BRUNEL UNIVERSITY.

VASCULAR REACTIVITY IN RENAL HYPERTENSION.

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angiotensin II was unlikely to be a stimulant of vascular supersensitivity since P.R.A.* was suppressed in both hypertensive models, and an angiotensin antagonist did not reverse the noradrenaline supersensitivity. The pathogenic significance of increased vascular reactivity in hypertension and future studies on this topic are discussed.

* P.R.A. is plasma renin activity

ABSTRACT

Increased vascular reactivity is a common observation in human and experimental hypertension. In this study, reactivity of the perfused mesenteric arterial/arteriolar vascular bed was examined during the development of renal and renal/salt hypertension in the rat.

Increased reactivity to noradrenaline was observed in tissues from rats 1-12 weeks after the induction of renal/salt hypertension. In the early (1-2 week) stages, preparations were supersensitive to noradrenaline but not to KCl. In the later (4-6 week) stages, the increase in noradrenaline reactivity was due to supersensitivity and another factor which increased KCl reactivity and was characterized by an elevated maximum response. The noradrenaline response potentiating effects of exogenous angiotensin II were attenuated in the early but not the later stages of hypertension. Vascular reactivity to noradrenaline in tissues from renal hypertensive rats was similar to that in renal/salt hypertensive rats, but with a slower time course.

The decay of noradrenaline responses in calcium-free conditions was slower in tissues from early renal/salt hypertensive rats, indicating that an increased availability of activator calcium was the mechanism of supersensitivity.

The characteristics of the α-adrenoceptor did not appear to differ in the renal/salt hypertensive rat as the pA_2 value for phentolamine did not change. Differences in the pA_2 value for indoramin were observed in tissues from hypertensive rats but probably resulted from the unusual properties of this drug.

The early supersensitivity to noradrenaline was partially attributable to the effects of a positive sodium balance. Endogenous

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CHAPTER 1

INTRODUCTION

The haemodynamic characteristic of hypertension in humans and in experimental animals is a raised peripheral resistance to the flow of blood (Freis, 1960). Several influences on the peripheral vasculature have been implicated as causes of increased resistance. Nervous and humoral influences on the blood vessels may increase resistance, however the evidence for hyper-activity of these systems is equivocal. There is a large body of evidence (Doyle, 1968) that the blood vessels of hypertensive patients respond more powerfully than normal to humoral and nervous activation. Increased "vascular reactivity" appears to be a more common finding in hypertension than a hyper-activity of the nervous or humoral control systems.

The aim of the present study is to examine the changes in reactivity of perfused resistance vessels from rats, during the development of hypertension. The perfused mesenteric vasculature preparation is used since it avoids the complications of a capillary and post-capillary resistance section. Increased reactivity in this preparation has been previously demonstrated but its nature has not been elucidated since full dose-response curve data during the development of hypertension was not obtained (McGregor and Smirk, 1968, 1970., Haeusler and Haefely, 1970., Armstrong, 1972., Haeusler and Finch, 1972 a,b). The important unanswered question is whether increased reactivity is a cause or a consequence of hypertension.

The experimental model used is the renal hypertensive rat, with and without salt-loading, since the development of hypertension and changes in reactivity can be accurately followed from a defined, operative

starting point. Renal hypertension was thought to be the best model for this type of study since hypertension induced by salt and desoxycorticosterone involves administration of an exogenous steroid which might influence vascular reactivity per se, while the spontaneously hypertensive rat develops hypertension rather slowly and lacks a suitable control animal.

LITERATURE REVIEW

Richard Bright (1836) first drew attention to the association of proteinuria and dropsy, with cardiac hypertrophy. He speculated that the cardiac hypertrophy might be due to some altered quality of the blood which led to either direct stimulation of the heart or to some alteration in the peripheral vessels resulting in a greater action being necessary to force the blood through them. The concept of blood pressure in clinical medicine was ill-defined at this time, but, Bright's suggestions laid the foundations of modern concepts of the pathogenesis of hypertension.

THE HAEMODYNAMICS OF HYPERTENSION IN HUMANS

The factors that determine the arterial blood pressure are, i) the cardiac output, ii) the peripheral resistance to the flow of blood and iii) the blood viscosity.

The blood viscosity is normal in hypertension (Pickering, 1936). Tibblin, Bergentz, Bjure and Wilhelmsen (1966) reported increased blood viscosity in hypertensive patients, but used an *in vitro* technique which may be an unreliable indicator of viscosity *in vivo* (Djojosugito, Folkow, Öberg and White, 1970).

When reliable methods for measuring cardiac output became available, no difference was found between normal and hypertensive subjects (Goldring and Chasis, 1944., Hickam and Cargill, 1948., Bolomey, Michie, Michie, Breed, Schreiner and Lavson, 1949., Werkö and Lagerlöf, 1949., Lewis, Houssay, Haynes and Dexter, 1953.,

Varnauskas, 1955., Judson, Hollander and Wilkins, 1957., Taylor, Donald and Bishop, 1957., Brod, 1960., Rowe., Castillo, Maxwell and Crumpton, 1961., Yurchak, Hood, Rolett, Hickler and Gorlin, 1964). It was concluded that increased vascular resistance to flow was the haemodynamic hallmark of hypertension (Freis, 1960., Page and McCubbin, 1966).

A recent clinical study by Frohlich, Tarazi and Dustan (1969) has confirmed that peripheral resistance and not cardiac output is raised in stable hypertension. However, patients with variable, but above average blood pressure, designated "labile hypertensives", were an exception, since they had increased cardiac output but no change in peripheral resistance.

THE EXPERIMENTAL INDUCTION OF HYPERTENSION IN ANIMALS

Experimental animal models of various high blood pressure conditions are required in order to examine the haemodynamics of the development of hypertension.

The earliest experimental studies were designed to determine the effect of nephrectomy and other manipulations of the kidney on the blood pressure. Grawitz and Israel (1879) induced hypertrophy of the heart, by partial nephrectomy of rabbits, which they attributed to the development of hypertension. Katzenstein (1905) made the first observations on the effects of partial constriction of the main renal arteries on the blood pressure, but his period of observation was short and the results were either negative or equivocal. There were numerous other attempts to induce hypertension by manipulation of the kidney or its circulation, the most successful being those of Chanutin and Ferris (1932), who induced chronic hypertension in rats by partial nephrectomy.

The great impetus to the modern study of hypertension was the demonstration by Goldblatt, Lynch, Hanzal and Summerville (1934) that the application of a clamp to the renal artery of a dog, with subsequent removal of the opposite kidney, resulted in chronic elevation of the blood pressure. Numerous procedures are now available which elevate the blood pressure in experimental animals. Most techniques involve some manipulation of the kidney or renal artery. Instead of a "Goldblatt" clamp, the kidney function can be impaired by a ligature (Grollman, 1944) or a cellophane capsule (Page, 1939). The removal of one kidney and the administration of desoxycorticosterone (D.O.C.) or other steroids (Grollman, Harrison and Williams, 1940), enucleation of the adrenals with unilateral nephrectomy (Skelton, 1955), the use of choline or potassium-free diets (Grollman and White, 1958) and infarction of the kidney (Loomis, 1946), all induce hypertension.

The spontaneously hypertensive rat (S.H.R.) has been developed by selective inbreeding (Smirk and Hall, 1958., Okamoto and Aoki, 1963) as a model of human essential hypertension. Dahl (1961) has developed a strain of pure bred salt-sensitive rats, which become hypertensive when salt loaded.

THE HAEMODYNAMICS OF EXPERIMENTAL HYPERTENSION

Renal hypertension has been commonly used for continuous haemodynamic studies, since the operative procedures provide an accurately defined starting point. Ledingham and Pelling (1967) and Ferrario, Page and McCubbin (1970), using rats and dogs, respectively, have shown that renal hypertension is characterized by an initial transient rise in cardiac output, followed by an increase in peripheral resistance, which sustains the hypertension. The transient rise in cardiac output is probably stimulated by an increased

plasma volume, due to impaired renal function, which would cause increased cardiac filling (Ledingham, 1971). However, prostaglandin $F_{2\alpha}$ may also play a role in stimulating venoconstriction and therefore in increasing venous return (McGiff, Crowshaw and Itskovitz, 1974).

The sustained rise in peripheral resistance which characterizes and maintains the high blood pressure in all stable hypertensive states, has attracted much research effort. Peripheral resistance, at a constant cardiac output, is determined by the diameter of the resistance vessels. The state of contraction, or tone, of these vessels is influenced by a complex interaction of neural and humoral influences, the effect of which is determined by the sensitivity of the vascular smooth muscle and the structure of the vessel. All these factors have been implicated as causes of increased peripheral resistance in hypertension.

POSSIBLE CAUSES OF INCREASED PERIPHERAL RESISTANCE IN HYPERTENSION

A. NERVOUS MECHANISMS

1. THE CENTRAL COMPONENT

The brain is the most oxygen sensitive organ served by the cardiovascular system. Teleologically, the brain should possess the dominant blood pressure regulating mechanism as protection against reduced blood flow and consequent ischaemia. Dickinson (1965), has suggested that vertebral artery disease, which causes increased cerebrovascular resistance to flow, could be the cause of human essential hypertension. This hypothesis has been extended to renal hypertension, in which levels of the vasoconstrictor agent angiotensin II may be increased (Gross, 1971). An infusion of angiotensin II into the vertebral artery produces hypertension, while the same dose, administered systemically is without a pressor

effect (Dickinson and Lawrence, 1963., Yu and Dickinson, 1965). However, Marshall (1966) found no tendency for patients with brain stem vasomotor centre ischaemia having higher blood pressures than those with ischaemia of the cerebral hemisphere.

Observations made with centrally acting antihypertensive agents such as α methyldopa support the hypothesis that the brain is involved in hypertension. α-Methylnoradrenaline, probably the active metabolite of α-methyldopa, is thought to stimulate the α-adrenoceptors in the brain-stem which mediate the reflex withdrawal of sympathetic drive (Henning and Rubenson, 1971). Catecholamine turnover is reduced in the brain-stem and hypothalamus of D.O.C.-saline hypertensive rats (Nakamura, Gerold and Thoenen, 1971 a) and this deficiency of catecholamines may be involved in the pathogenesis of this model of experimental hypertension. Conflicting results have been obtained for the S.H.R. since Louis, Krauss, Kopin and Sjoerdsma (1970) reported a deficiency of catecholamines but Nakamura, Gerold and Thoenen (1971 b) found no change in central catecholamine turnover.

2. THE PERIPHERAL COMPONENT

If the increased peripheral resistance in hypertension is due to excessive sympathetic vasomotor discharge, then sympathectomy should provide a cure. The extensive excision of the paravertebral sympathetic ganglia has been widely practised (Pickering, 1968) but the benefit of such an operation is still not clear since a properly controlled clinical trial has never been organized. Immediately after the operation, there is a considerable fall in arterial pressure, but in most patients the fall is not sustained (Platt and Stanbury, 1950).

Pharmacological sympathectomy, by the administration of large doses of the ganglion blocking agent hexamethonium, lowers the blood pressure of hypertensive patients more than in normotensive subjects (Doyle and Smirk, 1955). This would suggest an increased neurogenic component in the control of the blood pressure in the hypertensive subject. However, renal hypertension in rats can develop in the absence of a functional sympathetic nervous system, although a neurogenic component seems likely in the chronic renal hypertensive state (Dorr and Brody, 1966., Finch and Leach, 1970). The development of hypertension in the New Zealand strain of S.H.R . is prevented by immunosympathectomy (Clark, 1969), while catecholamine depletion by 6-hydroxy dopamine may or may not prevent the development of hypertension in the Japanese S.H.R. (Haeusler, Finch and Thoenen, 1972., Yamori, Yamabe, De Jong, Lovenberg and Sjoerdsma, 1972., Vapaatala, Hackman, Anttila, Vainionpää and Neuvonen, 1974).

Direct recording from or direct stimulation of sympathetic nerves in the Japanese S.H.R. has indicated that an increased vasoconstrictor discharge occurs in these animals (Okamoto, Nosaka, Yamori and Matsumoto, 1967., Iriuchijima, 1973). However, the levels of regulatory enzymes for catecholamine biosynthesis are decreased in the mesenteric arteries of the animals (Tarver, Berkowitz and Spector, 1971) and this would contra-indicate increased sympathetic activity.

Rates of excretion of noradrenaline and its metabolites, which may indicate sympathetic nervous activity, are usually normal in hypertensive patients (Brunjes, 1964). Gitlow, Mendlowitz, Wilk, Wolf and Naftchi (1964) suggest that essential hypertensives have an enhanced plasma noradrenaline clearance rate, whereas, De Quattro and Miura (1973) could find no evidence of abnormal noradrenaline

turnover in patients with primary hypertension. Patients with renal or renovascular hypertension have normal plasma noradrenaline clearance rates (Gitlow, Mendlowitz, Bertani, Wilk and Glabman, 1969). Louis, Doyle and Anavekar (1973) have demonstrated an increased catecholamine concentration in the plasma of hypertensive patients. Such an increase is unlikely to be the cause of hypertension, since plasma catecholamines can be increased without an elevation of the blood pressure (Engelman and Portnoy, 1970).

In experimental hypertension, the evidence for changes in catecholamine metabolism is equivocal. De Champlain has suggested that a reduction in noradrenaline storage, with a consequent increase in turnover rate, may be important in the pathogenesis of D.O.C.-salt hypertension in rats (De Champlain, Krakoff and Axelrod, 1968., De Champlain, Mueller and Axelrod, 1969). However, Louis (1970) has reported experiments on noradrenaline turnover in D.O.C.-salt and spontaneously hypertensive rats, indicating that noradrenaline plays only a contributory rather than a primary role in both forms of hypertension.

The second to second feedback control of vasomotor discharge is provided by the baroreceptors. Disturbances of the function of these pressure receptors can lead to hypertension in experimental animals (Krieger, 1964., Ferrario, McCubbin and Page, 1969., Cowley, Liard and Guyton, 1973). McCubbin, Green and Page (1956) have shown that the baroreceptors "reset" a few days after renal hypertension is induced, so that they tend to maintain rather than to suppress the elevated blood pressure. Disturbances of baroreceptor function are unlikely to maintain a stable hypertensive state alone, since resetting at normotensive levels could easily occur in the absence of stressful hypertensive stimuli. However,

secondary resetting of the baroreceptors may account for the inconclusive data which has been obtained on sympathetic function in hypertension. A sympathetic function in a hypertensive state, which is indistinguishable from the normotensive reference, may be abnormal in relation to the elevated blood pressure.

The first effective antihypertensive drugs were the ganglion blocking agents (Paton and Zaimis, 1949). The present day antihypertensive agents, guanethidine and α-methyldopa have their major effects on the sympathetic nervous system, whether peripheral or central in action. It is likely, therefore that there is a neurogenic component involved, at least in the maintenance of hypertension.

B. HUMORAL MECHANISMS

1. RENIN AND ANGIOTENSIN

Goldblatt, Lynch, Hanzal and Summerville (1934) demonstrated that persistent hypertension could be produced by constricting the renal arteries. It seemed obvious that the pressor substance, renin, which had been isolated from the kidney by Tigerstedt and Bergman (1898) was responsible. Renin itself is not pressor, but is a proteolytic enzyme (Helmer, 1971) produced mainly by the juxtaglomerular cells, which reacts with a substrate, present in the α globulin fraction of the plasma, to form the decapeptide angiotensin I. Angiotensin I is converted enzymically, in the lungs (Ng and Vane, 1968), to the octapeptide angiotensin II, which is the most potent natural pressor substance known (Gross and Bock, 1962).

It was assumed that increased plasma angiotensin II was the cause of experimental renal hypertension in animals, and its various naturally occuring counterparts in man. However, numerous studies

have failed to confirm this assumption, as both plasma renin and angiotensin II levels are often normal in chronic renal hypertension in various species, including man (Brown, Davies, Lever and Robertson, 1964).

Patients with renal artery stenosis and malignant hypertension have raised plasma renin levels (Brown, Davies, Lever and Robertson, 1966). However, normal levels are found in patients with chronic renal failure (Gutkin, Levinson, King and Lasker, 1969) and renal parenchymal disease (Brown, Davies, Lever and Robertson, 1965). Plasma angiotensin II concentrations are elevated in malignant hypertension (Catt, Cran, Zimmet, Best, Cain and Coghlan, 1971) and in patients with cirrhosis with ascites or juxtaglomerular cell hyperplasia, but this may be unrelated to the level of the blood pressure (Gocke, Gerten, Sherwood and Laragh, 1969).

