to the increased vascular resistance observed in hypertension. Folkow's model shows that such a structural change would be sufficient to increase the force generated, even though there was no change in the properties of the smooth muscle contained in the vessel wall (Folkow 1956).

There appears to be general agreement, on the basis of postmortem studies, that in the mesenteric bed of patients with essential hypertension the media to lumen ratio is increased (Short 1966) and that there is medial hypertrophy (Furuyama 1962; Suwa & Takahashi 1971 and Barrett 1963). Taken along with the findings from experimental and genetic animal models of hypertension, the findings in this study would support the concept that hypertension is associated with structural changes in the vasculature and studies in man have now confirmed these findings. The development of gluteal biopsies taken under local anaesthetic have enabled the study of isolated resistance arteries in wire myographs (Aalkjaer et al. 1986). Arteries have been studied from patients with untreated hypertension and compared to control volunteers (Korsgaard et al. 1991) and the patients with hypertension were found to have a greater media/lumen ratio (Aalkjaer et al. 1987b).

At all ages, except at 3 weeks, there was a greater absolute contractile response to high potassium in the SHR. This increase in contractile force could not be explained solely by the increase in media thickness since when the responses were expressed as media stress the results remained

unchanged at 3 weeks, greater at 6 and 12 weeks, and reduced at 18 weeks in the SHR. The force generated by high potassium containing noradrenaline was similar in the SHR at all ages except at 6 weeks where it was significantly greater. When this contractile response was expressed as media stress the increase force observed in the SHR could be accounted for by the increase of the media in the vessels. Mulvany and colleagues also showed that a high potassium and high potassium containing noradrenaline produced a greater contractile force in 24 week old SHRs when compared to WKYs (Mulvany et al. 1980b). However, in contrast to my study they were able to demonstrate that this increase in force appeared to be a direct result of the increased media volume (Mulvany et al 1978). On the other hand, in a later study of SHR and WKY animals at 6, 12 and 24 weeks Mulvany and his colleagues observed that the contractile force produced increased with age but that there was no significant difference between the SHR and WKY. This was irrespective of whether the contractile force was expressed as active tension or media stress but when the results were expressed as effective active pressure (contractile force /(diameter/2π)) there was a significant increase in the SHR at all ages (Mulvany et al. 1980b).

Noradrenaline produced a significantly greater absolute contractile force in all SHR animals, except at 3 weeks, when compared to the age matched WKY rats. When the contractile force was expressed as media stress the response in the SHRs at 6 and 18 weeks of age was similar suggesting that the increase in active tension observed was due to the increased media found in these vessels. However, in the 3 week old SHR the media thickness of the vessels and the active tension response to noradrenaline were similar to the age matched WKY animals but when the response was

expressed as media stress there was a significant decrease in the SHR animals. Additionally, at 12 weeks of age there was a significant increase in active tension and media stress when compared to age matched WKY animals. In patients with untreated hypertension it was demonstrated that the increase in maximum response of the vessels could be accounted for by the increase in media thickness (media stress, force per unit of smooth muscle cross section (Aalkjaer et al. 1987b). Therefore, while it may be observed that there are alterations in structure and function in these resistance vessels there is a variability which appears to be determined by the age chosen to study these animals.

However, considering structural changes and vasoconstriction of blood vessels in isolation may not fully reflect all the mechanisms underlying the development of hypertension. The endothelium may contribute to the increased vascular resistance. In this study acetylcholine induced, endothelium dependent, relaxation responses were comparable in mesenteric vessels from 3 week old SHR and WKY rats. However, at 6, 12 and 18 weeks, endothelium dependent relaxation evoked by high, but not low, concentrations of acetylcholine were significantly attenuated in the SHR. Endothelium derived relaxing factor diffuses towards vascular smooth muscle cells leading to the activation of guanyl cyclase and the resulting increase in intracellular levels of cyclic guanosine monophosphate is responsible for vascular smooth muscle relaxation (Holzman 1982; Rapoport & Murad 1983). The relaxation responses were more powerful than those observed in large and medium sized arteries (DeMey & Gray 1985; Owen & Bevan 1985; Aalkjaer et al. 1986).

The loss of endothelium dependent relaxation could result either from a functional alteration in the endothelial or smooth muscle cells or morphological changes in the vascular wall which could reduce diffusion of the endothelium derived relaxing factor towards the smooth muscle cells. This study favours the former explanation since the major reduction in acetylcholine relaxation occurred at 6 weeks of age, when structural change was not fully developed (Mulvany et al. 1980a). Moreover, the response to high rather than low concentrations of acetylcholine were impaired and the converse would be predicted if the change depended solely upon an increase in the diffusion pathway length for endothelium derived relaxing factors. Furthermore, although vascular hypertrophy takes several weeks to reverse following renal artery deconstriction in rats with Goldblatt hypertension (Lundgren 1974), endothelium dependent relaxation can be quickly restored by lowering the blood pressure (Lockette et al. 1986). Thus, it is more likely that the observed impaired relaxation was caused by a functional change in the resistance vessel leading to a decreased synthesis or release of endothelium derived relaxing factor, or possibly to the production of vasoconstrictor substances.

It is unlikely that the reduced relaxation responses in the adult SHR reflect a reduced sensitivity of the intracellular process because sodium nitroprusside, a nitric oxide donor and therefore a direct activator of guanyl cyclase (Rapoport & Murad 1983), relaxes preconstricted mesenteric resistance vessels from these animals (DeMey & Gray 1985). In this study the nitric oxide donor, sodium nitroprusside, showed that vessels from SHR and WKY animals produced similar relaxation responses. Both acetylcholine and sodium nitroprusside induced relaxation utilise the same final pathway (increasing cGMP) in vascular smooth muscle cells.

Other studies indicate that endothelial cells can synthesise and release contracting factors in addition to endothelium derived relaxing factor. These include endothelin, a constrictor peptide which has been sequenced and cloned (Yanagisawa et al. 1988) and a prostanoid, most probably thromboxane A_2 (Katusic & Vanhoutte 1985; Shirahase et al. 1987). Hence, vascular tone may be modulated by the release of dilator and constrictor vasoactive substances from the endothelium. However, in vitro studies (Vanhoutte 1987a) indicate that once endothelium derived contracting factor is produced, constriction predominates despite the continuous release of endothelium derived relaxing factor. This raises the possibility that the loss of endothelium dependent relaxation in pathological conditions such as hypertension may depend on the over-production of an endothelium derived contracting factor(s).

Other studies have shown decreased acetylcholine induced vasodilation using the preconstricted thoracic aorta in the SHR (Konishi & Su 1983; Winquist et al. 1984) and Luscher and Vanhoutte (Luscher & Vanhoutte 1986) have suggested that the impaired response may result from the simultaneous release of relaxing and contracting factors from the hypertensive vessels. My results confirm and extend this finding to the resistance artery, where depressed endothelium dependent relaxation in established hypertension may contribute to the elevated peripheral resistance by augmenting vasoconstriction.

3.6. CONCLUSIONS

1. The spontaneously hypertensive rat develops abnormalities in the endothelium dependent relaxation as the blood pressure increases with age.

2. The presence of a normal response to the nitric oxide donor suggests that this abnormality does not involve an abnormality of smooth muscle response or an increase in the diffusion pathway because of structural hypertrophy but indicates endothelial dysfunction.

3. It seems unlikely that endothelial dysfunction may be due to impaired EDRF release but the increased release of a contracting factor from the endothelium is an alternative explanation.

CHAPTER 4 ABNORMAL ENDOTHELIAL FACTOR IN HYPERTENSION : RELEASE OF CONTRACTING FACTORS

4.1. INTRODUCTION

Endothelial dependent relaxation is impaired in the mesenteric resistance vasculature of the spontaneously hypertensive rat and there is some evidence to suggest that this may be due to the release of an endothelial derived contracting factor. The endothelium is known to produce both relaxing and contracting factors, prostaglandins (PGI_2, PGE_2), thromboxanes and endothelin. Furthermore, endothelium derived contracting factors (EDCF) have been shown to be released in response to various stimuli (De Mey & Vanhoutte 1983; Miller & Vanhoutte 1985; Luscher & Vanhoutte 1986; Yanagisawa et al. 1988; Katusic & Vanhoutte 1985; Shirahase et al. 1987). However, acetylcholine stimulation of EDCF release has not so far been demonstrated.

The studies described in this chapter were designed to investigate the nature of the endothelial defect by observing the effects of the cyclooxygenase inhibitor, indomethacin, to block prostaglandin cyclooxygenase synthesis, a thromboxane A_2 antagonist, SQ29548, and a thromboxane A_2 synthesis inhibitor, Dazmegrel, on acetylcholine dependent relaxation.

4.2. METHOD and PROTOCOL

Female spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats were studied at 12 weeks of age. Third order mesenteric resistance arteries were mounted in a myograph. Morphological measurements were made using light microscopy. After normalisation a cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline the bathing medium was replaced several times with fresh physiological salt solution and the vessels allowed to return to baseline. The vessels were then maximally contracted with noradrenaline (10^{-5}M) and cumulative concentrations of acetylcholine (10^{-9}M to 10^{-4}M) were added and the relaxation observed. After further rinsing and a 20 minute interval the cyclo-oxygenase inhibitor, indomethacin (10^{-5}M), was added to the bath 5 minutes before the noradrenaline contraction and acetylcholine relaxation curves were repeated.

4.3. RESULTS

4.3.1. Vessel Morphology

The mesenteric vessel morphology of the 12 week old SHR and WKY animals are shown in Table 8. The internal diameter of the SHR (182 ± 7μm) was significantly reduced compared to the WKY (214 ± 5μm) ($p<0.01$) rats. The media thickness ($p<0.01$) and media to lumen ratio ($p<0.001$) were significantly greater in the SHR but the media volume was similar to that in the WKY animals (Table 8).

Table 8. Physical characteristics of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats used in the indomethacin study.

	No Animals	No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
WKY	11	22	214 ± 5	11.47 ± 0.43	8019 ± 321	5.52 ± 0.28
SHR	11	22	182 ± 7**	15.00 ± 0.86**	9474 ± 842	8.27 ± 0.45***

** $p<0.01$, *** $p<0.001$ SHR compared to WKY control

4.3.2. Contraction Studies

Noradrenaline caused a concentration dependent contraction of all mesenteric arteries. The absolute contractile response to noradrenaline was significantly greater in the SHR (3.90 ± 0.29mN/mm) compared to WKY (2.93 ± 0.10mN/mm) ($p<0.001$) (Table 9). Pre-treatment with indomethacin caused a significant reduction in the contractile response to noradrenaline in both the SHR (3.04 ± 0.29mN/mm) and WKY (1.40 ± 0.15mN/mm) ($p<0.001$) rats (Table 9, Figures 18 & 19). Interestingly, treatment with indomethacin reduced the response in the SHR to that observed in the WKY vessels before exposure to indomethacin (Figure 20). There was no difference in the sensitivity (ED_{50}) to noradrenaline in the SHR (3.82 ± 1.0μM) compared to the WKY (3.57 ± 0.64μM). However, treatment with indomethacin significantly reduced the sensitivity to noradrenaline in the WKY (13.42 ± 4.1μM) ($p<0.05$) (Table 10) but there was no change in sensitivity in the SHR (6.76 ± 1.3μM) treated with indomethacin (Table 9).

4.3.3. Relaxation Studies

Acetylcholine caused a concentration dependent relaxation of mesenteric resistance vessels, maximally contracted with noradrenaline (10^{-5}M). The maximum acetylcholine induced relaxation was significantly reduced in the SHR (26 ± 7%) compared to the WKY (39 ± 7%) ($p<0.001$) (Table 9, Figures 21 & 22). Concentrations of acetylcholine $>10^{-7}$M produced the previously observed recontraction in the SHR animals. Pre-treatment with indomethacin caused a significant increase in relaxation response in both the SHR (58 ± 5%, $p<0.001$) and WKY (69 ± 6%, $p<0.001$) rats. There was no difference in the relaxation response between the SHR and WKY after treatment with indomethacin (Figure 23).

Table 9. Contraction and relaxation properties of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats used in the indomethacin study.

	Maximum Noradrenaline Contraction		Noradrenaline sensitivity (ED_{50})		Maximum Acetylcholine Relaxation	
	control (mN/mm)	indomethacin (mN/mm)	control (μM)	indomethacin (μM)	control (%)	indomethacin (%)
WKY	2.93 ± 0.10	$1.40 \pm 0.15^{+++}$	3.573 ± 0.64	$13.419 \pm 4.1^{+}$	39 ± 7	$69 \pm 6^{+++}$
SHR	$3.90 \pm 0.29^{***}$	$3.04 \pm 0.29^{+++}$	3.824 ± 1.00	6.757 ± 1.30	$26 \pm 7^{***}$	$58 \pm 5^{+++}$

+ $p<0.05$, +++ $p<0.001$ compared to pre-antagonist treatment
*** $p<0.001$ SHR compared to WKY

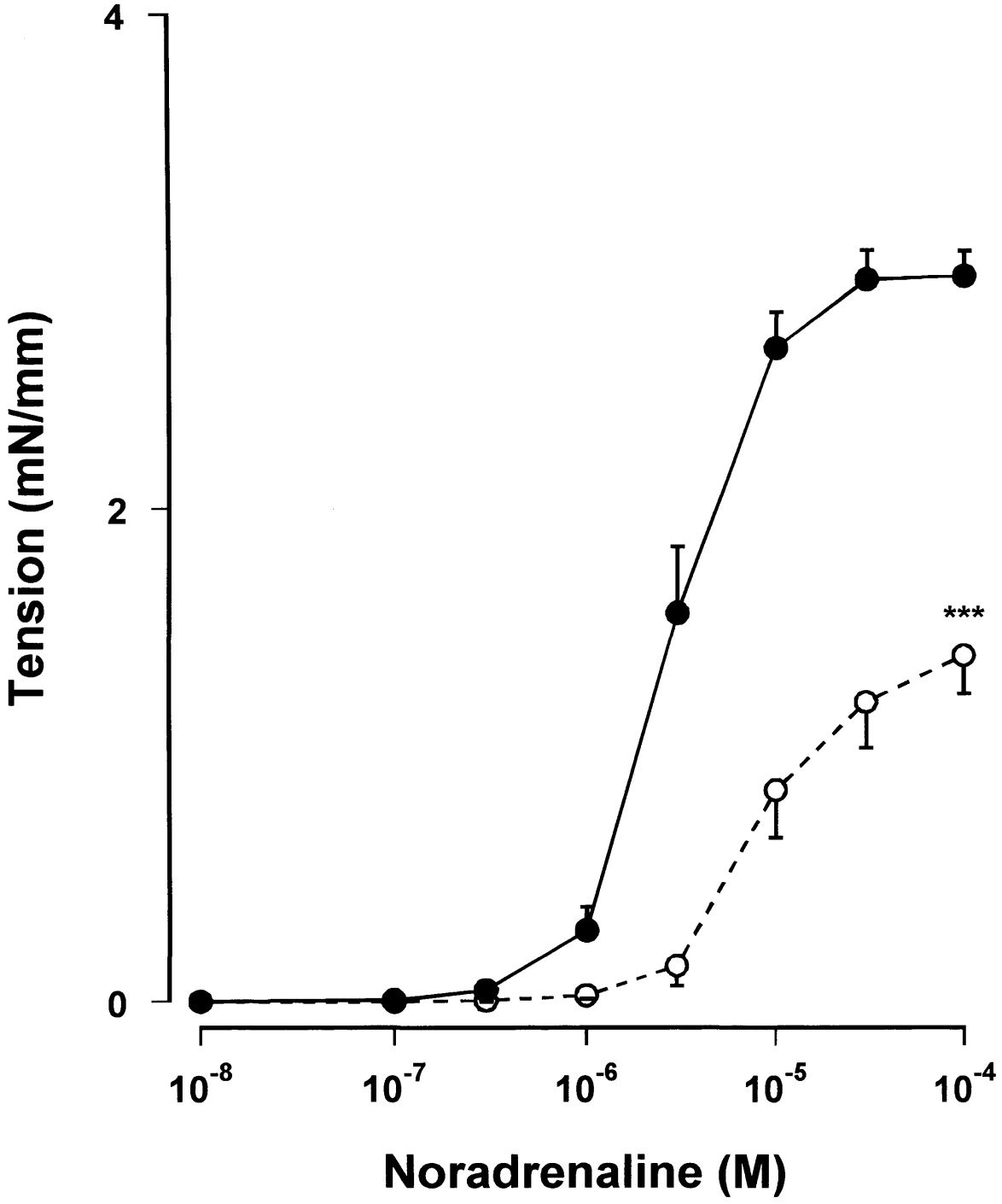

Figure 18 The noradrenaline contractile response of mesenteric vessels from WKY rats before (●) and after (○) incubation with indomethacin (10^{-5}M) ***p<0.001 denotes comparison between the two lines

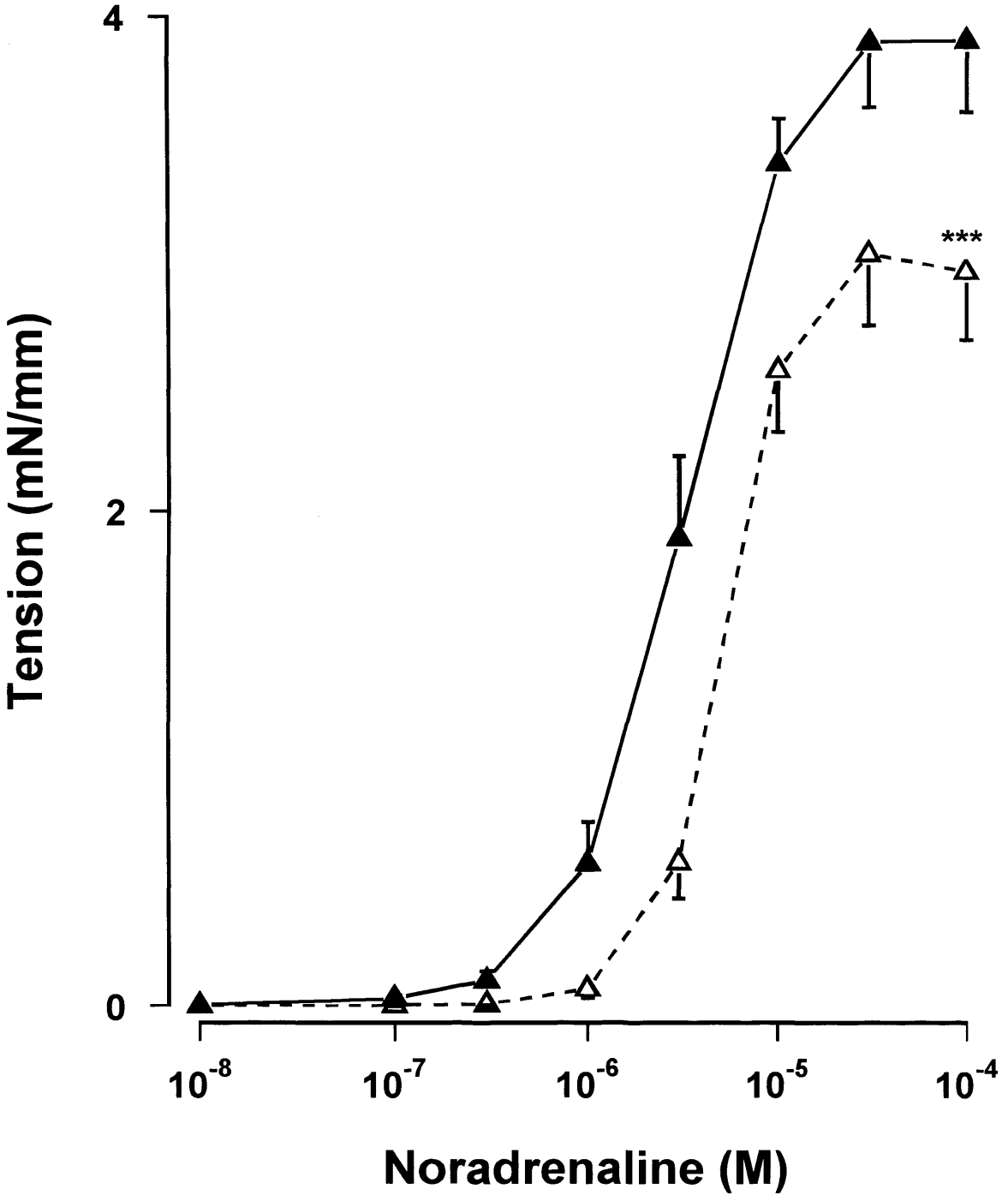

Figure 19 The noradrenaline contractile response of mesenteric vessels from SHR rats before (▲) and after (△) incubation with indomethacin (10^{-5}M) ***p<0.001 denotes comparison between the two lines

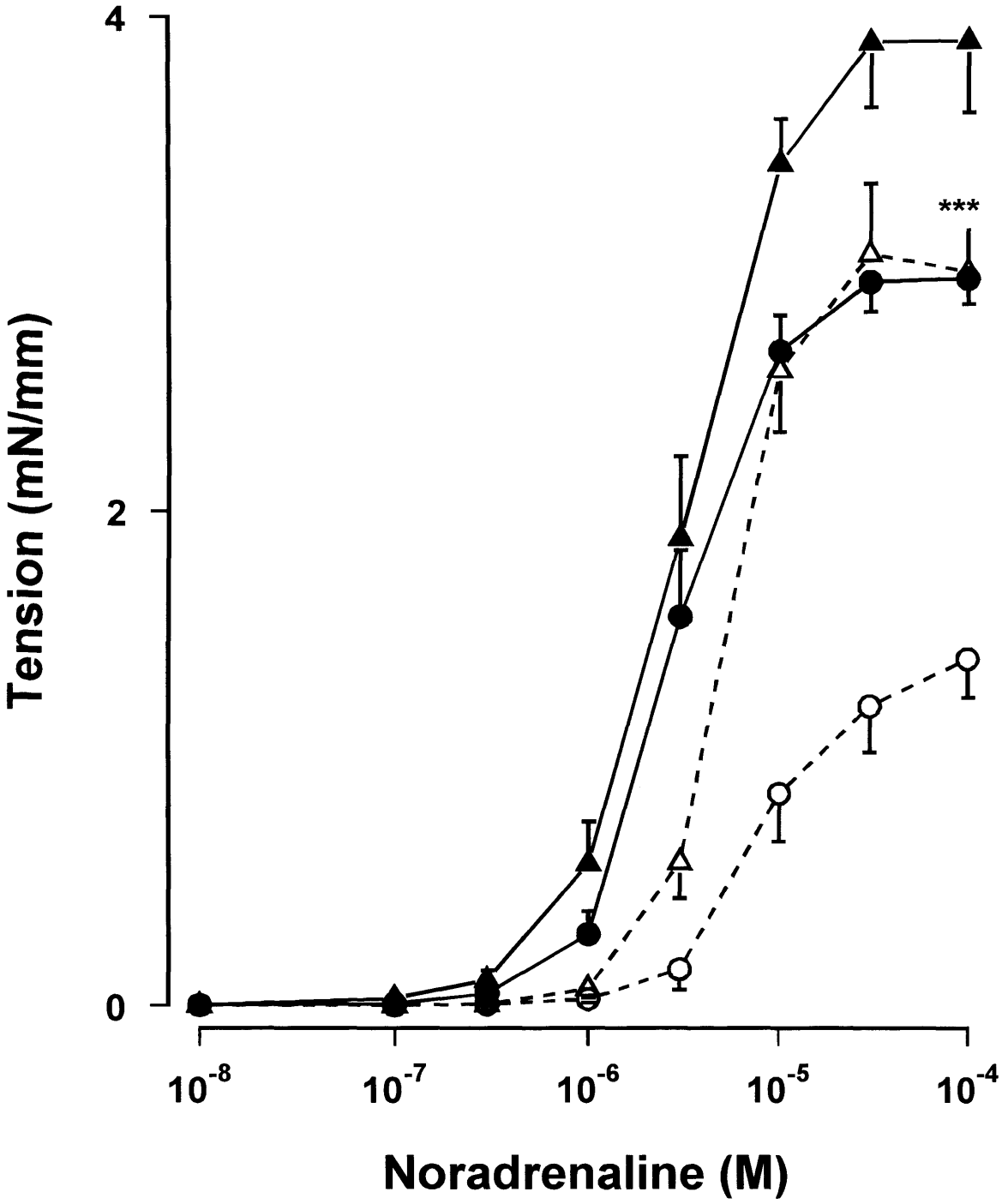

Figure 20 The noradrenaline contractile response of mesenteric vessels from SHR (triangles) and WKY (circles) rats before (closed symbols) and after (open symbols) incubation with indomethacin (10^{-5}M) ***p<0.001 denotes comparison with the WKY control line

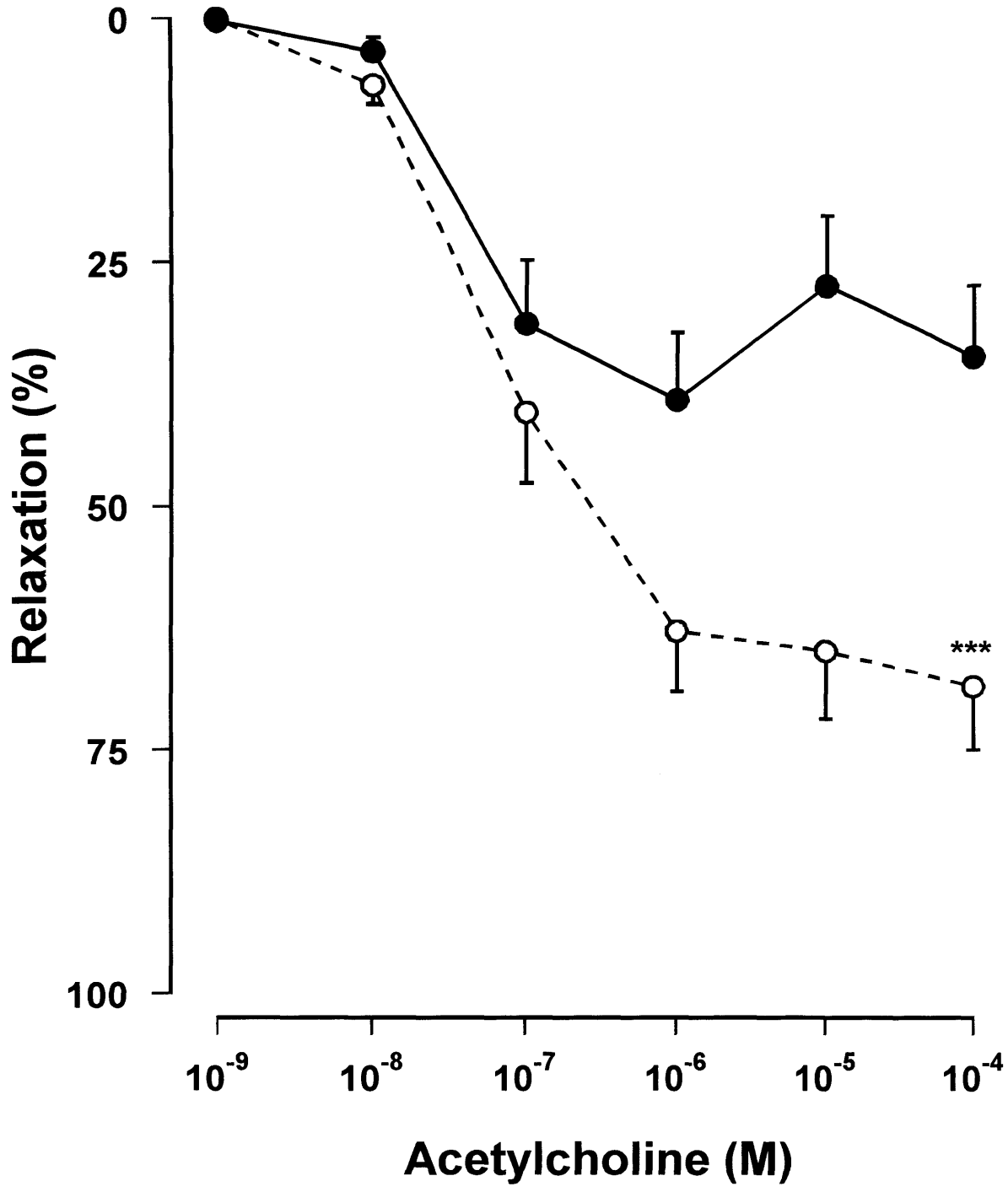

Figure 21 The acetylcholine relaxation response of mesenteric vessels from WKY rats before (●) and after (O) incubation with indomethacin (10^{-5}M) ***p<0.001 denotes comparison between the two lines

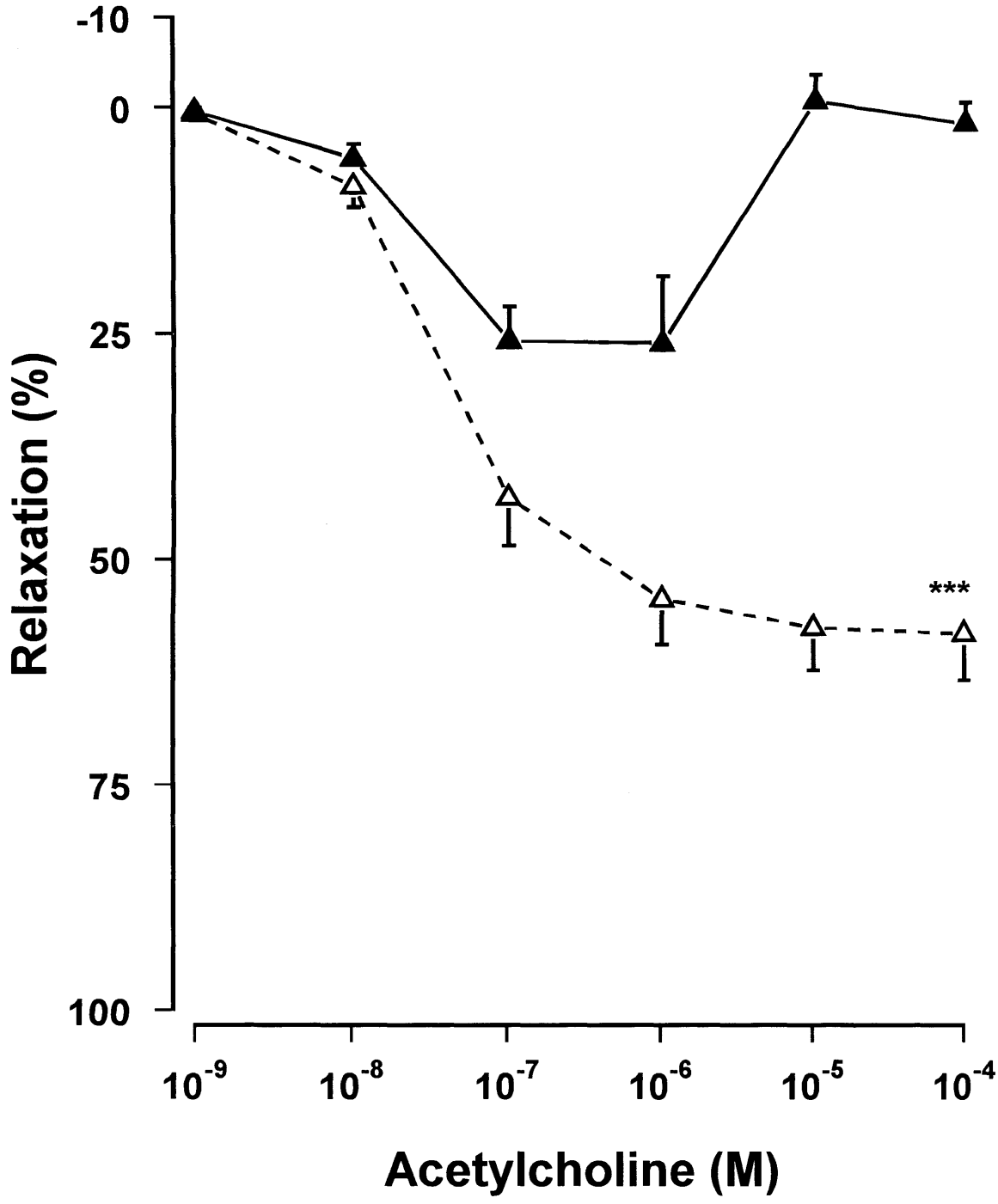

Figure 22 The acetylcholine relaxation response of mesenteric vessles from SHR rats before (▲) and after (△) incubation with indomethacin (10^{-5}M) ***p<0.001 denotes comparison between the two lines

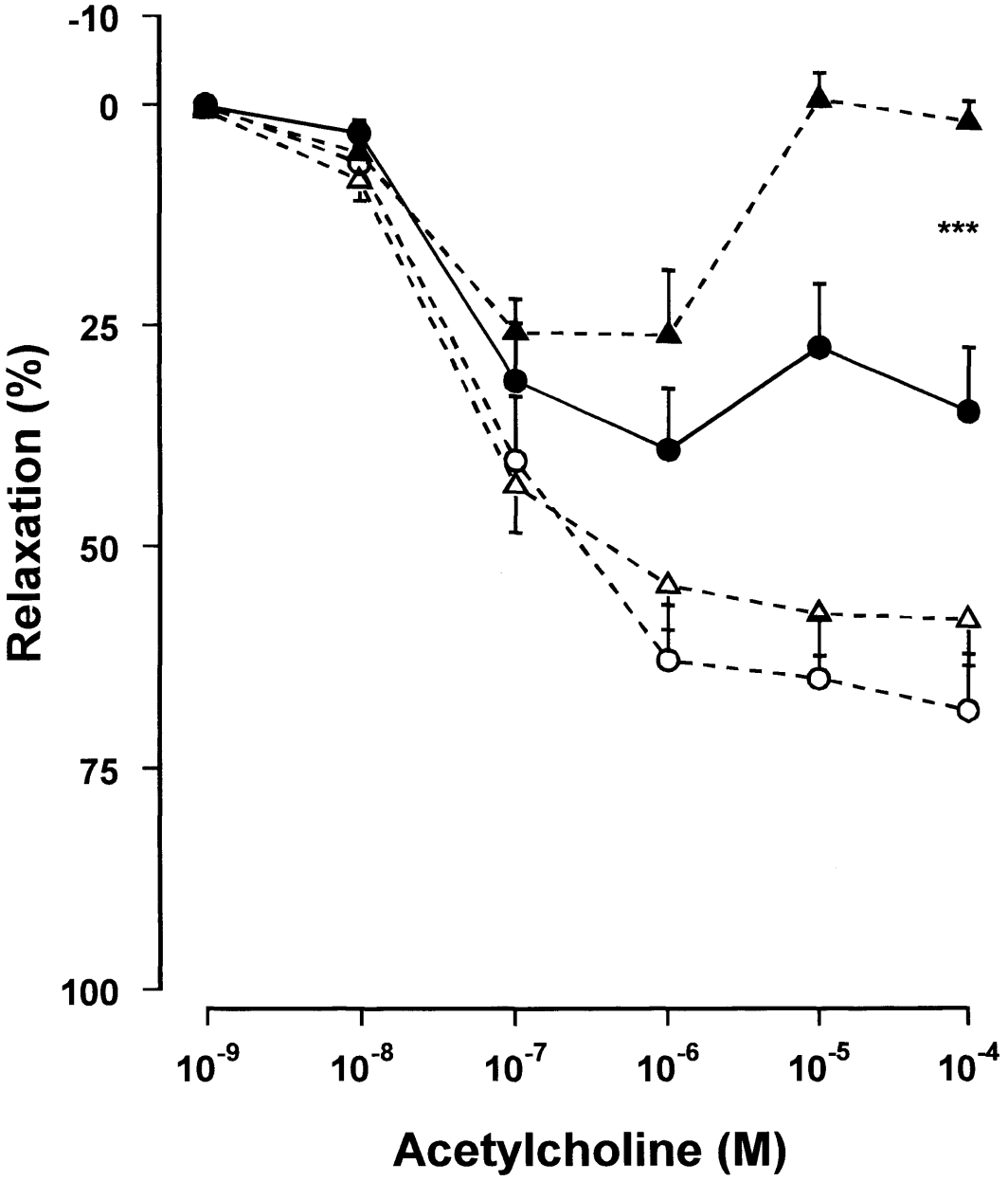

Figure 23 The acetylcholine relaxation response in the mesenteric vessels from SHR (triangles) and WKY (circles) rats before (closed symbols) and after (open symbols) incubation with indomethacin 10-5M

***** p<0.001 compared with WKY**

4.4. DISCUSSION

Pretreatment with indomethacin caused a reduction in the absolute contractile response to noradrenaline in both the SHR and WKY rats but the reduction observed in the WKY animals was much greater. The interesting feature in the SHR is that in the presence of indomethacin the SHR response was identical to the WKY response without indomethacin.

Indomethacin improved the acetylcholine induced relaxation in both the WKY and SHR. However, the WKY response appeared to be reduced compared to the previous study, though the WKY response was still significantly greater than that observed in the SHR. In the SHR the classical re-contraction was completely abolished by pre-treatment with indomethacin. Thus, vessels from both the SHR and WKY animals appear to release a prostaglandin contracting factor, although in the WKY this was less marked. This would suggest that the release of a vasoconstrictor prostaglandin in response to acetylcholine is greater in the SHR than in the WKY. The source of the vasoconstrictor substance remains to be established. Similar observations to these have been made using the thoracic aorta from adult SHR and in addition, acetylcholine was shown to cause endothelium dependent contractions which were inhibited by pretreatment with indomethacin (Luscher & Vanhoutte 1986). Moreover, Luscher et al. demonstrated a low basal but normal stimulated release of endothelium derived relaxing factor from the perfused adult SHR aorta using a two vessel bioassay system (Luscher et al. 1987b). The response to the NO donor, sodium nitroprusside, showed similar responses in the SHR and WKY, suggesting that the cGMP second messenger system in the SHR was functioning normally and that the increased vascular structure in the SHR was not influencing 'diffusion' of NO.

In conclusion, the abnormality of endothelium dependent vasodilation in the SHR does not appear to involve the reduced release of endothelium derived relaxing factor, or an increase in the length of the diffusion pathway due to structural hypertrophy. The improved response with indomethacin treatment suggests an production of a prostanoid contracting factor. However, indomethacin is a 'dirty' inhibitor in a sense because it interrupts all cyclooxygenase derived prostaglandins. Therefore, studies using a thromboxane A_2 antagonist and synthesis inhibitor were used to examine the possible role of the vasoconstrictor thromboxane A_2.

4.5. EFFECTS OF THROMBOXANE A_2 BLOCKADE

4.6. INTRODUCTION

The previous study, using the cyclooxygenase inhibitor indomethacin, suggested that a contracting prostaglandin may be overriding the effects of the endothelium derived relaxing factor. One possible candidate for this contracting factor is thromboxane A_2. Therefore, further studies were performed to ascertain whether thromboxane A_2 might be responsible using a thromboxane A_2 antagonist (SQ29548) and a thromboxane A_2 synthesis inhibitor (dazmegrel) were performed.

SQ29548 ([1S-[1α,2β(5Z),3β,4α]]-7-[3-[[2-[Phenylamino)-carbonyl] hydrazino] ethyl]-7-oxabicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid) is a competitive thromboxane A_2 receptor antagonist produced by Squibb Pharmaceuticals which has been shown to block the vascular and platelet effects of the prostaglandin endoperoxides and thromboxane A_2.
Preliminary studies are concerned with establishing the efficacy of SQ29548 as a thromboxane A_2 antagonist. The efficacy of dazmegrel (UK38485; 3-

(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid) has been established by Pfizer UK Ltd with in vitro studies using human platelet microsomal Tx-synthetase where $2x10^{-8}$M produced a half maximal inhibition.

4.7. METHOD and PROTOCOL

The efficacy of SQ29548 as a thromboxane A_2 antagonist was established using mesenteric resistance arteries from 12 week old WKY rats (10 vessels). A cumulative dose contraction response to thromboxane A_2 (10^{-8}M to $3x10^{-5}$M) was performed after which the vessels were rinsed and allowed to return to baseline. Twenty minutes later the thromboxane A_2 antagonist SQ29548 was added to the bath giving a final concentration of 10^{-7}M and the dose contraction to thromboxane A_2 was repeated. This procedure was repeated using SQ29548 10^{-6}M and 10^{-5}M.

4.8. RESULTS

Thromboxane A_2 produced a concentration dependent contraction with a maximum response of 2.14 ± 0.15mN/mm with a sensitivity (ED_{50}) of 3.181 ± 0.55μM. SQ29548, at all concentrations, caused a significant reduction in the contraction to thromboxane A_2 (10^{-7}M, Max: 1.96 ± 0.14mN/mm $p<0.01$; 10^{-6}M, Max: 1.88 ± 0.23mN/mm $p<0.001$; and 10^{-5}M, Max: 0 ± 0mN/mm $p<0.001$,)(Figure 24). SQ29548 10^{-7}M did not alter the sensitivity to thromboxane A_2 (ED_{50}: 4.33 ± 0.95μM) but SQ29548 10^{-6}M resulted in a significant decrease in sensitivity (ED_{50}: 11.476 ± 0.57μM $p<0.05$) and SQ29548 10^{-5}M totally abolished the contractile response to thromboxane A_2 (Figure 24).

This study confirmed that SQ29548 is a concentration dependent antagonist of thromboxane A_2 and the highest concentration (10^{-5}M) completely abolished the contractile response in isolated rat mesenteric resistance vessels.

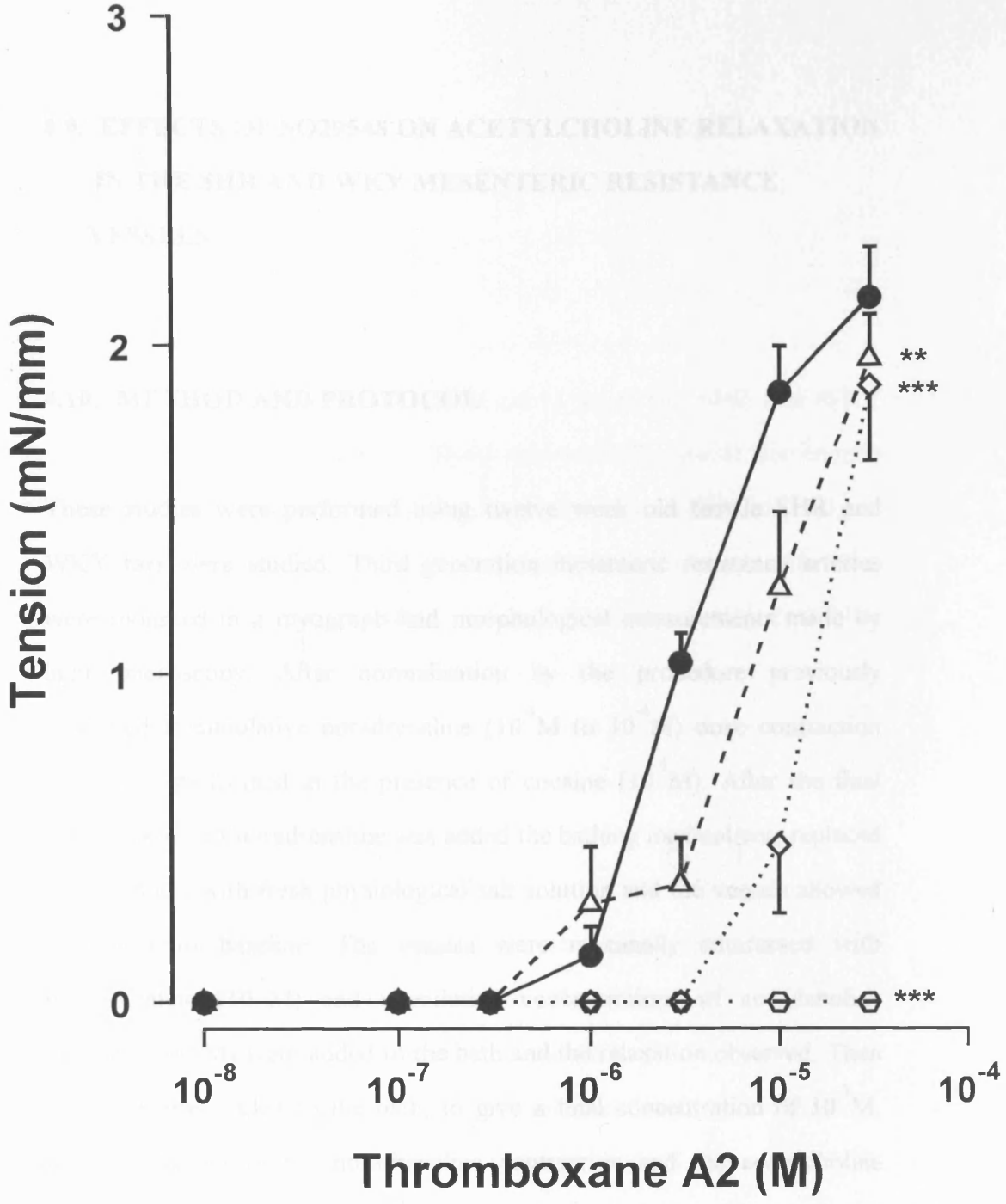

Figure 24 The thromboxane A_2 contractile response before (●) and after incubation with SQ29548 10^{-7}M (△) SQ29548 10^{-6}M (◇) and SQ29548 10^{-5}M (⬡) **p<0.01, ***p<0.001 denotes comparison with thromboxane A_2 control line

4.9. EFFECTS OF SQ29548 ON ACETYLCHOLINE RELAXATION IN THE SHR AND WKY MESENTERIC RESISTANCE VESSELS

4.10. METHOD AND PROTOCOL

These studies were performed using twelve week old female SHR and WKY rats were studied. Third generation mesenteric resistance arteries were mounted in a myograph and morphological measurements made by light microscopy. After normalisation by the procedure previously described a cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline was added the bathing medium was replaced several times with fresh physiological salt solution and the vessels allowed to return to baseline. The vessels were maximally contracted with noradrenaline (10^{-5}M) and cumulative concentrations of acetylcholine (10^{-9}M to 10^{-4}M) were added to the bath and the relaxation observed. Then SQ29548 was added to the bath, to give a final concentration of 10^{-7}M, immediately before the noradrenaline contraction and the acetylcholine relaxation curve was repeated. This procedure was repeated again in the presence of SQ29548 10^{-6}M and SQ29548 10^{-5}M. At the end of the experiment a cumulative dose contraction curve to noradrenaline was repeated after the addition of SQ29548 (10^{-5}M) to determine whether SQ29548 had an effect on the noradrenaline contractile response.

4.11. RESULTS

4.11.1. Vessel morphology

The morphology of mesenteric vessel of 12 week old SHR and WKY animals are shown in Table 10. There was no difference in the internal diameter of the SHR (197 ± 5μm) compared to the WKY (204 ± 5μm) rats. The media thickness, media volume and media to lumen ratio were significantly greater in the SHR compared to the WKY ($p<0.001$) (Table 10).

4.11.2. Contraction Studies

Noradrenaline caused a concentration dependent contraction of both WKY and SHR mesenteric resistance arteries. The contractile response to noradrenaline was significantly greater in the SHR (3.95 ± 0.26mN/mm) compared to the WKY (2.52 ± 0.13mN/mm) ($p<0.001$) (Table 9, Figure 25). There was no difference in sensitivity (ED_{50}) between the SHR (3.47 ± 1.3μM) and WKY (2.36 ± 0.68μM) animals. The maximum concentration of SQ29548 (10^{-5}M) had no significant effect on the contractile response to noradrenaline in either the SHR (4.14 ± 0.15mN/mm) or WKY (2.81 ± 0.19mN/mm) rats. Similarly, there was no alteration in the sensitivity in the SHR (5.81 ± 1.5μM) or WKY (6.17 ± 2.6μM) after treatment with SQ29548 (10^{-5}M) (Table 11, Figures 26 & 27).

4.11.3. Relaxation Studies

Acetylcholine produced a concentration dependent relaxation of mesenteric resistance vessels, maximally contracted with noradrenaline (10^{-5}M). The acetylcholine induced relaxation was significantly reduced in the SHR (37 ± 6%) compared to the WKY (71 ± 8%) ($p<0.001$) (Table 11, Figure 28). There was no alteration of the relaxation response to acetylcholine in the WKY after treatment with SQ29548 10^{-7}M (72 ± 9%), 10^{-6}M (74 ± 9%) and 10^{-5}M (69 ± 9%) (Table 11, Figure 29). However, SQ29548 10^{-6}M and 10^{-5}M caused a significant reduction in sensitivity (ED_{50}) in the WKY animals (Control 0.018 ± 0.004μM; SQ29548 10^{-6}M 0.077 ± 0.026μM, $p<0.05$; SQ29548 10^{-5}M 0.072 ± 0.017μM, $p<0.01$). SQ29548 caused a significant concentration dependent increase in the overall relaxation response to acetylcholine in the SHR ($p<0.001$) (Table 11, Figure 30). However, only pre-treatment with SQ29548 10^{-5}M caused a significant reduction in the sensitivity to acetylcholine (ED_{50}: 0.045 ± 0.10μM, $p<0.01$) compared to the untreated SHR (ED_{50}: 0.008 ± 0.002μM).

Table 10. Physical characteristics of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats used in the SQ29548 study.

	No Animals	No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
WKY	7	14	204 ± 5	13.08 ± 0.42	8909 ± 295	6.58 ± 0.37
SHR	8	16	197 ± 5	19.00 ± 1.10***	12785 ± 724***	9.87 ± 1.80***

*** $p<0.01$ SHR compared to WKY control

Table 11. Contraction and relaxation properties of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats before and after incubation with SQ29548.

	Maximum Noradrenaline Contraction		Noradrenaline sensitivity (ED_{50})		Maximum Acetylcholine Relaxation			
	control (mN/mm)	SQ (10^{-5}M) (mN/mm)	control (μM)	SQ (10^{-5}M) (μM)	control (%)	SQ 10^{-7}M (%)	SQ 10^{-6}M (%)	SQ 10^{-5}M (%)
WKY	2.52 ± 0.13	2.81 ± 0.19	2.358 ± 0.68	6.168 ± 2.60	71 ± 8	72 ± 9	74 ± 9	69 ± 9
SHR	3.95 ± 0.26***	4.14 ± 0.15	3.469 ± 1.30	5.805 ± 1.50	37 ± 6***	32 ± 7++	42 ± 6+++	46 ± 6+++

++ $p<0.01$, +++ $p<0.001$ compared to before incubation with SQ29548
*** $p<0.001$ SHR compared to WKY

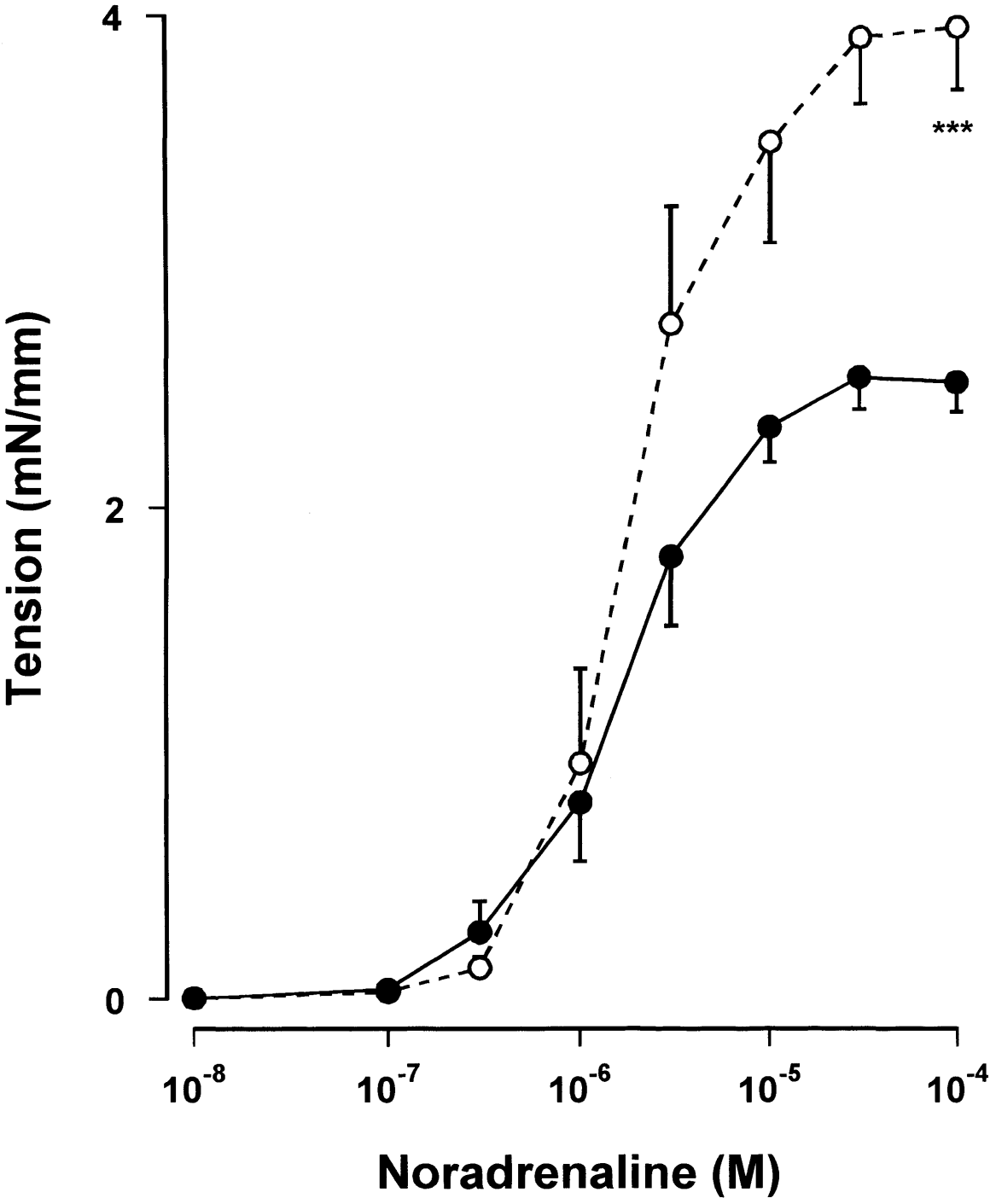

Figure 25 The noradrenaline contractile response of mesenteric vessels from SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

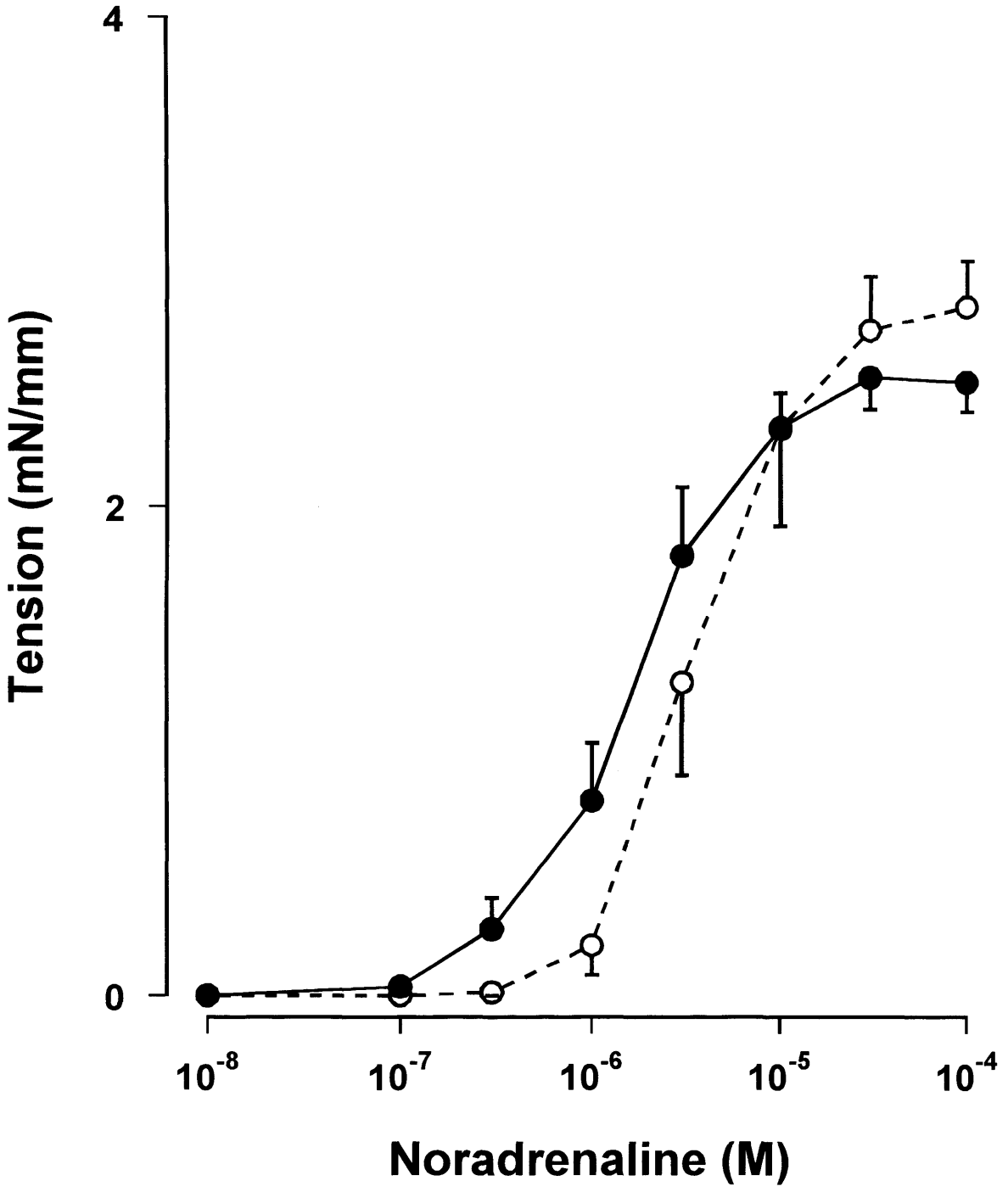

Figure 26 The noradrenaline contractile response of mesenteric vessels from 12 week WKY rats before (●) and after (O) incubation with SQ29548 (10^{-5}M)

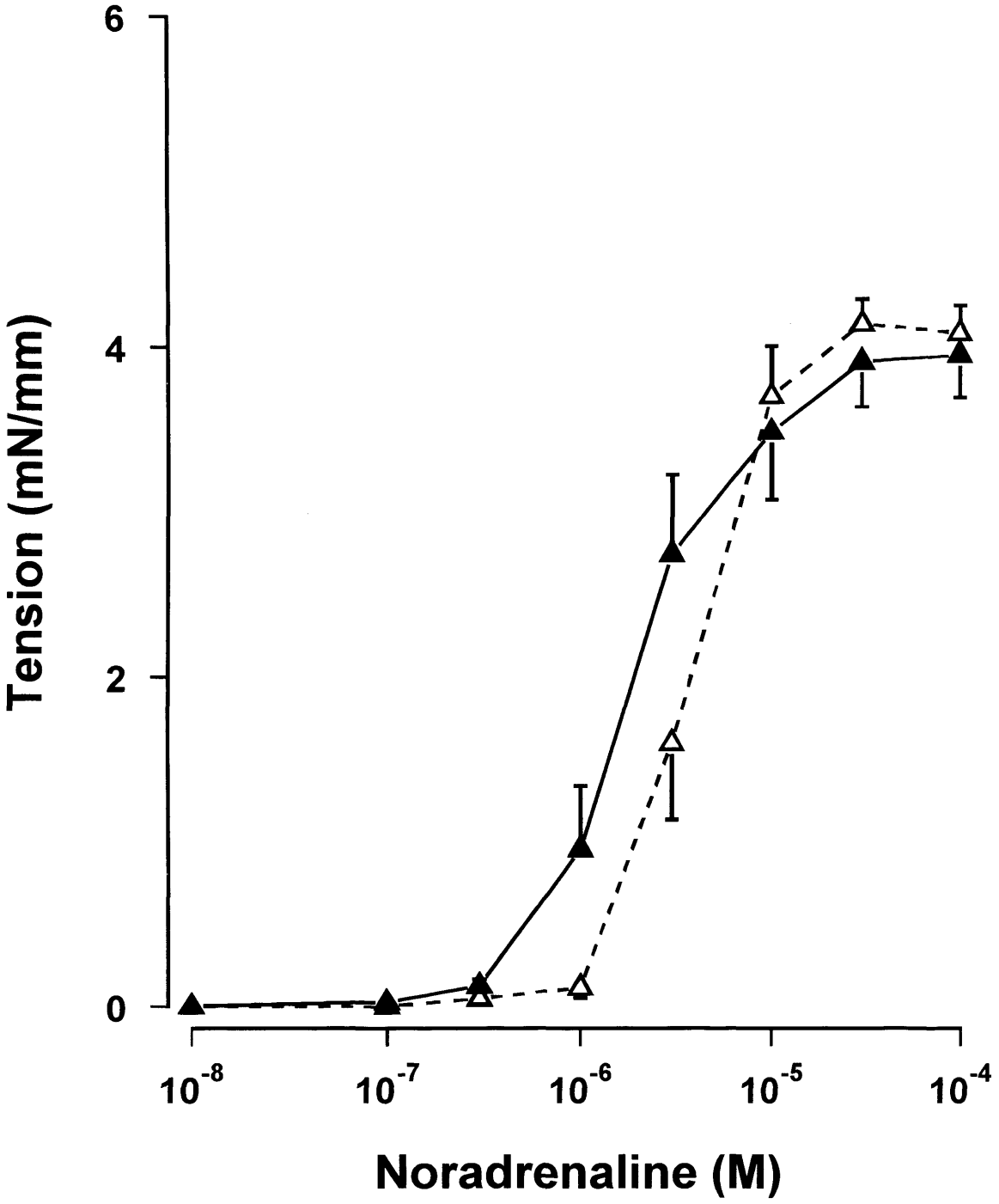

Figure 27 The noradrenaline contractile response of mesenteric resistance vessels from 12 week old SHR rats before (▲) and after (△) incubation with SQ29548 (10^{-5}M)

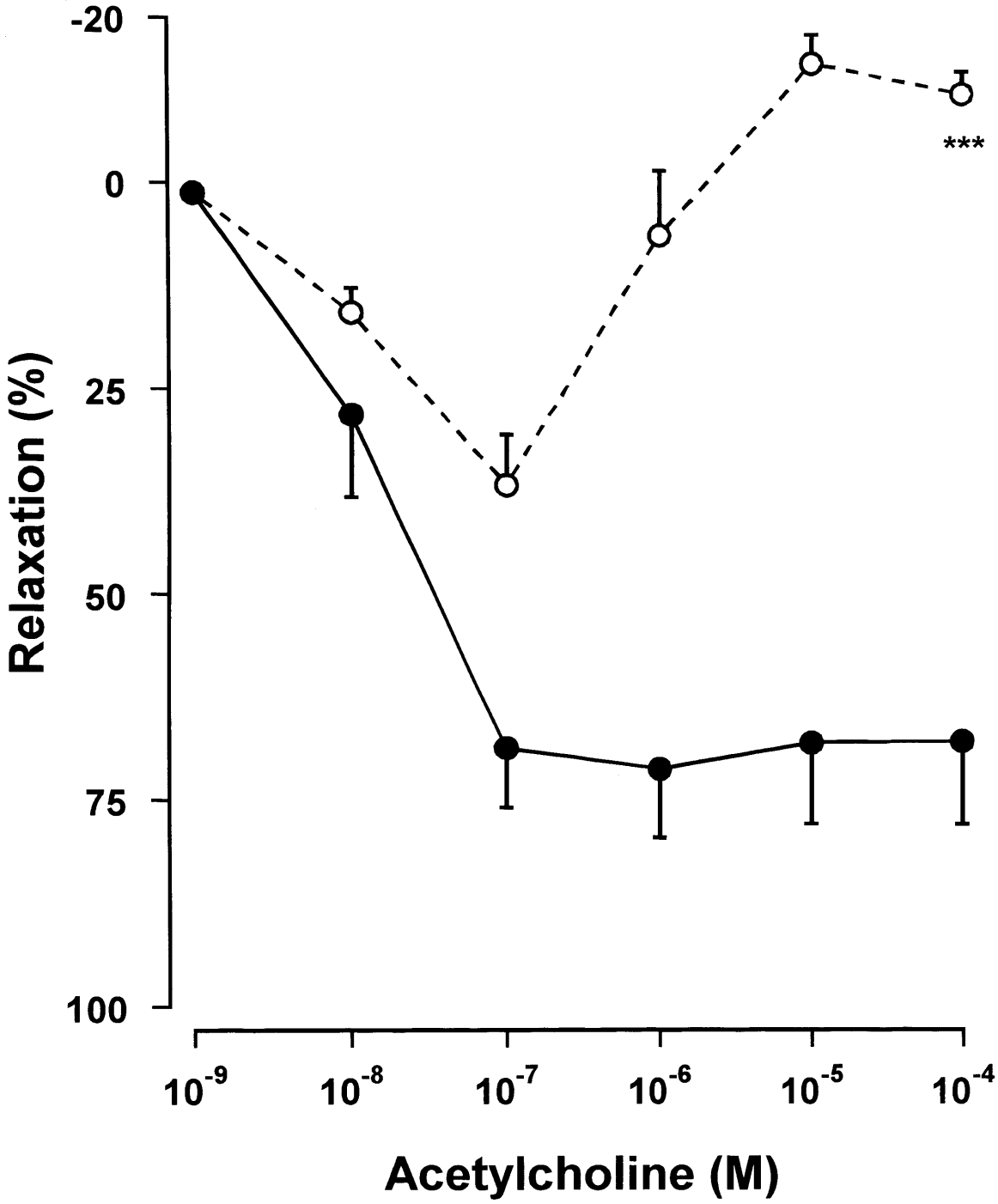

Figure 28 The acetylcholine relaxation response of mesenteric vessels from WKY (●) and SHR (O) rats ***p<0.001 denotes comparison between the two lines