If we consider non-renal forms of hypertension, plasma renin is suppressed in most hypertensive patients with aldosterone-secreting adenomas (Conn, Cohen and Rovner, 1964). Essential hypertensives have been divided into three sub-groups of low, high and normal plasma renin activities (Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard and Bühler, 1972).

In experimental animals, plasma renin activity is elevated in hypertension by clamping one renal artery with the contralateral kidney intact. If the contralateral kidney is removed, only an acute rise in plasma renin activity is observed (Gross, 1971) and subsequently the levels return to normal. Hypertension can be induced by overdosage with sodium retaining corticoids and dietary salt, even though renin has effectively disappeared from the blood (Gross and Sulser, 1957., Gross, 1960., Rosenthal and Hollander, 1973).

In the S.H.R. plasma renin activity increases after the development of hypertension suggesting that no direct relationship exists between the two parameters (De Jong, Lovenberg and Sjoerdsma, 1972). Conversely, Sen, Smeby and Bumpus (1972) found elevated plasma renin activities in the pre-hypertensive and early hypertensive stages and normal or subnormal activities during the established hypertensive phase in S.H.R. Koletsky, Shook and Rivera-Velez (1970) reported an abnormally low juxtaglomerular cell granulation, indicative of a suppressed renin synthesis, in kidneys from S.H.R. of 6 weeks to 12 months age. These contradictory findings may be due to the lack of a valid control animal.

Plasma renin and presumably angiotensin II levels are increased in some forms of hypertension, but the rise is often moderate, and the direct vasoconstrictor action alone probably insufficient to account for the elevated blood pressure (Peart, Robertson and Grahame-Smith, 1961). However, angiotensin II also has indirect pressor actions mediated by the sympathetic nervous system and adrenal glands. Angiotensin II stimulates the release of catecholamines from the adrenal medulla (Braun-Menendez, Fasciolo, Leloir and Munoz, 1939) and sensitizes the neurovascular effectors so that the effects of sympathetic vasomotor discharge are augumented (McCubbin and Page, 1963). Angiotensin II will also facilitate ganglionic transmission (Lewis and Reit, 1966).

The cross-perfusion experiments of Bickerton and Buckley (1961) first demonstrated that angiotensin II has cardiovascular effects which are mediated <u>via</u> the central nervous system. These effects are primarily due to an increased sympathetic discharge (Severs, Daniels, Smookler, Kinnard and Buckley, 1966., Ueda, Uchida, Ueda, Gondaria and Katayama, 1969). The central hypertensive effects

of a vertebral artery infusion of angiotensin II persist over 7 day infusion periods (Fukiyama, McCubbin and Page, 1971), indicating that this facet of the many actions of angiotensin could cause a stable hypertension.

Circulating angiotensin II appears to act on the brain in the area postrema, where there is no effective blood-brain barrier (Ueda, 1968). Endogenous non-circulatory, renin and angiotensin have also been demonstrated in brain tissue, and their distribution correlates with noradrenaline concentrations in different areas of the brain (Fischer-Ferraro, Nahmod, Goldstein and Finkielman, 1971). The conceptual division between neural and humoral influences on peripheral resistance vessels would therefore appear to be an over-simplification.

Recently, a new enzyme termed "tonin" has been found in rat submaxillary glands and many other tissues. This enzyme not only converts angiotensin I to angiotensin II but also cleaves angiotensin II directly from renin substrate (Boucher, Asselin and Genest, 1974). If this enzyme is of physiological significance, then previous studies using renin measurements as an index of angiotensin II levels may prove to be of limited value.

The role of angiotensin II in the pathogenesis of hypertension has been clarified by the recent development of specific angiotensin blocking agents (Marshall, Vine and Needleman, 1970). This direct approach to the angiotensin receptor, obviates many of the problems involved in the interpretation of results based on renin measurements. Results using these agents indicate that levels of angiotensin are elevated during acute renal artery constriction, and that angiotensin is involved in the pathogenesis of both acute one kidney and acute two kidney renal hypertension in rats (Davis,

Freeman, Johnson and Spielman, 1974). Increased levels of angiotensin II appear to contribute to the maintenance of the high blood pressure in chronic two kidney, but not chronic one kidney hypertensive rats (Davis, Freeman, Johnson and Spielman, 1974). Angiotensin antagonists have no effect on the blood pressure of normal, D.O.C. hypertensive or spontaneously hypertensive rats (Pals, Masucci, Denning, Sipos and Fessler, 1971).

In human hypertensives, the angiotensin antagonist, I-Sar-8-Ala angiotensin II, lowers the blood pressure in high renin essential, renal and malignant hypertension (Brunner, Gavras, Laragh and Keenan, 1973). Further studies using these specific antagonists should provide answers to many questions about the renin-angiotensin system, particularly with reference to hypertension.

2. RENAL ANTIHYPERTENSIVE AGENTS

An alternative hypothesis to that of excessive blood levels of pressor agents, is a deficiency of a humoral depressor agent in hypertension. These antihypertensive factors are linked with the non-excretory function of the kidney, for the following reasons. Renoprival hypertension, caused by total nephrectomy, can be promptly relieved by a renal transplant (Muirhead, Stirman, Lesch and Jones, 1956). In renoprival hypertension, the blood pressure is significantly reduced when intact renal tissue without excretory function is present (Muirhead, Stirman and Jones, 1960). Implantation of kidney tissue into the peritoneal cavity attenuates renal hypertension (Lauwers & Gomez, 1964), and renal homotransplantation in hypertensive patients usually reduces the blood pressure to normal (Kolff, Nakamoto, Poutasse, Straffon and Figueroa, 1964).

Attempts have been made to extract the antihypertensive factor from the kidney, and to identify it. Extracts of whole kidney contain some material that lowers the blood pressure in hypertensive animals (Grollman, Williams and Harrison, 1940). A neutral lipid from the renal medulla, with antihypertensive but no vasodepressor properties, was isolated by Muirhead, Daniels, Booth, Freyburger and Hinman (1965). The acidic fraction from the renal medulla contains potent vasodepressors including P.G.E.$_2$ (Daniels, Hinman, Leach and Muirhead, 1967). McGiff, Crowshaw and Itskovitz (1974) have suggested that pressor hormones constrict the blood vessels and release P.G$_{E_2}$ within the vascular bed, and in the kidney, which reduces or terminates the vasoconstrictor response. Finally, a phospholipid has been extracted from whole kidneys which inhibits the reaction between renin and its substrate (Sen, Smeby and Bumpus, 1968). These antihypertensive factors are of great interest, but there is, as yet, no substantial evidence to link them pathogenically with hypertension.

3. ADRENAL HORMONES

Adrenal cortical hormones may be involved in high blood pressure, since patients with Addison's disease are hypotensive while those with Cushing's syndrome are hypertensive (Cushing, 1932). A specific relationship between steroids and the production of hypertension was not established until Loeb, Atchley, Ferrebee and Ragan (1939) demonstrated the hypertensive effects of 11-deoxy-corticosterone when administered to patients with Addison's disease.

Conn (1955) made a major contribution to the study of steroid induced hypertension by describing a new hypertensive syndrome, caused by overproduction of aldosterone by an adenoma. Hypertension can also be caused by congenital adrenal hyperplasia

due to a deficiency of 11-hydroxylase or 17-hydroxylase, enzymes which are involved in the synthesis of cortisol (Kaplan, 1974).

In renal hypertensive patients, aldosterone levels are usually normal (Laragh, Sealey and Sommers, 1966), while excretion levels of aldosterone are elevated in malignant hypertension (Genest, Lemieux, Davignon, Koiw, Nowaczynski and Steyermark, 1956).

Currently, the work of Melby, Dale and Wilson (1971) has aroused interest in the possible participation of another mineralocorticoid, which might be secreted in patients with low renin essential hypertension. This mineralocorticoid, 18-OH deoxycorticosterone, has been found in elevated concentrations in about one third of low renin essential hypertensives (Brown, Ferriss, Fraser, Lever, Love, Robertson and Wilson, 1972).

The experimental model of steroid induced hypertension is the D.O.C.-salt hypertensive rat (Grollman, Harrison and Williams, 1940). Aldosterone secretion is stimulated in experimental renal hypertension where one renal artery is clamped and the other kidney left intact. However, if the contralateral kidney is removed, aldosterone levels are normal (Singer, Losito and Salmon, 1963). The adrenals are not necessary for the production of hypertension by experimental aortic co-arctation (Carretero, Enzmann, Polomski, Piwonska, Oza and Schork, 1973). However, the adrenal cortex is essential for the development of hypertension in the Japanese S.H.R. (Aoki, Takikawa and Hotta, 1973).

The mineralocorticoids do not raise the blood pressure by a direct constrictor action on the blood vessels. Water and sodium retention, stimulated by these steroids, probably increase blood volume which, in turn, would increase cardiac output (Borst and Borst

de Geus, 1963). Sodium retention and potassium excretion produce hypernatremia and hypokalemia, and these might also raise cardiac output by increasing myocardial strength (Haddy, 1974). The increased cardiac output would raise the arterial pressure, and the effect would be subsequently augmented by an increase in peripheral resistance. Sodium retaining steroids may have more direct effects on peripheral resistance, since alkalosis increases the resistance to flow through vascular beds (Haddy and Scott, 1965), and hypernatremia increases the sodium and perhaps water content of the aorta (Redleaf and Tobian, 1958a). An increased water content of the blood vessels would limit their distensibility and might also reduce their luminal diameter (Tobian, 1960). There is also evidence that D.O.C. potentiates the pressor effects of noradrenaline, (Raab, Humphreys and Lepeschkin, 1950) which could promote hypertension in the presence of a normal sympathetic discharge rate.

A chromaffin cell tumor, phaeochromocytoma, usually located in the adrenal medulla can cause hypertension by releasing excessive quantities of catecholamines into the blood stream (Beer, King and Prinzmetal, 1937). Phaeochromocytoma is perhaps the only example of hypertension in which we are fairly sure of the causative factor. The excessive plasma catecholamines cause vasoconstriction and, hence, an increase in peripheral resistance.

4. THE RENIN-ANGIOTENSIN-ALDOSTERONE-SODIUM SYSTEM

The humoral factors which may increase peripheral resistance in hypertension have been considered separately. A recurrent dilemma in work on the pathogenesis of hypertension is the difficulty of identifying single causative agents. This is particularly evident in the high, normal and sometimes low values found for such factors as renin and aldosterone. One possible explanation is that

they are not causative agents. Another, is that because of their interrelation and influence on sodium balance, a primary disturbance of one is followed by compensatory adjustment in the other.

It is very probable that sodium, renin and aldosterone are related in some way, although the exact nature of their interrelation is not established (Fraser, Brown, Chinn, Lever and Robertson, 1969). Brown, Fraser, Lever and Robertson (1971) have suggested that, sodium loss in some way stimulates the release of renin, and consequently elevates levels of angiotensin II (Fig. 1:1). Angiotensin II may raise the blood pressure by direct action on the blood vessels and/or stimulate aldosterone secretion. Elevation of the blood pressure would promote sodium loss by pressure diuresis, while aldosterone would stimulate sodium retention. Sodium retention, as previously discussed, would expand the plasma volume and increase the blood pressure. However, if renin was released in excess to the sodium balance, or the elevated blood pressure failed to promote sodium loss, then hypertension would result.

The action of angiotensin II, either in raising the blood pressure by increased peripheral resistance or by stimulating the secretion of aldosterone, may be regulated by sodium. In states of sodium depletion, the pressor effects of angiotensin II are diminished while the aldosterone effect is enhanced, but in sodium excess, the pressor response is enhanced and the aldosterone effect diminished (Fraser, Brown, Chinn, Lever and Robertson, 1969., Brown, Fraser, Lever, Robertson, James, McCusker and Wynn, 1968., Blair-West, Coghlan, Denton, Goding, Wintour and Wright, 1965). Therefore, a constant level of angiotensin II in the plasma could produce a progressively increasing stimulus to aldosterone secretion if there was progressive sodium depletion. Conversely, a normal or subnormal concentration of

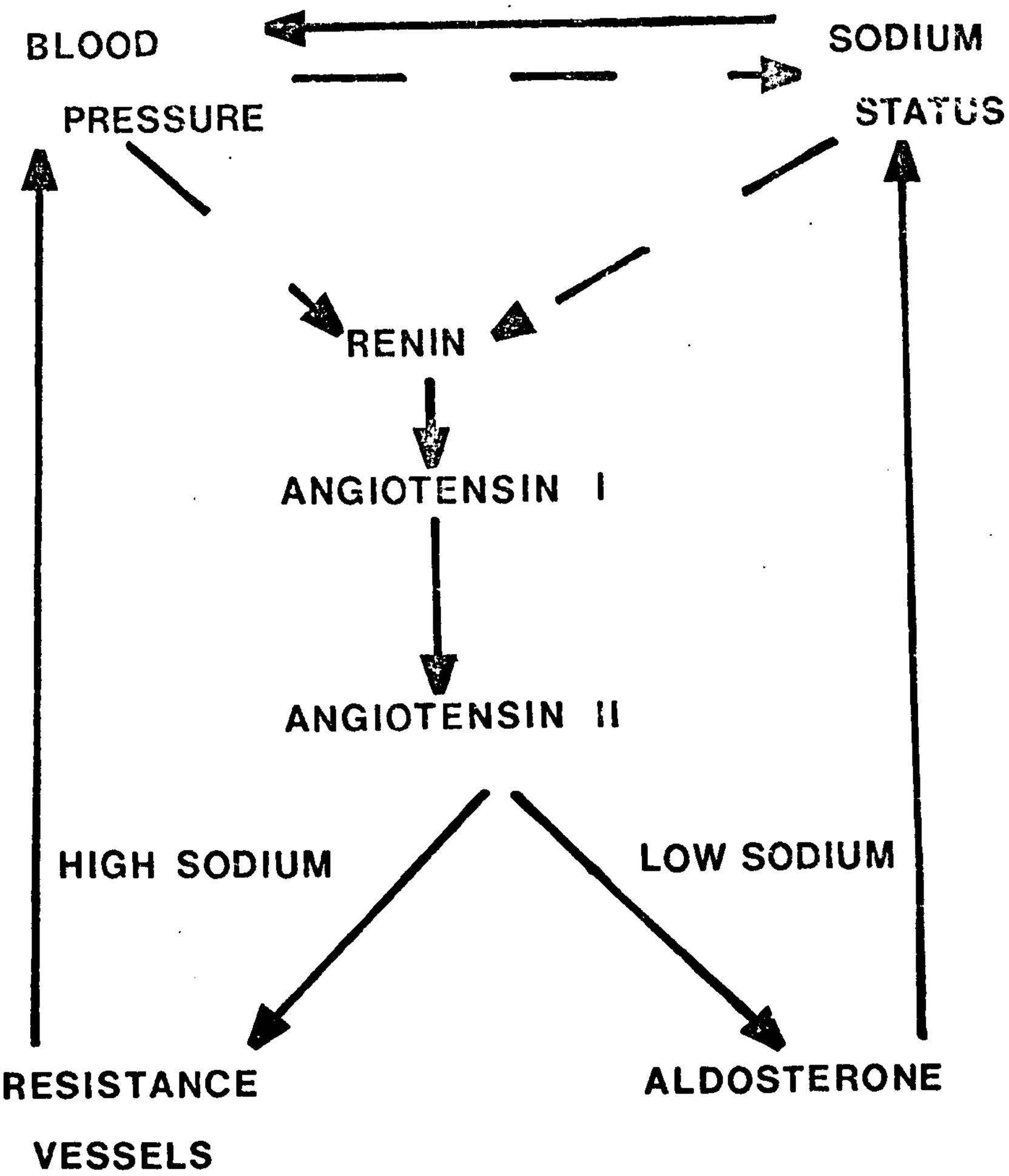


Fig. 1:1

A proposed scheme for the interrelationship of blood pressure the renin-angiotensin—aldosterone system and sodium balance.

Continuous arrows indicate stimulation, discontinuous arrows indicate inhibition. Both renin mediated pathways are hypertensive. See text for further explanation.

angiotensin could raise the blood pressure in a state of sodium retention.

C. BLOOD VESSEL STRUCTURE AND FUNCTION

1. AUTOREGULATION

Ledingham and Cohen (1964) have promoted the concept that the increased peripheral resistance of hypertension is due to autoregulation. This myogenic response to stretch by the resistance vessels could be stimulated by an early increase in cardiac output. Autoregulation is a protective mechanism against excessive tissue blood flow at high perfusion pressures. Evidence for this theory comes from patients with co-arctation of the aorta. The mean arterial pressure in the lower half of the body is normal, whereas, rostral to the co-arctation, it is raised. However, tissue blood flow in the upper and lower limbs remain normal, despite the marked disparity in perfusion pressure (Patterson, Shepherd and Whelan, 1957). Functional autoregulation is also suggested to be caused by structural changes in the vessel wall (Folkow, Hallbäck, Lundgren and Weiss, 1970 c) or by a reduction in the number of small arterioles supplying an organ (Hutchins and Darnell, 1974).