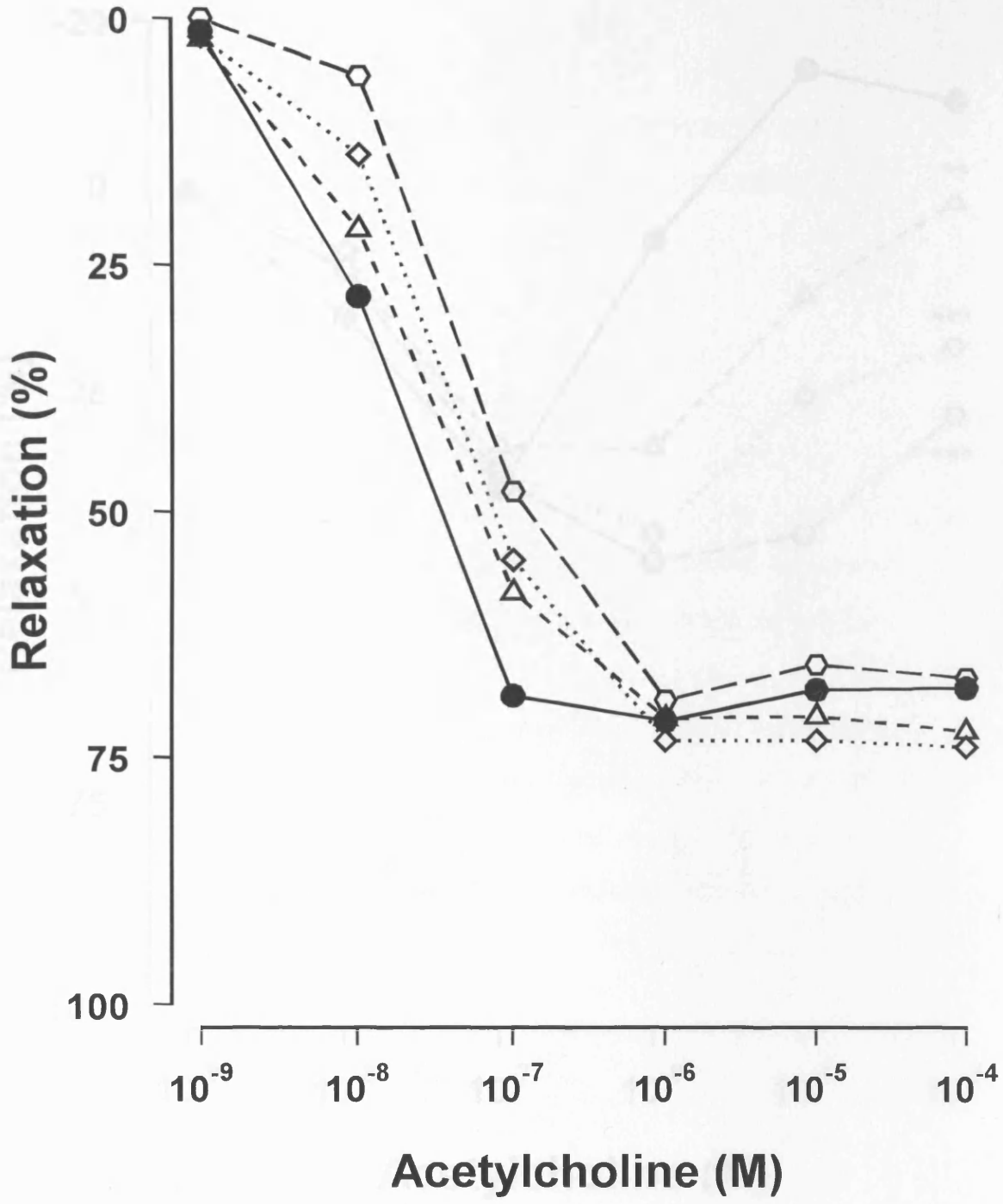

Figure 29 The acetylcholine relaxation response of mesenteric vessels from WKY rats before (●) and after incubation with SQ29548 10^{-7}M (△), SQ29548 10^{-6}M (◇) and SQ29548 10^{-5}M (○)

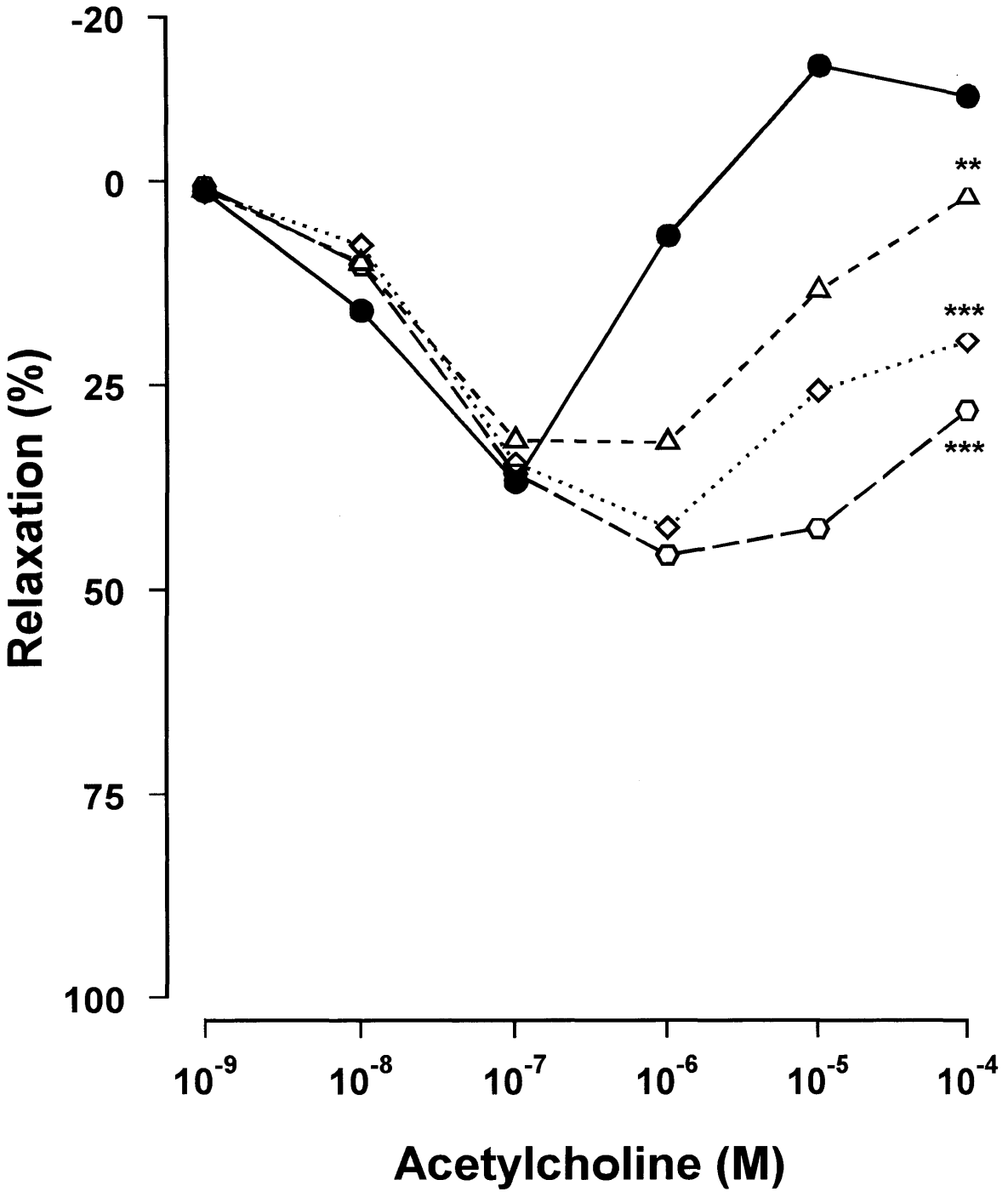

Figure 30 The acetylcholine relaxation response of mesenteric vessels from SHR rats before (●) and after incubation with SQ29548 10^{-7}M (△), SQ29548 10^{-6}M (◇) and SQ29548 10^{-5}M (⬡) **p<0.01, ***p<0.001 denotes comparison with the SHR control line

4.12. EFFECTS OF DAZMEGREL ON THE ACETYLCHOLINE RELAXATION IN THE SHR AND WKY MESENTERIC RESISTANCE ARTERY

4.13. INTRODUCTION

The previous study established that SQ29548 (10^{-5}M) is a potent antagonist producing complete inhibition of thromboxane A_2 induced contraction. Never the less, this concentration of SQ29548 failed to abolish all of the recontraction observed with acetylcholine induced release of endothelium derived relaxing factor in the SHR. To investigate the role of thromboxane A_2 release, further additional studies were performed using the thromboxane A_2 synthesis inhibitor dazmegrel to block the generation of thromboxane A_2.

4.14. METHOD and PROTOCOL

Twelve week old female SHR and WKY rats were studied as previously described in the SQ29548 study. After normalisation a cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline was added the bathing medium was replaced several times with fresh physiological salt solution and the vessels allowed to return to baseline. Then vessels were the maximally contracted with noradrenaline (10^{-5}M) and cumulative concentrations of acetylcholine (10^{-9}M to 10^{-4}M) were added to the bath and the relaxation observed. Dazmegrel was added

to the bath to achieve a final concentration of 10^{-7}M immediately before the noradrenaline contraction and the acetylcholine relaxation curves were repeated. This procedure was repeated again in the presence of dazmegrel 10^{-6}M and 10^{-5}M.

4.15. RESULTS

4.15.1. Vessel Morphology

The physical characteristics and mesenteric resistance vessel morphology of 12 week old SHR and WKY animals are shown in Table 12. The internal diameter of the SHR was significantly reduced (198 ± 6μm) compared to the WKY (244 ± 9μm) ($p<0.001$). The media thickness ($p<0.05$) and media to lumen ratio ($p<0.05$) were significantly greater in the SHR but the media volume was comparable to that in the WKY.

4.15.2. Contraction Studies

Noradrenaline caused a concentration dependent contraction in both the SHR and WKY mesenteric resistance arteries. The contractile response to noradrenaline was significantly greater in the SHR (2.96 ± 0.27mN/mm) compared to the WKY (1.96 ± 0.19mN/mm) ($p<0.001$) (Table 13, Figure 31). There was no difference in noradrenaline sensitivity (ED_{50}) between the SHR (1.82 ± 0.4μM) and the WKY (1.41 ± 0.19μM) (Table 13). Blockade of thromboxane A_2 synthesis using dazmegrel did not alter the maximum contraction to noradrenaline.

4.15.3. Relaxation Studies

Acetylcholine caused a concentration dependent relaxation of mesenteric resistance vessels, maximally contracted with noradrenaline (10^{-5}M). The acetylcholine induced relaxation was significantly reduced in the SHR (56 ± 9%) compared to the WKY (67 ± 5%) ($p<0.001$) (Table 13, Figure 32). There was significant change in the relaxation response to acetylcholine in the WKY after treatment with dazmegrel. Dazmegrel 10^{-6}M and 10^{-5}M caused a significant improvement in the overall relaxation response to acetylcholine ($p<0.05$) (Table 13, Figure 33) but this was associated with a reduction in acetylcholine sensitivity in the presence of dazmegrel 10^{-5}M (WKY control 0.045 ± 0.013μM; 10^{-5}M 0.219 ± 0.056μM, $p<0.05$). In the SHR dazmegrel caused a significant concentration dependent increase in the overall acetylcholine relaxation response ($p<0.05$) without an alteration in sensitivity (Table 13, Figure 34).

Table 12. Physical characteristics of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats used in the thromboxane A_2 synthesis inhibitor study with dazmegrel.

	No Animals	No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
WKY	12	20	244 ± 9	11.57 ± 0.22	9586 ± 420	4.67 ± 0.23
SHR	9	18	198 ± 6***	19.83 ± 1.93*	13639 ± 1389	10.02 ± 0.93*

* $p<0.05$, *** $p<0.001$ SHR compared to WKY control

Table 13. Contraction and relaxation properties of mesenteric vessels from 12 week old spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats used in the thromboxane A2 synthesis inhibitor study with dazmegrel

	Maximum Noradrenaline Contraction		Noradrenaline sensitivity (ED_{50})		Maximum Acetylcholine Relaxation			
	control	Dazmegrel	control	Dazmegrel	control	Dazmegrel		
						10^{-7}M	10^{-6}M	10^{-5}M
	(mN/mm)	(mN/mm)	(μM)	(μM)	(%)	(%)	(%)	(%)
WKY	1.96 ± 0.19		1.413 ± 0.19		67 ± 5	71 ± 6	67 ± 6^{+}	69 ± 6^{+}
SHR	2.96 ± 0.27***		1.818 ± 0.40		56 ± 9***	54 ± 8^{+}	56 ± 7^{+}	59 ± 8^{+}

+ $p<0.05$ compared to before incubation with dazmegrel

*** $p<0.001$ SHR compared to WKY

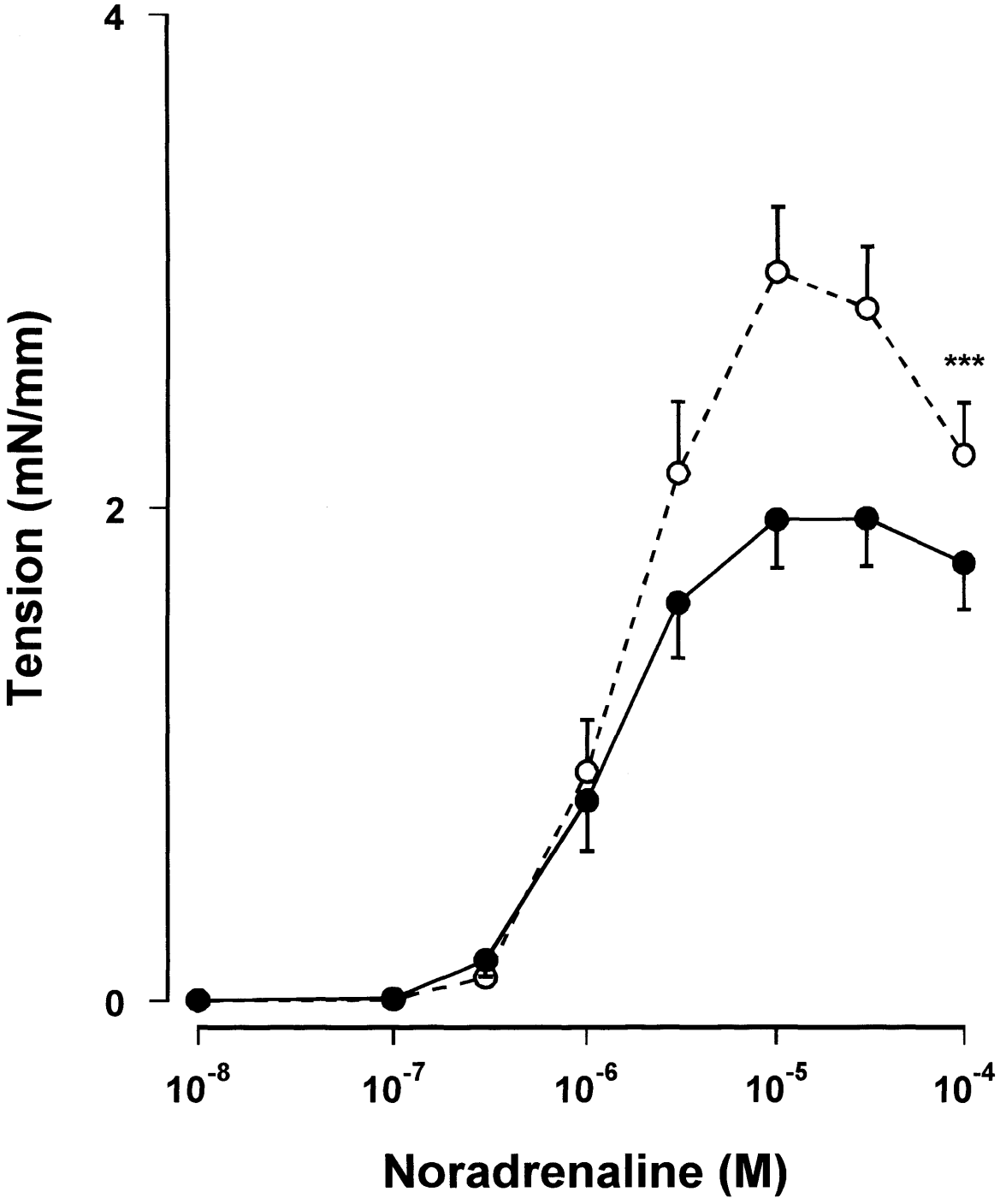

Figure 31 The noradrenaline contractile response of mesenteric vessels from 12 week old SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

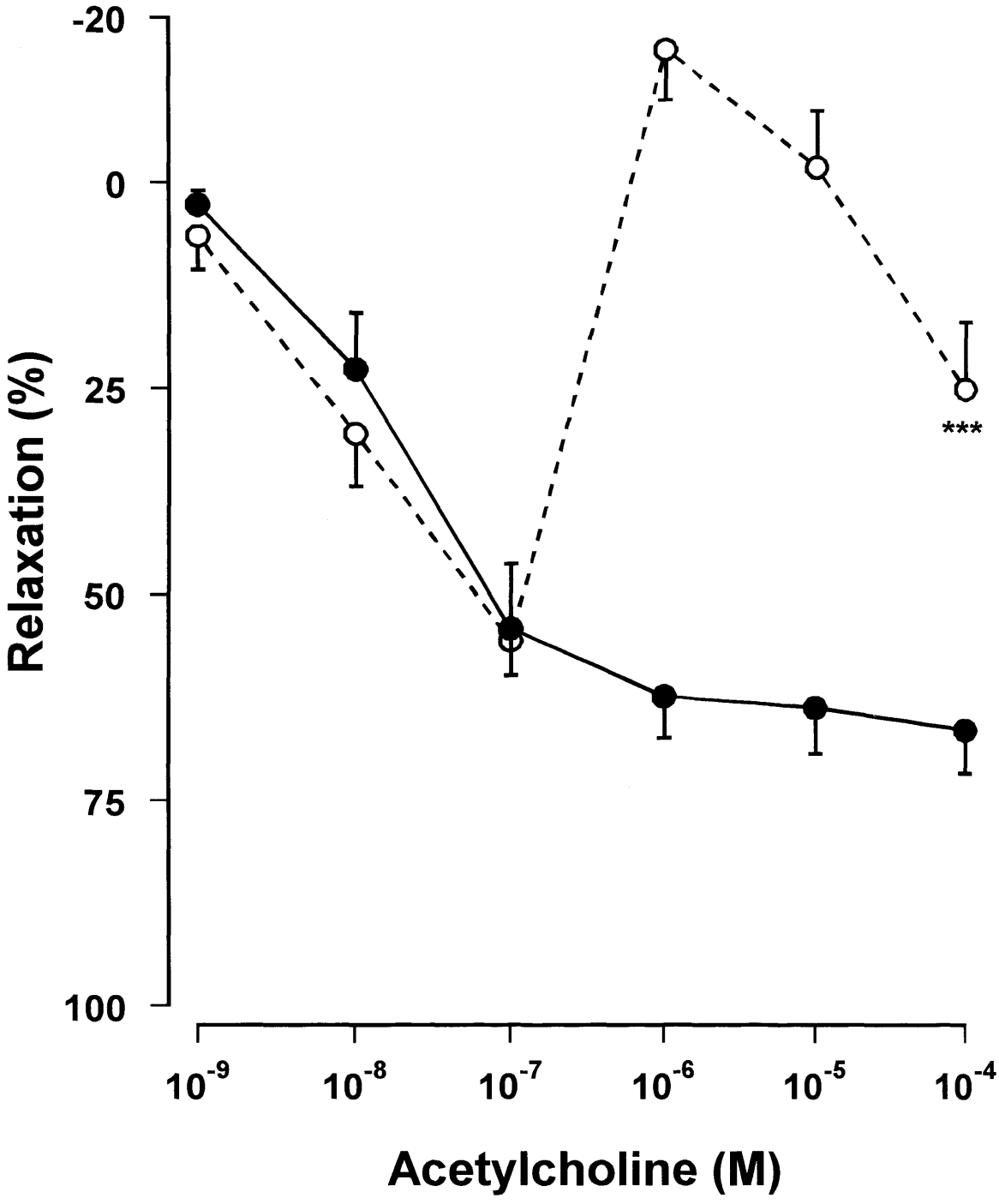

Figure 32 The acetylcholine relaxation response of mesenteric vessels from SHR (O) and WKY (●) rats ***p<0.001 denotes comparison between the two lines

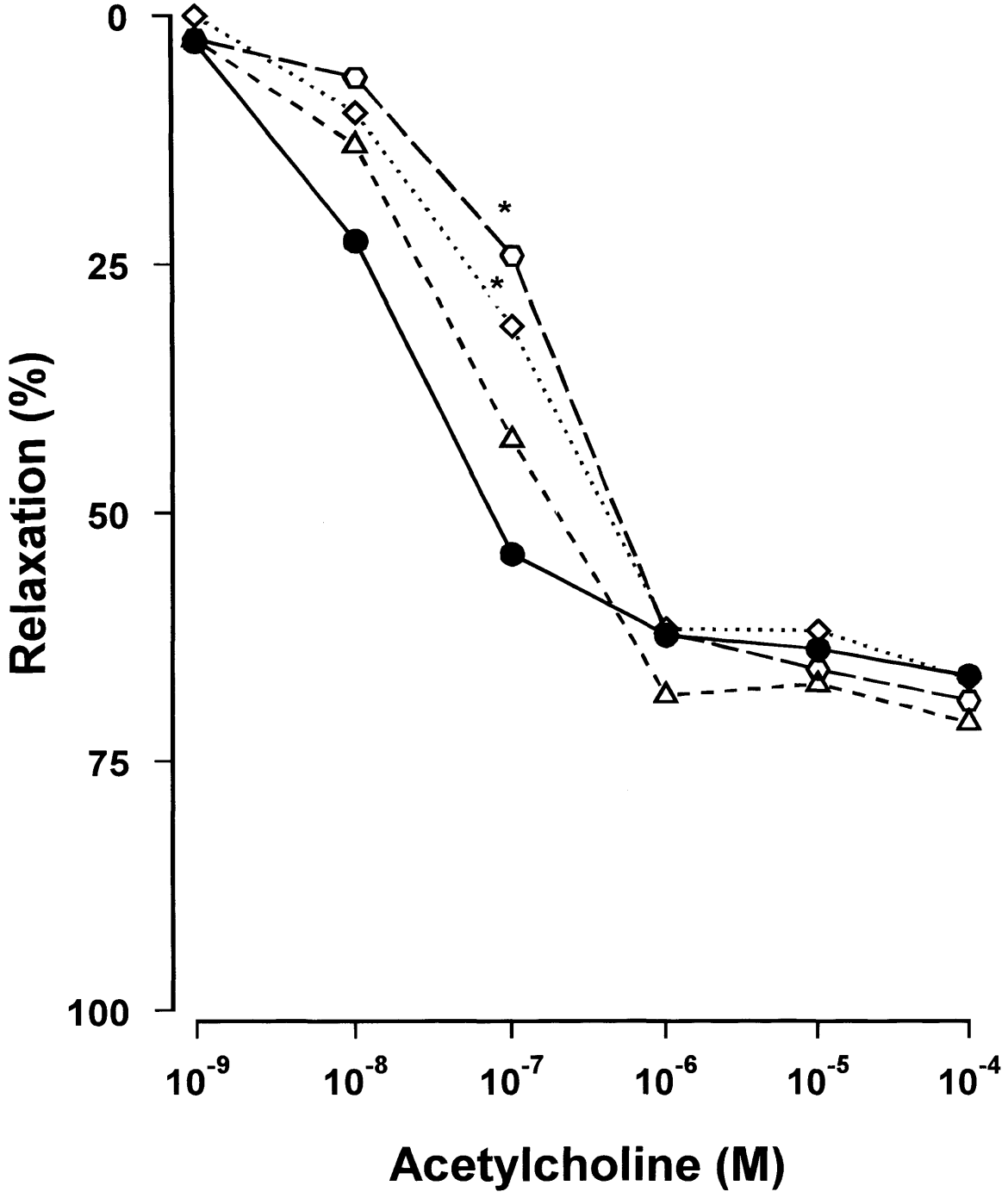

Figure 33 The acetylcholine relaxation response of mesenteric vessels from WKY rats before (●) and after incubation with dazmegrel 10^{-7} M (△), dazmegrel 10^{-6}M (◇)and dazmegrel 10^{-5}M (⬡) *p<0.05 denotes comparsion to the WKY control line

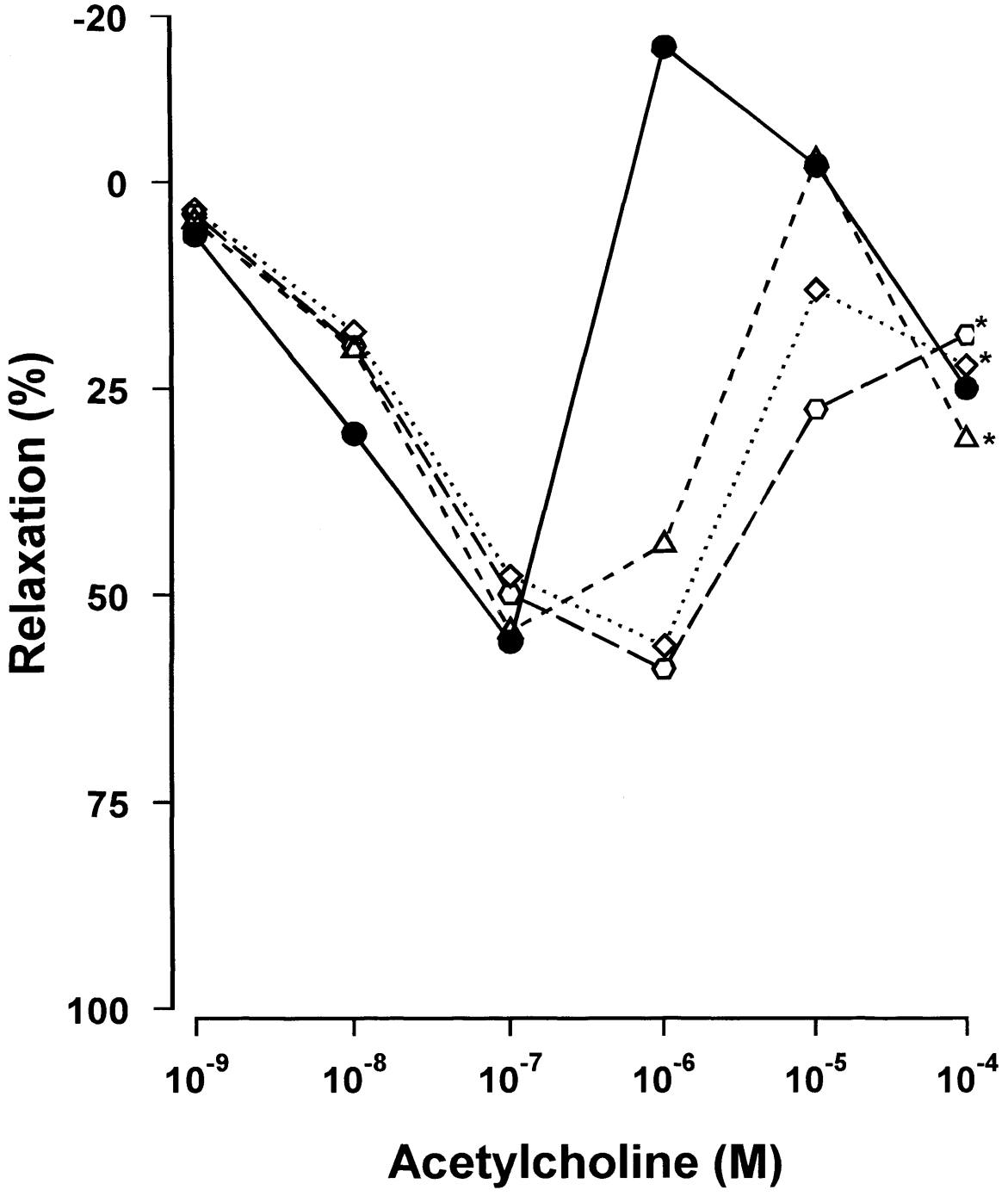

Figure 34 The acetylcholine relaxation response of mesenteric vessels from SHR rats before (●) and after incubation with dazmegrel 10^{-7}M (△), dazmegrel 10^{-6}M (◇) and dazmegrel 10^{-5}M (⬡) *p<0.05 denotes comparison with the SHR control line

4.16. DISCUSSION

This series of experiments were designed to examine whether increased amounts of thromboxane A_2 is released by SHR mesenteric resistance vessels in response to the endothelium dependent vasodilator, acetylcholine. The thromboxane A_2 contractile response was reduced by SQ29548 10^{-7} M & SQ29548 10^{-6}M and completely abolished by the presence of SQ29548 10^{-5}M. However, this concentration of SQ29548 improved but failed to totally abolish the re-contraction in the SHR. Similarly, use of the thromboxane A_2 synthesis inhibitor, dazmegrel, also failed to totally abolish the re-contraction observed with acetylcholine in the SHR. On the other hand, the relaxation responses were significantly improved with both compounds, but the improvement was not comparable to that observed using the cyclooxygenase inhibitor indomethacin suggesting that a constrictor prostaglandin other than thromboxane A_2 may be being released. One candidate could be prostaglandin H_2.

Prostaglandin H_2 (PGH_2) has a half life of approximately 5 minutes in aqueous solution (Hamberg et al. 1974) and is rapidly metabolized into PGI_2, $PGF_{2\alpha}$, PGD_2 and TXA_2. In aortic rings, acetylcholine increases the production of PGI_2 and PGE_2 (Chatty et al. 1990). Koga and colleagues (Koga et al. 1989) demonstrated that thromboxane A_2 release contributed to but did not fully explain the endothelium derived contraction from spontaneously hypertensive rat aorta. However, a role for other endothelium derived contracting factors such as angiotensin II (Kifor & Dzau 1987), endothelin (Yanagisawa et al. 1988) and oxygen free radicals (Katusic & Vanhoutte 1989) have been postulated. Endothelin is unlikely to be the cause of the endothelial abnormality in the SHR because the contractile response to endothelin is not inhibited by pre-treatment with

indomethacin (Yanagisawa et al. 1988). On the other hand it has been demonstrated that oxygen free radicals are generated during the conversion of PGG_2 to PGH_2 by activation of endothelial cyclooxygenase (Kontos et al. 1985) and it has been suggested that this could mediate endothelium dependent contraction observed in the basilar artery of the dog (Katusic & Vanhoutte 1989). However, pre-treatment with free radical scavengers have no effect on the endothelium dependent contraction to acetylcholine in the aorta of the SHR, indicating that EDCF is chemically distinct from oxygen free radicals (Auch-Schwelk et al. 1989).

Ito and colleagues (1991) have demonstrated that inhibition of TXA_2/PGH_2 receptors with ONO-3708 completely abolished contraction to acetylcholine in aorta from the SHR. They concluded that acetylcholine induced contractions were unlikely to be due to the superoxide anion concomitantly produced by the cyclooxygenase pathway and that the re-contraction observed was possibly due to PGH_2.

It is apparent that in some forms of experimental hypertension e.g. induced by salt or angiotensin II, TXA_2/PGH_2 may play a pivital role in elevating blood pressure (Mistry & Nasjletti 1988). It has been suggested that there may be a relative decrease in antihypertensive eicosanoids and in some cases a coincidental increase in prohypertensive eicosanoids (Quilley at el. 1990). In both SHR and Goldblatt one kidney one clip hypertensive rats the increase in PGI_2 formation correlated with a rise in blood pressure. Moreover, studies of human and experimental hypertension have shown that a decrease in blood pressure normalised the urinary excretion of PGE_2 and vascular prostaglandin synthesis.

Human hypertensive subjects, Dahl salt rats and spontaneously hypertensive rats all exhibit an impaired compensatory increase in prostaglandin synthesis when put on a high salt diet. Enhanced TXA_2 synthesis has been documented in the SHR and in other studies there appears to be an imbalance of pro- versus anti-hypertensive prostanoids. The ratio of glomerular PGE_2/PGI_2 to TXA_2 is lower in the SHR than WKY despite an overall increase in prostanoid synthesis. In the SHR, Shibouta et al. have demonstrated enhanced renal release of TXA_2 in response to angiotensin II during development of hypertension (Shibouta et al. 1981) and both urinary and serum TXB_2 levels were higher in the SHR, suggesting a role for TXA_2 in the development of hypertension. The most convincing evidence for a role of TXA_2 in hypertension was provided by Shibouta and colleagues (1982). They demonstrated that a TXA_2 receptor antagonist, pinane TXA_2, reversed the increase in renal vascular resistance and decreased glomerular filtration rate in six week old SHRs. The response to thromboxane A_2 synthase inhibitors may not provide an accurate reflection of the role of TXA_2 in hypertension since precursor endoperoxides may be diverted to antihypertensive prostanoids or may exert a vasoconstrictor effect of their own by interaction with TXA_2/PGH_2 receptors (Quilley et al. 1989).

The present series of studies using a TXA_2 receptor antagonist or a TXA_2 synthase inhibitor has not confirmed or disproved the hypothesis that impaired endothelial dependent relaxation in the SHR is a result of an abnormal release of thromboxane A_2. Further studies using combined TXA_2/PGH_2 receptor antagonist may help elucidate whether the prostaglandin contracting factor in hypertension is TXA_2 or PGH_2.

4.17. CONCLUSIONS

1. Acetylcholine induced relaxation in both the SHR and WKY is associated with the release of an endothelial derived contracting factor which can be inhibited by pre-treatment with the cyclooxygenase inhibitor, indomethacin. The effect of the prostaglandin contracting factor is overridden in the WKY but in the SHR increased amounts are released overcoming the endothelium dependent relaxation.

2. It appears that abnormal endothelial function in the SHR is caused by the release of increased amounts of contracting prostaglandins. Since acetylcholine and shear stress share the same G protein system it is likely that the dysfunction may play an important role in blood pressure elevation in the SHR.

3. Treatment with the thromboxane A_2 antagonist SQ29548 did not alter the contractile response to noradrenaline in either the SHR or WKY but SQ29548 significantly improved the acetylcholine relaxation response in the SHR.

4. The thromboxane A_2 synthesis inhibitor, dazmegrel, improved the acetylcholine relaxation response in the SHR but failed to prevent the recontraction observed at the high concentrations of acetylcholine.

5. These results are in keeping with the view that the endothelial contracting factor is thromboxane A_2 or another prostaglandin such as PGH_2 which can be partially inhibited by thromboxane A_2 antagonists.

CHAPTER 5 GOLDBLATT TWO KIDNEY ONE CLIP HYPERTENSION STUDIES IN THE ACUTE AND CHRONIC PHASES

5.1. INTRODUCTION

Richard Bright in 1836 was the first to recognise the association between renal disease and hypertension. His observations stimulated the search for the mechanism by which the kidney might produce these effects. In 1898 Tigerstedt and Bergmann discovered renin and established a mechanism to link renal disease and cardiac hypertrophy, though its importance was not immediately appreciated because other studies failed to repeat their results (Pickering 1968) and a reproducible model of chronic experimental renal hypertension was lacking.

There followed many attempts to induce chronic hypertension by partial nephrectomy (Cash 1924 and Chautin & Ferris 1932), irradiating the kidneys (Hartman et al. 1926) and banding the renal vein in combination with wrapping the kidneys in a cellophane membrane (Pederson 1927). However, it was Goldblatt and his colleagues (1934) who developed a reliable model by constricting the renal arteries in a dog with adjustable silver clamps. This work produced an experimental model of hypertension and provided the stimulus for others to make detailed biochemical studies of the renin angiotensin system (Braun-Menedez et al. 1940; Page & Helmer 1940 and Skeggs et al. 1954). Goldblatt's technique was later modified for use in other species and five years after Goldblatt's original observations there were three major experimental renovascular models of hypertension (Rytand 1938 and Page 1939).

5.1.1. Goldblatt hypertension

Goldblatt's experiments in dogs involved either bilateral constriction of the renal arteries or unilateral constriction with a contralateral nephrectomy. In 1938 Pickering and Prinzmetal induced hypertension in rabbits and it soon became clear that constriction of one renal artery, the other kidney being untouched, produced transient hypertension of a few days/weeks duration in the dog (Goldblatt et al. 1934) and rabbit (Pickering & Prinzmetal 1938b). The failure of what was later to be called the Goldblatt two kidney one clip hypertension in the dog was attributed to the antihypertensive action of the contralateral kidney. However, subsequent studies showed that the transient hypertension in the dog was overcome by the development of an effective collateral circulation with vessels growing into the kidney from perivascular tissues (Cerque & Sanaan. 1939)

In 1939 Wilson and Byrom adapted the Goldblatt technique for the rat by the use of a silver clip, to constrict the renal artery, and it compared favourably with the hypertension seen in man induced by unilateral renal ischaemia. In addition, Brown and colleagues (1976) proposed an additional classification of this renovascular hypertension, based on the level of plasma renin and the response to corrective surgery. In this study the period following application of the constricting clip until 6 weeks after clipping was referred to as the acute phase and the period after 16 weeks, the chronic phase (Thurston et al. 1980b).

5.1.2. Haemodynamic measurements

In the established phase, all forms of renovascular hypertension are characterised by an elevated peripheral vascular resistance in the face of a normal or reduced cardiac output (Ferrario & Page 1978). The majority of studies in the Goldblatt two kidney one clip model indicate that hypertension is maintained by an elevated peripheral vascular resistance (Bianci et al. 1972; Averill et al. 1976; Hallback-Nordlander et al. 1979 and Russell et al. 1983). One study, of both anaesthetised and conscious dogs, reported a small increase in cardiac output, extracellular fluid volume and extra plasma volume 24 hours after renal artery constriction (Bianci et al 1972). However, all these variables returned to normal within seven days except for the blood pressure and peripheral vascular resistance, which remained elevated. In the two kidney one clip hypertensive rat the elevated peripheral vascular resistance is associated with a reduction in cardiac index in both the early and chronic phases (Russell et al 1983). The vascular capacitance also is reduced, with no change in compliance (Edmunds et al 1989 and Yamamoto & Ogino 1982). On the other hand, mean circulatory filling pressure is increased and this helps maintain cardiac output in the face of increased vascular resistance (Edmunds et al 1989).

The increased peripheral resistance of early phase experimental renal hypertension could be due to a neurohumoral mechanism causing a stimulating increase in vascular smooth muscle tone, but in the established phase there may be reinforcement by structural change within the blood vessels. Vascular hypertrophy accentuates the decrease in lumen diameter, producing an elevated resistance despite normal levels of vasoconstrictor activity (Folkow et al 1973). Structural changes develop rapidly, thus left ventricular hypertrophy can be demonstrated within seven days of renal

artery constriction in the rat and vascular change after approximately three weeks (Lundgren & Weiss 1979). Recently these indirect observations, using the isolated perfused hindlimb preparation, have been confirmed by in vitro morphological measurements of isolated mesenteric resistance arteries from hypertensive rats four weeks after renal artery constriction (Mulvany & Korsgaard 1983). However, structural vascular change cannot be the sole determinant of the raised peripheral resistance, because removal of the constricting clip in two kidney one clip hypertension results in a rapid fall in blood pressure, to normal levels within twenty four hours (Ferrario 1974; Russell et al. 1983 and Thurston et al 1980b), whereas structural vascular changes take several weeks to regress after reversal of hypertension (Lundgren & Weiss 1979 and Mistry et al 1983).

In summary, the elevated blood pressure of two kidney one clip hypertension depends on an increased peripheral vascular resistance with a moderate reduction in cardiac output.

The previous study in the spontaneously hypertensive rat indicated that structural and functional alterations may contribute to the raised vascular resistance. Therefore, this study was designed to examine the development of structural changes in the vasculature and functional alterations during the acute (< 6 weeks) and chronic (> 16 weeks) phases of renovascular hypertension

5.2. METHODS

5.2.1. Induction of Hypertension

Nine week old white female Wistar rats, weighing 170-190g were used throughout. Goldblatt two kidney one clip hypertension was induced by placing a constricting silver clip around the left renal artery under ether anaesthesia, the contralateral kidney being left undisturbed. A loin incision was made in the abdomen, the left kidney exposed and lifted clear of the body wall. The kidney, renal artery and vein were carefully cleaned of fat and connective tissue with cotton buds. The artery was separated from the vein using a small pair of forceps and a silver clip with an internal diameter of 0.2mm was placed around the main renal artery. The clips were made from annealed silver ribbon which was cut into 15mm lengths and bent around a steel feeler gauge to obtain a precise internal diameter. In order to facilitate the clipping process clips were arranged to have one short and one long arm. After clipping, the kidney was replaced taking care not to occlude the artery. The fat was replaced, the wound sutured in layers and the animal allowed to recover. The contralateral kidney remained untouched. A control group of rats underwent a sham operation where the surgical procedure was identical except that a non-constricting strip of silver was placed between the renal artery and the vein. This procedure has been described in detail by Byrom (1969). The rats were maintained on standard laboratory chow with water ad libitum. Indirect anaesthetised systolic blood pressure was measured at intervals after surgery. When a clipped animal attained a systolic blood pressure greater than or equal to 150mmHg it was considered to be hypertensive. Sham animals also had their systolic blood pressure measured, at intervals, and on no occasion exceeded 130mmHg

As hypertension developed the animals were divided into two groups. The study of hypertension over a four to six week period was regarded as the acute Goldblatt two kidney one clip phase and hypertension at sixteen weeks post-operation was considered to be the chronic Goldblatt two kidney one clip phase. The sham controls were studied in parallel with the acute and chronic animals.

5.2.2. Measurement of Blood Pressure

Systolic blood pressure was measured under light ether anaesthesia using a tail cuff method (Swales & Tange 1970) at varying intervals during the development of hypertension (See Chapter 2). Animals with a systolic blood pressure ≥150mmHg were considered to be hypertensive. At least 24 hours was allowed to elapse after blood pressure measurements before the animals were culled for resistance vessel studies.

5.3. PROTOCOL

Animals were studied together with age matched controls at 4-6 weeks (acute) and 16 weeks (chronic) after induction of hypertension. Animals were killed by stunning followed by cervical dislocation. The heart was removed, cleaned of blood clots and fat before being weighed (See Chapter 2). Arterial resistance vessels were taken from the superior mesenteric bed which supplies the jejunum at a point 8-10cm from the pylorus. The third generation branch was used in all preparations. Arteries were cleaned of fat and surrounding tissue before being mounted in a myograph. The vessels were maintained in physiological salt solution at 37°C and gassed with $5\%CO_2/95\%O_2$ to achieve a pH of 7.4.

After equilibrating for 60 minutes the vessel morphology was determined using water immersion light microscopy. Media cross sectional area (equivalent to media volume per unit length) was calculated from the media thickness and the internal circumference (See chapter 2). The length tension characteristics for each vessel was determined and the vessel set to $L_{0.9}$. This is 90% of the internal diameter that the relaxed vessel would have had in vivo and under a transmural pressure of 100mmHg (13.3kPa). After the vessels were normalised a further 60 minutes was allowed to elapse before they were stimulated three times with a high potassium physiological solution (KPSS) followed by KPSS containing noradrenaline (10^{-5}M). The vessels bathing medium was replaced after each contraction and the vessels allowed to return to baseline. A cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline the bathing medium was replaced several times with fresh physiological salt solution and the vessels allowed to return to baseline.

The vessels were then maximally contracted with noradrenaline (10^{-5}M) and cumulative concentrations of acetylcholine (10^{-9}M to $3x10^{-5}$M) were added and the relaxation observed. Finally, the vessels were maximally contracted with noradrenaline and cumulative concentrations of sodium nitroprusside (10^{-9}M to 10^{-4}M) were added and the relaxation observed. Two vessels were studied from each animal and the results averaged. The relaxation response was expressed as the percentage decrease from noradrenaline maximal contraction.

5.4. RESULTS - ACUTE HYPERTENSION

5.4.1. Physical Characteristics

The blood pressure, body weight, heart weight and heart to body weight ratio of the acute Goldblatt two kidney one clip (2K1C) and sham operated controls (sham) are shown in Table 14. The systolic blood pressure was significantly higher in the hypertensive animals (166 ± 5 mmHg, $p<0.001$) compared to sham operated controls (105 ± 3 mmHg). The body weight of the hypertensive animals (253 ± 9g) was similar to the sham operated controls (253 ± 8g). The heart weight (clip: 1.0670 ± 0.048g; sham: 0.8657 ± 0.028g, $p<0.01$) and the heart to body weight ratio (clip: 0.422 ± 0.013%; sham: 0.345 ± 0.010, $p<0.001$) both were significantly increased in the hypertensive animals compared to the sham operated controls (Table 14).

5.4.2. Vessel Morphology

The vessel morphology is shown in Table 15. The internal diameter of the mesenteric resistance arteries of the acute hypertensive animals (220 ± 10μm) was similar to the sham operated controls (217 ± 9μm). The media volume (clip: 11448 ± 1000μm^3; sham: 7512 ± 412μm^3, $p<0.001$), media thickness (clip: 15.85 ± 1.20μm; sham: 10.62 ± 0.62μm, $p<0.01$) and media to lumen ratio (clip: 7.95 ± 0.89%; sham: 5.07 ± 0.45%, $p<0.01$) were all significantly greater in the hypertensives animals compared to the sham operated controls (Table 15).

5.4.3. Contraction Studies

High potassium and noradrenaline potassium contractions

123mM Potassium (KPSS) contraction

Stimulation with a high potassium solution produced a similar contractile response in the vessels from the acute hypertensive animals (3.97 ± 0.25mN/mm) compared to the sham operated controls (3.48 ± 0.12mN/mm) (Table 16). When the contractile response was expressed as media stress, the contraction per unit volume of smooth muscle, there was a slight reduction in the response from the hypertensive animals (259 ± 18mN/mm^2) compared to the sham operated controls (334 ± 12mN/mm^2) (Table 16).

Noradrenaline potassium contraction

Stimulation with 123mM potassium containing noradrenaline (10^{-5}M) caused a small increase in the absolute contractile response in the hypertensive animals (5.42 ± 0.55mN/mm) compared to the sham operated controls (4.57 ± 0.23mN/mm) (Table 16). However, when the response was expressed as media stress again the responses in the hypertensive animals (354 ± 45mN/mm^2) were less than those of the sham operated controls (437 ± 45mN/mm^2) (Table 16).

Noradrenaline contraction response

Noradrenaline produced a concentration dependent contraction of mesenteric resistance vessels. The induction of renovascular hypertension resulted in a significant increase in the absolute contractile response to noradrenaline compared to sham operated control (clip: 5.42 ± 0.52mN/mm; sham: 4.41 ± 0.25mN/mm, $p<0.001$) (Table 17, Figure 35)

but this was not associated with an alteration in the sensitivity (ED_{50}) (Table 17).

When the contraction response was expressed as media stress the hypertensive animals (359 ± 39 mN/mm^2, $p<0.05$) generated a significantly smaller force per unit mass of smooth muscle compared to sham the operated controls (423 ± 23 mN/mm^2) (Table 17, Figure 36).

5.4.4. Relaxation Studies

Acetylcholine relaxation response

Acetylcholine produced a concentration dependent relaxation of mesenteric resistance vessel segments, maximally contracted with noradrenaline (10^{-5}M). Acetylcholine induced relaxation was significantly reduced in the hypertensives animals (22 ± 8%, $p<0.001$) compared to the sham operated controls (51 ± 5%) (Figure 37). In the hypertensive animals there was a small but non-significant reduction in acetylcholine sensitivity (ED_{50}) when compared to the sham operated control (Table 17).

Sodium Nitroprusside relaxation response

Sodium nitroprusside produced a concentration dependent relaxation of maximally contracted (noradrenaline 10^{-5}M) mesenteric resistance vessel segments. The sodium nitroprusside induced relaxation was similar in the hypertensives animals (89 ± 9%) compared to the sham operated controls (87 ± 5%) (Figure 38) and there was no alteration in the sensitivity to sodium nitroprusside (clip: 1.20 ± 0.58μM; sham: 0.52 ± 0.44μM) (Table 17).

Table 14. Physical characteristics of Goldblatt acute and chronic two kidney one clip hypertensive (clip) and sham operated control (sham) rats.

		No Animals	Blood Pressure (mmHg)	Body Weight (grams)	Heart Weight (grams)	Heart:Body Weight Ratio (%)
Acute						
	SHAM	12	105 ± 3	253 ± 8	0.8657 ± 0.028	0.345 ± 0.010
	CLIP	12	166 ± 5***	253 ± 9	1.0670 ± 0.048**	0.422 ± 0.013***
Chronic						
	SHAM	9	113 ± 5	240 ± 5	0.7878 ± 0.018	0.329 ± 0.007
	CLIP	9	158 ± 3***	249 ± 6	1.0220 ± 0.0603**	0.408 ± 0.023**

** $p<0.01$ *** $p<0.001$ Compared to the Sham operated control

Table 15. Mesenteric vessel morphology of Goldblatt acute and chronic two kidney one clip hypertensive (clip) and sham operated control (sham) rats.

		No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
Acute						
	SHAM	21	217 ± 9	10.62 ± 0.62	7512 ± 412	5.07 ± 0.45
	CLIP	23	220 ± 10	15.85 ± 1.20***	11448 ± 1000**	7.95 ± 0.89**
Chronic						
	SHAM	18	225 ± 9	9.53 ± 0.46	7054 ± 501	4.19 ± 0.32
	CLIP	18	205 ± 10	16.23 ± 1.40***	10508 ± 788**	8.97 ± 1.20**

** $p<0.01$ *** $p<0.001$ Compared to Sham operated control

Table 16. Tension and media stress responses to high potassium and potassium containing noradrenaline (10^{-5}M) in mesenteric vessels from Goldblatt acute and chronic two kidney one clip hypertensive (clip) and sham operated control (sham) rats.

	123mM Potassium		123mM Potassium + Noradrenaline (10^{-5}M)	
	Active Tension (mN/mm)	Media Stress (mN/mm^2)	Active Tension (mN/mm)	Media Stress (mN/mm^2)
Acute				
SHAM	3.48 ± 0.12	334 ± 12	4.57 ± 0.23	437 ± 20
CLIP	3.97 ± 0.25	259 ± 18	5.42 ± 0.55	354 ± 45
Chronic				
SHAM	3.05 ± 0.12	327 ± 12	4.05 ± 0.27	368 ± 46
CLIP	2.96 ± 0.11	197 ± 11***	4.19 ± 0.22	270 ± 29***

*** $p<0.001$ compared to the Sham operated control

Table 17. Noradrenaline sensitivity and the maximum contractile response to noradrenaline expressed as tension and media stress. Sensitivity and maximum response to acetylcholine and sodium nitroprusside in mesenteric vessels from Goldblatt acute and chronic two kidney one clip hypertensive (clip) and sham operated control (sham) rats.

	Noradrenaline Maximum		Noradrenaline	Acetylcholine		Sodium nitroprusside	
	Active Tension (mN/mm)	Media Stress (mN/mm^2)	sensitivity (ED_{50}) (μM)	Relaxation (%)	Sensitivity (ED_{50}) (μM)	Relaxation (%)	Sensitivity (ED_{50}) (μM)
Acute							
SHAM	4.41 ± 0.25	423 ± 23	1.773 ± 0.38	51 ± 5	0.068 ± 0.015	87 ± 5	0.52 ± 0.44
CLIP	5.42 ± 0.52***	359 ± 39*	1.599 ± 0.58	22 ± 8***	0.222 ± 0.160	89 ± 9	1.20 ± 0.58
Chronic							
SHAM	3.76 ± 0.19	397 ± 17	2.347 ± 0.75	46 ± 9	0.108 ± 0.049	75 ± 5	1.08 ± 0.35
CLIP	4.19 ± 0.25***	277 ± 25***	0.893 ± 0.19	16 ± 4***	0.063 ± 0.023	77 ± 9	1.88 ± 1.02

* $p<0.05$ *** $p<0.001$ compared to sham operated control

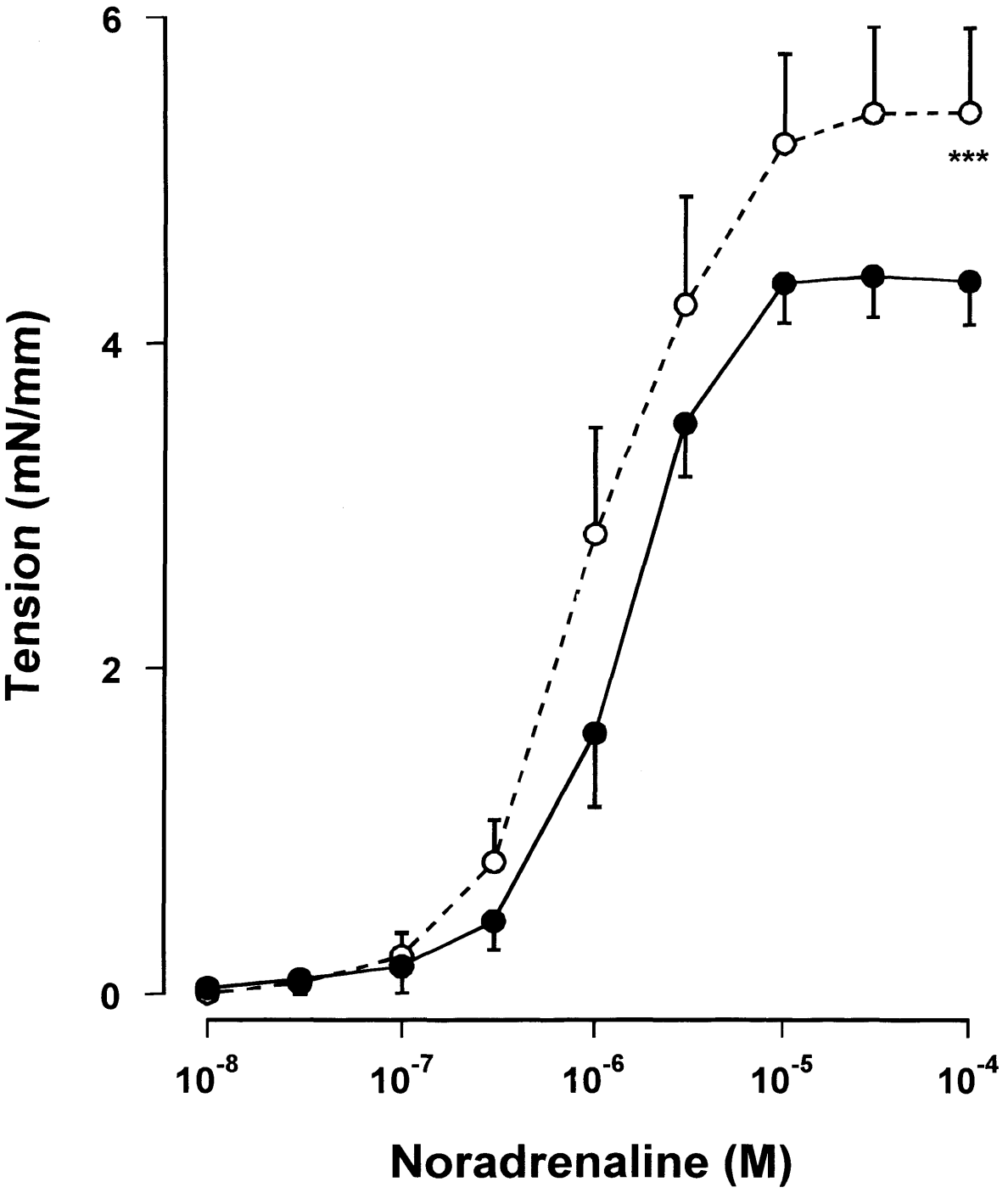

Figure 35 The noradrenaline contractile response of mesenteric vessels from acute Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

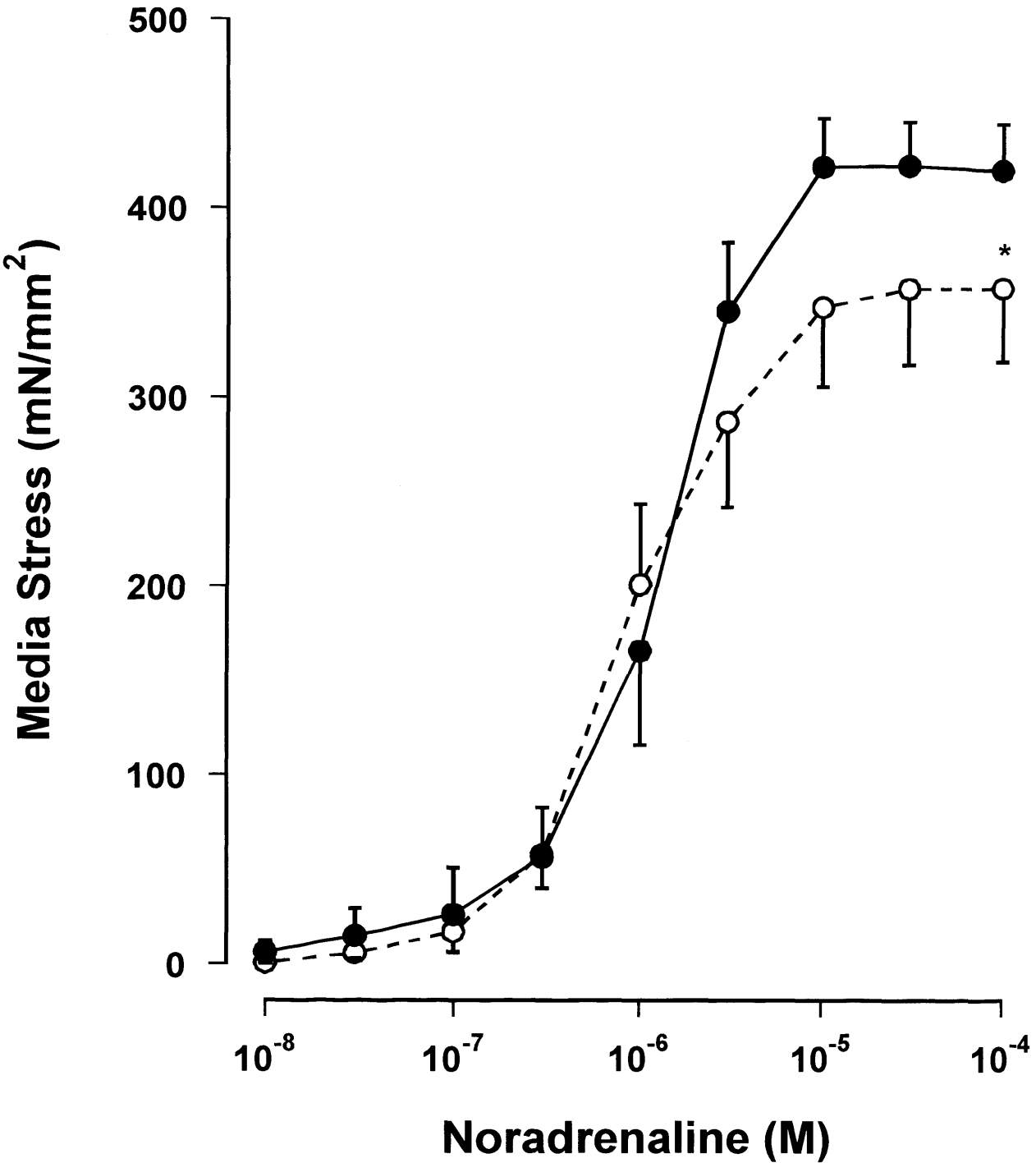

Figure 36 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from acute Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats
***p<0.05 denotes comparison between the two lines**

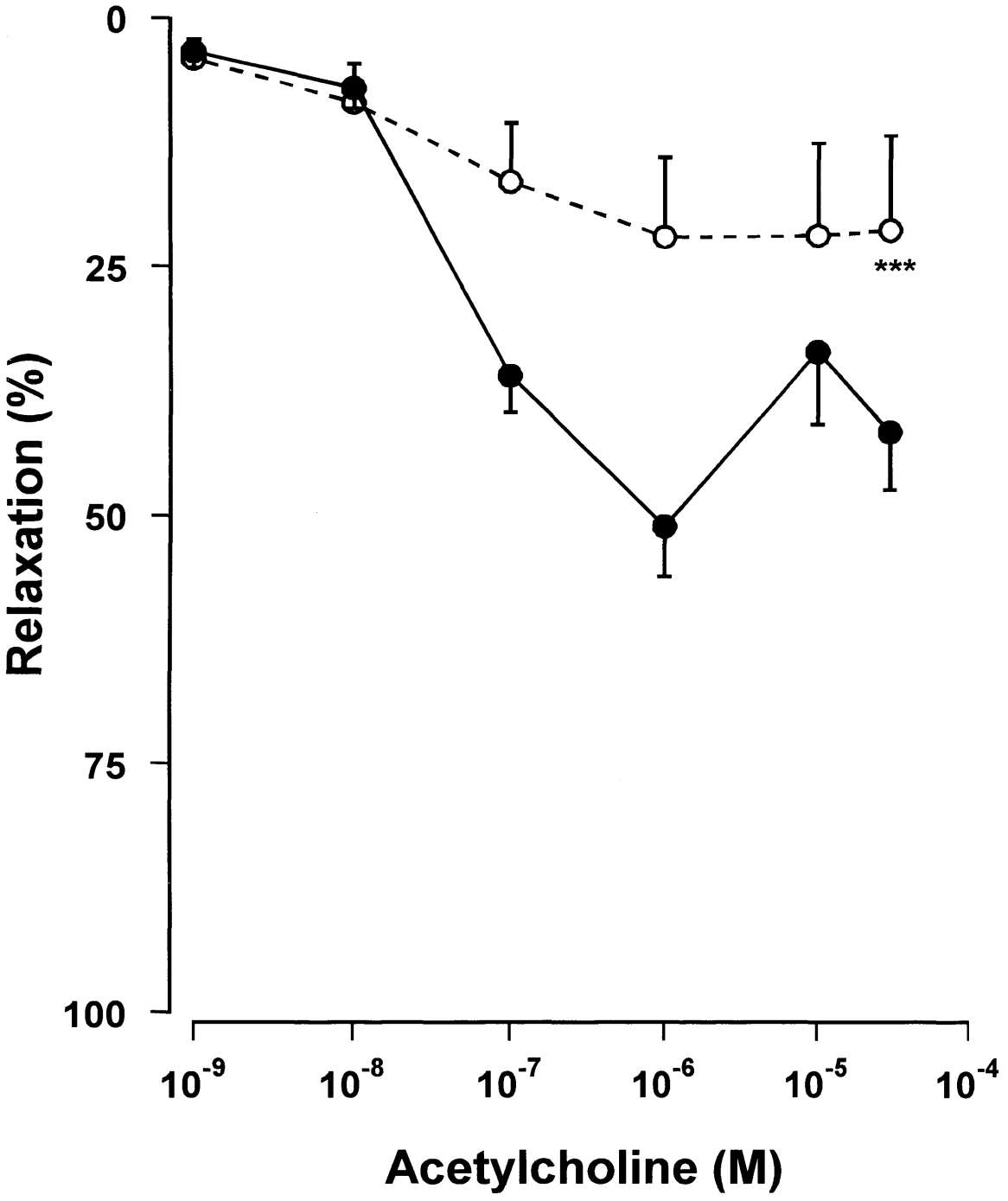

Figure 37 The acetylcholine relaxation response of mesenteric vessels from acute Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

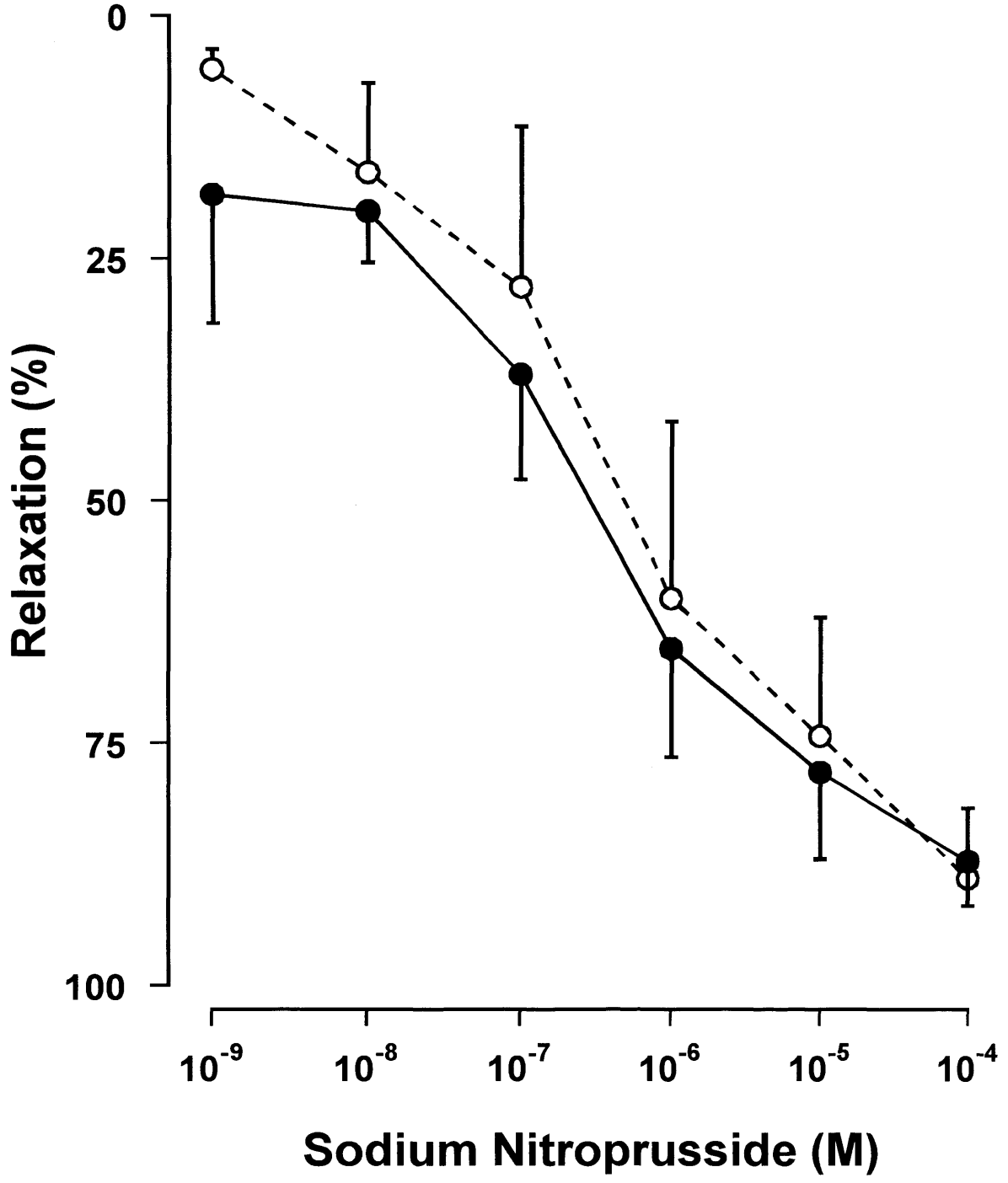

Figure 38 The sodium nitroprusside relaxation response of mesenteric vessels from acute Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats

5.5. RESULTS - CHRONIC HYPERTENSION

5.5.1. Physical Characteristics

The blood pressure, body weight, heart weight and heart to body weight ratio of the chronic Goldblatt two kidney one clip (2K1C) and sham operated controls (Sham) are shown in Table 14. The systolic blood pressure was significantly higher in the hypertensive animals (158 ± 3mmHg, $p<0.001$) compared to sham operated controls (113 ± 5 mmHg). The body weight of the hypertensive animals (249 ± 6g) was similar to the sham operated controls (240 ± 5g). The heart weight (clip: 1.0220 ± 0.0603g; sham: 0.7878 ± 0.018g, $p<0.01$) and heart to body weight ratio (clip: 0.408 ± 0.023g; sham: 0.329 ± 0.007g, $p<0.01$) were both significantly increased in the chronic hypertensive animals compared to the sham operated controls.