Olmsted and Page (1965), however, found that renal hypertension in dogs was associated with an early rise in peripheral resistance, associated with a fall in cardiac output. Their results were therefore not compatible with the autoregulation theory.

2. VASCULAR REACTIVITY

Attempts to demonstrate increased autonomic nervous activity or increased amounts of circulating pressor substances in all types of hypertension have not been generally successful. An alternative possibility is, that in hypertension the resistance vessels might

respond to stimuli of a normal magnitude by an excessive constriction, either as a primary defect, or as a secondary sustaining mechanism. In order to examine this possibility it is important to know whether the resistance vessels contract more powerfully in hypertensive subjects than in normal people, whether this phenomenon is specific to certain agonists or generalized, and whether it precedes or follows the hypertension.

Two types of test have been used to examine this hypothesis in humans, i) systemic pressor responses to various stimuli, "cardiovascular reactivity", and ii) studies of constriction in regional vascular beds, "vascular reactivity".

i) Cardiovascular reactivity in humans

Cardiovascular reactivity is defined as the degree with which the heart and peripheral vascular system respond to quantitated stimuli.

a) Reflex stimuli

Early studies on cardiovascular reactivity involved the use of reflex stimuli with the measurement of the evoked pressor response. Hines and Brown (1933) found enhanced pressor responses to cold in hypertensive patients, but Pickering and Kissin (1936) did not confirm these results. Later investigations by Alam and Smirk (1938) and Russek and Zohman (1945) showed that although larger pressor responses to cold frequently occurred in hypertensive patients, some had responses in the normal range.

Exercise, emotional or pain stimuli produced rises in blood pressure which were often greater in hypertensive patients (Barath, 1928., Alam and Smirk, 1938., Wolff, 1951).

It was generally assumed that the rise in blood pressure to reflex pressor stimuli was due to vasoconstriction. However, Boyer, Fraser and Doyle (1960) have shown that cold immersion of the foot causes a pressor response in normal subjects which is mainly due to a rise in cardiac output, whereas, in the hypertensive subject, the response is largely due to vasoconstriction. Evidence for cardiovascular hyperreactivity from experiments using reflex pressor stimuli is therefore probably not a reliable indication of the degree of vasoconstriction.

b) Chemical stimuli

Systemic pressor responses to adrenaline and noradrenaline were found to be normal in hypertensive patients (Pickering and Kissin, 1936; and Gombos, Hulet, Bopp, Goldring, Baldwin and Chasis 1962). Conversely, Goldenberg, Hines, Baldwin, Greeneand Roh (1948), Judson, Epstein and Wilkins (1950) and Staquet, Demanet and Basténie (1965) found that hypertensive subjects were more sensitive to injected noradrenaline than normotensive volunteers.

The first comparison of the effects of angiotensin in hypertensive and normal subjects was made by Doyle and Black (1955). They found that a slightly larger pressor response occurred in hypertensive patients, and that this was greatly augmented by ganglion blockade.

Kaplan and Silah (1964) have studied pressor responses to angiotensin in a variety of hypertensive states. Patients with essential hypertension exhibited hyperreactivity to angiotensin whereas, those with renovascular and malignant hypertension were hypo-reactive. This specific lack of sensitivity was attributed to high endogenous levels of angiotensin. Roguska, Simon and Del Greco

(1968) have suggested that this lack of sensitivity to angiotensin may also be partly due to non-specific hyporeactivity of the vasculature.

Evidence for the effects of chemical vasoconstrictor stimuli in inducing pressor responses is obviously conflicting. The differences in results are probably due to the different starting blood pressures and baroreceptor sensitivities of normotensive and hypertensive subjects. The use of intra-arterial infusions of vasoconstrictor agents has permitted examination of the responses of the blood vessels without the complications introduced by the general circulation.

ii) Vascular reactivity in the peripheral circulation of hypertensive patients

Vascular reactivity is defined as the degree with which the vasculature responds to quantitated stimuli.

Duff (1957) demonstrated increased reactivity to intra-arterial adrenaline, using the hand blood flow of hypertensive and normotensive individuals. Adrenaline constricts blood vessels in the skin and dilates those in the muscles and is therefore not the ideal agonist for studies of vascular reactivity.

Doyle, Fraser and Marshall (1959) studied the response of the forearm circulation and found increased reactivity to intra-arterial noradrenaline, 5-hydroxytryptamine and angiotensin in hypertensive subjects. When the results were corrected for the initial state of resistance in the forearm, responses to noradrenaline and 5-hydroxy-tryptamine were still significantly greater in the hypertensive group, but responses to angiotensin were no different from the normotensive control.

Increased vascular reactivity to noradrenaline in essential hypertensive patients has been demonstrated in the skin (Bárány, 1963), striated muscle (Moulton, Spencer and Willoughby, 1958), the digit (Mendlowitz, Naftchi, Wolf and Gitlow, 1965) and in the conjunctiva (Lee and Holze, 1951). Scroop and Whelan (1968), in contrast, could find no difference in hand blood vessel reactivity to angiotensin or noradrenaline in essential hypertensives.

Folkow, Grimby and Thulesius (1958) and Sivertsson (1970) have studied resistance to flow in the forearm and hand vascular beds of normotensive and essential hypertensive subjects. They found that resistance to flow at maximal vasodilation, due to heating and ischaemia of the beds, was greater in hypertensive than normotensive patients. These results were confirmed by Conway (1963).

Dose-response curves of resistance responses to intra-arterial noradrenaline exhibited a steeper slope than control but with no change in the vasoconstrictor threshold dose. Folkow, Hallbäck, Lundgren, Sivertsson and Weiss (1973) have claimed that such changes in the noradrenaline dose-response curve, together with an increase in basal resistance to flow, are caused by an increase in vascular wall/lumen ratio, due to medial hypertrophy. Folkow and his group have suggested that essential hypertension is triggered by intermittent bouts of hypothalamic neurohumoral stimulation which increase cardiac output in genetically predisposed persons. The increased blood flow so produced is reduced by increased peripheral resistance due to structural changes in the resistance vessels.

This theory does not explain why hypertension and medial hypertrophy do not occur in many patients with increased cardiac output due to a hyperdynamic circulation, marked nutritional anaemia,

hyperthyroidism, and Pagets' disease (Page, 1974). It is also partly contradicted by evidence from studies on intact in vivo arterioles in human hypertensives, which indicate that the increased wall/lumen ratio is due to a persistent shortening of the smooth muscle and not to hypertrophy or hyperplasia of the media (Short, 1966).

Studies in patients with renal or renovascular hypertension have indicated that vascular reactivity to noradrenaline is at the upper limit of the normal range, as assessed in digital and hand blood vessels (Mendlowitz, Naftchi, Wolf and Gitlow, 1965., Scroop and Whelan, 1968).

Perhaps the most interesting studies of regional vascular reactivity have been those in the healthy offspring of hypertensive parents. Doyle and Fraser (1961 a,b) found that an infusion of noradrenaline into the brachial artery induced greater vasoconstriction in the forearm of the normotensive offspring of hypertensive patients than in a group of students whose parents were normotensive. Davis and Landau (1966) found that hypertensive patients were more sensitive to adrenaline applied to the conjunctiva than normotensive individuals, and that 70% of normotensive first degree relations of hypertensive patients also had enhanced sensitivity.

It is therefore probable that in inherited hypertension, vascular hyperresponsiveness may precede the rise in blood pressure and may therefore be of importance in the pathogenesis of the disease.

The use of isolated vascular preparations from human hypertensives would provide the most detailed evidence on vascular reactivity. Because of the lack of fresh blood vessels from patients who are not receiving antihypertensive therapy, vessels from

experimental animals have been widely used.

There are, however, two recent studies on isolated vessels from human hypertensives. Horwitz, Clineschmidt, Van Buren and Ommaya (1974) have studied the responses of temporal artery strips from hypertensive patients whose antihypertensive medication had been withdrawn for 2 weeks. Responses to noradrenaline or phenylephrine were identical in vessels from hypertensive and normotensive subjects, over the full dose range used. The α-adrenoceptors in the "hypertensive" and "normotensive" arteries were also identical, as judged by the pA_2 and K_b values for phentolamine. Ettinger, Seibel and Riecker (1970) perfused small arteries from patients with essential hypertension. In contrast to the work using arterial strips, they found larger than normal responses to noradrenaline in vessels from hypertensive subjects.

iii) Cardiovascular reactivity in animals with experimental hypertension

a) Renal hypertension

The first demonstration of increased cardiovascular reactivity in experimental hypertension was the report by Ogden, Brown and Page (1940) of increased sensitivity in the renal hypertensive rabbit to the pressor effects of vasopressin. Phelan, Eryetishir and Smirk (1962) and Phelan (1966) showed that the renal hypertensive rat (R.H.R.) exhibited exaggerated cardiovascular responses to adrenaline and vasopressin, but, like Conway (1955), could only demonstrate hyperreactivity to noradrenaline in the ganglion blocked animal. In contrast, Page and Taylor (1949) and Page, Kaneko and McCubbin (1966) found little or no change in cardiovascular reactivity to a variety of pressor agents in chronic renal hypertensive dogs, with the exception that tyramine induced responses were increased.

In acute renal hypertensive dogs, they found increased cardiovascular reactivity to adrenaline, noradrenaline and 5-hydroxytryptamine, but responses to angiotensin were subnormal.

Recent studies have not clarified the situation. Shibayama, Mizogami and Sokabe (1971) using pithed, decerebrate, vagotomized R.H.R. found no changes in reactivity to noradrenaline, whereas Finch (1971), reported increased blood pressure responses to noradrenaline and tyramine in the pithed R.H.R.

b) <u>D.O.C. hypertension</u>

Evidence for cardiovascular hyperreactivity in the D.O.C. hypertensive animal is also contradictory. Masson, Page and Corcoran (1950) using dogs and rats, and Shibayama, Mizogami and Sokabe (1971) using rats, found no changes in cardiovascular responses to adrenaline, noradrenaline and angiotensin. However, increased cardiovascular reactivity to noradrenaline in D.O.C. hypertensive rats has been demonstrated by Sturtevant (1956) and Finch (1971).

c) <u>The spontaneously hypertensive rat</u>

In the New Zealand strain of S.H.R., Smirk and Hall (1958) and Phelan (1968) have demonstrated increased pressor responses to adrenaline and vasopressin. Like the R.H.R., hyperreactivity to noradrenaline could be unmasked by ganglion blockade.

In the Japanese strain of S.H.R., the study by Shibayama, Mizogami and Sokabe (1971) indicated no changes in noradrenaline pressor responses but increased reactivity to angiotensin. Dupont and Sassard (1974), in contrast, demonstrated exaggerated responses to noradrenaline and normal responses to angiotensin, in ganglion blocked S.H.R.

d) Adrenal regeneration and salt induced hypertension

Rats with adrenal-regeneration hypertension (Gardner and Honoré, 1964 a,b) show enhanced pressor responses to vasopressin but not to angiotensin, noradrenaline and adrenaline. Conversely, rats with hypertension induced by chronic salt ingestion exhibit increased reactivity to angiotensin, noradrenaline, adrenaline and vasopressin (Honoré and Gardner, 1966 a,b). Dahl, Heine and Tassinari (1964) found increased cardiovascular reactivity to noradrenaline and angiotensin in a strain of rats that were prone to the development of hypertension on salt ingestion. This increased reactivity occurred even when the rats were maintained in a normotensive state by a low salt diet, indicating that the increased reactivity preceded the development of hypertension.

Studies involving the measurement of the response to various drugs as a change in the blood pressure of the whole animal, have their limitations. Reflex and direct effects of sympathomimetic substances on cardiac output and hormonal influences on the blood vessels will affect the reactivity. Interpretation of such results is complicated because the cardiovascular reactivity is dependant on the stage and type of hypertension, on the pressor drug used, on the level of anaesthesia, if used, and on the starting blood pressure. In order to determine vascular reactivity without the complications of the intact cardiovascular system, investigations on isolated blood vessels have been made.

iv) Vascular reactivity in isolated preparations from experimental hypertensive animals

The use of isolated vascular preparations to examine the phenomenon of increased vascular reactivity in hypertension, obviates many of the problems of the *in vivo* approach.

Two methods have been used; arterial strips or rings and perfused vascular beds. Both have advantages and disadvantages; the arterial strip or ring can provide a direct tension record uncomplicated by anatomical changes in the vascular structure. The technique is, however, limited to fairly large arteries in the rat. Perfusion techniques are usually necessary to examine the behaviour of the arterioles, which are the major site of peripheral resistance (Freis, 1960), but changes in the structure of these vessels can complicate interpretation of the results.

The stimulating agent usually employed is exogenous noradrenaline. Sympathetic nerve stimulation to release endogenous noradrenaline, would be a more physiological stimulus. Few investigations have attempted this approach since excision of the required vascular tissue often damages the nerve supply, making comparisons between the "hypertensive" and "normotensive" preparation difficult.

v) The determinants of vascular reactivity

The term, "vascular reactivity" in its widest sense, means "the ability of the vessel to respond". To understand and sub-divide this rather nebulous term, a consideration of the events which link the application of a vasoactive stimulant to the haemodynamic response is required (see Fig. 1:2).

a) The drug-receptor interaction

It is widely accepted that the interaction between a drug and a specific receptor follows the law of mass action (Clark, 1933). The receptor occupancy (Ariens, 1964) or the rate of receptor occupancy (Paton, 1961) determines the degree of the stimulus to respond. A modification of the receptors could cause a change in the reactivity,

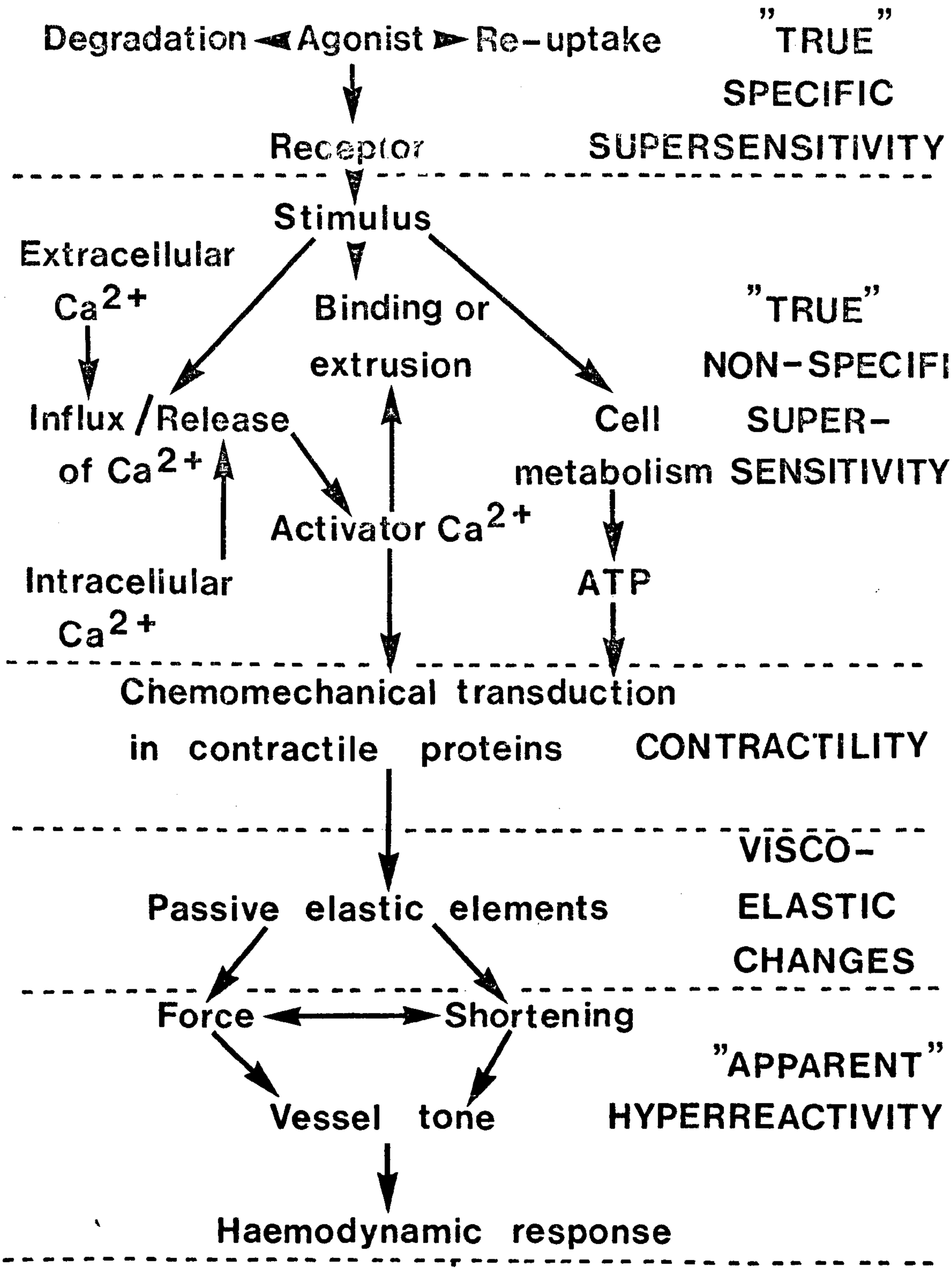


Fig. 1:2

A scheme of the events which link the application of a vaso-active stimulant to the haemodynamic response.