5.5.2. Vessel Morphology

The vessel morphology is shown in Table 15. The internal diameter of the mesenteric resistance vessels of the chronic hypertensives animals (205 ± 10μm) were similar to the sham operated controls (225 ± 9μm) (Table 15). The media thickness (clip: 16.23 ± 1.40μm; sham: 9.53 ± 0.46μm, $p<0.001$), media volume (clip: 10508 ± 788μm^3; sham: 7054 ± 501μm^3, $p<0.01$) and media to lumen ratio (clip: 8.97 ± 1.20%; sham: 4.19 ± 0.32%, $p<0.01$) all were significantly greater in the hypertensive animals compared to the sham operated controls (Table 15).

5.5.3. Contraction Studies

5.3.1. High potassium and noradrenaline potassium contractions

123mM Potassium (KPSS) contraction

Stimulation with a high potassium solution produced a similar absolute contractile response in the vessels from the hypertensive animals (2.96 ± 0.11mN/mm) compared to the sham operated controls (3.05 ± 0.12mN/mm) (Table 16). However, when the contractile response was expressed as media stress, the contraction per unit volume of smooth muscle, the response in the hypertensive animals was significantly reduced (197 ± 11 mN/mm^2, $p<0.001$) compared to the sham operated controls (327 ± 12mN/mm^2) (Table 16).

Noradrenaline potassium contraction

Stimulation with 123mM potassium containing noradrenaline (10^{-5}M) caused a small increase in absolute contractile response in the hypertensive animals (4.19 ± 0.22mN/mm) compared to the sham operated controls (4.05 ± 0.27mN/mm) (Table 16). However, when the response was expressed as media stress the response in the hypertensive animals (270 ± 29mN/mm^2, $p<0.001$) was significantly less than in the sham operated controls (368 ± 46mN/mm^2) (Table 16).

Noradrenaline contraction response

Noradrenaline produced a concentration dependent contraction of mesenteric resistance vessels. There was a significant increase in the absolute contractile response in the hypertensive animals (4.19 ± 0.25mN/mm, $p<0.001$) compared to the sham operated controls (3.76 ± 0.19mN/mm) (Table 17, Figure 39) but this was not associated with an alteration in the noradrenaline sensitivity (ED_{50}) (Table 17). When the

contraction responses were expressed as media stress the hypertensive animals (277 ± 25 mN/mm^2, $p<0.001$) generated a significantly smaller force compared to the sham operated controls (397 ± 17 mN/mm^2) (Figure 40).

5.5.4. Relaxation Studies

Acetylcholine relaxation response

Acetylcholine produced a concentration dependent relaxation of mesenteric resistance vessels, maximally contracted with noradrenaline (10^{-5}M). Acetylcholine induced relaxation was significantly reduced in the hypertensives animals (16 ± 4%, $p<0.001$) compared to the sham operated controls (46 ± 9%) (Figure 41). In the hypertensive animals there was a small but non-significant increase in acetylcholine sensitivity (ED_{50}) compared to the sham operated controls (Table 17).

Sodium Nitroprusside relaxation response

Sodium nitroprusside produced a concentration dependent relaxation of maximally contracted (noradrenaline 10^{-5}M) mesenteric resistance vessel segments. The sodium nitroprusside induced relaxation was similar in the hypertensives (77 ± 9%) compared to the sham operated controls (75 ± 5%) (Figure 42) animals with no difference in the sodium nitroprusside sensitivity (clip: 1.88 ± 1.02μM; sham: 1.08 ± 0.35μM) (Table 17).

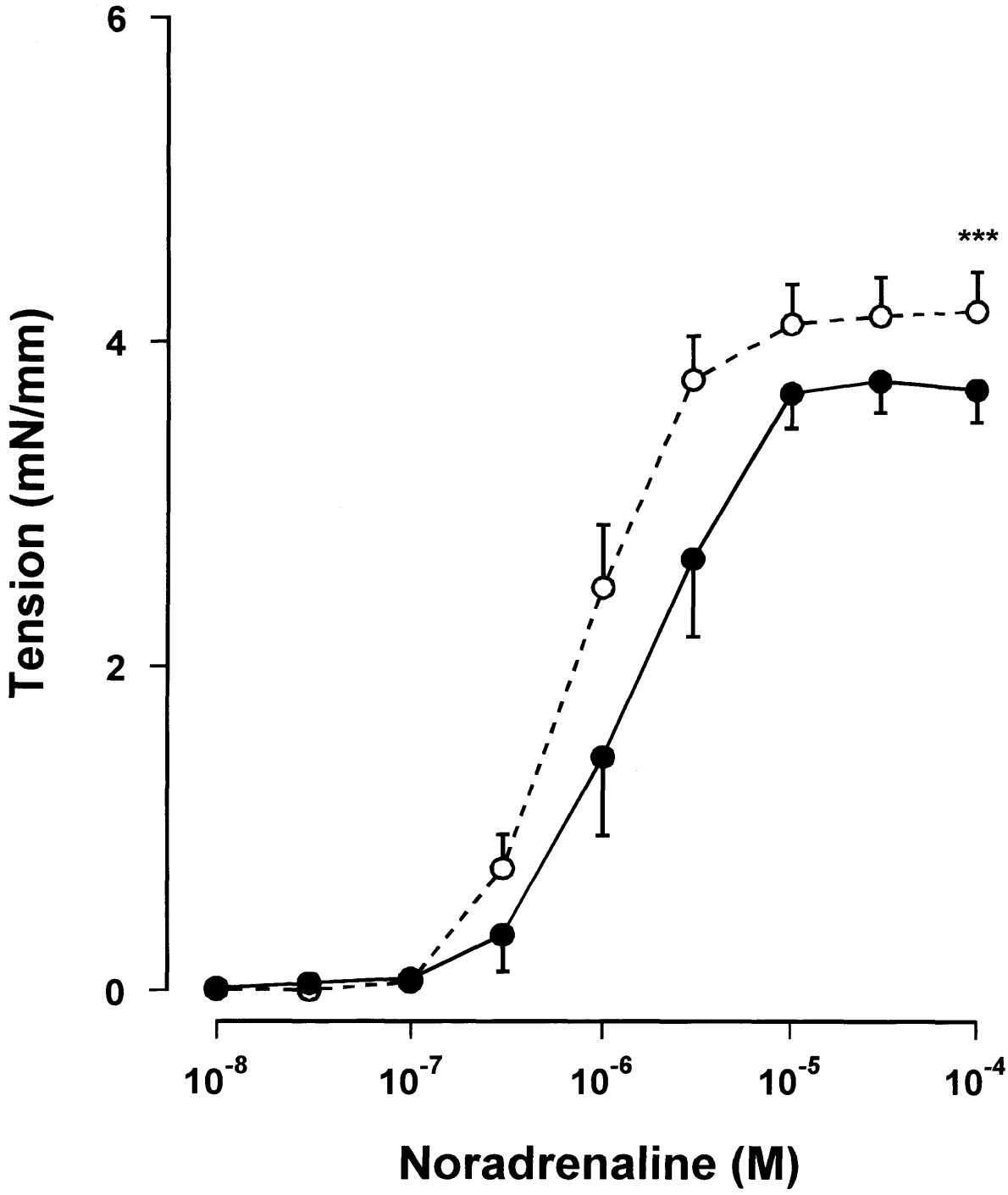

Figure 39 The noradrenaline contractile response of mesenteric vessels from chronic Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

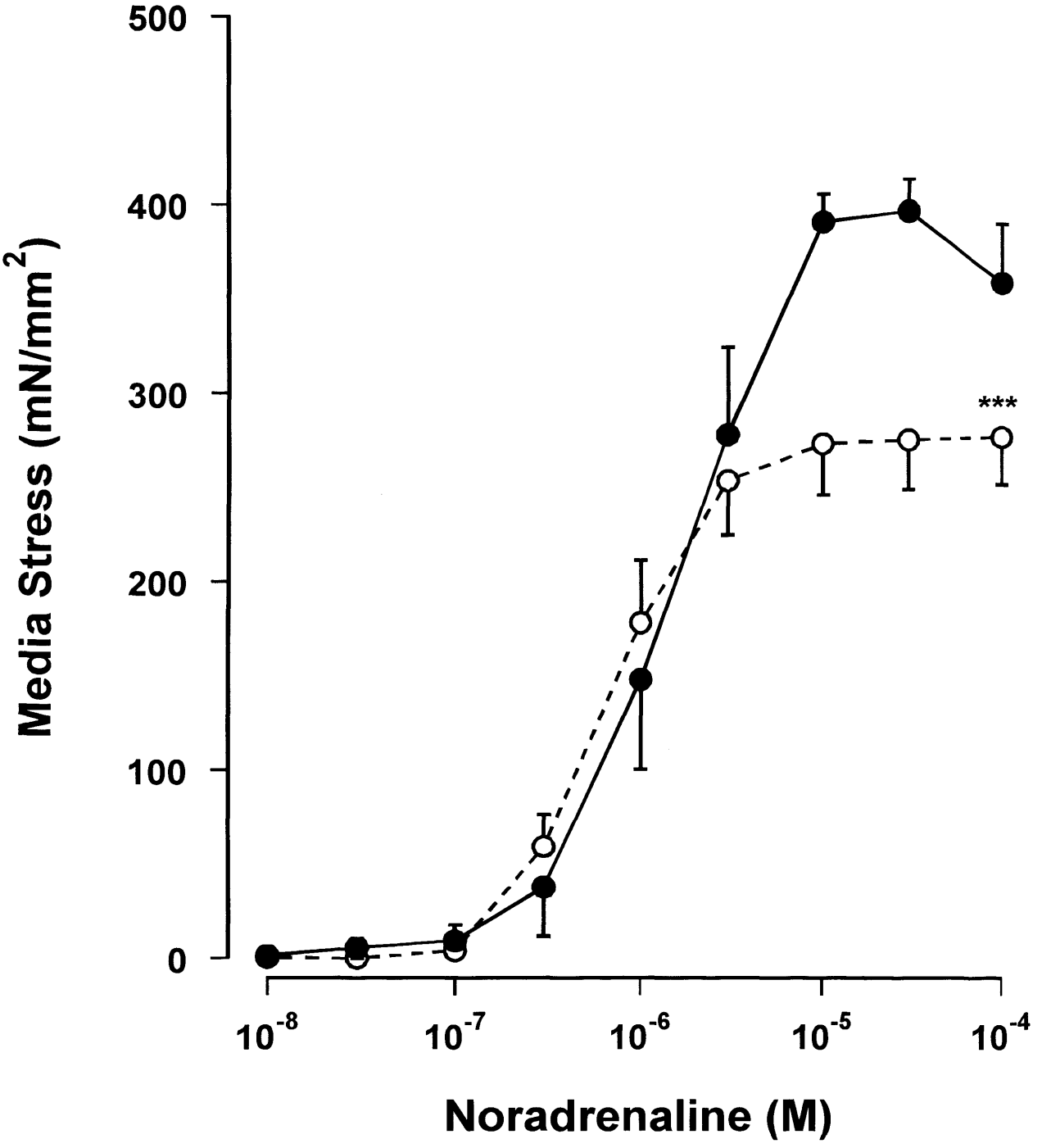

Figure 40 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from chronic Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats *p<0.05 denotes comparison between the two lines

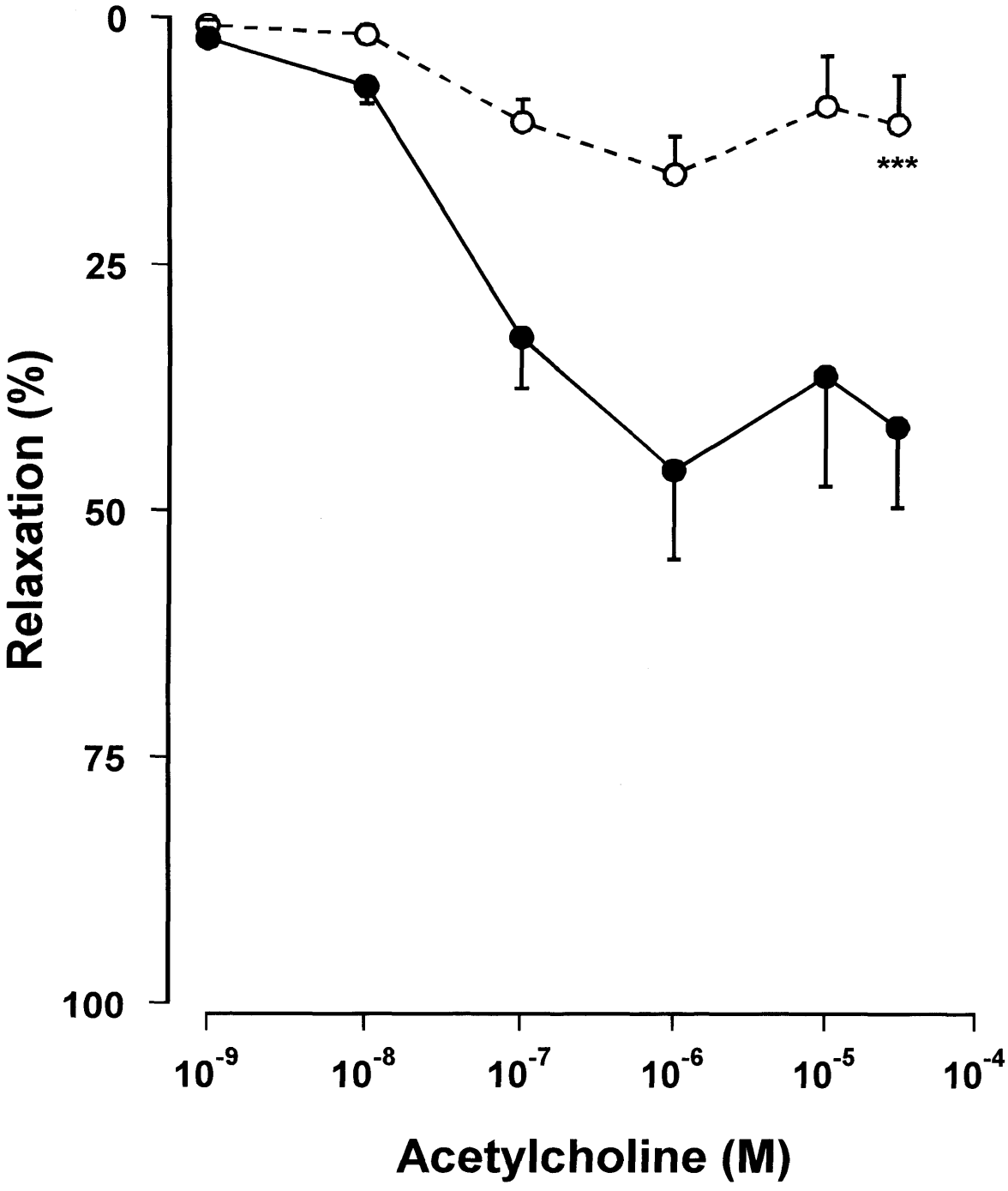

Figure 41 The acetylcholine relaxation response of mesenteric vessels from chronic Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

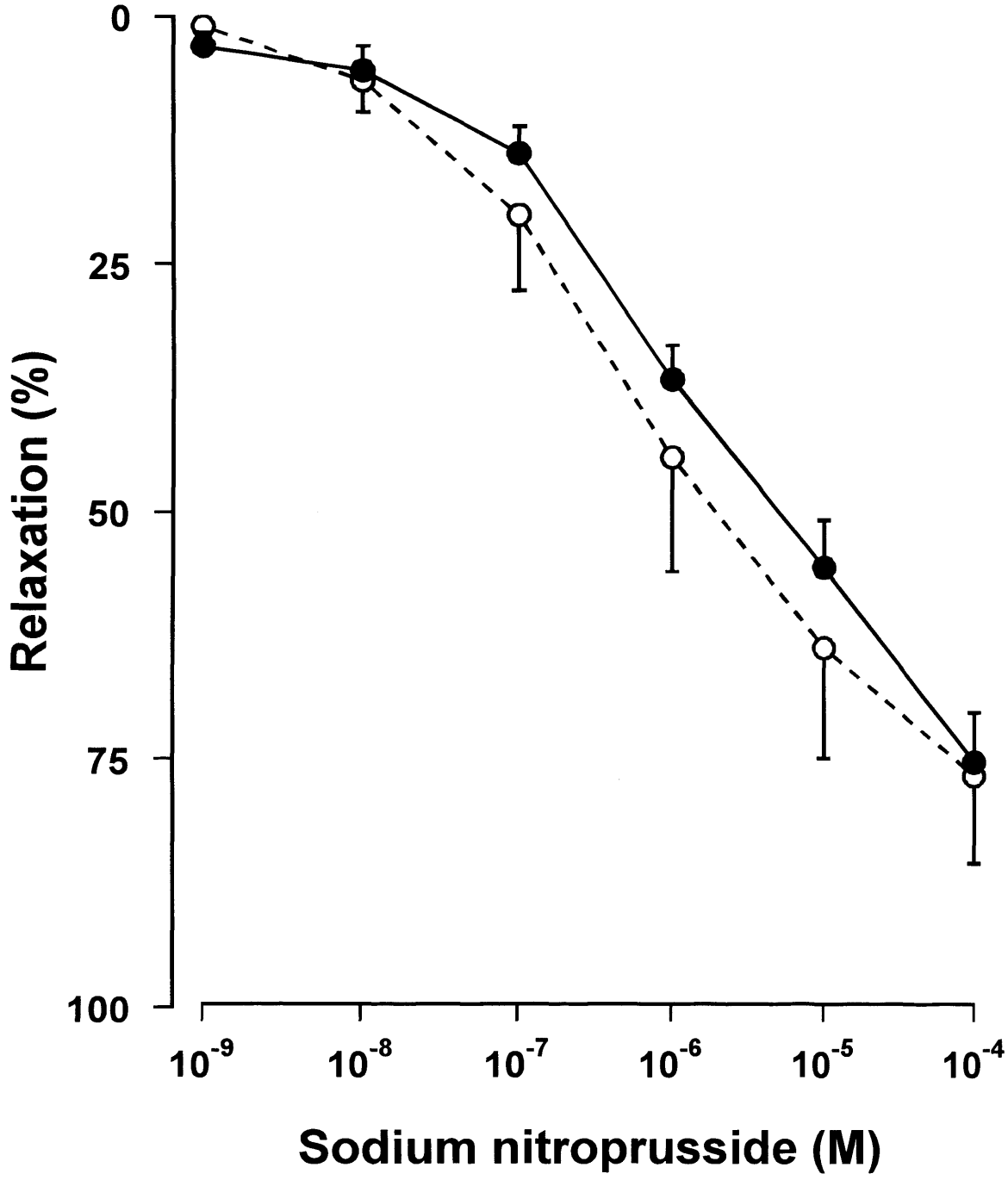

Figure 42 The sodium nitroprusside relaxation response of mesenteric vessels from chronic Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats

5.6. DISCUSSION

The majority of studies in the two kidney one clip model of hypertension indicate that the raised blood pressure is maintained by an elevated peripheral vascular resistance (Bianci et al. 1972; Averill et al. 1976; Hallback-Nordlander et al. 1979 and Russell et al. 1983). The elevated peripheral vascular resistance is associated with a reduction in cardiac index in both the early and chronic phases (Russell et al. 1983). Vascular capacity is reduced but this is not associated with a change in vascular compliance (Edmunds et al. 1989 and Yamamoto & Ogino 1982). The increased peripheral vascular resistance of early phase experimental renal hypertension could be due to neurohumoral mechanisms causing a functional increase in vascular smooth muscle tone but in the established phase there may be reinforcement by structural changes within the blood vessels.

The present study has demonstrated evidence of vascular smooth muscle growth which did not appear to be associated with an encroachment of the lumen within six weeks. In the acute and chronic phases the cross sectional area of the vessels from the hypertensive animals was increased by 52% and 49% respectively. It is often accepted that vascular growth is, in part, an adaptive process which follows the increase in blood pressure. However, Lymm et al. (1994) demonstrated increased DNA synthesis in the aorta and mesenteric vessels before the blood pressure rose in the Goldblatt two kidney one clip hypertensive rats. DNA synthesis reached a plateau at 14 days and had fallen to normal by 28 days.

Interestingly, they also showed that there was no increase in DNA synthesis in subcutaneous arteries over the 28 day period. However, morphological

measurements of the subcutaneous vessels showed that there was some evidence of re-modeling. It has been suggested that vascular hypertrophy may lead to an elevated peripheral vascular resistance in the presence of normal levels of vasoconstrictor activity (Folkow et al. 1973). Some studies, despite the presence of structural changes, have shown increased reactivity of blood vessels to a variety of vasoactive hormones in experimental models of hypertension (Collis & Alps 1975 and Davis et al. 1986). Moreover, there is evidence that the increased vasoconstrictor response precedes structural alterations (Hashimoto et al. 1987; Meininger et al. 1984 and Stacy et al. 1987). For example Carretero and Gulati (1978) showed that angiotensin II antagonists in two kidney one clip hypertensive animals were most effective in the early, renin-dependent, phase of hypertension. By contrast, in the present study, vascular responses to high potassium and high potassium containing noradrenaline failed to elicit a significant increase in the absolute contractility in acute phase hypertension although the responses were mildly elevated. Expressing these responses as media stress, thus correcting for the increase in cross sectional area, resulted in a small non-significant reduction of the vascular responses. Furthermore, in the chronic phase not only was there no increase in the absolute contractile response, when the responses were expressed as media stress the responses of vessels from the hypertensive animals were significantly less than the controls. Deng and Schiffrin (1991) also demonstrated significantly reduced potassium induced contractions, in Goldblatt two kidney one clip hypertension whether expressed as absolute contractile responses (active tension mN/mm) or media stress (mN/mm^2).

On the other hand the response to cumulative concentrations of noradrenaline was significantly greater in both acute and chronic phase hypertension although the sensitivity to noradrenaline was unchanged.

Never the less, when the results were expressed as media stress, again the responses of the vessels from the hypertensive animals were significantly less than those from the controls. Similarly, Deng and Schiffrin (1991) showed that noradrenaline produced similar active tension responses in hypertensive animals compared to sham operated controls but that this response of hypertensives was reduced when expressed as media stress. Moreover, even the present study and that reported by Deng and Schiffrin (1991) failed to demonstrate evidence of exaggerated intrinsic smooth muscle reactivity in renal hypertensive animals during the acute phase of Goldblatt hypertension.

Morphological and contraction studies do not provide a complete view of hypertension associated changes in the peripheral vasculature. Previous studies in the spontaneously hypertensive rats indicate that the endothelium may play an important role in determining vascular resistance. Similarly in this study, endothelium dependent relaxation was reduced by 43% in the early phase and 35% in the chronic phase of hypertension. However, unlike observations in the spontaneously hypertensive rat, the relaxation of the vessels from the Goldblatt two kidney one clip hypertensive animals continued with each increasing concentration of acetylcholine. At no stage was there a re-contraction of the vessels with higher concentrations ($>10^{-7}$M) of acetylcholine. However, the vessels from the sham operated controls elicited a minor re-contraction with high concentrations of acetylcholine ($\geq 10^{-5}$M).

Impaired endothelium dependent relaxation could result from a functional alteration in the endothelium, morphological changes in the vascular wall, which could reduce the diffusion of endothelium derived relaxing factor towards the smooth muscle cells or a change in smooth muscle cell function

as discussed in Chapter 3. The normal response to the nitric oxide donor, sodium nitroprusside, showed that there was no alteration seen in the vessels from the hypertensive animals during the acute or chronic phases of hypertension. Thus, the maximum response and the sensitivity (ED_{50}) to sodium nitroprusside were similar in the hypertensive animals and the sham operated controls. This would suggest that morphological alteration of the vascular smooth muscle cells could not account for the decreased endothelium dependent relaxation. Moreover, the smooth muscle cell cGMP response is similar to controls in both the acute and chronic phases of hypertension.

The present study was not designed to investigate the release of an abnormal endothelial relaxation dependent on an endothelial derived contracting factor as observed in the spontaneously hypertensive rat. However, in a later study Bennett et al. (1993) showed that acetylcholine induced relaxation was significantly improved after incubation with indomethacin in ten weeks but not four weeks after induction of Goldblatt two kidney one clip hypertension. They concluded that impairment of endothelium derived relaxation developed in response to a prolonged exposure to high blood pressure and that this depended on the increased formation of a cyclooxygenase endothelial derived contracting factor. Thus, in the later stage of Goldblatt two kidney one clip hypertension the development of endothelial dysfunction may contribute to the enhanced vascular constriction and to the maintenance of chronic hypertension (Bennett et al 1993).

5.7. CONCLUSIONS

1. Two kidney one clip hypertension results in an increased vascular mass and cardiac hypertrophy after six weeks with little further change in the chronic phase at sixteen weeks.

2. The contractile response to noradrenaline is increased in the acute and chronic phases of hypertension but this increase in contractility can not be attributed to the increase in wall mass alone.

3. Endothelium dependent but not endothelium independent relaxation is reduced during the acute and chronic phases of two kidney one clip hypertension.

4. Surgical reversal of hypertension by removal of the constricting clip could offer an opportunity to explore whether the structural vascular changes are the sole determinant of the increased peripheral vascular resistance in established hypertension.

CHAPTER 6 SURGICAL REVERSAL OF GOLDBLATT TWO KIDNEY ONE CLIP HYPERTENSION

6.1. INTRODUCTION

Several studies have shown that the blood pressure response to surgical correction varies according to the phase of hypertension and whether the ischaemic kidney is removed or renal artery unclipping performed. Thus, removal of the ischaemic kidney during the acute phase of hypertension (< 6 weeks), returns blood pressure to normal (Wilson and Byrom 1941; Koletsky and Rivera-Velez 1970; Gross 1971; Thurston and Swales 1974). However, excision of the ischaemic kidney in chronic hypertension (>16 weeks) produces only a partial fall in blood pressure and many studies have reported persistent blood pressure elevation (Wilson and Byrom 1941; Grollman et al. 1943; Koletsky and Rivera-Velez 1970; Thurston and Swales 1974; Thurston et al. 1980b). By contrast, most studies have demonstrated that removal of the constricting clip restores blood pressure to normal (Gross 1971; Thurston et al. 1980a and 1980b), although Floyer (1951) noted persistent hypertension, all be it at a reduced level occurring in only 50% of rats. Although total peripheral resistance falls with the removal of the clip, cardiac output also falls, albeit transiently after unclipping (Funder et al 1970; Ledingham & Cohen 1962). Haemodynamic studies, using radioactive microspheres, indicate that nephrectomy and unclipping cause a profound fall in peripheral vascular resistance (Russell et al. 1983) (Table 18).

Table 18 The haemodynamic effect of unclipping chronic Goldblatt two kidney one clip hypertension in the rat.
Reproduced from H Thurston (1994) Text of hypertension. ed. JD Swales, Blackwell Scientific Publ, Oxford 477-493

	Normals	Intact	24hrs after unclipping	60 days after unclipping
Direct BP mmHg	123 ± 3.4	173 ± 8.6	122 ± 6.5**	131 ± 7.4**
Peripheral resistance mmHg/ml/min/100g	4.5 ± 2.3	9.9 ± 1.9	4.4 ± 0.3**	4.6 ± 0.5**
Cardiac index ml/min/100g	28 ± 1.8	20.3 ± 2.2	29 ± 2.3*	30 ± 2.7
Heart rate beats/min	474 ± 12	435 ± 11	440 ± 15	445 ± 16
plasma renin concentration ng AI/ml/hr	84 ± 20	109 ± 42	-	53 ± 16

* $p<0.05$ compared to intact Goldblatt two kidney 1 clip
** $p<0.01$ compared to intact Goldblatt two kidney 1 clip

Plasma renin concentration is increased during the acute phase of Goldblatt two kidney one clip hypertension but declines towards normal levels as time passes (Godfrey et al. 1985). There is a direct relationship between plasma renin and blood pressure elevation in the acute but not the chronic phase of hypertension (Swales et al. 1983). Removal of the ischaemic kidney or surgical removal of the restricting clip results in a dramatic fall in plasma renin to sub-normal levels in both the acute and chronic phases (Russell et

al. 1983; Thurston et al. 1980b and Brice et al. 1983) and this is associated with a positive sodium balance (Thurston et al. 1980b). Moreover, saline infusion effects the blood pressure fall or the final blood pressure (Otsuka et al. 1979). Thus, these studies argue against the role for sodium retention in the maintenance of the elevated blood pressure or the reduction of plasma renin in the chronic phase of two kidney one clip model of hypertension (Gavras et al. 1975).

A fall in blood pressure can be demonstrated within two hours of unclipping (Hallback-Nordlander et al. 1979) although the residual effects of anaesthesia remain uncertain. Blood pressure is normal 1 day after surgical reversal of hypertension in both the acute and chronic phases of hypertension (Thurston et al. 1980b) despite the fact that indirect studies indicate that structural changes in the vessel wall are not reversed until three weeks after normalisation of blood pressure (Lundgren 1974). This has led to the suggestion that smooth muscle tone may be subnormal following renal artery unclipping (Hallback-Nordlander et al. 1979).

Reversal of hypertension by unclipping is associated with a natriuresis with no change in plasma renin in one kidney one clip hypertension (Ledingham & Cohen 1962; Muirhead & Brookes 1980; Godfrey et al. 1985). Exchangeable sodium, which is raised, returns to normal (McAreavey et al. 1984) and sodium balance becomes slightly negative seven days after removing the clip (Godfrey et al. 1985). If the salt and water losses are replaced (Muirhead & Brookes 1980) or prevented by uterocaval anastamosis (Floyer 1955) blood pressure still falls again indicating that the sodium and water losses are not responsible for the reversal of hypertension with unclipping.

Removal of the ischaemic kidney or the constricting clip causes a fall in peripheral resistance while cardiac output increases or remains normal (Russell et al. 1983). This would be in keeping with the removal of a renal constrictor system or an activation of a vasodilator system. In the acute phase of two kidney one clip hypertension, plasma renin is elevated and inhibition of the renin angiotensin system with an angiotensin II antagonist or a converting enzyme inhibitor produces a partial fall in blood pressure. However, in the chronic phase only inhibition of converting enzyme partially reduces the blood pressure. Many studies have been undertaken to assess the role of the renin angiotensin system in hypertension and to study the effects of renal artery clipping in the presence of pharmacological blockade. Infusion of saralasin or captopril had no influence on the pattern of the fall in blood pressure (Russell et al. 1982b). Additionally, neither aprotinin nor indomethacin influenced the reversal of hypertension after unclipping (Russell et al. 1982a).

It has been suggested that an alteration in vascular reactivity to angiotensin II may maintain the elevated blood pressure in renovascular hypertension (Brown et al. 1979). Increased vascular reactivity has been demonstrated using isolated tissue (Folkow et al. 1973) and arterial strips (Bandick & Sparks 1970; Holloway & Bohr 1973) in rats. A specific increase in responsiveness has also been demonstrated in isolated, perfused untouched kidney (Collis & Vanhoutte 1978). Studies of vascular responsiveness in the isolated blood perfused hindlimb have demonstrated increased reactivity to angiotensin II and noradrenaline in chronic hypertension (Mistry et al. 1983). Removal of the renal artery clip resulted in a partial normalisation of the pressor response at 60 days which coincided with the regression of structural vascular hypertrophy. In the acute phase of hypertension angiotensin II response was reduced but could be restored to normal by

administration of the ACE inhibitor captopril or 24 hours after unclipping. However, the fall in blood pressure, after unclipping, could only be prevented by high infusion rates of angiotensin II. Therefore, this would suggest that there are mechanisms other than angiotensin II involved in the blood pressure response to unclipping.

Recently attention has focused on whether the development of structural change can be prevented or reversed by lowering the blood pressure. Early studies of the effects of antihypertensive therapy in the spontaneously hypertensive rat has demonstrated that resistance artery hypertrophy developed despite lowering the blood pressure to the normal range (Christensen et al. 1988). However, treatment with the angiotensin converting enzyme inhibitors captopril and perindopril prevented structural changes from occurring and in the case of perindopril, a longstanding effect on blood pressure was observed when therapy was discontinued (Christensen et al. 1989; Harrap et al. 1986).

By contrast, no study has examined the effects of surgical reversal of renovascular hypertension on the structural and functional changes in the resistance vasculature . This study was designed to investigate the changes in mesenteric vascular structure before and 1 and 75 days after reversing renovascular hypertension by removing the constricting clip from the renal artery.

6.2. METHODS

6.2.1. Induction of Hypertension

Goldblatt two kidney one clip hypertension was induced as previously described (See Chapter 6).

6.2.2. Reversal of Hypertension

Goldblatt two kidney one clip rats with sustained hypertension for 4-6 weeks (i.e. a blood pressure >150mmHg), and age matched sham operated controls were selected for study. A loin incision was made under ether anaesthesia and the left kidney re-exposed. The fat and connective tissue were carefully dissected clear of the kidney using cotton buds and the renal artery silver clip exposed. The jaws of the clip were prized apart, taking care not to damage the renal artery or vein and the silver clip removed. Control animals underwent a similar sham procedure to remove the non-constricting silver strip. The wound was re-sutured in layers after which the animal was allowed to recover from the anaesthetic.

Those animals which were not used in the 1 day reversal study group had their blood pressure measured 24 hours after operation to confirm that blood pressure returned to control levels within 24 hours of renal artery unclipping.

6.3. PROTOCOL

Experimental hypertensive and age matched sham control rats were studied 4-6 weeks after renal artery constriction and 1 and 75 days following surgical reversal of hypertension.

Animals were killed by stunning followed by cervical dislocation. The heart was removed, cleaned of blood clots and fat before being weighed. Mesenteric arterial resistance vessels were taken from the superior mesenteric bed which supplies the jejunum at a point 8-10cm from the pylorus. The third generation branch was used in all preparations. Arteries were cleaned of fat and surrounding tissue before being mounted in a myograph. The vessels were maintained in physiological salt solution at 37°C and gassed with $5\%CO_2/95\%O_2$ to achieve a pH of 7.4.

After equilibrating for 60 minutes, at minimal tension (0.1mN), the vessel length and morphology was measured using water immersion light microscopy (See Chapter 2). Media cross sectional area (equivalent to media volume per unit length) was calculated from the media thickness and the internal circumference. The vessels were normalised as described in Chapter 2 and the internal diameter was set to $L_{0.9}$, which is 90% of the internal diameter the vessel would have had in vivo and under a transmural pressure of 100mmHg (13.3kPa). After a further 60 minute equilibrium period the vessels were stimulated three times with a high potassium physiological solution (KPSS) followed by KPSS containing noradrenaline (10^{-5}M). The vessels bathing medium was replaced after each stimulation and allowed to return to baseline. A cumulative noradrenaline (10^{-8}M to 10^{-4}M) dose contraction curve was performed in the presence of cocaine (10^{-6}M). After the final concentration of noradrenaline the bathing medium was replaced several times with fresh physiological salt solution and the vessels allowed to return to baseline.

Finally, the vessels were maximally contracted with noradrenaline (10^{-5}M) and a cumulative acetylcholine (10^{-9}M to $3x10^{-5}$M) concentration relaxation

curve was performed. Two vessels from each animal were studied and the results averaged.

6.4. RESULTS

6.4.1. Physical Characteristics

The blood pressure, body weight, heart weight and heart to body weight ratio before and after surgical reversal of hypertension are shown in Table 19. Before surgical reversal of hypertension the systolic blood pressure in the Goldblatt two kidney one clip hypertensive group was significantly higher than that in the sham operated controls ($p<0.01$). Removal of the renal artery clip caused a rapid fall in blood pressure of the hypertensive rats within 24 hours, the blood pressure returning to that in the control rats (Table 19). Sham operation did not alter the blood pressure of the control animals. The heart weight ($p<0.01$) and heart to body weight ratio ($p<0.001$) (Table 19) were both significantly greater in the hypertensive group compared to sham operated controls. One day after surgical reversal of hypertension, the heart weight was still significantly greater ($p<0.001$) in the clipped animals but by 75 days it had decreased to that in the sham operated controls. Similarly, the heart to body weight ratio was significantly higher ($p<0.001$) in the hypertensive animals compared to sham operated controls. One day after reversing hypertension the heart to body weight ratio was unchanged and remained higher than in the controls ($p<0.05$) even 75 days after surgical reversal of hypertension

7.4.2. Vessel Morphology

The internal diameter of the mesenteric resistance arteries of the hypertensive rats before, 1 and 75 days after reversal was similar to that in the sham operated controls (Table 20). Before surgical reversal of hypertension the media volume ($p<0.001$), media thickness ($p<0.01$) and media to lumen ratio ($p<0.01$) were significantly greater in the hypertensive animals compared to sham operated controls (Table 20). One day after surgical reversal of hypertension the media volume ($p<0.001$), media thickness ($p<0.01$) and media to lumen ratio ($p<0.05$) was unchanged and all were significantly increased (Table 20). However, 75 days after reversing hypertension there was significant regression of the media volume, media thickness and media to lumen ratio and none of these parameters were significantly different from that in the sham operated controls (Table 20).

6.4.3. Contraction Studies

High potassium and noradrenaline potassium contraction

123mM Potassium (KPSS) contraction

Stimulation with a high potassium salt solution produced a similar absolute contractile response (mN/mm) in the vessels from the hypertensive rats and their sham operated controls before (clip: 3.97 ± 0.25; sham: 3.48 ± 0.12), 1 day (clip: 3.34 ± 0.22; sham: 3.40 ± 0.18) and 75 days (clip: 3.58 ± 0.19; sham: 3.43 ± 0.13) after surgical reversal of hypertension (Table 20). When the responses were expressed as media stress (mN/mm^2), the contraction per unit volume of smooth muscle, there was no difference between the hypertensive (259 ± 18) and sham (334 ± 12) operated control animals before reversal. However, the media stress developed was significantly

reduced in the hypertensives, 1 day (clip: 206 ± 18; sham: 300 ± 15, $p<0.001$) and 75 days (clip: 237 ± 11; sham: 274 ± 9, $p<0.05$) after reversal of hypertension (Table 21).

Noradrenaline potassium contraction

Stimulation with 123mM potassium containing noradrenaline (10^{-5}M) caused a greater increase in the absolute contractile response (mN/mm) in the hypertensive group compared to the sham (clip: 5.42 ± 0.55; sham: 4.57 ± 0.23). After surgical reversal there was no difference at 1 day (clip: 4.23 ± 0.54; sham: 4.39 ± 0.36) and 75 days (clip: 4.39 ± 0.38; sham: 4.25 ± 0.23) (Table 21). When the contractile responses was expressed as media stress (mN/mm^2) there was a significant reduction in the hypertensive animals compared to sham operated controls (clip: 354 ± 45; sham: 437 ± 20). One day after surgical reversal the media stress response remained significantly reduced in the hypertensive (259 ± 46) animals compared to sham operated controls (410 ± 26, $p<0.05$). However, 75 days after surgical reversal of hypertension the contraction expressed as media stress was similar in the hypertensive (294 ± 23) animals compared to sham operated controls (343 ± 18) (Table 21).

Noradrenaline contractile response

Noradrenaline produced a concentration dependent contraction of mesenteric resistance vessels. The induction of renovascular hypertension resulted in a significant increase in the absolute contractile response (mN/mm) to noradrenaline in the hypertensive (5.42 ± 0.52, $p<0.001$) rats compared to sham operated controls (4.41 ± 0.25) (Figure 43). One day after surgical reversal of hypertension the contractile response of the unclipped animals (4.26 ± 0.52) was similar to that in the sham operated controls (4.57 ± 0.37) (Figure 44). Similarly, 75 days after surgical reversal

of hypertension the response to noradrenaline in the unclipped animals (4.24 ± 0.34) (Figure 45) was not significantly different the sham operated controls (4.27 ± 0.36). There was no alteration in the sensitivity to noradrenaline (ED_{50}) in any of the groups compared to their sham operated controls (Table 22).

When the noradrenaline contractile response was expressed as media stress, the contraction per unit volume of smooth muscle (mN/mm^2), vessels from the hypertensive animals (359 ± 39, $p<0.05$) generated a significantly smaller force compared to sham operated controls (423 ± 23) (Figure 46). One day after surgical reversal the contractile response, expressed as media stress remained significantly reduced , in the unclipped animals (267 ± 42, $p<0.001$) compared to the sham operated controls (392 ± 27) (Figure 47). However, 75 days after surgical reversal the contractile response in the unclipped animals (286 ± 20) was not significantly different from the sham operated controls (338 ± 31) (Table 22, Figure 48).

6.4.4. Relaxation Studies

Acetylcholine relaxation response

Acetylcholine caused a concentration dependent relaxation of mesenteric resistance vessels maximally contracted with noradrenaline ($10^{-5}M$). Before surgical reversal of hypertension acetylcholine induced relaxation was significantly reduced in the hypertensives animals (22 ± 8%, $p<0.001$) compared to the sham operated controls (51 ± 5%) (Figure 49). One day after surgical reversal of hypertension the relaxation response in the hypertensive animals (29 ± 5%) was similar to that in the sham operated controls (37 ± 4%) (Figure 50). Seventy five days after surgical reversal of hypertension the relaxation response in the unclipped animals (26 ± 8%)

was similar to the sham operated controls (17 ± 7%) (Figure 51). There was a non-significant reduction in acetylcholine sensitivity (ED_{50}) in the hypertensive rats before and 1 day after reversal but 75 days acetylcholine sensitivity was the same as sham operated controls (Table 22).

Table 19. Physical characteristics of Goldblatt two kidney one clip acute hypertensive (clip) and sham operated control (sham) rats.

	No Animals	Blood Pressure (mmHg)		Body Weight (grams)	Heart Weight (grams)	Heart:Body Weight Ratio (%)
		pre-unclip	post-unclip			
Before unclipping						
SHAM	12	105 ± 3		253 ± 8	0.8657 ± 0.028	0.345 ± 0.010
CLIP	12	166 ± 5***		253 ± 9	1.0670 ± 0.048**	0.422 ± 0.013***
24 hrs unclip						
SHAM	11	112 ± 4	110 ± 8	251 ± 5	0.7867 ± 0.010	0.315 ± 0.005
CLIP	10	171 ± 4***	123 ± 10++	249 ± 6	1.0030 ± 0.043***	0.404 ± 0.015***
75 days unclip						
SHAM	10	106 ± 3	111 ± 3	323 ± 15	0.9318 ± 0.031	0.292 ± 0.010
CLIP	10	181 ± 8***	113 ± 5+++	302 ± 8	0.9940 ± 0.033	0.331 ± 0.013*

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ Compared to Sham operated control

++ $p<0.01$, *** $p<0.001$ Compared to pre-unclip blood pressure

Table 20. Mesenteric vessel morphology of Goldblatt two kidney one clip acute hypertensive (clip) and sham operated control (sham) rats.

	No Vessels	Vessel Diameter (μm)	Media Thickness (μm)	Media Volume (μm^3)	Media:Lumen Ratio (%)
Before unclipping					
SHAM	21	217 ± 9	10.62 ± 0.62	7512 ± 412	5.07 ± 0.45
CLIP	23	220 ± 10	15.85 ± 1.20***	11448 ± 1000**	7.95 ± 0.89**
24 hrs unclip					
SHAM	22	195 ± 10	11.62 ± 0.70	7502 ± 594	6.29 ± 0.48
CLIP	20	198 ± 17	19.09 ± 1.90**	12298 ± 985***	11.63 ± 2.10*
75 days unclip					
SHAM	20	209 ± 11	12.64 ± 0.46	9163 ± 526	5.95 ± 0.33
CLIP	20	209 ± 7	15.27 ± 1.20	11123 ± 820	7.16 ± 0.68

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ Compared to Sham operated control

Table 21. The contractile response to high potassium and potassium containing noradrenaline (10^{-5}M) expressed as active tension and media stress in mesenteric vessels from Goldblatt two kidney one clip acute hypertensive rats (clip) and sham operated controls (sham).

	123mM Potassium		123mM Potassium + Noradrenaline (10^{-5}M)	
	Active Tension (mN/mm)	Media Stress (mN/mm^2)	Active Tension (mN/mm)	Media Stress (mN/mm^2)
Before unclipping				
sham	3.48 ± 0.12	334 ± 12	4.57 ± 0.23	437 ± 20
clip	3.97 ± 0.25	259 ± 18	5.42 ± 0.55	354 ± 45
24 hrs unclip				
sham	3.40 ± 0.18	300 ± 15	4.39 ± 0.36	410 ± 26
clip	3.34 ± 0.22	206 ± 18***	4.23 ± 0.54	259 ± 46*
75 days unclip				
sham	3.43 ± 0.13	274 ± 9	4.25 ± 0.23	343 ± 18
clip	3.58 ± 0.19	237 ± 11*	4.39 ± 0.38	294 ± 23

* $p<0.05$, *** $p<0.001$ compared to sham operated control

Table 22. Noradrenaline sensitivity and the maximum contractile response to noradrenaline expressed as tension and media stress. Acetylcholine sensitivity and the maximum relaxation response in mesenteric vessels from Goldblatt two kidney one clip acute hypertensive rats (clip) and sham operated controls (sham)

	Noradrenaline Maximum		Noradrenaline	Acetylcholine	
	Active Tension (mN/mm)	Media Stress (mN/mm^2)	Sensitivity (ED_{50}) (μM)	Relaxation (%)	Sensitivity (ED_{50}) (μM)
Before unclipping					
sham	4.41 ± 0.25	423 ± 23	1.773 ± 0.38	51 ± 5	0.068 ± 0.015
clip	5.42 ± 0.52***	359 ± 39*	1.599 ± 0.58	22 ± 8***	0.222 ± 0.160
24 hrs unclip					
sham	4.57 ± 0.37	392 ± 27	1.880 ± 0.16	37 ± 4	0.062 ± 0.360
clip	4.26 ± 0.52	267 ± 42***	2.261 ± 0.49	29 ± 5	0.145 ± 0.088
75 days unclip					
sham	4.27 ± 0.36	338 ± 31	1.205 ± 0.17	17 ± 7	0.082 ± 0.040
clip	4.24 ± 0.34	286 ± 20	1.123 ± 0.23	26 ± 8	0.064 ± 0.019

** $p<0.01$, *** $p<0.001$ compared to sham operated control

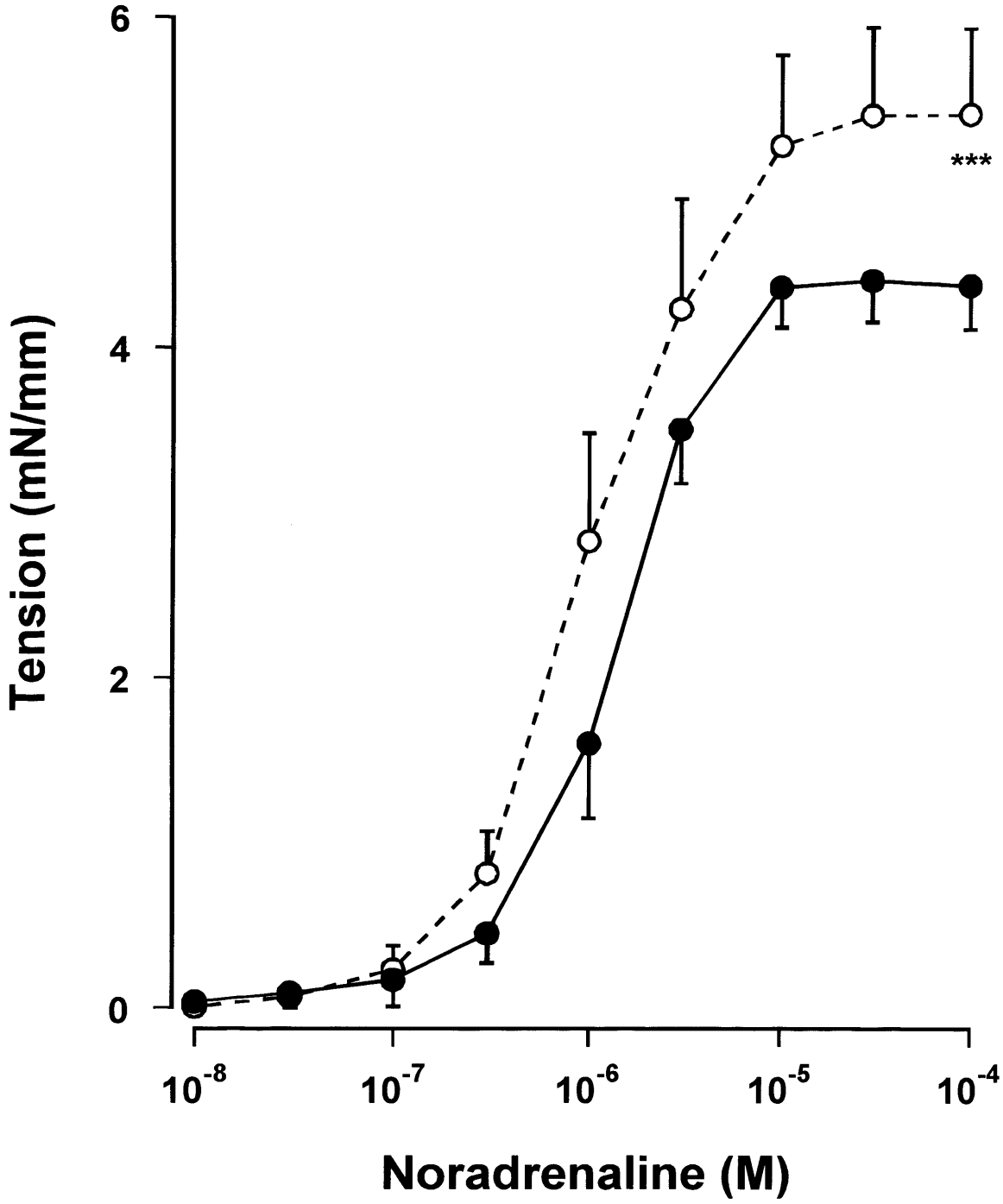

Figure 43 The noradrenaline contractile response of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

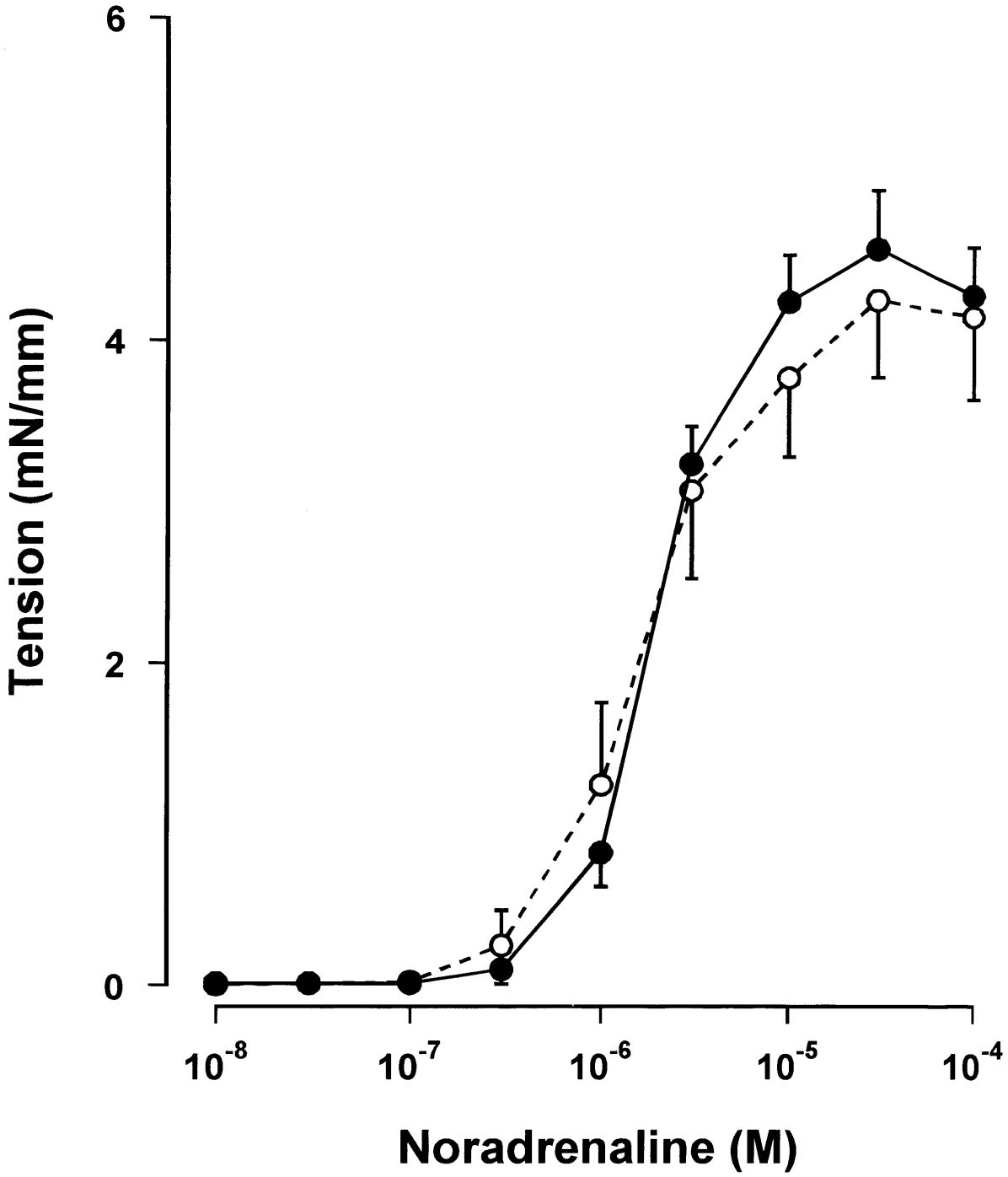

Figure 44 The noradrenaline contractile response of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 1 day after surgical reversal of hypertension

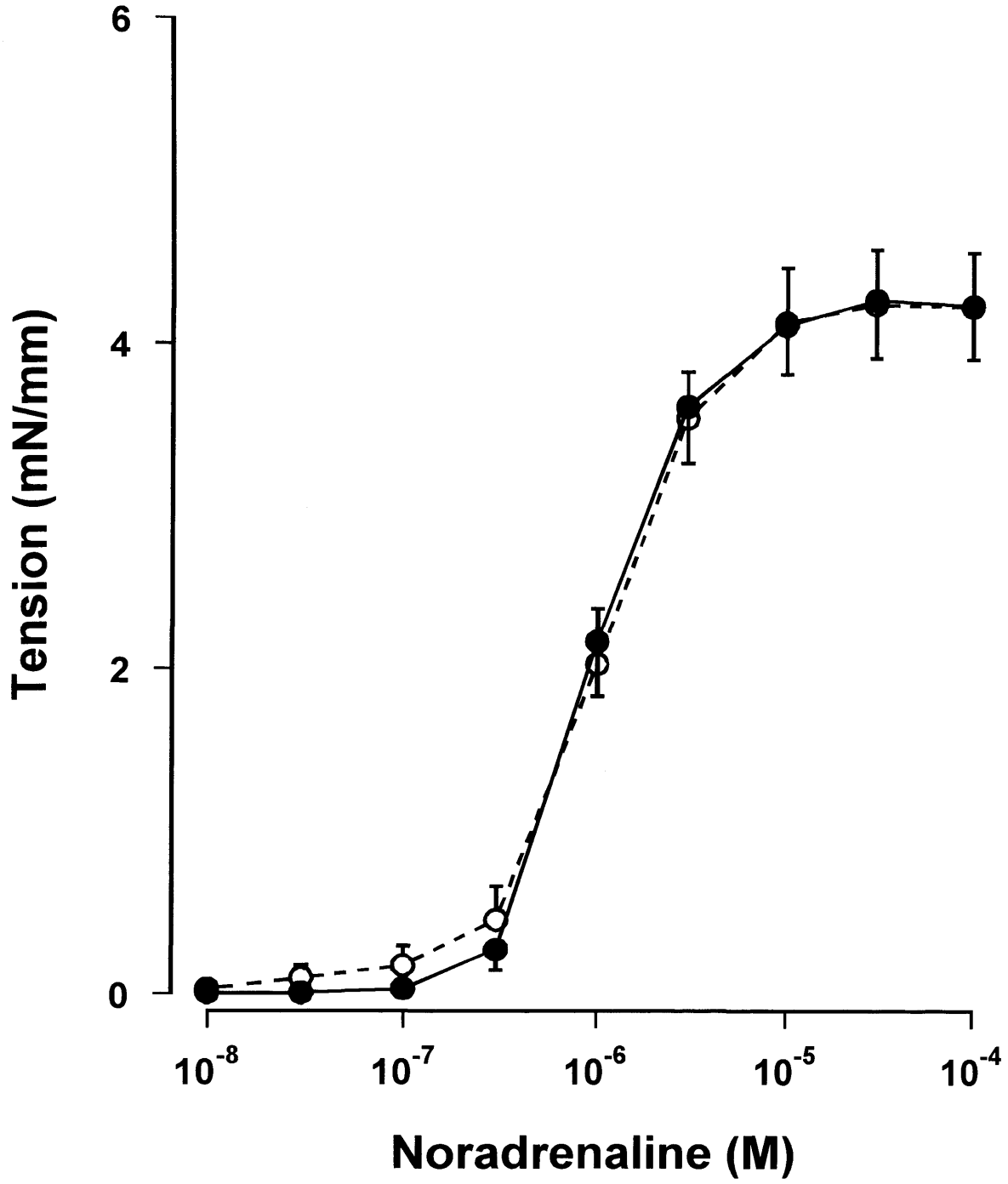

Figure 45 The noradrenaline contractile response of mesenteric bessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 75 days after surgical reversal of hypertension

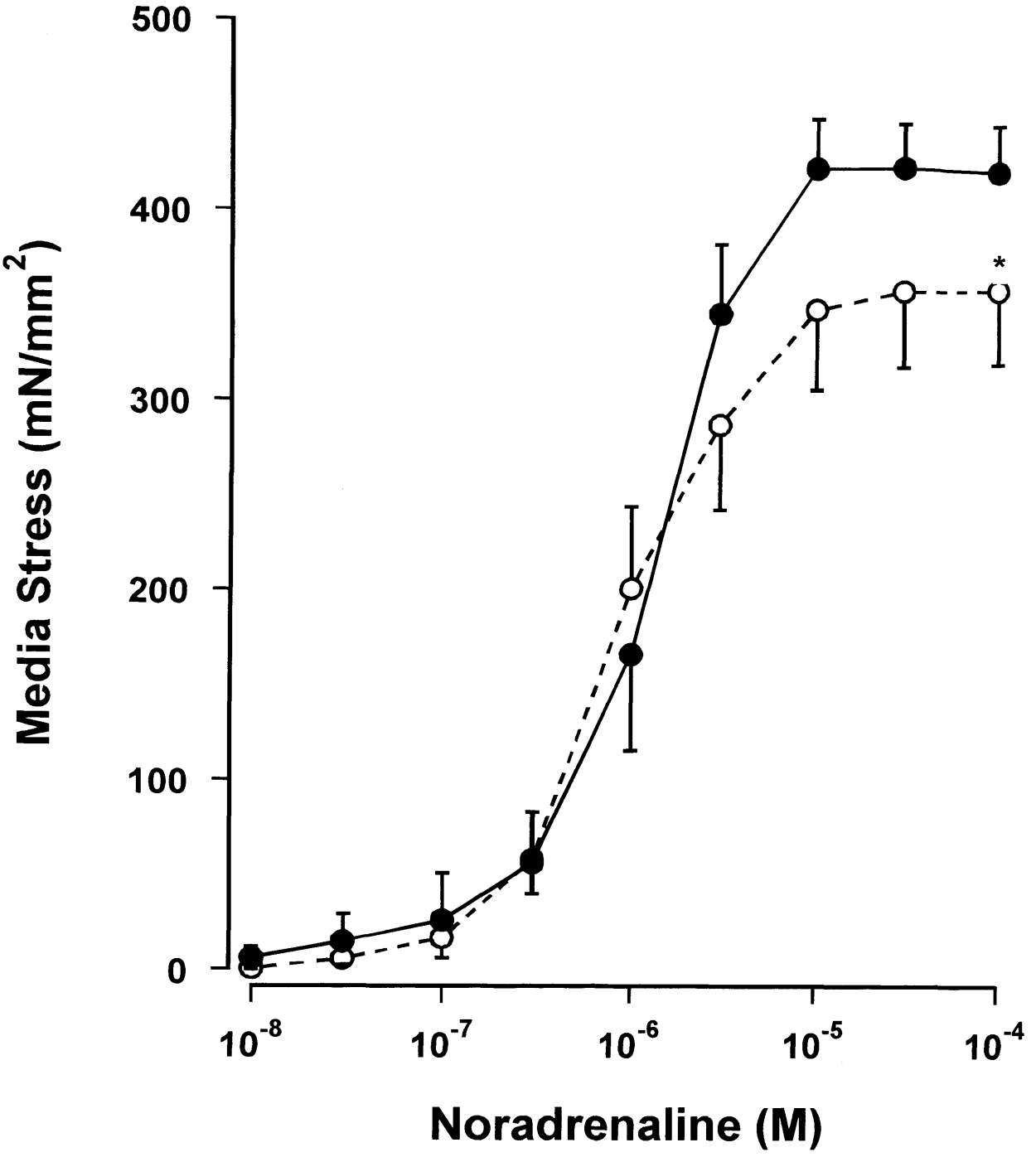

Figure 46 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats *p<0.05 denotes comparison between the two lines

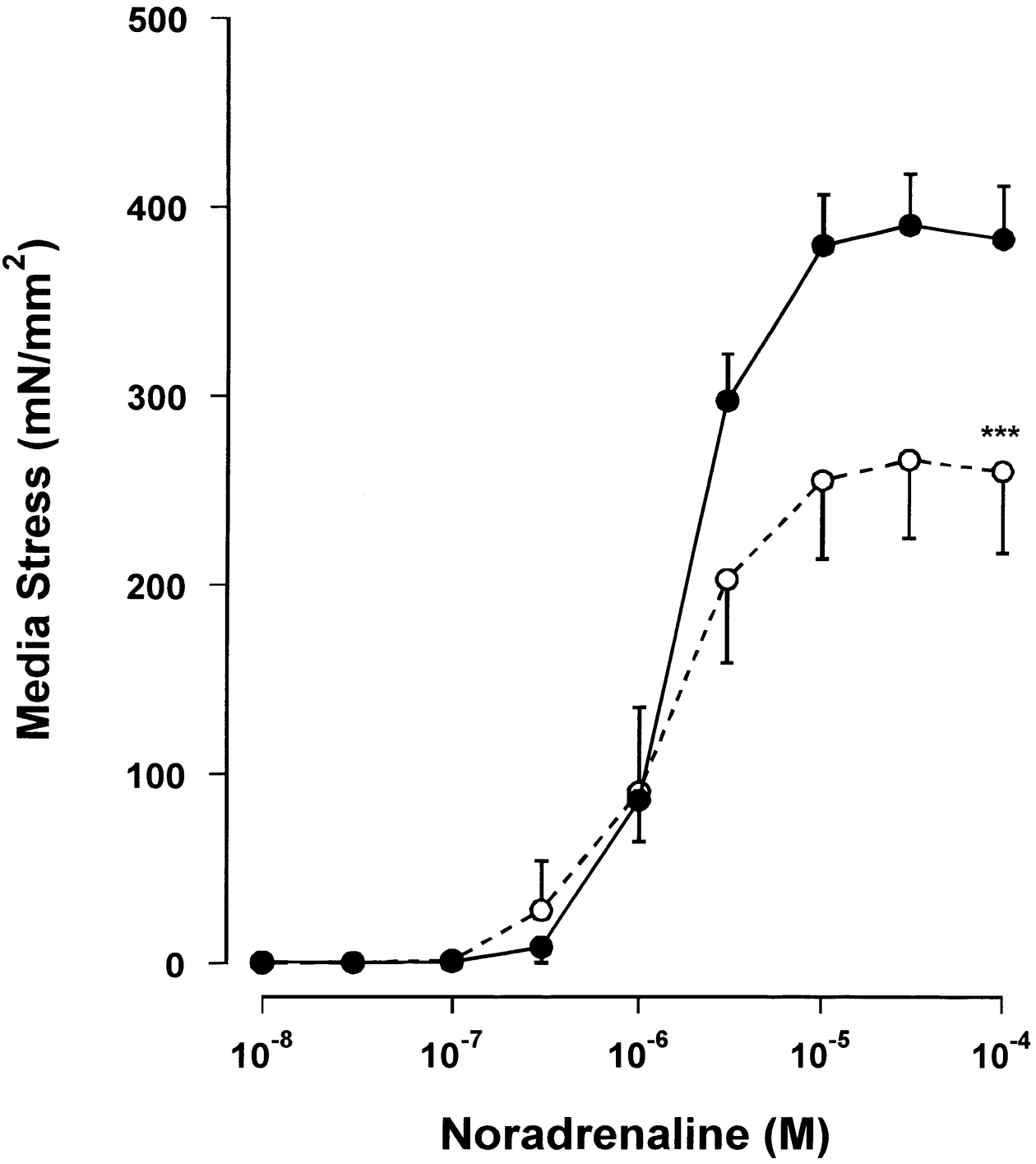

Figure 47 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 1 day after surgical reversal of hypertension ***p<0.001 denotes comparison between the two lines