(Adapted from Johansson, 1974).

which will be defined as "true specific supersensitivity". This phenomenon would be experimentally characterized by a shift of the dose-response curve to the left, for agonists stimulating a single receptor type. The maximum response of the vascular tissue would be normal but the threshold dose for stimulation would be lowered. Such a true specific supersensitivity could, however, also be caused by changes in the neuronal uptake or enzymatic decomposition of the agonist in the tissue.

b) The supply of ATP and calcium to the contractile proteins

The possibility that the supply of ATP to the contractile filaments may cause changes in reactivity is unlikely. Evidence suggests that the intracellular calcium concentration is the rate-limiting step for contraction (Waugh, 1962., Bohr, 1964., Hinke, Wilson and Burnham, 1964). The intracellular [Ca^{++}] may be increased to a level that will activate the contractile proteins, either by increasing the rates of accumulation, or by decreasing the rates of elimination or sequestration of Ca^{++}.

Activator Ca^{++} may come from either intracellular or extracellular sources (Hinke, 1965). In the resting smooth muscle the concentration of activator Ca^{++} is less than 10^{-7}M, although the concentration of Ca^{++} in the extracellular pool is greater than 10^{-3}M (Bohr, 1973). The plasma membrane of the cell must therefore maintain a 10,000 fold concentration difference if the vascular smooth muscle is to remain relaxed. Three sites have been implicated as the intracellular bound Ca^{++} pool, the sarcoplasmic reticulum (Devine, Somlyo and Somlyo, 1972), the mitochondria and the plasma membrane with its surface vesicles (Fitzpatrick, Landon, Debbas and Hurwitz, 1972., Baudouin, Meyer, Fermandjain, and Morgat, 1972).

An increase in the permeability of the plasma membrane, or a reduction in the calcium binding ability of the intracellular Ca^{++} pool could cause an increase in reactivity. Such a change would be characterized by a shift to the left of the dose-response curve to all agonists utilizing the affected calcium pool. Such a change, without an increase in maximal tension development will be termed "true non-specific supersensitivity".

c) The contractile proteins

An increase in actomyosin synthesis could lead to hypertrophy of the vascular smooth muscle. This would be evidenced by an increase in the absolute contractile force exerted by the vessel, without any change in the degree of muscle shortening. Such a change in "contractility" would be characterized by a greater maximum tension development, to all agonists, without any shift in the dose % maximum response curve.

d) The passive elastic elements

To exert tension, or shorten, the contractile filaments of the smooth muscle cell must "take up the slack" in its passive elastic elements. The amount of "slack" will be determined by the resting length and tension of the muscle under investigation. Changes in the passive elastic elements can affect the dose-response curve to any agonist. An increase in the elastic component of a vascular tissue would tend to shift the dose-response curve to the right.

e) Mechanical factors in intact blood vessels

In the intact vessel, there are additional mechanical factors which complicate the analysis of the contractile response. One factor which can have a marked influence on vascular reactivity is the wall/lumen ratio. For a given degree of shortening of the smooth muscle in the outer media, the lumen will be reduced more in a

thick walled vessel than in a thin walled vessel. Consequently, if hypertensive disease is associated with an increase in wall/lumen ratio due to hypertrophy (Folkow, Hallbäck, Lundgren and Weiss, 1970c) or swelling (Tobian, 1960) of the vascular wall, this structural change will lead to augmented responses in the intact vessel. A structurally based "apparent hyperreactivity" would be characterized by a steeper dose-response curve with no change in the vasoconstrictor threshold dose. If the increase in wall/lumen ratio was due to medial hypertrophy, an elevation of the maximum response would occur. A greater resistance to flow at maximum vasodilation would indicate that the increase in wall thickness had narrowed the luminal diameter (Folkow, Hallbäck, Lundgren & Weiss, 1970c)

It would be ideal if all studies on vascular reactivity used the same criteria and definitions of the phenomenon. This is not the case, for a variety of reasons. Early investigators did not realize the importance of analysing full dose-response data and concepts of the factors determining the vascular response were, in retrospect, primitive. Studies on responses from arterial strips cannot directly determine whether apparent hyperreactivity is present. Studies in perfused intact vessels cannot distinguish between contractility or viscoelastic changes when a change in the wall/lumen ratio has occurred. Also, there often appears to be a complex of these various influences which summate to cause increased vascular reactivity. The participation of individual influences is therefore extremely difficult to ascertain.

If the factors which can increase vascular reactivity are divided into two groups comprising a) specific and non specific supersensitivity, and b) contractility, viscoelastic influences and structural changes, the significance of early work on vascular

reactivity is clearer. A change in the sensitivity of the vascular smooth muscle preceding the development of hypertension could be the primary event in increasing peripheral resistance and blood pressure. A change in the structure of the vessel wall would probably be a defensive reaction against the effects of high blood pressure and therefore secondary to hypertension.

(vi) Vascular reactivity in arterial strips and rings from renal hypertensive rats

a) Aortic preparations

Redleaf and Tobian (1958a) used helically cut aortic strips from R.H.R. and found that they responded to noradrenaline with weaker contractions than normal. Mallov (1959, 1961) improved the methodology by cutting matched saw-tooth strips of aortae from renal hypertensive and normotensive rats. He found that the aortae from R.H.R. exerted less tension than normal to noradrenaline stimulation, even though the strips from hypertensive animals contained more alkali-soluble nitrogen, thought to be of muscular origin.

Gordon and Nogueira (1962), related the noradrenaline induced contractile tension of aortic strips from renal hypertensive and normotensive rats to the width and length of the strips. Strips from R.H.R. produced more tension than those from control animals. This contradiction of the previous studies was explained on the grounds that "hypertensive" aortae had a greater optimum resting tension than the "normotensive" strip. The low resting tensions used by the previous investigations could therefore have masked the increased reactivity. Gordon (1962) suggested that increased aortic reactivity preceded the development of hypertension. Gordon did not prove that aortic reactivity was increased in pre-hypertensive animals, but defined

(1973) also suggested that an increase in available calcium was the mechanism of increased reactivity. Nephrotensin, a pressor agent which is present in the blood of acute but not chronic R.H.R. (Grollman, 1970., Grollman and Krishnamurty, 1971) was proposed as the cause of this change in calcium regulation.

b) Preparations from carotid and femoral arteries

Moerman, Herman, Bogaert and De Schaepdryver (1969) used carotid artery strips from renal hypertensive dogs to investigate vascular reactivity. Strips from renal hypertensive dogs were more sensitive than normal to noradrenaline, as assessed by the $ED_{50\%}$ dose.

Studies using femoral artery strips (Bandick and Sparks, 1970., Holloway and Bohr, 1973) from acute renal hypertensive rats have also demonstrated increased reactivity to noradrenaline, adrenaline and potassium chloride. Both groups of investigators found a greater degree of spontaneous activity in arteries from hypertensive than from normotensive rats. Bandick and Sparks (1970) found that the "hypertensive" strips were less extensible than normal, whereas, Holloway and Bohr (1973) could demonstrate no difference in passive stiffness. Both groups suggested that the increased reactivity and spontaneous activity in femoral arteries from acute R.H.R. was due to an increase in membrane permeability, probably involving calcium.

c. Preparations from arterioles

Bohr (1961) reported studies on strips of small resistance vessels from R.H.R. These arteriolar strips were more sensitive than normal to adrenaline, noradrenaline and angiotension. This technique of direct tension recording from strips of arterioles is ideal for studies of vascular reactivity, since the resistance vessels

contribute the major proportion of peripheral resistance. Technical difficulties have apparently inhibited further investigations using arteriolar strips.

vii) Reactivity in arterial strips from D.O.C. hypertensive rats

a) Aortic preparations

Studies by Redleaf and Tobian (1958a) Mallov (1959, 1961) and Massingham and Shevde (1971) have demonstrated reduced reactivity to noradrenaline in aortic strips from D.O.C. hypertensive rats. Tension development to KCl and the resting potential of the vascular smooth muscle were found to be normal (Massingham and Shevde, 1971).

b) Preparations from femoral and carotid arteries

Extensive investigations of femoral artery reactivity in D.O.C. hypertension have been made by Bohr's group (Bohr and Sitrin, 1970, Holloway, Sitrin and Bohr, 1972., Holloway and Bohr, 1973). They have shown that the femoral artery strips from D.O.C. hypertensive rats respond to lower concentrations of adrenaline and KCl than do normal preparations. Preparations from hypertensive rats exhibited a greater tendency to develop spontaneous activity and this was exaggerated by high sodium bathing solutions. This, together with evidence that a higher than normal concentration of calcium was required to stabilize the "hypertensive" artery, led to the suggestion that a change in membrane permeability had occurred.

Hansen, Abrams and Bohr (1974) investigated the paradox that strips from D.O.C. hypertensive rats were more sensitive than normal to agonists, as assessed by threshold and $ED_{50\%}$ dose, but produced less tension at high doses of agonist. Partial occlusion of one iliac artery was used to protect one femoral artery from the high blood pressure. The ability to develop tension, however, was

equally reduced in strips of femoral arteries from occluded and non-occluded limbs. Iliac occlusion per se seemed to have weakened the vascular smooth muscle. Responses at different initial tension showed that "hypertensive" strips developed less tension than normal at any level of passive tension. Contradicting the suggestion of Gordon and Nogueira (1962), that arteries from hypertensive rats might adapt to the higher pressure so that a greater passive tension would be required for their complete activation. The decrease in the ability to develop tension was suggested by Hansen, Abrams and Bohr (1974) to be a functional result of ultrastructural disruption of the muscle filaments (Gardner and Matthews, 1969).

Since previous studies had indicated that ion permeability of the vascular smooth muscle membrane might be altered in hypertension, Bohr (1974) examined the effects of various ions on carotid artery strips from D.O.C. hypertensive rats. Reactivity to barium was less than normal, while responses to strontium exceeded the controls. These differences were attributed to an altered ability to bind calcium in the artery cell wall.

viii) Reactivity in arterial strips from spontaneously hypertensive rats

a) Aortic preparations

Studies on aortic strips and rings from the Japanese and New Zealand strains of S.H.R. have demonstrated a reduced ability to develop tension when stimulated with noradrenaline (Spector, Fleisch, Maling and Brodie, 1969., Levy, 1973, Massingham and Shevde, 1971). The reduction in contractility may precede the development of hypertension (Shibata, Kurahashi and Kuchii, 1973).

Field, Janis and Triggle (1972) and Janis and Triggle

(1972) demonstrated increased tension development to low doses of noradrenaline; however, responses to high doses of the agonist were less than normal. In contrast, Hallbäck, Lundgren and Weiss (1971) reported that tension development in the aorta of the S.H.R. was normal over the full noradrenaline dose-response curve. Clineschmidt, Geller, Govier and Sjoerdsma (1970) have shown that the choice of control animals greatly influences the interpretation of results from aortae of S.H.R. Aortic reactivity to noradrenaline in the S.H.R. was identical with results from aortae of one strain of control rats, but less than the reactivity in another strain.

An impairment of the α-adrenoceptors cannot account for the generally low contractility reported for noradrenaline responses. The affinity and the number of α-adrenoceptors in the aortae from S.H.R. have been reported as normal (Clineschmidt, Geller, Govier and Sjoerdsma, 1970., Janis and Triggle, 1972).

Responses to KCl, like those to noradrenaline, are reduced in the aorta of the S.H.R. (Spector, Fleisch, Maling and Brodie, 1969., Massingham and Shevde, 1971., Grollman and Krishnamurty, 1973., Levy, 1973., Shibata, Kurahashi and Kuchii, 1973). Field, Janis and Triggle (1972) however, suggested that a greater tension is developed at low dose levels. Responses to 5-hydroxytryptamine (Spector, Fleisch, Maling and Brodie, 1969) angiotensin, ouabain (Shibata, Kurahashi and Kuchii, 1973) and prostaglandin E_2 (Levy, 1973) are also reduced in the aortae from the S.H.R.

Spector, Fleisch, Maling and Brodie (1969) have reported that aortae from S.H.R. relax more than normal when stimulated with isoprenaline. Relaxation induced by papaverine and nitroglycerin were, however, found to be of normal magnitude (Shibata, Kurahashi

and Kuchii, 1973).

The rate of decay of aortic responses, on washing noradrenaline or KCl from the tissues, was found to be slower than normal in preparations from S.H.R. (Field, Janis and Triggle, 1972., Grollman and Krishnamurty, 1973). However, the rate of decay of responses in calcium-free conditions was found to be normal by Field, Janis and Triggle, 1972) but faster than normal by Shibata, Kurahashi and Kuchii (1973).

Shibata, Kurahashi and Kuchii (1973) demonstrated differences between the profile of the noradrenaline induced response of the aortae from the S.H.R. and the normotensive rat. The normal response to noradrenaline comprised a fast and a slow phase, but, in aortae from the S.H.R., the slow phase was reduced or absent. The S.H.R. aorta was found to contract when exposed to Mn^{++}, Co^{++} and La^{+++} ions which were without contractile effects in control tissues.

The majority of investigators suggest that there is a change in the musculature of the S.H.R. aorta, which impairs contraction (Spector, Fleisch, Maling and Brodie, 1969., Levy, 1973). This defect may be due to a change in the calcium permeability of the cell membrane (Shibata, Kurahashi and Kuchii, 1973., Tenner, 1973).

b) <u>Preparations from femoral and carotid arteries</u>

Holloway and Bohr (1973), using femoral artery strips, have confirmed that La^{+++} contracts vascular smooth muscle from the S.H.R.

This result was also reported by Bohr (1974) using the carotid artery. Holloway and Bohr (1973) found that femoral arteries

from S.H.R. had a greater tendency to develop spontaneous activity than control tissues, but exhibited no change in threshold or tension development to KCl or adrenaline.

ix) Vascular reactivity in intact perfused blood vessels from renal hypertensive rats

a) Perfused hind-quarters or hind-limb preparations

McQueen (1956) demonstrated that responses to noradrenaline of the perfused hind-quarters preparation were increased 2 or 3 days after the induction of renal or renoprival hypertension in rats. The reactivity to 5-hydroxytryptamine and pitressin was also increased (McQueen, 1957). The increased reactivity of renoprival hypertension was dependent on the adrenal glands or an adequate salt intake, while the ischaemic kidney was implicated in the increased reactivity of renal hypertension (McQueen, 1956).

McQueen (1961) suggested that an increase in vascular wall thickness had occurred, since the noradrenaline response of the isolated preparation, at a constant flow rate, was related to the initial perfusion pressure. Water-logging of the vascular wall was suggested to be the mechanism involved (Tobian and Binion, 1954) since medial hypertrophy was unlikely to cause such a rapid increase in reactivity.

Reserpine treatment lowered the blood pressure of the R.H.R. to normal, but did not completely reverse the changes in reactivity of the perfused hind-quarters preparation (McQueen, 1961). The increased reactivity was therefore probably not solely a consequence of the increased blood pressure.

Laverty (1961), using the perfused hind-limb preparation, also demonstrated increased reactivity to noradrenaline in the R.H.R.

Oono (1966) and Baum and Shropshire (1967a) confirmed this result but only in tissues from the chronic R.H.R. Reactivity in preparations from the acute R.H.R. was not significantly different from normal. Baum and Shropshire (1967a) demonstrated that responses to lumbo-sympathetic nerve stimulation were only increased in the early stages of hypertension. In contrast, Tripod and Bein (1960) found a reduction in reactivity to noradrenaline in perfused hind-limb preparations from chronic renal hypertensive rats, but responses to adrenaline, histamine and angiotensin were elevated.