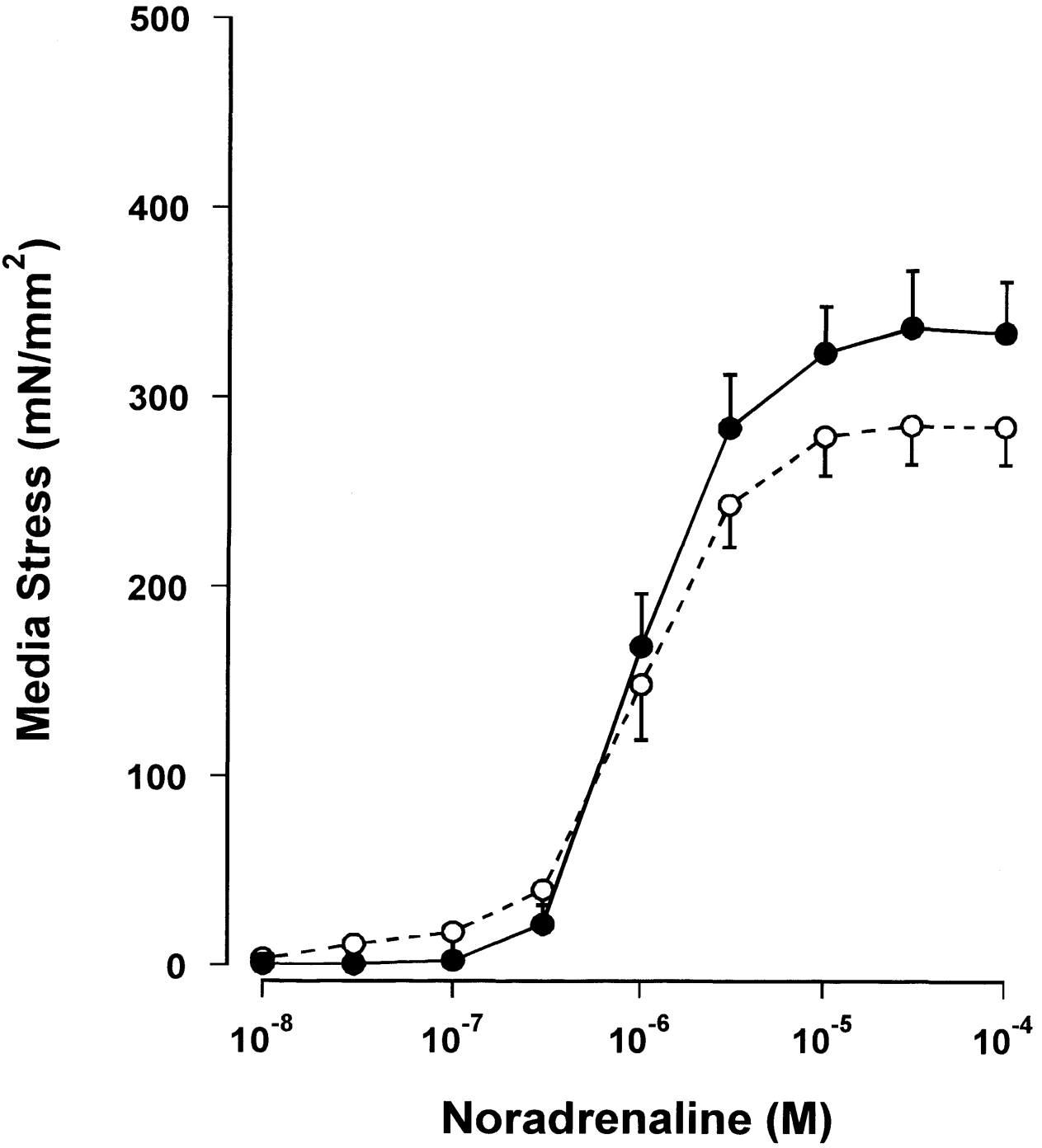

Figure 48 The noradrenaline contractile response expressed as the media stress of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 75 days after surgical reversal of hypertension

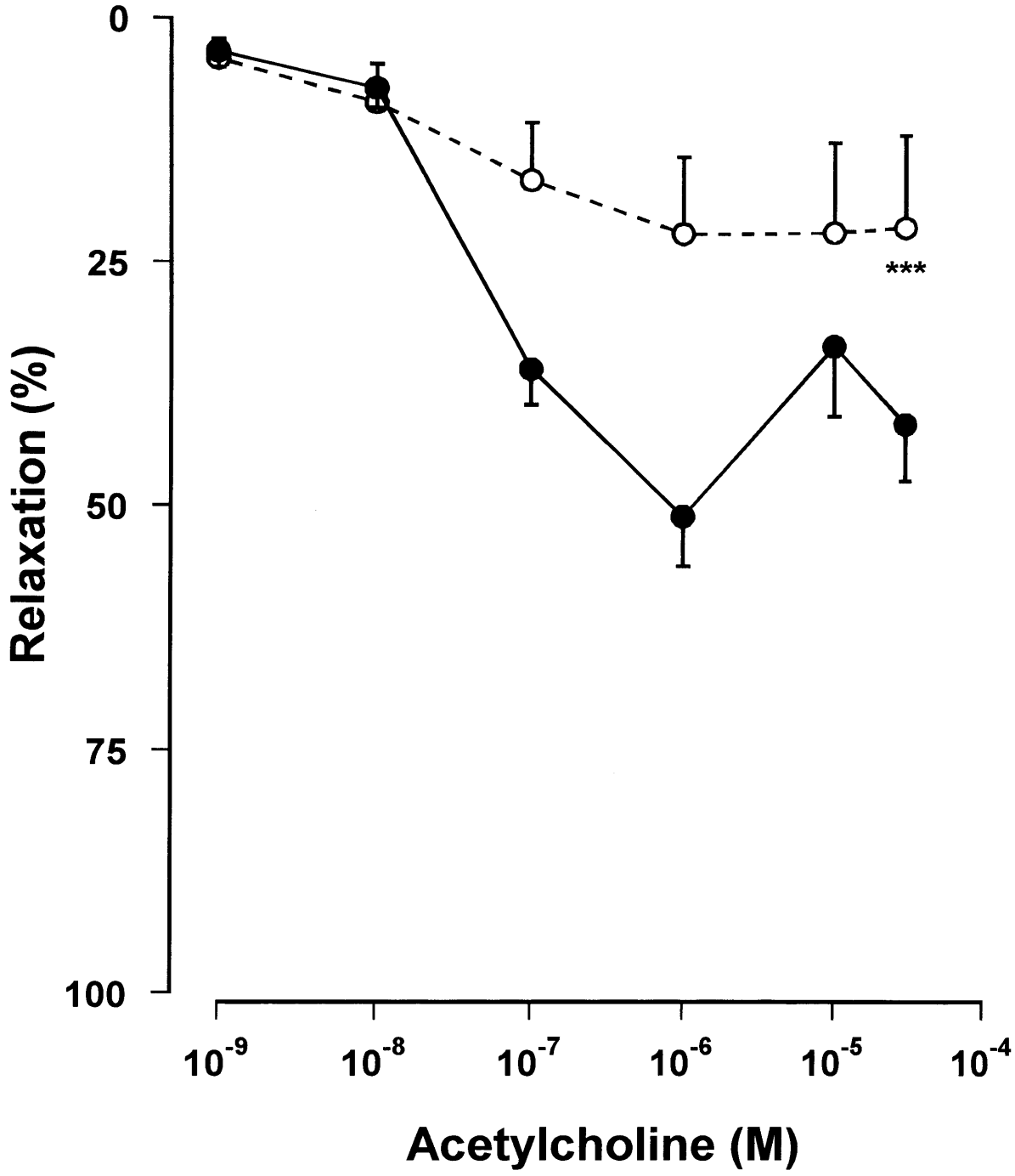

Figure 49 The acetylcholine relaxation response of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats ***p<0.001 denotes comparison between the two lines

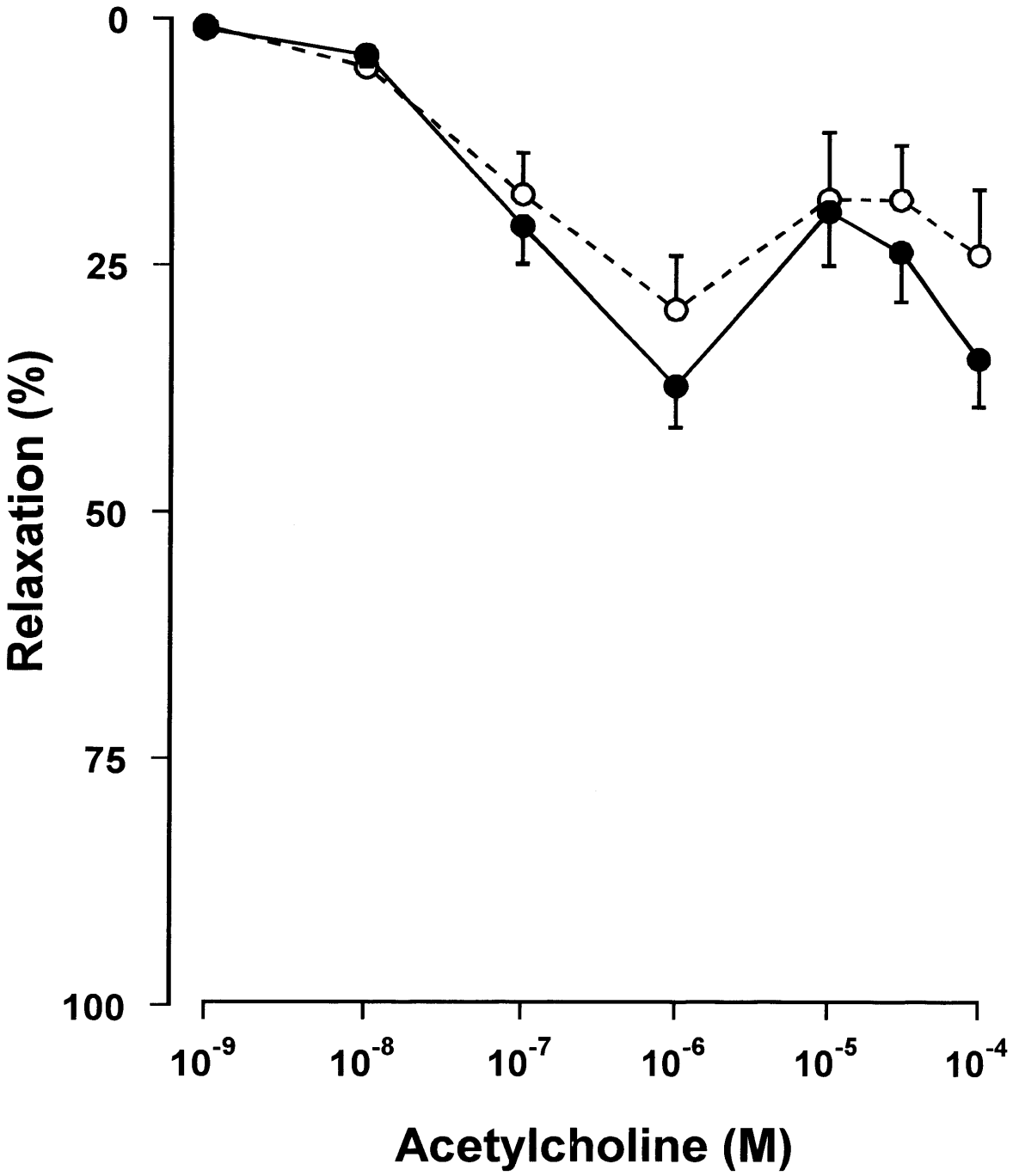

Figure 50 The acetylcholine relaxation response of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 1 day after surgical reversal of hypertension

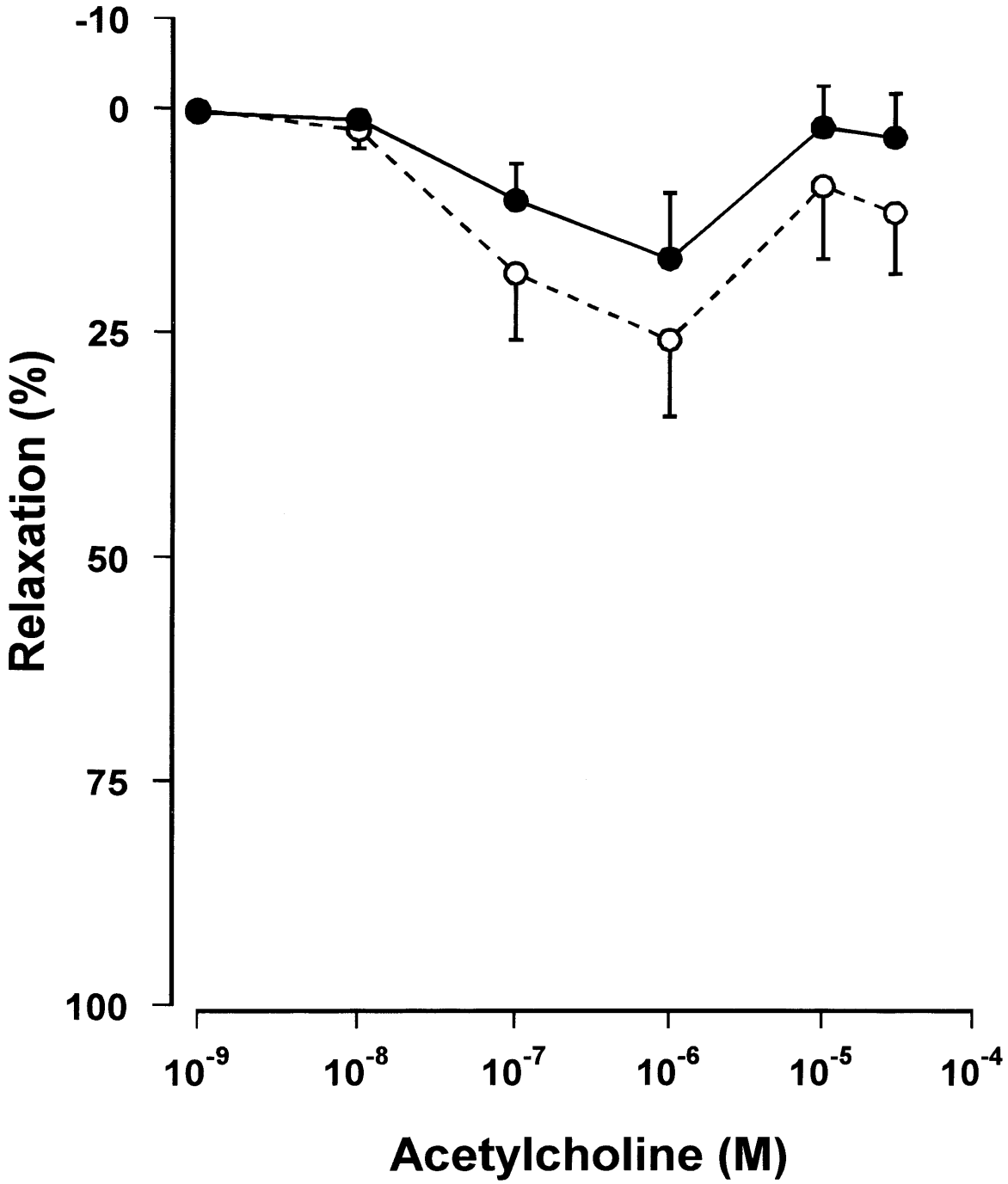

Figure 51 The acetylcholine relaxation response of mesenteric vessels from Goldblatt 2 kidney 1 clip hypertensive (O) and sham operated control (●) rats 75 days after surgical reversal of hypertension

6.5. DISCUSSION

Reversal of renovascular hypertension by removal of the renal artery constricting clip resulted in a rapid fall in blood pressure, within a 24 hour period, to pre-clipping levels, thus confirming previous observations (Gull & Sutton 1872; Furuyama 1962; Suwa & Takahashi 1971 and Lee et al. 1983). This occurred despite the persistence of structural vascular changes. Thus, induction of renovascular hypertension resulted in a 52% increase in medial cross sectional area in the mesenteric resistance vessels one day after surgical reversal of hypertension the cross sectional area was unchanged (64%). By contrast, seventy five days after unclipping there was no significant difference between the unclipped hypertensives and age matched sham controls. This is in keeping with Lundgren and Weiss (1979) who reported that structural vascular changes take several weeks to resolve after reversal of hypertension.

Similarly, after reversal of hypertension there was still evidence of cardiac hypertrophy and even after seventy five days the heart to weight ratio remained significantly greater in the hypertensive group. This may be due, in part, to the persistence of collagen and other intracellular matrix and this has been reported by other workers. Such intracellular material shows far less regression than vascular smooth muscle hypertrophy (Wolinsky 1971 & 1972 and Folkow 1978).

Folkow (1978) has shown that the duration of hypertension may be linked to the amount of intracellular material, mainly collagen, added to arterial walls. Collagen and elastin molecules can be synthesised and secreted by vascular smooth muscle exposed to an increased load. The addition of non-

contractile elements could provide local support to relieve the vascular walls from the strain of a raised blood pressure (Wolinsky 1971 & 1972).

Other studies have shown that the relationship between blood pressure and structure of resistance arteries is not clear cut. Treatment of young spontaneously hypertensive rats with antihypertensive drugs failed to prevent the development of abnormal resistance vessels structure (Weiss & Lundgren 1978; Nyborg & Mulvany 1984; Jespersen et al. 1985; Smeda et al. 1988; Christensen et al. 1989 and Smeda & Lee 1991). However, the failure to prevent structural change was attributed to the fact that although the mean blood pressure returned to normal levels the pulse pressure did not. Recently, treatment with angiotensin converting enzyme (ACE) inhibitors has been shown to fully normalise blood pressure and vascular structure in a variety of vascular beds (Lee et al. 1991; Hajdu et al. 1991 and King et al. 1992).

It has been suggested that vascular hypertrophy may maintain an elevated peripheral vascular resistance in the presence of normal levels of vasoconstrictor activity (Folkow et al. 1973). However, vessels from the established phase of Goldblatt two kidney 1 clip hypertension failed to show an enhanced response to high potassium and high potassium containing noradrenaline. Moreover, expressing responses with a correction for the increase in cross sectional area (media stress) suggests that the contractile response was decreased in the hypertensive animals. One day following reversal of hypertension the potassium stimulation elicited a similar response in the sham and unclipped animals. However, when corrected for cross sectional area the response to potassium or potassium containing noradrenaline was significantly reduced (31% and 37% respectively). Thus, potassium contractile responses to potassium were

significantly reduced before there was evidence of regression of vascular structure. This indicates wall mass cannot account for the elevated peripheral vascular resistance prior to unclipping because the blood pressure fell to normal levels one day after reversal of hypertension (Lundgren & Weiss 1979). By seventy five days the contractile response of vessels from the unclipped and sham animals were similar but the potassium responses appeared to be lower (14%) in the unclipped animals when expressed as media stress.

The contractile response to noradrenaline, was significantly increased in the hypertensive animals although again when corrected for cross sectional area the media stress was significantly less (15%). One day after reversal of hypertension noradrenaline contractility was similar to controls and the media stress response was further reduced (32%). At 75 days, vascular structure had resolved, the contractile response to noradrenaline was similar in the unclipped and sham operated animals whether you expressed in absolute terms or as media stress. These observation are not in keeping with the concept of a vascular amplifier since an increase in the contractile response would have been expected one day after reversal of hypertension. However, the haemodynamic amplifier properties of vessels tend to be underestimated in the wire myograph.

In addition, previous studies have shown, in this model, that when a vasoconstrictor mechanism is suddenly removed there will be a rapid fall in blood pressure (2-4 hours) and a decrease in vascular contractility. It has been postulated that the previously clipped kidney may release a powerful vasodilator and the candidate put forward for this has been medullipin (Folkow and Lever 1992). Gothberg and colleagues showed that a reduction of angiotensin II stimulated medullipin release and suggested that

the combined effects of exogenous angiotensin II withdrawal and medullipin release may cause a decrease of vasoconstrictor tone to noradrenaline.

Endothelium dependent relaxation was significantly decreased by 43% in the hypertensive animals but one day after reversal of hypertension the endothelium dependent relaxation was similar in the unclipped and sham animals, albeit both groups showed a reduced response. Seventy five days after reversal of hypertension the response to acetylcholine was similar in both groups but the absolute responses were decreased. Spontaneously hypertensive rat studies have shown that age is an important determinant of endothelium dependent relaxation. Thus, Koga et al. (1989) showed that endothelium dependent relaxation decreased with age in normotensive and spontaneously hypertensive rats and that this alteration was associated with an increase in the release of an endothelial derived contracting factor, probably thromboxane A_2 or some other prostanoid. Also, Lee et al. (1987) studied the effects of age on endothelium dependent relaxation and concluded that relaxation was reduced with age and that there was a greater reduction in hypertensive (SHR and DOCA) rats compared to normotensive controls. However, they demonstrated that nitrovasodilator induced relaxation also was decreased in the adult spontaneously hypertensive rat compared to age matched normotensive controls and that responses decreased with age in both hypertensive and normotensive groups. By contrast, Imaizumi and colleagues (1990) found no age dependency of endothelial dependent relaxation in man. They measured forearm blood flow in three groups of normotensive volunteers, classified by age and showed that acetylcholine induced relaxation was similar in all three groups. In the present study, the response to acetylcholine was decreased as the animals became older and it is not clear what effect ether

anaesthesia had on endothelial dependent relaxation within the first twenty four hours post surgery.

Reversal of hypertension and the subsequent regression in the resistance vessel structure suggest that the changes observed following the development of hypertension are reversible if blood pressure is controlled or reversed. Functional abnormalities, particularly endothelial derived relaxing factors, also could be normalised after reversal of hypertension. This would imply that there is possibly an on-going feedback mechanism or pathway that is interrupted or weighted during hypertension. Possibly part of the imbalance may be due to either factors such as a reduction in endothelium modulation of contractions or the release of an additional contracting factor during vascular relaxation.

6.6. CONCLUSIONS

1. Surgical removal of the constricting clip lowers blood pressure in the Goldblatt two kidney one clip model of hypertension and led to regression of vascular hypertrophy to normal within seventy five days. The regression of cardiac hypertrophy was incomplete even at seventy five days.

2. The increased contractile response to noradrenaline during hypertension was abolished one day after reversal of hypertension and remained unchanged at seventy five days. Correcting the contractile response for media cross sectional area showed a reduced response to noradrenaline at both one and seventy five days after reversal of hypertension.

4. Endothelial derived relaxing factor was decreased in Goldblatt two kidney one clip hypertension but one day after surgical reversal of hypertension there was no significant difference between the unclipped hypertensives and controls. Similarly, seventy five days after reversal of hypertension endothelial derived relaxing factor responses were similar in unclipped hypertensives and controls.

CHAPTER 7 STUDIES WITH THE PEPTIDE ENDOTHELIN-1

7.1. INTRODUCTION

Many mechanical and neurohumoral stimuli are capable of inducing endothelium dependent vasoconstriction (Furchgott 1984; Vanhoutte et al. 1986; Harder 1987). Yanagisawa et al. 1988a have described an endothelial derived vasoconstrictor polypeptide and named it endothelin. Endothelin is derived from a 203 amino acid peptide precursor, pre-proendothelin, which is cleaved after translation to form proendothelin. In the presence of a converting enzyme, located within the endothelial cells, proendothelin (or big endothelin) is cleaved to produce a 21 amino acid peptide, endothelin (Yanagisawa et al. 1988a). Endothelin is a potent vasoconstrictor peptide, acting on a variety of blood vessels, and in low concentrations induces a sustained rise in arterial blood pressure in the rat. Endothelin has a relative mass of 2492 and is characterised by two intrachain disulfide rings. This type of structure is unknown in mammalian peptides but is often found in sub-mammalian venoms. In fact endothelin has considerable chemical similarity to alpha scorpion toxins and the snake venom sarafotoxin (Yanagisawa et al. 1988a).

There are now 3 related mammalian endothelin peptides encoded by distinct genes in man as well as in rat and pig (Inoue et al. 1989). The have been named endothelin-1 (human/porcine), endothelin-2 and endothelin-3 (rat endothelin). Preproendothelin mRNA expression has been demonstrated in cultured bovine glomerular capillary endothelial cells (Marsden et al. 1989), suggesting that the microvasculature is capable of producing endothelin.

Agents such as thrombin, adrenaline and the calcium ionophore A23187, which stimulate phosphoinositide turnover, has been shown to increase mRNA levels (Yanagisawa et al. 1988a). In addition, phorbol esters up-regulate preproendothelin expression in human umbilical vein endothelial cells suggesting that there may be a role for protein kinase C in its vascular actions (Yanagisawa et al. 1989). Moreover, cultured endothelial cells exposed to increased shear stress increases expression of preproendothelin in cultured endothelial cells (Kurihara et al. 1989) which may be of significance for the transduction of changes in vascular flow rates and blood viscosity in autoregulatory responses. Although endothelin induces a potent and long acting vasoconstrictor response there is evidence of heterogeneity when vascular beds are compared (Yanagisawa et al 1988b).

The mechanisms underlying endothelin induced smooth muscle contraction are not precisely known but in large blood vessels vasoconstrictor responses are largely dependent on the presence of extracellular calcium and can be attenuated by pre-treatment with calcium channel antagonists (Yanagisawa et al. 1988a; Hughes et al. 1988). However, others have suggested that endothelin may act partly by stimulating phospholipase C causing phosphoinositol hydrolysis and protein kinase C activation (Griendling et al. 1989; Kasaya et al. 1989; Marsden et al. 1989).

The effects of endothelin, at the microvascular level, is supported by in vivo studies showing decreased blood flow in rabbit skin (Brain et al. 1988) and concentration-dependent arteriolar vasoconstriction after topical application on the hamster cheek pouch (Brain 1989). In addition, endothelin also has contractile effects on tracheal smooth muscle (Uchida et al. 1988) and cardiac myocytes (Hu et al. 1988). The pressor effects of endothelin have been confirmed in ganglion-blocked rats (Wright & Fozard 1988) and that

study demonstrated a long lasting profound vasoconstriction in the cerebral, mesenteric, renal and hindquarter beds (Wright & Fozard 1988).

Most in vitro studies have been confined to large or medium sized arteries and since changes in vascular resistance are dependent on small vessels these studies were designed to investigate the effects of endothelin-1 in isolated human resistance vessels.

7.2. GENERAL METHODS

7.2.1. Preparation of Human tissue

Surgical samples of omental fat were obtained from patients undergoing routine operations at Leicester Royal Infirmary. The fat samples were collected in cold (4°C) calcium free physiological salt solution (Appendix A) and transported to the laboratory on ice. Resistance arteries (<300μm) were dissected free from surrounding fat and tissue before being mounted as ring preparations in a myograph. The vessels were bathed in physiological salt solution, heated to 37°C and gassed with 5%CO_2/95%O_2 to achieve a pH of 7.4. After equilibrating for 60 minutes the length tension characteristics for each vessel were determined. The internal diameter was set to $L_{0.9}$ which is 90% of that which the vessel would have had in vivo under a transmural pressure of 100mmHg (13.3kPa). Following this normalisation procedure groups of vessels were randomly selected for a number of experimental protocols.

7.3. COMPARISON OF THE CONTRACTILE RESPONSE TO NORADRENALINE AND ENDOTHELIN-1

A cumulative dose contraction curve to noradrenaline (10^{-8}M to 10^{-4}M) was performed in the presence of cocaine (10^{-6}M). The vessels were then rinsed in fresh physiological salt solution and allowed to return to baseline. After a 20 minute interval a cumulative dose contraction curve to endothelin-1 (10^{-11}M to 10^{-7}M) was performed. Each concentration of endothelin-1 was added when the previous dose had either reached a plateau or if after 3-5 minutes no response was recorded. The vessels were then rinsed and allowed to return to baseline. Only one dose contraction curve to endothelin-1 was performed on each pair of vessels because preliminary studies demonstrated tachyphalaxis which persisted for over two hours.

7.3.1. Results

Ten resistance arteries (276 ± 21μm) were obtained from normotensive males and females aged 33-70. Both agents (noradrenaline and endothelin-1) produced a concentration dependent contraction but the endothelin-1 maximum contraction of 2.95 ± 0.52mN/mm was significantly greater than that produced by noradrenaline (2.04 ± 0.34mN/mm, $p<0.001$). There was a 1,000 fold greater sensitivity to endothelin with an ED_{50} of 6.5 ± 1.26nM compared to 2.1 ± 0.7μM for noradrenaline ($p<0.001$) (Figure 52). Endothelin washed out slowly and repeat response curves were limited by tachyphylaxis. At least two hours needed to elapse before a full response could be obtained after washing out the first curve. Therefore, in subsequent studies a single dose contraction response to endothelin-1 was performed.

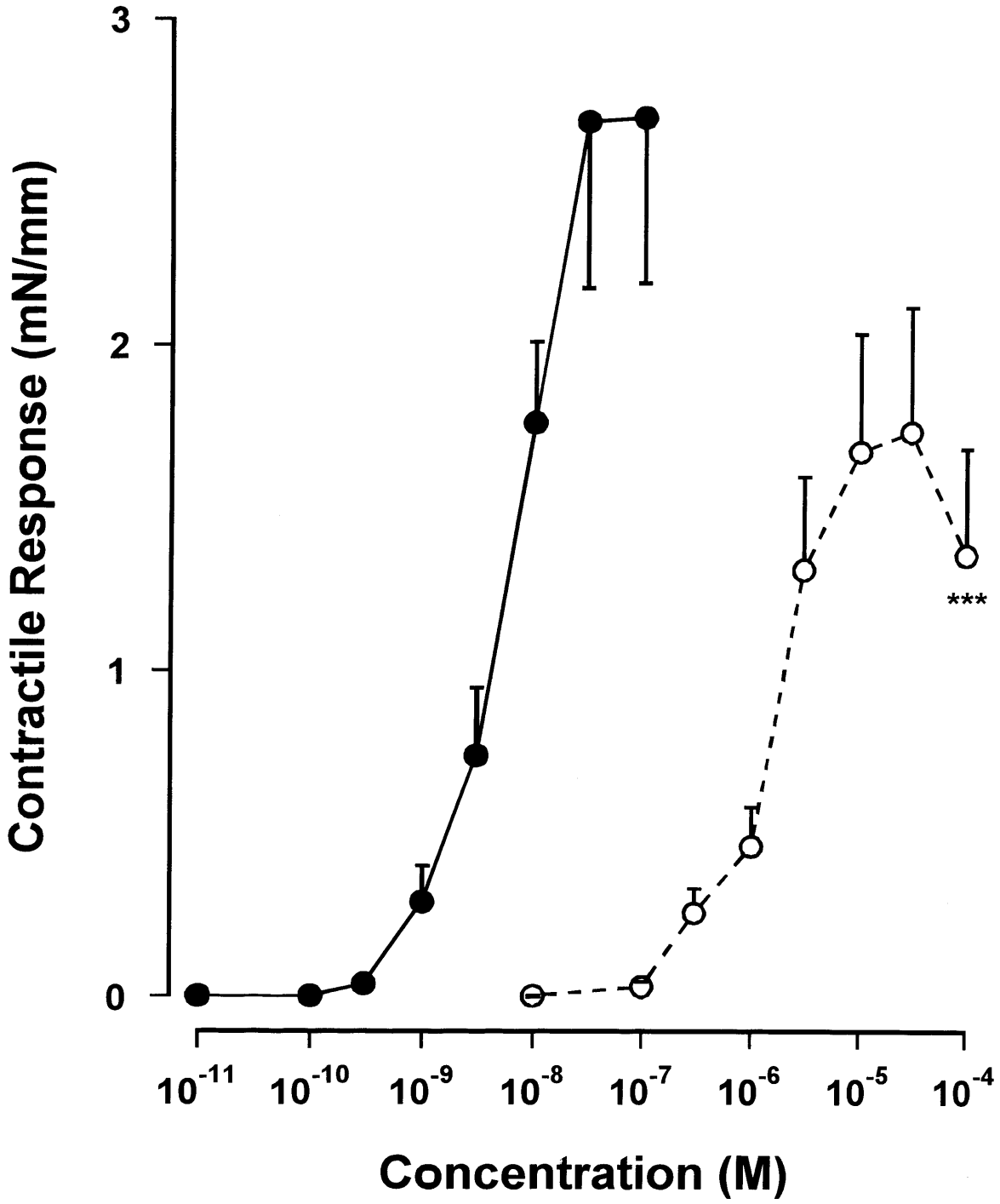

Figure 52 The contractile response of human omental resistance vessels to endothelin-1 (●) and noradrenaline (O) ***p<0.001 denotes comparison between the two lines

7.4. EFFECTS OF CALCIUM CHANNEL BLOCKADE WITH NICARDIPINE

A cumulative dose contraction curve to noradrenaline (10^{-8}M to 10^{-4}M) was performed in the presence of cocaine (10^{-6}M). The vessels were then rinsed in fresh physiological salt solution and allowed to return to baseline. After a 20 minute interval a cumulative dose contraction curve to endothelin-1 (10^{-10}M to $3x10^{-8}$M) was performed in the presence or absence of the calcium channel antagonist nicardipine (10^{-5}M). Nicardipine (10^{-5}M) was added to the bath 5 minutes before the cumulative endothelin-1 dose contraction curve in normal physiological salt solution.

7.4.1. Results

Twenty four resistance arteries (260 ± 40μm) were obtained from normotensive males and females aged 29 to 79 years. Noradrenaline produced a concentration dependent contraction with a maximum response of 1.72 ± 0.39mN/mm. Endothelin-1 produced a concentration dependent contraction in all vessels with a maximum response of 2.95 ± 0.52mN/mm with an ED_{50} of 6.50 ± 1.26nM. In the presence of nicardipine (10^{-5}M) the maximum response to endothelin-1 (1.26 ± 0.20mN/mm) was significantly reduced ($p<0.01$) but there was no alteration in the sensitivity (ED_{50} 8.33 ± 0.90nM) (Figure 53).