Nolla-Panades (1963) examined the effect of high blood pressure on reactivity, by co-arctation of the rat aorta anterior to the origins of the renal arteries. Blood pressure rostral to the co-arctation was elevated, but, caudal to the co-arctation the pressure was normal. Reactivity to noradrenaline in the perfused hind-quarters was increased, even though this area was normotensive. The basal perfusion pressure of the isolated preparation and noradrenaline response amplitude were correlated. The study showed that although a change in the vessel wall probably had occurred, it could not be a consequence of the high blood pressure.

Demura, Fukuchi, Takahashi and Goto (1965) related increased reactivity in the perfused hind-quarters preparation from renal hypertensive rats, to the ionic composition of the aorta. Reactivity to noradrenaline and angiotensin was increased in the R.H.R., and in rats treated with D.O.C., angiotensin and NaCl, aldosterone, angiotensin and NaCl, and angiotensin alone. All animals with increased reactivity also exhibited increased aortic contents of Na^+ and K^+ ions. Rats with aminonucleoside induced nephrosis or carbon tetrachloride induced liver cirrhosis exhibited reduced reactivity and reduced aortic K^+ ion content. Increased reactivity therefore appeared to be

associated with an increased K^+ ion content of the vasculature. The pathogenic implications of changes in vascular K^+ content were unclear, since changes in reactivity did not always parallel changes in the blood pressure.

Lundgren, Hallbäck, Weiss and Folkow (1974) studied the changes in the noradrenaline dose-response curve of the perfused hind-quarters preparations, during the development of renal hypertension in the rat. Hypertension was induced by clipping one renal artery with the contralateral kidney left untouched. The blood pressure of the rats stabilized at hypertensive levels two weeks after the application of the "Goldblatt" clip. Changes in the dose-response curve, i.e. increased slope, maximum response and the basal resistance to flow stabilized after 2-3 weeks. The first signs of increased reactivity, as evidenced by changes in the noradrenaline dose-response curve, occurred 7 days after the operation, but the blood pressure was elevated after 3 days. These results indicated that apparent hyperreactivity in renal hypertension was a consequence and not a primary cause of the high blood pressure.

Removal of the constricting clip from the renal artery reduced the blood pressure of the rats to normal levels in one day (Lundgren, 1974 a). Reversion of the noradrenaline dose response curve and basal resistance to flow took 3 weeks. This result would suggest that medial hypertrophy in the vasculature has no significant role in the maintenance of hypertension.

Lundgren (1974 a) argued that the removal of the renal artery clip would cause a reduction in cardiac output, which would lower the blood pressure (Ledingham, 1971). This explanation is erroneous since Ledingham, (1971) was discussing the one kidney R.H.R. while Lundgren

(1974 a) used the two kidney model.

Lundgren (1974 b) investigated the effects of propranolol on the development of renal hypertension and changes in vascular design. Neither pretreatment nor treatment in established hypertension with propranolol had any effect on blood pressure or changes in reactivity. If a rise in cardiac output was responsible for the structural adaptation of the blood vessels (Folkow, 1971), then this could not have been mediated by β adrenoceptors.

Haeusler and Finch (1972 a) and Armstrong (1972) have demonstrated that reactivity to 5-hydroxytryptamine is increased in the perfused hind-quarter preparation from the R.H.R. Dose-response curves to 5-hydroxytryptamine in "hypertensive" preparations, were steeper and had a greater maximum response than normal. Reactivity to noradrenaline was also increased, but to a lesser degree. Hyperreactivity could not be solely due to a structural change in the vasculature, even though there was an increase in basal resistance to flow (Haeusler and Finch, 1972 b).

An equal degree of hyperreactivity to all agonists would have resulted if a structural vascular adaptation was the only cause of the increased reactivity. However, the differences in reactivity of the hind-quarters vascular bed may have been because noradrenaline and 5-hydroxytryptamine constrict the precapillary vessels while 5-hydroxytryptamine also dilates the post-capillary vessels (Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, 1973).

b) <u>The perfused mesenteric vasculature preparation</u>

McGregor and Smirk (1968, 1970), Armstrong (1972) and Haeusler and Finch (1972 a) demonstrated hyperreactivity to noradrenaline and 5-hydroxytryptamine but not Ca^{++} using the perfused mesenteric

vasculature preparation from the R.H.R. The increase in response amplitude to 5-hydroxytryptamine was much greater than the increase in noradrenaline reactivity.

The dose-response curves of the "hypertensive" tissues were steeper and achieved greater maximum responses than the control curves (Armstrong, 1972., Haeusler and Finch, 1972 a). The threshold vasoconstrictor dose of 5-hydroxytryptamine was lower in "hypertensive" tissues, which would suggest that some change in vascular sensitivity to this agonist had occurred. Since the mesenteric vasculature preparation consists of precapillary vessels only, the difference in hyperreactivity between noradrenaline and 5-hydroxytryptamine could not be explained by the post-capillary vasodilator action of the latter.

Despite the greatly increased reactivity to 5-hydroxytryptamine, studies using 5-hydroxytryptamine antagonists have shown no causal relationship between this agonist and the high blood pressure (Armstrong, 1972., Haeusler and Finch, 1972 a).

x) Vascular reactivity in intact perfused blood vessels from D.O.C. hypertensive rats

a) Perfused hind-quarters or hind-limb preparations

Demura, Fukuchi, Takahashi and Goto (1965) and Baum and Shropshire (1967b) have demonstrated increased reactivity to noradrenaline and angiotensin in perfused hind-limb and hind-quarters preparations from D.O.C. hypertensive rats. In contrast, Oono (1966) could find no change in reactivity to these agonists.

Haeusler and Finch (1972 b) and Armstrong (1972) also demonstrated increased reactivity to noradrenaline and to 5-hydroxytryptamine in perfused hind-quarters preparations from D.O.C.

hypertensive rats. Response curves to both agonists were steeper than normal with a greater maximum response. The basal resistance to flow was increased in "hypertensive" preparations (Baum and Shropshire, 1967 b), Haeusler and Finch, (1972 b) which could have been due to a medial hypertrophy. Haeusler and Finch (1972 b), but not Armstrong (1972), reported a lowered threshold to noradrenaline and 5-hydroxytryptamine in "hypertensive" preparations, which could indicate supersensitivity of the blood vessels.

b) The perfused rat tail preparation

Beilin and Wade (1970) studied the perfused rat tail preparation from D.O.C. hypertensive rats and found increased reactivity to noradrenaline but not to angiotensin. The basal resistance to flow of the "hypertensive" preparations was less than that of the control preparation. Perfused tails from rats with hypertension that persisted after withdrawal of D.O.C. behaved somewhat differently (Beilin and Ziakus, 1971). Basal resistance to flow was increased and response curves to both noradrenaline and to 5-hydroxytryptamine were steeper than normal with a greater maximum response.

Beilin and Wade (1970) and Beilin and Ziakus (1971) suggested that in D.O.C. hypertension, the D.O.C. had some specific effect on the α-adrenoceptor or on noradrenaline metabolism, while in the post-D.O.C. hypertensive phase, a structural change had occurred in the blood vessels.

c) The perfused rat caudal artery preparation

Hinke (1965) made a rigorous study of the changes in the structure and reactivity of the isolated, perfused caudal artery preparation from the D.O.C. hypertensive rat.

Histological examination of the arteries revealed fragmentation

of the internal elastic lamina, degeneration of the inner half of the tunica media and an increase in artery wall mucopolysaccharides. Wall hypertrophy was demonstrated, particularly in arteries from rats in the early stages of hypertension. "Hypertensive" arteries also exhibited an increased resistance to flow and a reduced internal diameter, indicating that the wall hypertrophy had reduced lumen diameter.

The work performed by the arteries was assessed by the variation of wall tension with radius, at a constant pressure. Arteries from hypertensive rats, constricted with noradrenaline and vasopressin, performed more work than normal. This increase in the amount of work performed could not have been due to a simple increase in vascular wall/lumen ratio. Muscle fibre hypertrophy could have increased the work output, but the histological evidence indicated an expansion of the extracellular space as the mechanism of wall hypertrophy. Hinke (1965) suggested that a change in cell membrane permeability could have caused the increase in sensitivity of the "hypertensive" vessel.

Hinke (1966) further investigated the caudal artery by abolishing and re-establishing contractions to noradrenaline or potassium by manipulation of the $[Ca^{++}]$ in the perfusion fluid. Responses in "hypertensive" arteries were more difficult to abolish in zero $[Ca^{++}]$ perfusion fluid than in normal vessels. Less Ca^{++}, than normal, was required to re-establish contractions in "hypertensive" arteries. The caudal arteries from hypertensive rats contained more Na^{+}, K^{+}, Mg^{++} and Ca^{++} than normal. These results indicated that the "hypertensive" artery was more sensitive to noradrenaline than normal because of an increase in the efficiency of Ca^{++} utilization.

d) The perfused mesenteric vasculature preparation

Dose-response curves to 5-hydroxytryptamine and to noradrenaline are steeper than normal, with a greater maximum response, in perfused mesenteric vasculature preparations from D.O.C. hypertensive rats (Armstrong, 1972., Haeusler and Finch, 1972 b). These results suggest an increase in vascular wall/lumen ratio. However, the inequality of the increases in reactivity to the two agonists and the normal resistance to flow at maximum vasodilation did not support this mechanism (Haeusler and Haefely, 1970., Haeusler and Finch, 1972 b).

The normal reactivity of depolarized "hypertensive" preparation to Ca^{++} provided further evidence against a structural vascular change as the sole cause of hyperreactivity. In addition, vasoconstrictor threshold doses to 5-hydroxytryptamine (Armstrong, 1972) or to both 5-hydroxytryptamine and noradrenaline (Haeusler and Finch, 1972 b), were lowered in the "hypertensive" preparation, indicating that some degree of supersensitivity was also present.

Finch (1974) has shown that reduction of the blood pressure of D.O.C. salt hypertensive rats, by 4 weeks of antihypertensive therapy, does not alter the increased reactivity of the mesenteric vasculature preparation to noradrenaline and 5-hydroxytryptamine. Finch (1974) suggested that these results seriously questioned the role of increased vascular reactivity in the maintenance of hypertension. However, the vasodilator hydrallazine was included in the anti-hypertensive treatment. This drug would counteract the haemodynamic effects of increased reactivity *in vivo* but this effect would not be apparent in the isolated mesenteric vasculature preparation.

e) The perfused renal artery preparation

Perfused renal arteries from D.O.C. hypertensive rats

responded normally to noradrenaline or 5-hydroxytryptamine (Haeusler and Finch, (1972 b). The basal resistance to flow was less than normal, probably because of compensatory dilation in response to the unilateral nephrectomy procedure used by Haeusler and Finch (1972 b).

xi) Vascular reactivity in intact perfused blood vessels from spontaneously hypertensive rats

a) Perfused hind-quarters or hind-limb preparations

Folkow's group have reported that vascular hyperreactivity occurs in the perfused hind-quarters preparation as well as in the systemic vascular bed of the Japanese S.H.R. (Folkow, Hallbäck, Lundgren and Weiss, 1970 a, b, c). The increased reactivity was characterized by an increase in the slope and maximum response of the noradrenaline dose-response curve, with an increase in basal resistance to flow, but, with no change in vasoconstrictor threshold dose (Folkow, Hallbäck, Lundgren and Weiss, 1970 b,c).

Folkow, Hallbäck, Lundgren and Weiss (1970 c) have suggested that these changes were caused by medial hypertrophy in the blood vessels leading to an increase in the wall/lumen ratio. The major site of this change in wall/lumen ratio was the precapillary resistance section of the hind-quarters vascular bed (Folkow, Hallbäck, Lundgren, Weiss, Albrecht and Julius, 1974). Further evidence supporting an increase in vascular wall thickness was demonstrated by the resistance vessels of the S.H.R. being less distensible than normal (Hallbäck, Lundgren and Weiss, 1974).

Hallbäck and Folkow (1974) have suggested that the S.H.R. responds to environmental stimuli with exaggerated pressor responses. Elimination of the pressure load prevents the development of

vascular hyperreactivity in the S.H.R. (Folkow, Gurèvich, Hallbäck, Lundgren and Weiss, 1971). It was therefore suggested that the exaggerated pressor responses trigger the gradual structural adaptation of the resistance vessels (Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, 1973). If such a mechanism is of primary importance in the pathogenesis of high blood pressure in the S.H.R., then the time course of the structural vascular adaptation should be rapid. Regional hypotension, caused by aortic ligation in the young S.H.R. stimulated regression of the structural vascular adaptation within 3-7 days and was complete in 3 weeks (Folkow, Hallbäck, Lundgren and Weiss, 1973). In the old S.H.R., regression of vascular changes had a similar time course, but was less complete, probably because of increased collagen and elastin in the vessels (Weiss and Hallbäck, 1974). Vascular hypertrophic changes in the S.H.R. appear to regress rapidly; but there is no evidence to date on the speed of development of these changes.

Evidence suggests that the S.H.R. may have a genetic disposition towards hypertrophic changes in response to load, since immunosympathectomy or anti-hypertensive drug treatment reduced the blood pressure to normal without complete regression of the vascular changes (Folkow, Hallback, Lundgren and Weiss, 1971., 1972., Weiss, 1974). The resistance vessels of the S.H.R. hypertrophy to a greater extent than those from normal rats, in response to the same pressure load (Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, 1973).

Laverty (1961) has demonstrated that increased reactivity to noradrenaline also occurs in the perfused hind-limb preparation from the New Zealand strain of S.H.R. Increased peripheral resistance in the S.H.R. hind-limb was initially suggested to be wholly of neurogenic origin (Laverty and Smirk, 1961). However, later

studies (Laverty, McGregor and McQueen, 1968) indicated that a structural change in the vascular wall was also involved.

Haeusler and Finch (1972 b) have shown that the increased resistance to flow, in the systemic or hind-quarters circulation of Japanese S.H.R., develops with age. Dose-response curves of the perfused hind-quarters to noradrenaline were steeper than normal, with an elevated maximum response, but, with no change in vaso-constrictor threshold dose, Armstrong (1972) reported similar results for the New Zealand strain of S.H.R.

Haeusler and Finch (1972 b) found that there was no change in the 5-hydroxytryptamine dose-response curve in the perfused hind-quarter preparation from the Japanese S.H.R. Armstrong (1972), in contrast, reported an increase in reactivity to this agonist which exceeded that for noradrenaline responses, using the New Zealand S.H.R.

b) <u>The perfused mesenteric vasculature preparation</u>

Reactivity to noradrenaline, angiotensin, 5-hydroxytrypt-amine, KCl but not to Ca^{++} is increased in the perfused mesenteric vasculature preparation from the S.H.R. (McGregor and Smirk, 1968, 1970; Haeusler and Haefely, 1970; Armstrong, 1972; Haeusler and Finch, 1972 a,b). Resistance to flow was increased in preparations from the New Zealand strain (McGregor and Smirk, 1968) but not from the Japanese strain of S.H.R. (Haeusler and Finch, 1972 b). The dose-response curves to noradrenaline and 5-hydroxytryptamine exhibited the characteristics of an increased vascular wall/lumen ratio (Haeusler and Finch, 1972 b, Armstrong, 1972).

Some aspects of the hyperreactivity did not fit the hypothesis of an increased wall/lumen ratio. The vasoconstrictor threshold to 5-hydroxytryptamine was lower in preparations from the S.H.R. (Armstrong,

1972; Haeusler and Finch, 1972 a) and the increase in reactivity to this agonist greatly exceeded that to noradrenaline (McGregor and Smirk, 1970., Haeusler and Haefely, 1972 a., Armstrong, 1972).

Finch (1974) has shown that 4 weeks of anti-hypertensive treatment lowers the blood pressure of the S.H.R. but does not affect reactivity to noradrenaline. Similarly, Wood and Clark (1974) have shown that sympathectomy by 6-OH-dopamine lowers the blood pressure of S.H.R. but does not abolish the increased mesenteric vasculature reactivity to noradrenaline and 5-hydroxytryptamine. These results contradict a similar study (Weiss, 1974) in which some regression of the vascular changes in the perfused hind-quarters preparation were found.

c) <u>The perfused renal artery and renal vascular bed</u>

In the perfused renal vascular bed of the S.H.R. resistance to flow was found to be lower than normal (Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, 1973) while it was normal in the perfused renal artery (Haeusler and Finch, 1972 a). Folkow, Hallbäck, Lundgren, Sivertsson and Weiss, (1973) have suggested that the renal vascular bed of the S.H.R. resembles the hind-quarters preparation in its response to agonists, while Haeusler and Finch (1972 a,b) have reported normal or below normal reactivity to noradrenaline and 5-hydroxytrypt-amine in the perfused renal artery.

A SUMMARY OF THE EVIDENCE FOR INCREASED VASCULAR REACTIVITY IN HYPERTENSION

Studies on human hypertensive patients have shown that increased reactivity to noradrenaline is a more common observation, especially in essential hypertension, than are changes in the humoral and neurogenic influences on the peripheral resistance.

Evidence for increased reactivity in experimental hypertension is

dependant on the type of preparation employed.

Investigations using large artery strips have shown that a true non-specific supersensitivity may occur in acute renal and D.O.C. hypertension. As these hypertensive states progress, the vascular changes converge with those in the S.H.R., as impaired arterial contractility becomes the dominant factor.

In the perfused preparation, involving true resistance vessels, the evidence is different. Increased reactivity is a common observation and the controversy is whether it is caused by changes in the vessel structure or sensitivity. The degree of structural change may depend on the segment of the vascular tree which is studied. In complete vascular beds, such as the hind-quarters, some narrowing of the vessel lumen seems to occur. In the arterial-arteriolar bed, such as the mesenteric vasculature preparation, luminal narrowing is apparently absent. Contractility of the resistance vessels is increased, in direct contrast to the reduction in contractile strength observed in the larger arteries.

The opposite directions of the contractility changes in the aorta and in smaller arteries and arterioles, during hypertension, can be explained from the results of studies with tritiated thymidine. True hypertrophy of smooth muscle cells, as evidenced by the increased incorporation of tritiated thymidine has been demonstrated in arterial and arteriolar smooth muscle, but not in the aorta (Crane and Dutta, 1963). Increases in vascular smooth muscle mass have been detected in the mesenteric and coronary arteries, but not in the aorta of experimental hypertensive animals (Fischer and Llaurado, 1967). It is likely, therefore, that medial hypertrophy and consequently increased contractility do not occur in the aorta of hypertensive animals. Degenerative changes

in the smooth muscle cells (Gardner and Matthews, 1969) with reduced contractility are therefore more prominent in the aorta than in smaller arteries.

CHAPTER 2

GENERAL METHODS

In this study, blood pressure was recorded from conscious normotensive and renal hypertensive (Grollman, 1944) rats and reactivity to agonists was examined in the perfused mesenteric vasculature preparation. This chapter describes these methods.

1. THE RAT PERFUSED ISOLATED MESENTERIC VASCULATURE PREPARATION

a) INTRODUCTION

The perfused preparations commonly used for the evaluation of vascular reactivity are the hind-quarters (McQueen, 1956., Folkow, Hallbäck, Lundgren and Weiss, 1970 a, b, c), the hind-limb (Laverty, 1961), the tail (Beilin and Wade, 1970) and the mesenteric vasculature of the rat (McGregor and Smirk, 1968, 1970). The mesenteric vasculature was chosen for the present study since it contains true resistance vessels without the complications of a capillary and post-capillary vascular section.

b) METHODS

The technique for the perfusion of the mesenteric vasculature is a modification of that described by McGregor (1965).

Female Charles River rats (150-300 g) were anaesthetized by intraperitoneal injection of pentobarbitone sodium (60 mg/kg). The abdomen was opened by midline incision and the ileum and colon were exposed. The superior mesenteric artery, which accompanies the vein in adipose tissue, was identified and cannulated. The perfusion pump was in operation during the cannulation process. The perfused area was identified by blanching, and included an area of ileum and caecum. Since the ileal mesentery was the only area to be used, the other branches of the artery (caecal, ileo-colic, colic and pancreatico-duodenal) were tied off. The perfused mesentery with the ileum still

attached was removed from the rat. The mesentery was severed close to the ileum so that only arteries and arterioles were perfused, obviating possible interference from the activity of the intestinal smooth muscle.

The mesenteric vessels were placed in a water-jacketed glass cup, and perfused with Kreb's solution for 1 h to equilibrate.

Kreb's perfusion solution of the following composition (mM): Na Cl, 118.4; KCl, 4.7; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Ca Cl_2, 2.5; Mg SO_4, 1.2; Glucose, 11.0; bubbled with 5% CO_2 in O_2, was normally used. Mesenteric vasculature preparations were perfused at a rate of 2 ml/min except when otherwise stated.

The perfusion apparatus consisted of a heating coil maintained at 37°C, a Watson-Marlow H.R. flow inducer pump and 'Y' junction polyethylene tube, one arm of which led to a Bell and Howell 4-327-L221 pressure transducer and the other via a short length of rubber tubing (for injection of drugs) to a portex cannula O.D. 0.75 mm (Fig. 2:1).

Under conditions of constant flow, constrictor responses caused increases in perfusion pressure which were recorded on a Devices M.2. recorder (Fig. 2:2). Constrictor responses were measured from the perfusion pressure baseline preceding each response.

Agonist drugs were injected at 2 min intervals for noradrenaline or 4 min intervals for 5-hydroxytryptamine and KCl. Preparations were dosed with one of these agonists until there was no further increase in responses to a standard dose. Dose-response data was then obtained by increasing doses of the agonist from the threshold to near the maximum dose and then reducing the doses down to the threshold. The two responses obtained for each dose level were averaged, minimizing the influence of the previous agonist dose.

Fig. 2:1

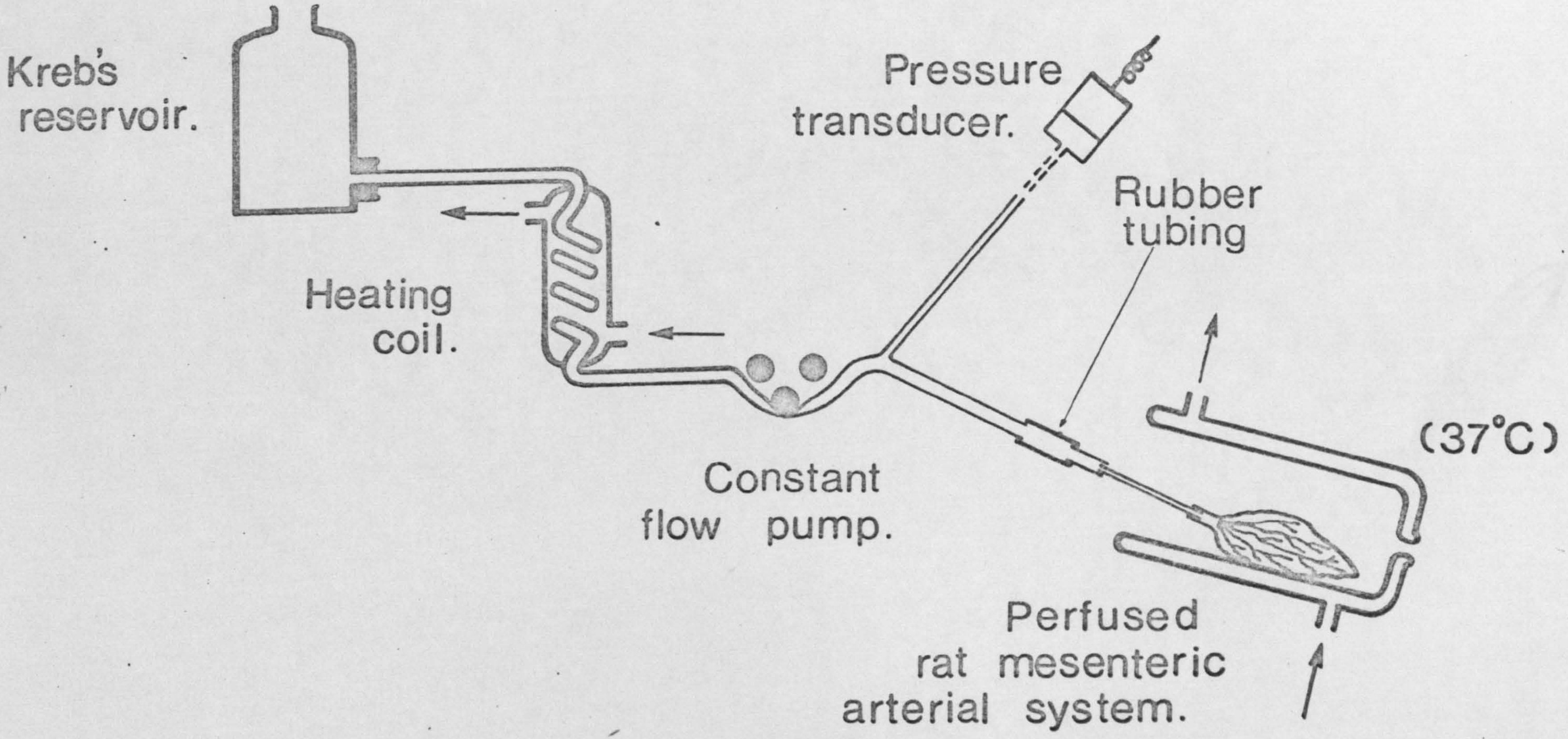

Kreb's reservoir.
Heating coil.
Pressure transducer.
Rubber tubing
(37°C)
Constant flow pump.
Perfused rat mesenteric arterial system.

Fig. 2:1

Fig. 2:1

A diagram of the apparatus for the rat perfused mesenteric vasculature preparation.

Thick arrows indicate the flow of water at 37°C. The Krebs solution is pumped from the reservoir via a heating coil to a Y-junction. One arm, including an injection port of rubber tubing, supplies the perfused tissue, the other connects to a pressure transducer. Increased resistance to flow in the mesentery causes an increased back-pressure which is recorded by the transducer.

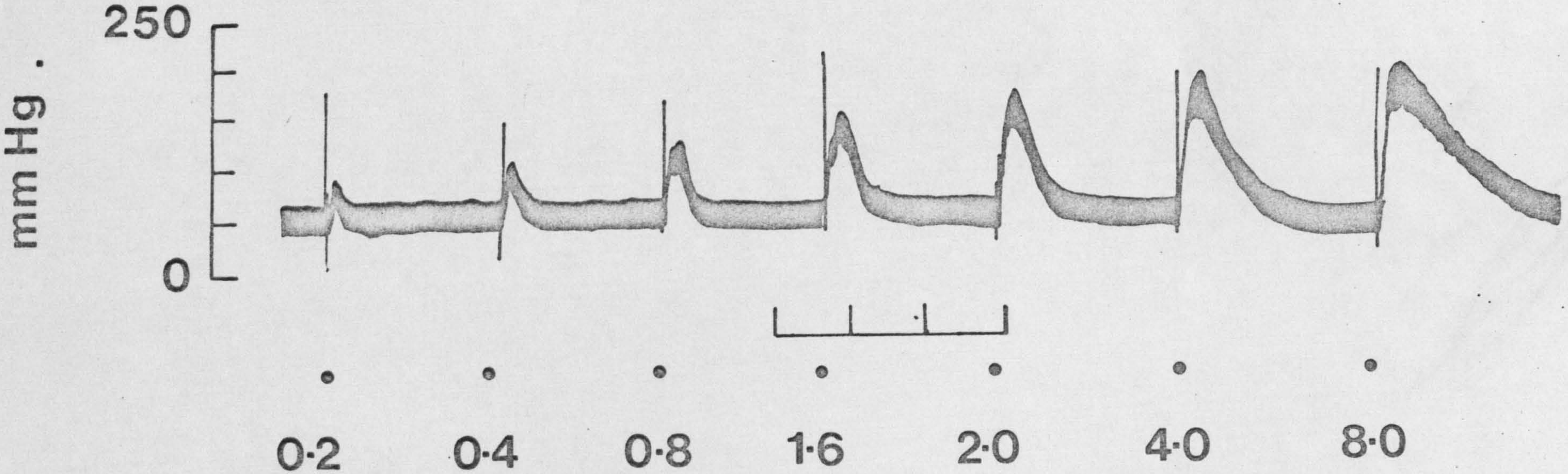


Fig. 2:2

Figure 2:2

Noradrenaline induced constrictor responses (mm Hg) of the rat isolated perfused mesenteric vasculature. The horizontal axis represents minute intervals. Note the initial injection artifact followed by a rapid constrictor response which returns to a stable baseline.

Bipolar platinum electrodes, placed around the superior mesenteric artery were occasionally used to stimulate peri-arterial sympathetic nerves. Square wave stimuli of 40 V, 0.5 ms width at a frequency of 40 Hz were applied for periods of 10 s.

Antagonist drugs and angiotensin II amide were added to the Kreb's solution and perfused for periods of 15 min to 1.5 h.

i) Histology

Sections of terminal vessels of the mesenteric vasculature preparation, i.e. at the point of entry into the ileum, were examined histologically to identify the nature of the vessels involved. The sections were stained with haematoxylin and eosin or with Gomori aldehyde fuchsin.

ii) Drugs used

The drugs used throughout this study were:- angiotensin II amide (Hypertensin, Ciba), Sar^1 Ile^8 angiotensin II (Beckman), desipramine hydrochloride (Geigy), disodium ethylenediaminetetra-acetic acid (Na_2E.D.T.A., B.D.H.), ethylene bis-(aminoethyl)-tetraacetic acid (E.G.T.A., Koch-Light), guanethidine sulphate (Ciba), indoramin hydrochloride (Wyeth), (±) isoprenaline sulphate (Burroughs Wellcome), (-) noradrenaline bitartrate (Koch-Light), papaverine sulphate (B.D.H.), phentolamine mesylate (Ciba), potassium chloride (B.D.H.), 5-hydroxytryptamine (serotonin creatinine sulphate, Koch-Light). Doses refer to base.

iii) Statistical Analysis

Significant differences were assessed using the Student's t test. Significant differences are indicated on figures, throughout this study, by stars:-

$p < 0.05$ is indicated by ✪ $p < 0.02$ is indicated by ✪✪

p <0.01 is indicated by ★ p <0.001 is indicated by ★★

Unless stated otherwise, mean values ± S.E.M. are given throughout this study.

c) RESULTS

i) The baseline perfusion pressure

The mean minimum perfusion pressures recorded before and after cannulation of the tissue were 21.2 ± 0.5 (due to the cannula, n = 6) 38.8 ± 1.1 (cannula and tissue, n = 6) respectively.

Isoprenaline (0.5-1.0 µg) and papaverine (1 µg) reduced the baseline perfusion pressure by 2-4 mm Hg, indicating that the tissue was virtually atonic. The resistance to flow was due to the calibre and elasticity of the mesenteric arterioles.

ii) The stability of the preparation

The baseline perfusion pressure remained constant for at least six h. The tissue showed full sensitivity to noradrenaline after about 1 h of dosing with this agonist and remained stable for 4-5 h.

iii) The sensitivity of the preparation

Tissues usually gave recordable constrictor responses at 0.02 µg of noradrenaline (n = 15), 0.1 µg of 5-hydroxytryptamine (n = 6) and 200 µg of KCl (n = 10 Figs. 2:3, 4, 5).

Maximum responses were evoked by 8.0 - 16.0 µg of noradrenaline, 3.0 - 12.0 µg of 5-hydroxytryptamine and 2.0 - 8.0 mg. of KCl (Figs. 2:3, 4, 5).

Typical constrictor responses to noradrenaline are shown in Fig. 2:2.