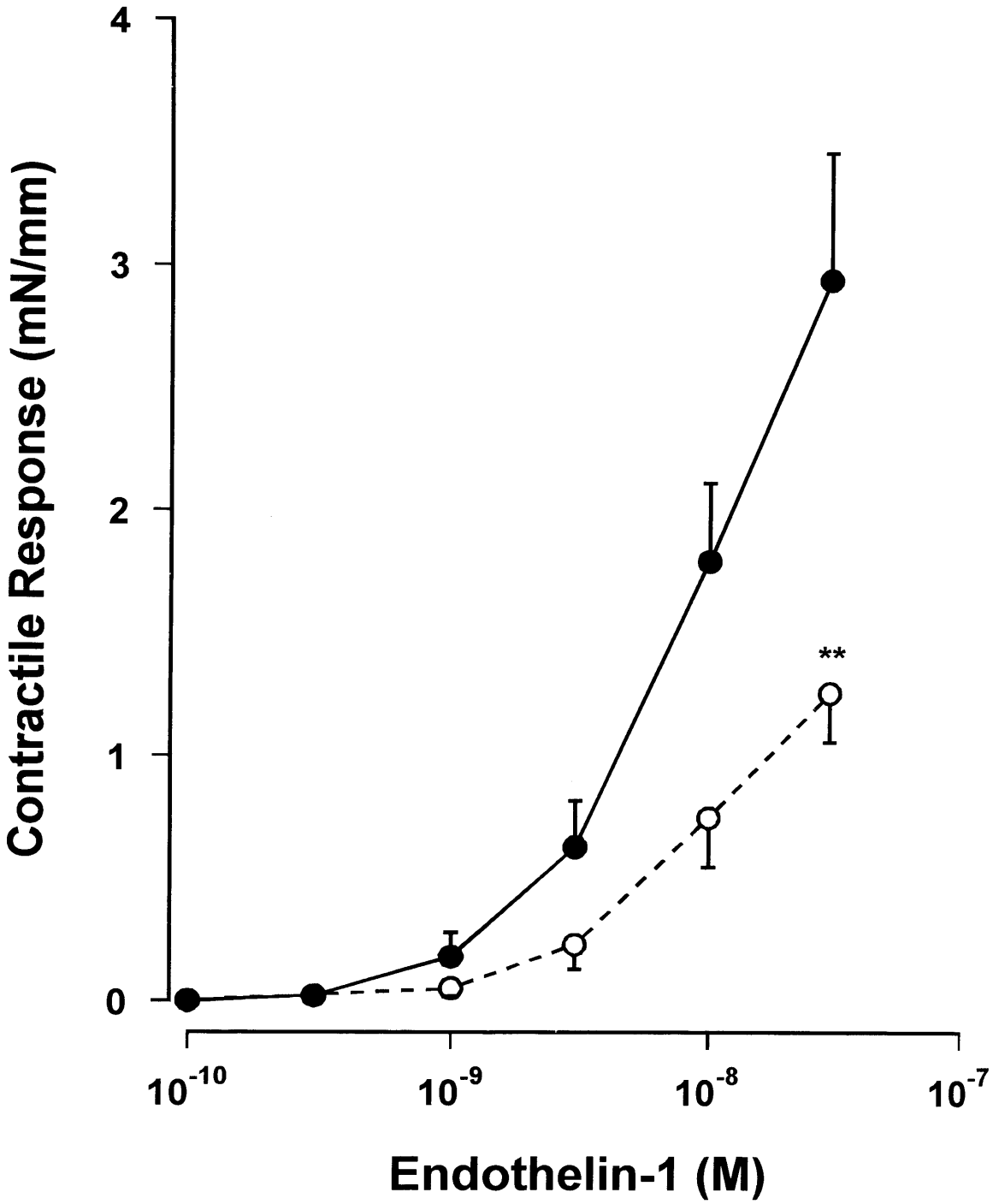

Figure 53 The contractile response of human omental resistance vessels to endothelin-1 in the presence (O) and absence (●) of nicardipine (10^{-5}M) **p<0.01 denotes comparison between the two lines

7.5. DEPLETION OF VASCULAR CALCIUM STORES

Endothelin-1 (10^{-11}M to $3x10^{-8}$M) cumulative dose contraction curves were performed before (N=10) or after (N=10) the intracellular calcium depletion. Extracellular calcium was removed from the bath by replacing the normal physiological salt solution with calcium free physiological salt solution. Internal calcium stores were depleted by repeatedly stimulating the vessels with noradrenaline (10^{-5}M) after which the bath was rinsed at least 3 times with calcium free physiological salt solution. The stimulation with noradrenaline and rinsing in calcium free medium was repeated until no contraction was observed (1-2 hours). Calcium (2.5mM) was re-introduced to the bath at the end of the endothelin-1 contraction curve and a further contraction was recorded.

7.5.1. Results

Twenty resistance arteries (260 ± 24μm) were obtained from normotensive males and females aged 30 to 75 years. In the presence of extracellular calcium endothelin-1 caused a concentration dependent contraction with a maximum response of 2.95 ± 0.52mN/mm and an ED_{50} of 6.5 ± 1.26nM. Calcium depletion attenuated the response to endothelin-1 with the maximum contractile response being 0.92 ± 0.22mN/mm ($p<0.001$) but the sensitivity was only moderately reduced (12.15 ± 1.4nM). Re-introduction of calcium to the bath resulted in a significant increase of the maximum endothelin-1 contraction (2.78 ± 0.41, $p<0.001$), returning the response to the control level (Figure 54).

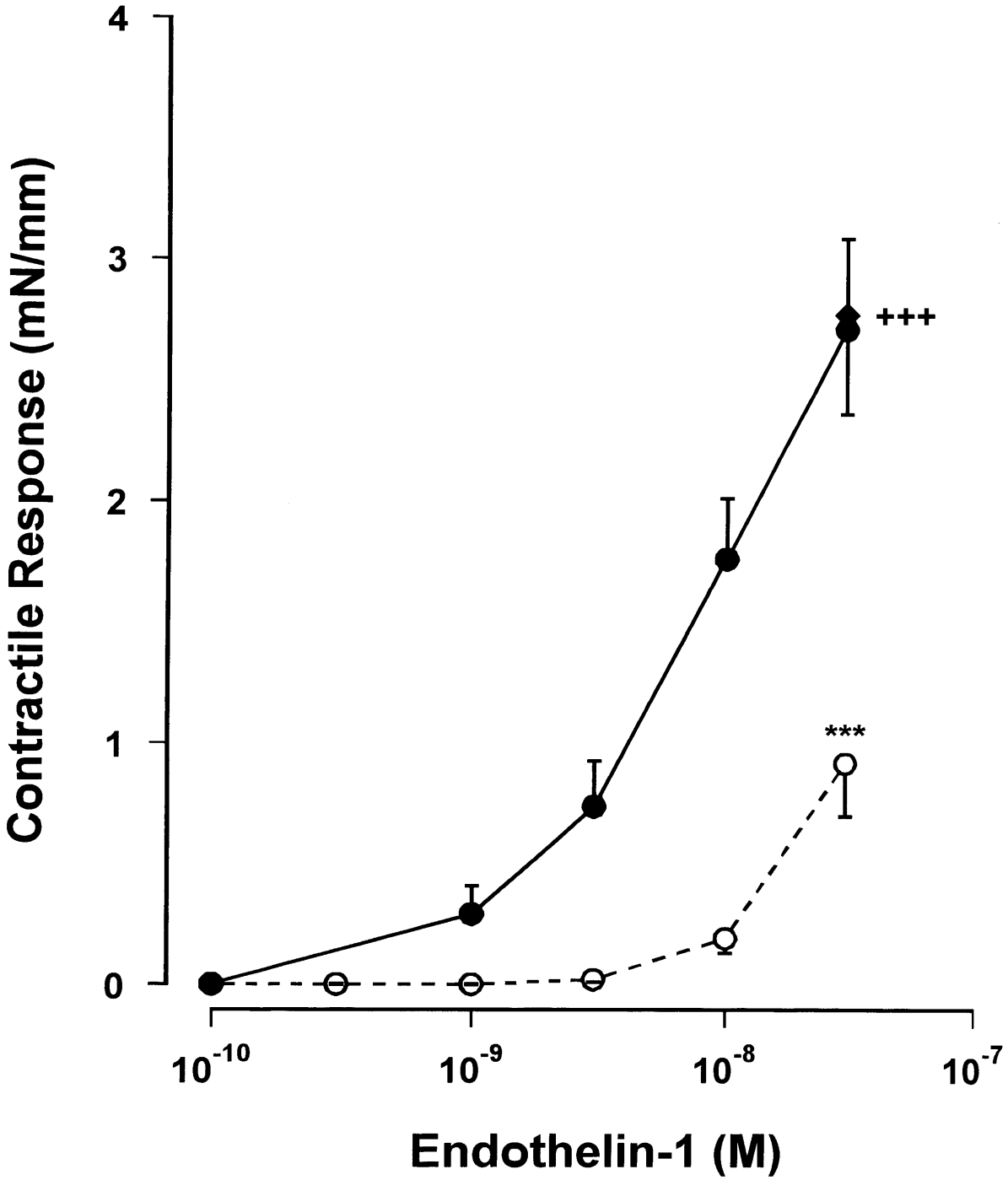

Figure 54 The contractile response of human omental resistance vessels to endothelin-1 before (●) and after (O) depletion of calcium stores and the effect of calcium re-introduction (◆) ***p<0.001 denotes the comparison between before and after calcium depletion +++p<0.001 denotes the compariosn of the maximum response before and after re-introduction of calcium

7.6. EFFECTS OF INTRACELLULAR CALCIUM DEPLETION AND PROTEIN KINASE C INHIBITION

7.6.1. Introduction

A further series of experiments were performed to examine the effects of combining protein kinase C inhibition with calcium depletion. Human omental resistance arteries were studied in a myograph as previously described. The vessels were studied before or after calcium store depletion, before and after incubation with the protein kinase C antagonist, H7, and combined calcium store depletion with protein kinase C inhibition.

As previously described, depletion of external and internal calcium using repeated noradrenaline or caffeine stimulation in calcium free PSS was completed within 1 hour. The protein kinase C inhibitor, H7 is an isoquinoline sulphonamide and is a direct inhibitor of protein kinase C with a 2-20 fold selectivity over cyclic nucleotide-dependent protein kinase and myosin light kinase respectively (Hidaka & Hagiwara 1987).

7.6.2. Protocol

The vessels were arbitrarily assigned to one of the following protocols and all experiments were performed over the same time period.

1. Control (normal PSS)

Fourteen resistance arteries were studied in the presence of calcium. A cumulative dose contraction curve to noradrenaline (10^{-8}M to $3x10^{-5}$M) and endothelin-1 (10^{-10}M to $3x10^{-8}$M) was performed in normal physiological salt solution (PSS).

2. Protein kinase C inhibition

Eight resistance arteries were studied in the presence of calcium. A cumulative dose contraction curve to noradrenaline was performed. The protein kinase C inhibitor H7 (10^{-4}M). H7 was added to the bath 20 minutes prior to a cumulative endothelin-1 dose contraction curve in normal PSS.

3. Calcium store depletion (noradrenaline)

Nine resistance arteries were studied. A cumulative dose contraction curve to noradrenaline was performed in normal PSS. External and partial internal calcium depletion was achieved by incubating the vessels in a Ca^{2+} free physiological salt solution and stimulating the vessels with noradrenaline (10^{-5}M) until a contraction no longer occurred. Then the vessels were rinsed in calcium free physiological salt solution and a cumulative dose contraction curve to endothelin-1 performed.

4. **Calcium store depletion (noradrenaline combined with caffeine)**

Ten resistance arteries were studied. A cumulative dose contraction curve to noradrenaline was performed in normal PSS. External and partial internal calcium depletion was achieved by incubating the vessels in a Ca^{2+} free physiological salt solution and stimulating the vessels with a combination of noradrenaline (10^{-5}M) and caffeine (10^{-4}M) until a contraction no longer occurred. Then the vessels were then rinsed in calcium free physiological salt solution and a cumulative dose contraction curve to endothelin-1 performed.

5. Combined protein kinase C inhibition and calcium store depletion

Eight resistance arteries were studied. A cumulative dose contraction curve to noradrenaline was performed in normal PSS. External and

internal Ca^{2+} depletion was achieved using noradrenaline stimulation after which a cumulative endothelin-1 dose contraction curve was performed in the presence of H7 (10^{-4}M)

6. **Effects of calcium re-introduction**

In each study where the vessels were depleted of calcium, the calcium level was restored (2.5mM) after the response to the last concentration of endothelin-1 ($3x10^{-8}$M) had been observed and any further contraction was recorded.

7.6.3. Results

The patient characteristics, details of the internal diameter and the number of vessels used for the different protocols is shown in Table 23. Omental tissue samples from 31 patients (16 males and 15 females) aged 24-79 years (mean 56 ± 3) were used in this study. All the patients were normotensive with a mean blood pressure of 131 ± 4 / 76 ± 2mmHg and none of the patients were diabetic. The control group comprised of a greater number of females but the groups were otherwise well matched and there was no significant difference between the mean internal diameters of the vessels studied (Table 24).

Noradrenaline produced a concentration dependent contraction in all vessels. The response to noradrenaline was similar in all groups and there was no alteration in sensitivity (ED_{50}) to noradrenaline (Table 24).

In the presence of external calcium, endothelin-1 induced a concentration dependent contraction with a maximum tension of 2.72 ± 0.40mN/mm and an ED_{50} of 4.97 ± 1.35nM. In the control group endothelin-1 produced a

1000 fold greater contraction than noradrenaline whose maximum response in the same vessels was 1.74 ± 0.39mN/mm and whose ED_{50} was 2.27 ± 0.58μM (Table 24, Figure 55). The endothelin-1 contraction was markedly reduced in vessels exposed to protein kinase C inhibitor H7 (10^{-4}M) with a maximum tension of 1.50 ± 0.41mN/mm ($p<0.05$) but there was no alteration in the sensitivity (ED_{50}) to endothelin-1 (7.28 ± 2.82nM).

Removing external calcium with partial depletion of intracellular calcium stores, by repeated noradrenaline stimulation, also resulted in significant attenuation of the contractile response to endothelin-1 reducing the maximum tension to 0.92 ± 0.22mN/mm ($p<0.05$) and this was associated with a decrease in the sensitivity (ED_{50}) (12.15 ± 1.40nM) ($p<0.05$).

Interestingly, when the same calcium depletion regime involving a combination of noradrenaline and caffeine stimulation in calcium free physiological salt solution was used, the maximum contraction to endothelin-1 was greater but still significantly reduced compared to that in the control group. The maximum tension generated was 1.48 ± 0.22mN/mm ($p<0.05$) but in this case there was no alteration in the endothelin-1 sensitivity (ED_{50}) (6.11 ± 0.89nM). When the calcium depletion regime was combined with protein kinase C inhibition, the contractile response to endothelin-1 was completely abolished (Table 24, Figure 55).

Re-introducing calcium (2.5mM) into the bath resulted in the response to endothelin-1 being restored in the calcium depleted vessels being, the maximum contraction being similar to that observed in normal physiological salt solution (Table 24). However, when calcium depletion with protein kinase C inhibition, calcium re-introduction resulted in a further contraction

which was comparable to the response of endothelin-1 in normal physiological salt solution in the presence of H7 (1.51 ± 0.20mN/mm) (Table 24).

Table 23. Patient characteristics and internal diameter of human omental vessels in the calcium depletion and protein kinase C inhibition study.

	No Patients	Sex M:F	Age (years)	Blood Pressure (mmHg)	MAP (mmHg)	No Vessels	Vessel Size (μm)
ET Control	7	1:6	64 ± 7	137 ± 5 / 78 ± 4	98 ± 4	14	227 ± 21
ET + H7 (10^{-4}M)	4	2:2	60 ± 8	133 ± 5 / 83 ± 3	103 ± 5	8	236 ± 19
ET + Ca^{2+} free (NA)	5	3:2	56 ± 6	130 ± 11 / 76 ± 7	94 ± 8	9	260 ± 24
ET + Ca^{2+} free (Caffeine)	5	3:2	52 ± 10	125 ± 5 / 68 ± 2	87 ± 3	10	272 ± 14
ET + H7 + Ca free	4	2:2	68 ± 5	138 ± 11 / 78 ± 5	100 ± 6	8	232 ± 13

Table 24. Maximum contractile response and sensitivity (ED_{50}) to noradrenaline and endothelin-1 of human omental vessels before and after protein kinase C inhibition, calcium store depletion and combined protein kinase C inhibition with calcium store sepletion.

	Max Noradrenaline (mN/mm)	Noradrenaline ED_{50} (μM)	Max Endothelin (mN/mm)	Endothelin ED_{50} (nM)	Ca^{2+} re-introduced (mN/mm)
ET Control	1.74 ± 0.39	2.270 ± 0.58	2.71 ± 0.51	5.751 ± 1.10	-
ET + H7 (10^{-4}M)	1.68 ± 0.20	0.696 ± 0.23	1.51 ± 0.20*	7.279 ± 1.40	-
ET + Ca^{2+} free (NA)	1.77 ± 0.68	4.050 ± 1.20	0.92 ± 0.29*	12.007 ± 1.40*	2.81 ± 0.51
ET + Ca^{2+} free (Caffeine)	2.43 ± 0.59	1.294 ± 0.35	1.47 ± 0.24*	6.111 ± 0.89	3.13 ± 0.24
ET + H7 + Ca free	1.68 ± 0.53	0.575 ± 0.18	0 ± 0*	0 ± 0*	1.47 ± 0.47

* $p<0.05$ compared to endothelin control (corrected for multiple comparisons)

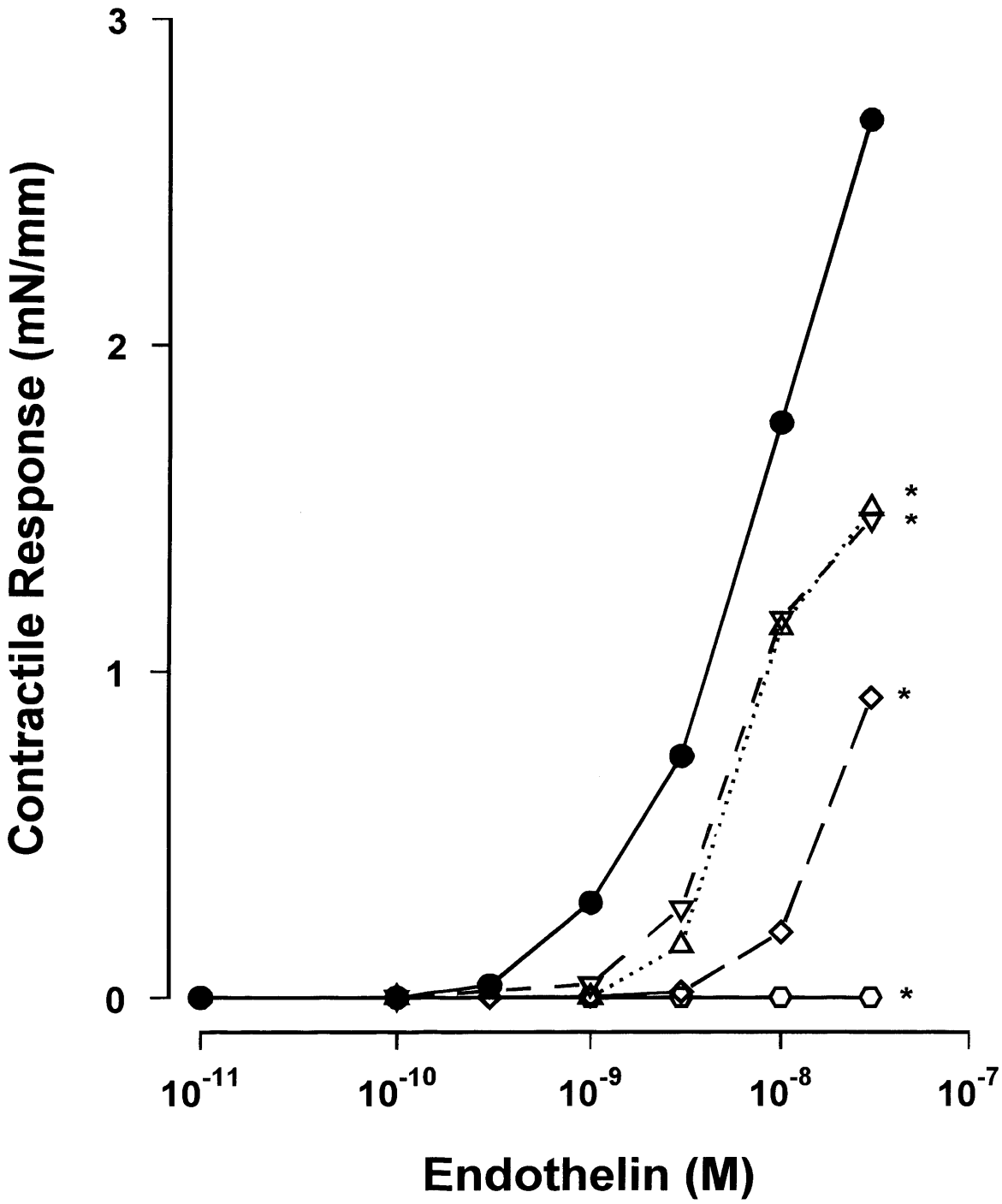

Figure 55 The endothelin-1 contractile response of human omental resistance vessels in normal PSS (●), calcium store depletion with noradrenaline (◇), calcium store depletion with caffeine (▽), in the presence of H7 in normal PSS (△) and calcium store depletion combined with H7 (⬡) *p<0.05 denotes comparison of the whole response compared to endothelin-1 in normal PSS

7.7. DISCUSSION

These studies demonstrate that endothelin-1 causes a powerful concentration dependent contraction of isolated human omental resistance arteries. In contrast to early reports, using large or medium sized vessels (Yanagisawa et al. 1988b), the contractile response was not abolished by pre-treatment with the calcium channel antagonist nicardipine or by partial depletion of the internal calcium stores in a calcium deficient medium. However, with both these procedures, the residual contraction occurred mainly with high concentrations of endothelin-1 and the maximum response was less than 50% of that observed in the presence of extracellular calcium.

The residual contraction may depend on calcium mobilisation from other intracellular stores, since endothelin-1 has been shown to induce a rise in intracellular calcium in cultured vascular smooth muscle cells, even after repeated noradrenaline stimulation in a calcium free medium (Kai et al. 1989). However, these calcium transients were abolished by repeated stimulation with caffeine in the absence of extracellular calcium (Kai et al. 1989). In this study repeated noradrenaline and caffeine stimulation should ensure that the calcium transients were abolished. Nevertheless, it appears that endothelin-1 may elicit vascular smooth muscle contraction by two mechanisms. In the presence of extracellular calcium, endothelin-1 caused increased calcium entry inducing a rapid sustained rise in intracellular calcium and contraction in a concentration dependent manner (Hirata et al. 1988; Kodama et al. 1989).

Endothelin-1 does not appear to interact directly at the dihydropyridine binding site of the L type calcium channel but causes membrane depolarisation by opening a non-specific channel which is permeable to

calcium and magnesium (Van Renterghem et al. 1988). The resulting depolarisation induced brings the membrane potential near to the threshold for the L type channel and as a result substantial amounts of calcium enter the vascular smooth muscle cells.

Endothelin-1 has been reported to cause vascular smooth muscle contraction in the absence of extracellular calcium. Thus, in a calcium-free medium, the contractile response of a porcine coronary artery strip has been shown to be little affected but this was associated with only a transient rise of intracellular calcium (Kodama et al. 1989). Moreover, endothelin-1 increases intracellular calcium in cultured aortic smooth muscle cells, even after repeated stimulation by noradrenaline in a calcium-free medium suggesting that a variety of calcium stores may be involved (Kai et al. 1989). However, these calcium transients were abolished by repeated stimulation by caffeine (Kai et al. 1989). Similarly, in the porcine coronary artery, when the internal calcium stores were depleted by repeated stimulation with potassium (Kasaya et al. 1989) or caffeine and histamine (Kai et al. 1989) the maximum contractile response was reduced to 28% of control and was not associated with a change in internal calcium (Kai et al. 1989). It has been suggested that the residual contraction depends on stimulation of the phosphoinositide signaling pathways, causing activation of protein kinase C, a phosphorylating enzyme with a low calcium requirement. Moreover, contractile responses after calcium depletion were obtained only with high concentrations of endothelin-1 which are required to stimulate phosphoinositol turnover in the porcine coronary artery (Kasaya et al. 1989).

Our results, using the isolated human omental resistance artery, are in keeping with those obtained using the larger muscular arteries and suggest

that the residual contraction found after external and internal calcium depletion may not involve a change of intracellular calcium. When these vessels were pre-treated with a protein kinase C inhibitor (H7) in the presence of external calcium, the maximum contraction caused by endothelin-1 at high concentrations, was reduced to about 60% of the control. However, H7 pre-treatment completely abolished the contraction in the calcium deficient vessels suggesting that the calcium-independent component of the response depends on protein kinase C activation. However, it should be noted that while the protein kinase C inhibitor H7 was used in this study other more specific inhibitors are now available. Since this study was performed the specificity of H7 has been called in to question and it may well inhibit more than the protein kinase C pathway.

The present study shows that in addition to calcium-dependent contraction, involving the activation of dihydropyridine-sensitive voltage dependent calcium channels, endothelin-1 may also activate protein kinase C via the phosphoinositide signaling pathway.

7.8. CONCLUSIONS

1. In human omental resistance arteries, endothelin-1 is 1,000 fold more potent than noradrenaline.

2. Dihydropyridine channel blockade with nicardipine, or partial removal of internal calcium stores and external calcium, or protein kinase C inhibition blocked the contraction by low concentrations of endothelin-1 but only partially inhibited the contraction produced by high concentrations of endothelin-1.

3. A combination of protein kinase C inhibition (H7) and intracellular calcium deficiency abolished the contractile response to endothelin-1.

4. These studies suggest that endothelin-1 may elicit vascular smooth muscle contraction by two mechanisms. A calcium dependent contraction, at low concentrations with activation of protein kinase C via the phosphoinositide signaling pathway by high concentrations of endothelin-1.

CHAPTER 8 CONCLUSIONS

The aim of the experiments in this thesis has been an attempt to evaluate the alteration in vascular structure and function during the on-set and established phase of hypertension.

The preliminary experiments in Chapter 3 were designed to investigate age related structural and functional changes in the spontaneously hypertensive rat. The study was conducted at four different stages in the development of hypertension. The blood pressure was demonstrated to increase with age in the spontaneously hypertensive rat and the morphological studies confirmed the findings of other investigators. The study went on to demonstrate that the spontaneously hypertensive rat had an abnormality in the endothelium dependent relaxation as the blood pressure increased with age. The normal relaxation response to a nitric oxide donor suggested that the abnormality was not due to an increase in the diffusion pathway but perhaps an endothelial dysfunction and there was some evidence to suggest that the endothelial dysfunction might be due to the release of a contracting factor from the endothelium. This led to a second series of experiments to determine whether a contracting prostaglandin was being released by the vascular endothelium.

To ascertain whether in vessels from the spontaneously hypertensive rat a contracting prostaglandin was being released the cyclooxygenase inhibitor indomethacin was used. The successful inhibition suggested that there was a contracting prostaglandin being released as the acetylcholine induced endothelial relaxation response in the spontaneously hypertensive rat was restored to normal. This result pointed to the assumption that the

endothelium released a prostaglandin which is normally overridden in the WKY but in the SHR increased amounts are released overcoming the endothelium dependent relaxation.

Further experiments attempted to ascertain the prostaglandin involved in this process. Treatment with a thromboxane A_2 (SQ29548) antagonist significantly improved the acetylcholine relaxation response in the spontaneously hypertensive rat. Also, the thromboxane A_2 synthesis inhibitor, dazmegrel, improved the acetylcholine relaxation response but both failed to entirely prevent the re-contraction observed at high concentrations of acetylcholine. This suggested that the endothelial contracting factor being released was not thromboxane A_2 but another prostaglandin. A likely candidate could be PGH_2 which can be partially inhibited by thromboxane A_2 antagonists.

The second part of this thesis (Chapter 5) was designed to study the development of structural and functional changes during the acute and chronic phases of renovascular hypertension. The Goldblatt two kidney one clip model of hypertension was selected and studied six and sixteen weeks after renal artery constriction. The morphological results showed that there was a rapid structural alteration of the resistance vasculature which was established after six weeks. Moreover, there was little further development of structural alteration during the chronic phase. Contractile responses to noradrenaline were increased in both the acute and chronic phases of hypertension. However, the results indicated that this increase in response could not be attributed to the increase in wall mass alone. Endothelium dependent relaxation was again reduced in this model of hypertension. However, unlike the responses seen in the spontaneously hypertensive rat, there was no observed re-contraction with high doses of acetylcholine.

Therefore, this did not suggest that a contracting prostaglandin was being released. This led on to a second series of experiments to determine whether the structural vascular changes were the sole determinant of the increased peripheral vascular resistance in established hypertension. To achieve this surgical reversal of renovascular hypertension was studied after 1 and seventy five days.

Surgical removal of the constricting renal clip lowered the blood pressure in the Goldblatt two kidney one clip model of hypertension which confirmed other recorded observations. Vascular hypertrophy regressed to normal within the seventy five days but regression of cardiac hypertrophy was incomplete. These findings confirmed those of Folkow and while this study did not ascertain the composition of the cardiac hypertrophy it has been reported that this is mainly due to collagen deposits which take longer to resolve after the blood pressure is lowered. Again, there was an increased contractile response during hypertension and this was abolished one day after reversal while the vascular structural changes remained un-resolved. Correcting the contractile response for media cross sectional area showed a decreased response to noradrenaline at both one and seventy five days after reversal of hypertension. Interestingly, the endothelial dependent relaxation response 1 day after surgical reversal of hypertension was similar to controls. There was reduction in the maximum response to acetylcholine in both the unclipped and sham operated controls and this deterioration appeared to be age related.

The final series of experiments (Chapter 7) were designed to investigate the effects of the novel peptide endothelin-1. A series of pharmacological experiments were carried out using human omental vessels. The initial study compared the potency of endothelin-1 and noradrenaline. Endothelin-

1 was found to be 1,000 fold more potent than noradrenaline. This study also determined that tachyphalaxis occurred with exposure to endothelin-1. There then followed a series of experiments to determine which pathways were involved in endothelin-1 induced contractions. Initially, the dihydropyridine channel blocker, nicardipine, was used and this was found to partially block the endothelin-1 induced contraction. Similarly, partial removal of internal calcium stores and external calcium, or protein kinase C inhibition blocked the contractions by low concentrations of endothelin-1 but only partially inhibited the contraction by high concentrations. Combination treatment of protein kinase C inhibition and intracellular calcium depletion abolished the contractile response to endothelin-1. This series of experiments suggested that endothelin-1 may elicit vascular smooth muscle contraction by two mechanisms. A calcium dependent contraction, at low concentrations with activation of protein kinase C via the phosphoinositide signaling pathway by high concentrations of endothelin-1.

There were many questions raised by the findings of this thesis. What is the role of the endothelium in modulating resistance artery contraction? What is the effect of endothelial modulation in genetic and renovascular hypertension? Does the duration of hypertension effect endothelium dependent relaxation and does any defect in endothelium dependent responses contribute to the development of hypertension or is it merely a result of elevated blood pressure? Another area of research that would be of interest is the effect of antihypertensive therapy on resistance artery structure and function. In addition, a lot of attention is now being paid to endothelial dependent hyperpolarising factor(s). Some early indications suggest that this relaxing factor may play a crucial role in peripheral vascular resistance.

APPENDIX A

PHYSIOLOGICAL SALT SOLUTION

Physiological salt solution (PSS) contains the following compounds:

Physiological salt solution (PSS)

Compound	Molarity (mM)	grams/litre
NaCl	118	6.90
KCl	4.5	0.34
$CaCl_2$	2.5	0.37
$MgSO_4.7H_2O$	1.0	0.25
KH_2PO_4	1.0	0.14
$NaHCO_3$	25	2.10
Glucose	6.0	1.08

HIGH POTASSIUM PHYSIOLOGICAL SALT SOLUTION (KPSS)

A high potassium PSS was achieved by substituting sodium chloride with potassium chloride in equimolar quantities.

High Potassium Physiological Salt Solution (KPSS)

Compound	Molarity (mM)	grams/litre
KCl	122.5	9.26
$CaCl_2$	2.5	0.37
$MgSO_4.7H_2O$	1.0	0.25
KH_2PO_4	1.0	0.14
$NaHCO_3$	25	2.10
Glucose	6.0	1.08

CALCIUM DEPLETED PHYSIOLOGICAL SALT SOLUTION

Calcium Depleted Physiological salt solution

Compound	Molarity (mM)	grams/litre
NaCl	118	6.90
KCl	4.5	0.34
$MgSO_4.7H_2O$	1.0	0.25
KH_2PO_4	1.0	0.14
$NaHCO_3$	25	2.10
Glucose	6.0	1.08

EQUIPMENT and SUPPLIERS

(All equipment is UK based unless stated)

Myograph (model 400A)	JP Trading, Aarhus, Denmark
Kistler Morse transducers	JP Trading, Aarhus, Denmark
Stainless steel wire (40μm diameter)	JP Trading, Aarhus, Denmark
Technival 2 Stereomicroscope	Carl Zeiss Jena Ltd
Olympus stage microscope	Olympus Ltd
Filar micrometer eyepiece (x8)	Carl Zeiss Ltd
Water immersion objective lens (x25)	Leitz Ltd
Flat bed 2 channel recorder	Fisons Ltd
Heidolph T50 water heater & circulator	Scientific Industries Ltd
Large water bath	Grant instruments Ltd
Suction Pump	BDH Ltd
$5\%CO_2/95\%O_2$	BOC Ltd
Stainless steel wire for clipping	Thessco Ltd
Sutures	Ethicon Ltd
Blood pressure equipment	Medical Physics, LRI
Sphygmomanometer	Richardons Ltd
IBM PC clone (286) & printer	DA Computers Ltd, Leicester

Surgical Instruments

3" opthalmic scissors (Hans Geuder)	Altomed Ltd
Watchmaker forceps size 5	Richardsons Ltd
Assorted dissecting instruments	Richardsons Ltd

APPENDIX B

Publications arising from the work in this thesis:

Papers

Watt PAC, Baker AR, Thurston H (1991). Calcium independent and dependent endothelin-1 induced contraction of human resistance vessels. J Human Hypertension 5: 145-148

Watt PAC, Baker AR, Thurston H (1989). Vasoconstrictor actions of endothelin-1 in human resistance vessels. J Hypertension 7 (suppl 6): s134-s135 1989.

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