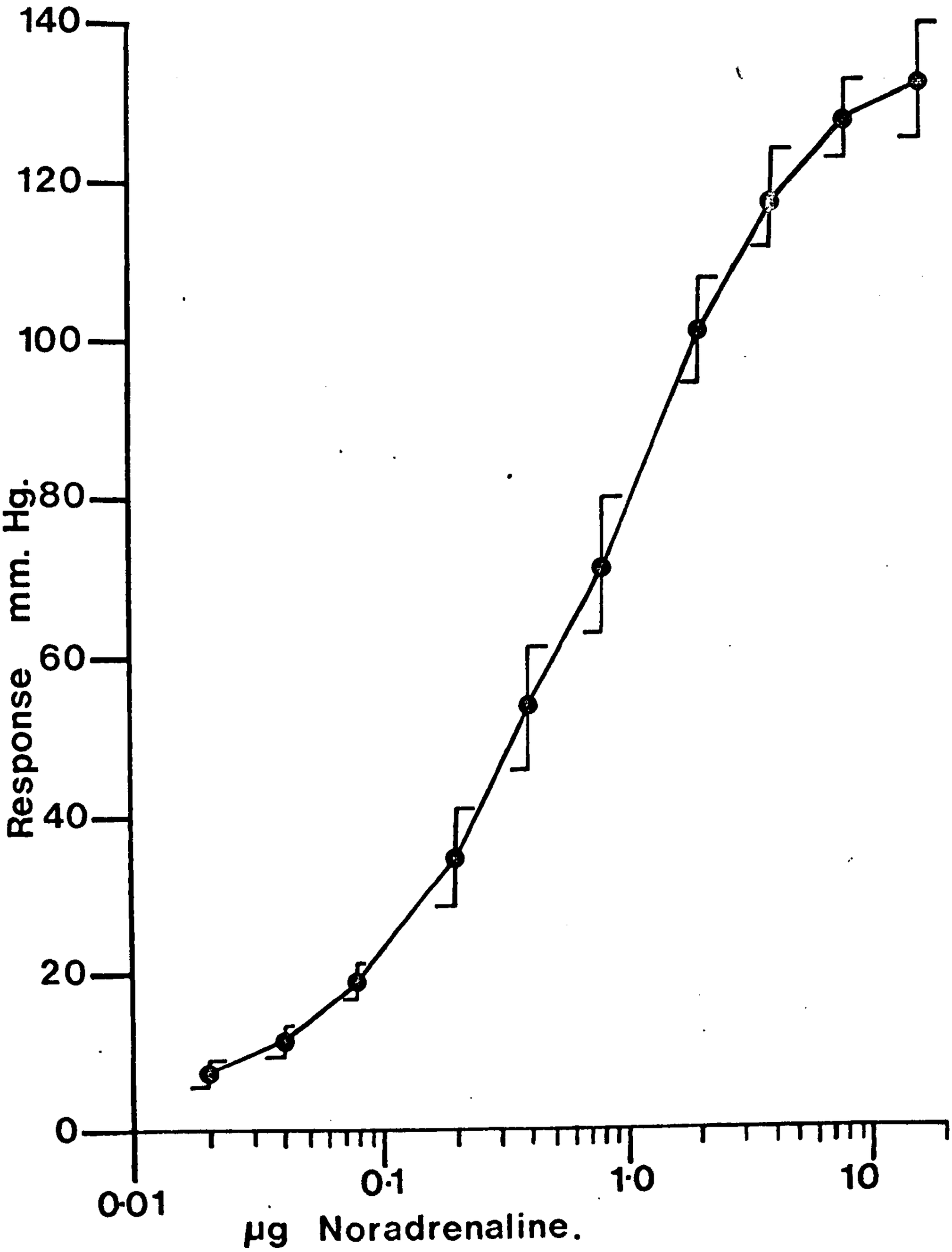


Fig. 2:3

The mean noradrenaline dose-response curve for mesenteric vasculature preparations (n = 9-19). The threshold for stimulation was 0.02 µg and the maximum response was evoked by 8 - 16 µg of

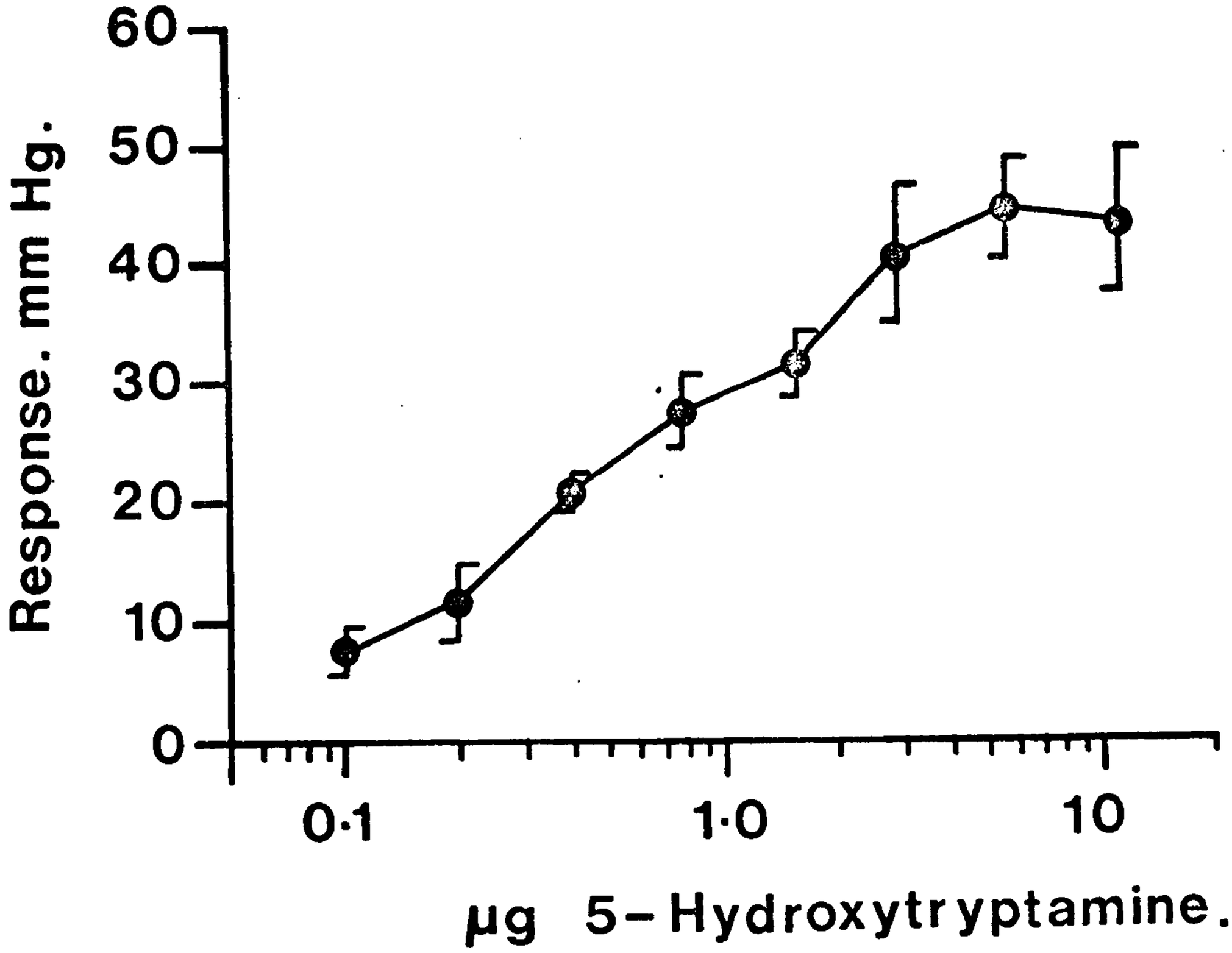


Fig. 2:4

The mean 5-hydroxytryptamine dose-response curve of 6 mesenteric vasculature preparations. The threshold for stimulation was 0.1 µg and the maximum response occurred at 3 - 12.0 µg of 5-hydroxytryptamine. Note the shallow gradient of the dose-response line.

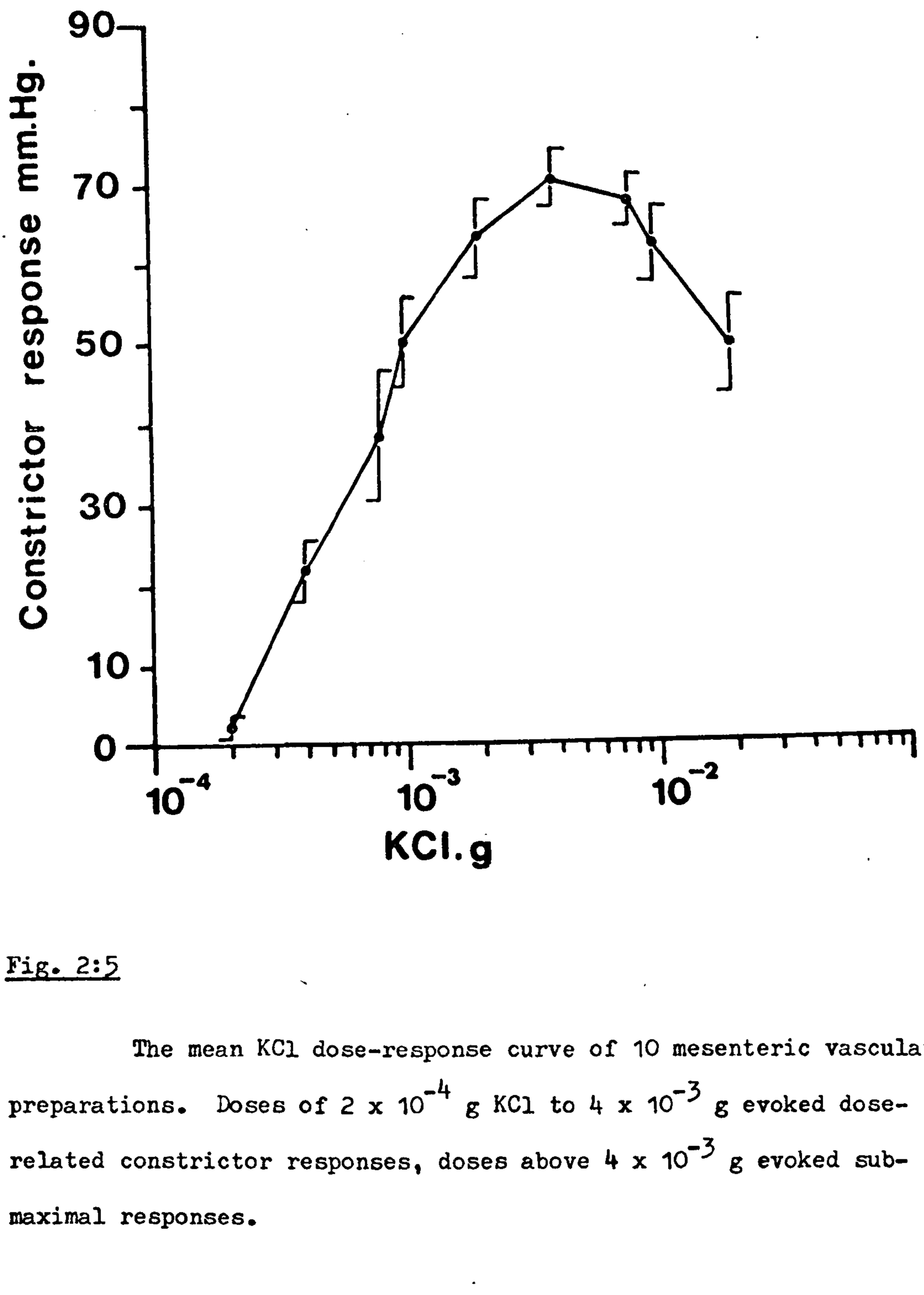


Fig. 2:5

The mean KCl dose-response curve of 10 mesenteric vasculature preparations. Doses of 2×10^{-4} g KCl to 4×10^{-3} g evoked dose-related constrictor responses, doses above 4×10^{-3} g evoked sub-maximal responses.

iv) The specificity of the preparation

Tachyphylaxis to 5-hydroxytryptamine (continuous infusion of 0.57×10^{-5}M) had no effect on responses to noradrenaline.

v) Adrenergic neurone stimulation by potassium chloride

Potassium chloride is a depolarizing agent which will stimulate adrenergic nerves. The α-adrenoceptor blocking agent indoramin (10^{-6}M) had no effect on KCl induced responses (n = 2), indicating that under the conditions of these experiments, there was no indirect adrenergic component of the mesenteric vasculature response.

vi) The dimensions and structure of the terminal mesenteric vessels

Arterial/arteriolar vessels were identified in sections of the mesenteric vasculature preparation (Figs. 2:6, 7). The external diameter of these vessels ranged from 20 - 300 μm. The smallest vessels had 2 - 3 layers of medial smooth muscle cells. All the vessels had internal elastic laminae, indicating that they were not precapillary metarterioles.

vii) The innervation of the preparation

Peri-arterial nerve stimulation evoked responses of 25 - 80 mm Hg (n = 4). Guanethidine (5×10^{-6}M) abolished these responses but had no effect on responses to exogenous noradrenaline (n = 2).

d) DISCUSSION

A perfused mesenteric vasculature preparation from the rat has been described. The preparation consists of arteries and arterioles which are innervated by sympathetic nerves and constrict in response to noradrenaline, 5-hydroxytryptamine and KCl.

Fig. 2:6

Section of a terminal arteriole from the rat mesenteric vasculature preparation, stained with haematoxylin and eosin (x 100). Note, there are two to three layers of medial muscle cells.

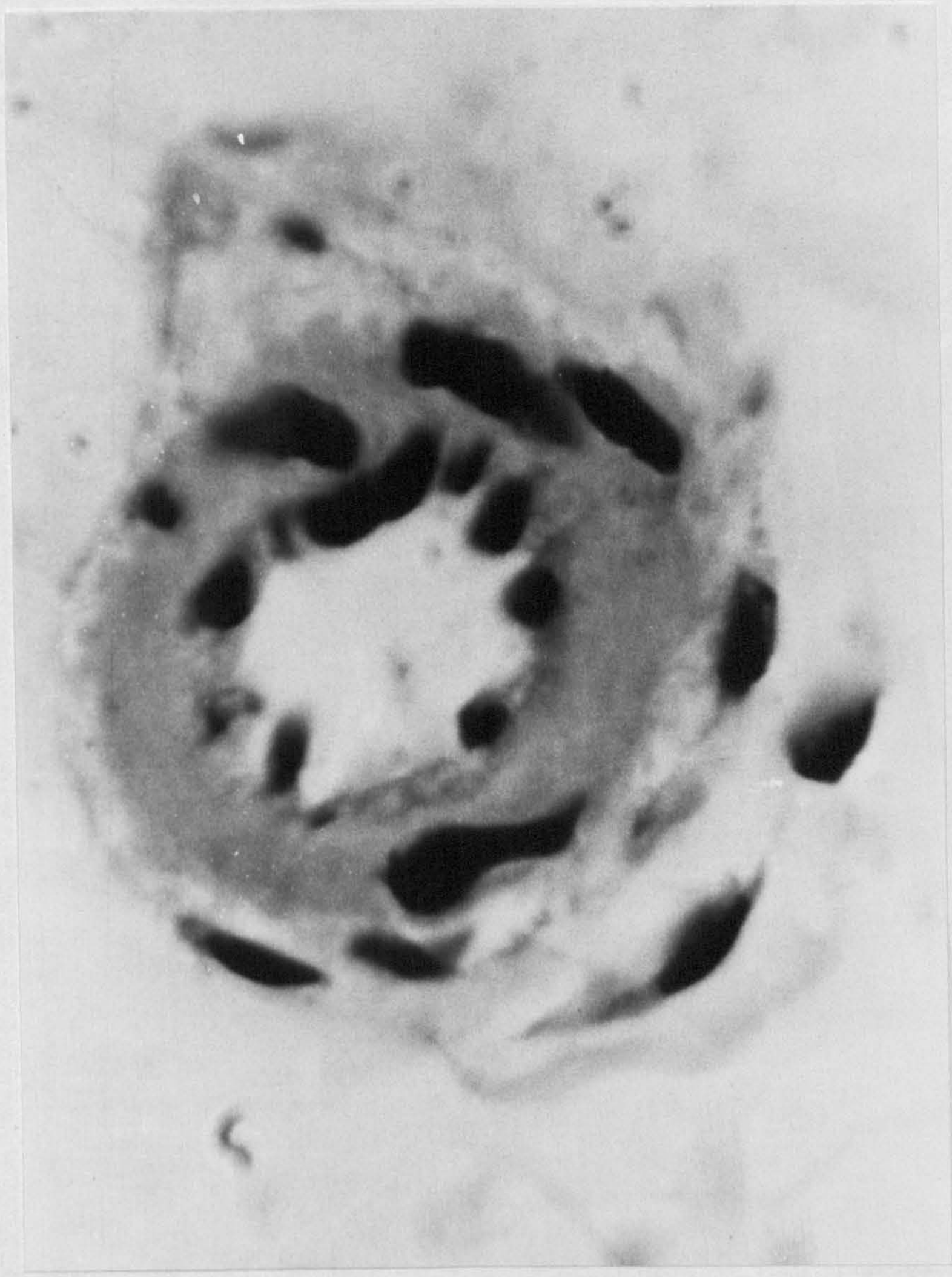
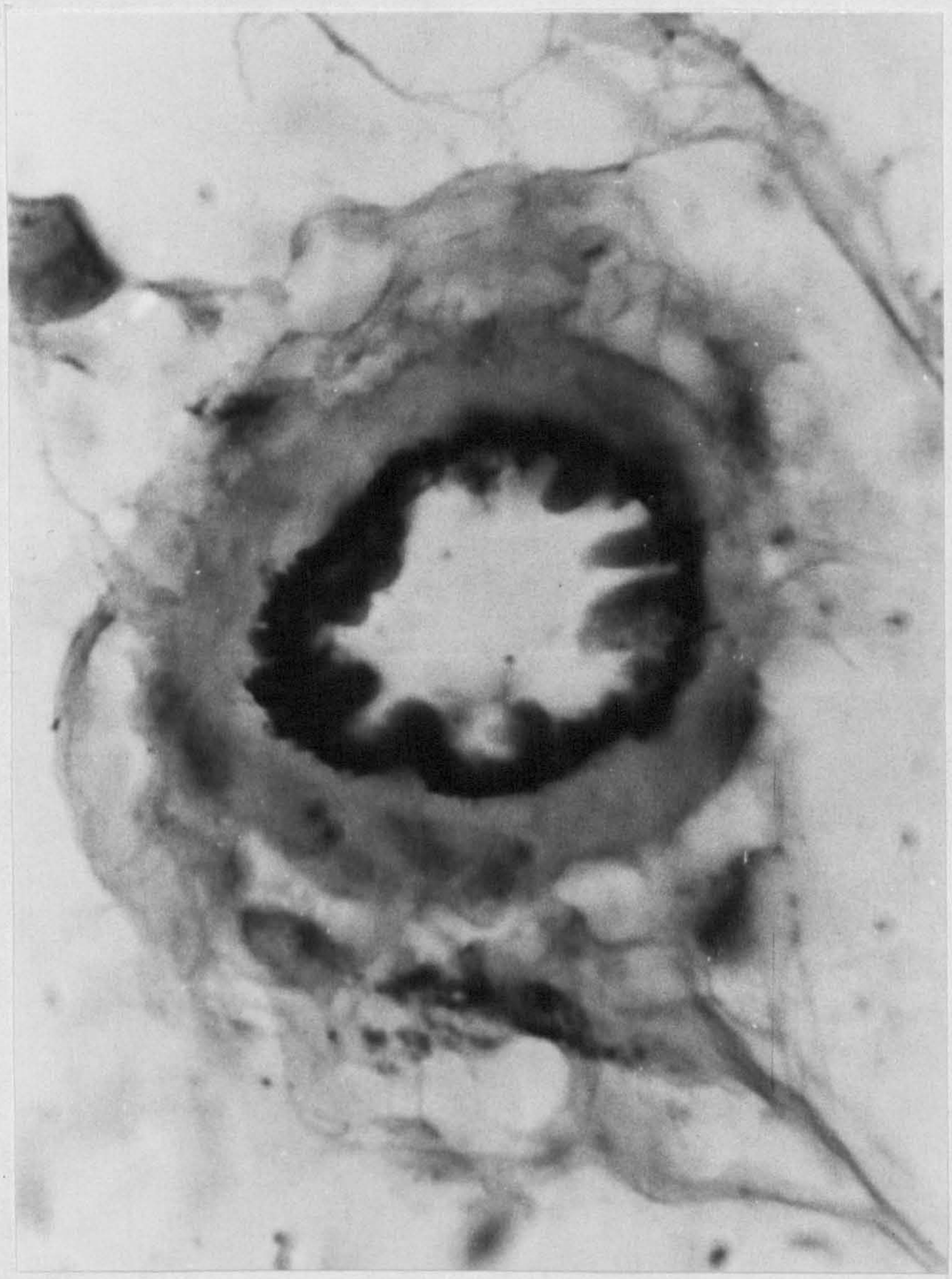


Fig. 2:7

Section of a terminal arteriole from the rat mesenteric vasculature preparation, stained with Gomori aldehyde fuchsin for elastic tissue (x 100). Note, the heavily stained internal elastic lamina.



Since the preparation was intended for use in studies on vascular reactivity, it was important to know which elements of the vascular tree were present. Severing of the mesentery from the ileum ensured that only the afferent vessels were perfused.

The distinction between a small artery and an arteriole is vague. Furness and Marshall (1974) sub-divided the afferent mesenteric vessels of the rat on the basis of their internal diameters. Principal arteries had internal diameters of 80 - 350μm, small arteries ranged from 30 - 40μm and terminal arterioles had internal diameters of 18 - 30μm. Maximov and Bloom (1957) classified arterioles as having a maximum diameter of 300μm, whereas Ham (1957) drew a dividing line between arteries and arterioles at 100μm diameter. A classification of arteries and arterioles based on the diameter of the fixed preparation is further complicated by the influence of the method of preservation (Cowdry, 1938). Benninghoff (1930) classified arterioles into three groups on a structural basis. The smallest, group 1 arterioles, had no elastic lamina and one layer of muscle cells. Group 2 arterioles had an elastic lamina, and group 3 arterioles had an elastic lamina and several layers of muscle cells.

The results of the present study indicate that the mesenteric vasculature preparation contains arterioles whether they are classified on size or structure. The mesenteric arterioles are probably of the group 3 type (Benninghoff, 1930). Histological evidence, and the lack of basal tone in the preparation indicate that the preparation does not contain precapillary metarterioles since these vessels exhibit spontaneous tone (Folkow and Neil, 1971), and have no internal elastic lamina (Ham, 1957).

The mesenteric vasculature preparation is innervated by

sympathetic adrenergic nerves, which are sensitive to blockade with guanethidine. The sympathetic innervation of this tissue has also been demonstrated pharmacologically by McGregor (1965) and Malik and Ling (1969) and histologically by Furness and Marshall (1974).

The dose-response curves of the mesenteric vasculature preparation to noradrenaline, 5-hydroxytryptamine and KCl were similar to those reported for this preparation by Haeusler and Haefely (1970) and Haeusler and Finch (1972a). Slight quantitative differences between the present results and those previously reported may be due to the higher perfusion rates used by Haeusler and Haefely (1970) and Haeusler and Finch (1972a).

Mesenteric vasculature responses to noradrenaline were rapid and stable over long periods. The absence of cross-tachyphylaxis between noradrenaline and 5-hydroxytryptamine responses demonstrated that noradrenaline was stimulating α-adrenoceptors and not a combination of α- and 5-hydroxytryptamine receptors (Innes, 1962).

Dose-response curves to KCl were steeper than those to noradrenaline. At high doses of KCl the responses were depressed, probably because of impaired repolarization of the smooth muscle cells.

The dose-response curves to 5-hydroxytryptamine had very shallow slopes. A rather flat dose-response curve for 5-hydroxytryptamine has also been demonstrated by Haeusler and Finch (1972a).

2. THE INDIRECT RECORDING OF SYSTOLIC BLOOD PRESSURE IN UNANAESTHETIZED RATS

a) INTRODUCTION

The recording of the blood pressure of conscious experimental animals is a pre-requisite of any study of hypertension. The implantation

of an indwelling arterial catheter to enable the direct measurement of systolic and diastolic blood pressure is ideal (Weeks and Jones, 1960., Popovic, Sybers and Popovic, 1968) but untenable when the blood pressures of a large number of animals is to be followed over long periods.

The tail cuff method of Gerold and Tschirky (1968) was used in the present study to measure the systolic blood pressure of the rats.

b) METHOD

Rats were pre-heated in perspex water jackets or restrained in perspex holders in a heating box (39°C) prior to blood pressure recording.

The pressure applied by an occluding cuff around the rats tail was measured using a Bell and Howell 4-327-L221 pressure transducer, and recorded using a Devices M2 recorder. Pulsations of the tail artery were recorded by a pneumatic pulse-transducer (E. and M. Instruments). The pressure applied by the cuff, which just occluded the tail artery, was taken as the systolic blood pressure. The sensitivity of the pulse transducer was constant throughout the study, so that the pulse would re-appear at the same tail artery blood flow in each animal.

c) RESULTS

A typical experimental record is shown in Fig. 2:8. The pressure in the tail-cuff is raised until pulsations of the tail artery cease. The occluding pressure is then slowly released through a valve. The cuff pressure at which the tail artery pulsations re-appear is taken as the systolic blood pressure. Three readings were taken for each animal and averaged. These readings were usually constant over the 2-3 min recording period indicating that reactive hyperaemia was not interfering with the measurements.

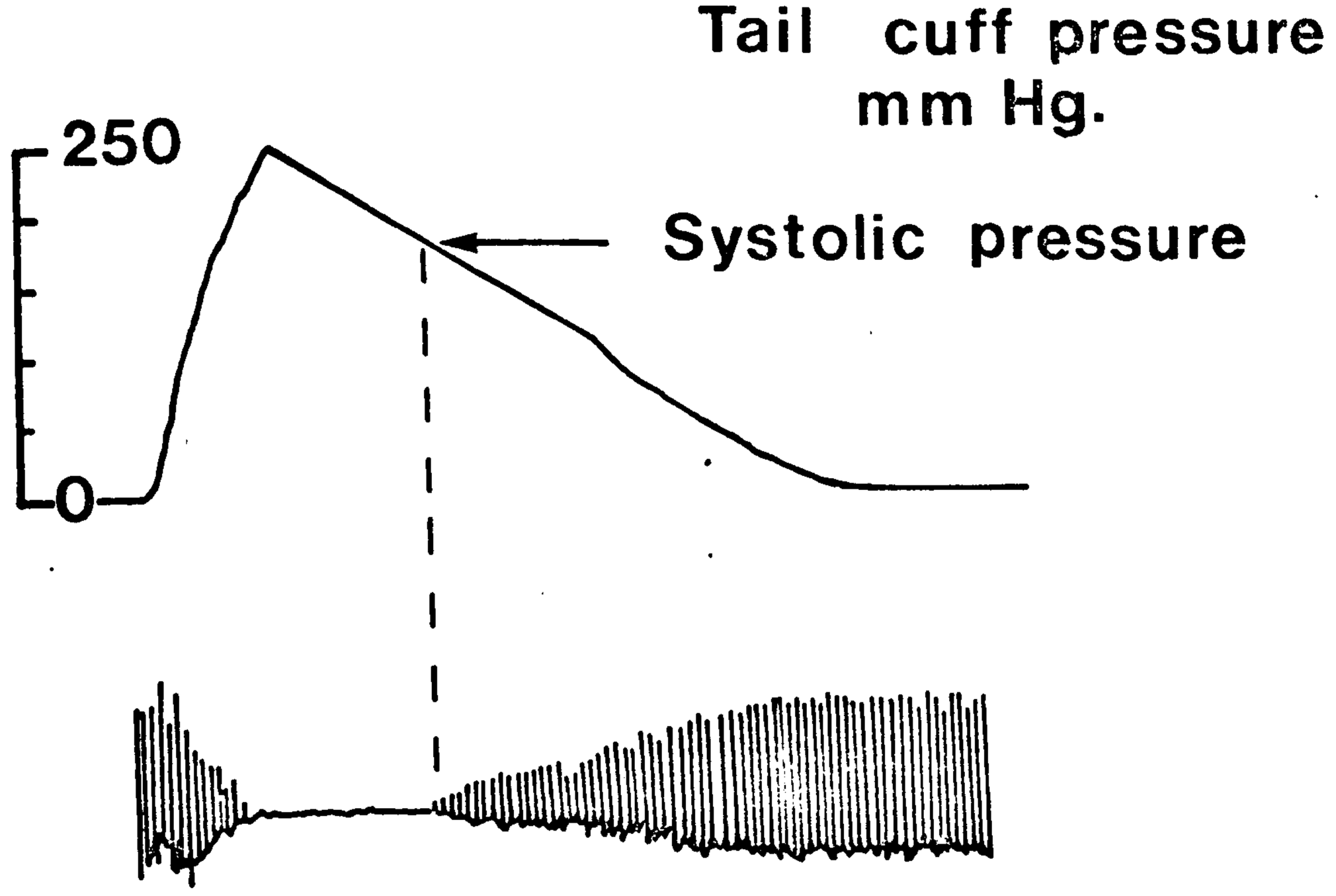


Fig. 2:8

A typical indirect blood pressure measurement record. The pulsations of the tail artery of the preheated rat are shown in the lower trace, and the occlusion cuff pressure in the upper trace. The cuff pressure is increased until the tail artery is occluded (pulsations cease) and then decreased via a slow release valve until pulsations re-commence. The occlusion cuff pressure (175 mmHg) at which pulsations re-commence is taken as the systolic blood pressure. Note, this rat had a systolic blood pressure of 175 mmHg i.e. was hypertensive.

In order to examine whether the procedure was stressful, and whether the stress affected the blood pressure, mean results from 7 rats obtained on their first and third exposure to the technique were compared. The average blood pressure at the first measurement was 116.0 ± 4.5. The third mean blood pressure reading (three weeks after the first) was 114.9 ± 4.3. There was no significant difference between these mean values.

d) DISCUSSION

The method described provides a rapid non-invasive technique for the measurement of systolic blood pressure in unanaesthetized rats.

A previous investigation of this method in which simultaneous recordings of blood pressure were made directly from an indwelling carotid cannula (Staniforth, 1970., personal communication) has shown that pre-heating of the rats causes a small (5 mm Hg) fall in the systolic blood pressure. This was unavoidable since pre-heating was necessary to obtain a measurable tail artery pulse.

The major problem involved in recording the blood pressure of conscious animals is that the stress of the procedure may cause temporary hypertension. The procedure used in thisstudy did not raise the blood pressure of the rats. Mean pressures were no higher when the rats were first introduced to the technique than when they had become accustomed to it.

Staniforth (1970, personal communication) found that a constant correction factor of 23 mmHg should be added to the indirect reading to achieve parity with the direct measurement. This correction factor was omitted from the present study, since changes in blood pressure rather than absolute values are important in hypertension (Pickering, 1968).

3. THE PRODUCTION OF RENAL HYPERTENSION BY THE METHOD OF GROLLMAN (1944)

a) INTRODUCTION

Renal hypertension in experimental animals can be produced by a variety of methods:-

1. Partial ablation of the kidney, (Chanutin and Ferris, 1932).
2. Constriction of the renal artery, (Goldblatt, Lynch, Hanzal and Summerville, 1934).
3. Encapsulation of the kidney in silk or cellophane causing an inflammatory invasion of the renal cortex, (Page, 1939).
4. Compression of the kidney by a figure-of-eight ligature, (Grollman, 1944).

All of these methods have advantages and disadvantages, the common difficulty being to impair renal function sufficiently to produce hypertension but not so much as to cause death by renal insufficiency. The "Goldblatt - clip" method is the most commonly used, but the Grollman method was judged to be technically simpler. The Grollman method is an established technique in this institute for the evaluation of novel antihypertensive agents. Further information on the characteristics of this type of renal hypertension would therefore be advantageous.

b) METHOD

Female Charles River rats, 130-180 g were anaesthetized with a fluothane-oxygen mixture. The right kidney was exposed retroperitoneally by lumbar incision, and the adrenal gland pushed away from its attachment to the kidney. A silk ligature was passed under both poles of the kidney in a figure-of-eight fashion to compress the parenchyma (Fig. 2:9).

ligature

A diagram of the figure-of-eight ligature around the kidney.

Note, the ligature compresses the kidney parenchyma.

Fig. 2:9

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One week after the application of the ligature, contralateral nephrectomy was performed. After the removal of the contralateral kidney, some of the rats were given 0.9% saline to drink ad libitum. These rats were termed "renal/salt hypertensive rats". Some animals were given tap-water to drink ad libitum and were termed "renal hypertensive rats".

i) Sham operations

These were identical to the operative procedure outlined above except that a) the constricting ligature was not applied to the kidney, b) the contralateral kidney was not removed.

c) RESULTS

The tightness of the figure-of-eight ligature was found to be the dominant factor in the "Grollman" renal hypertension. If the ligature was too loose, the rats did not become hypertensive. If the ligature was too tight the rats died of renal insufficiency 1 - 2 weeks after nephrectomy. The optimum tightness of the ligature was found, by trial and error, to be that which compressed the kidney without distorting the organ.

The mean systolic blood pressures of the successfully operated rats with or without salt-loading are shown in Fig. 2:10. The application of the "figure-of-eight" ligature caused a significant rise in systolic blood pressure which continued after removal of the contralateral kidney. Salt-loading with 0.9% saline had no effect on the rise in blood pressure during the first week after contralateral nephrectomy. From 2 - 4 weeks after nephrectomy the salt-loading exacerbated the hypertension. The blood pressure of the renal/salt hypertensive rats reached a plateau betw 170 and 180 mmHg 2 weeks after nephrectomy, but it took 3 - 4 weeks for the blood pressures of the renal hypertensive rats to stabilize.

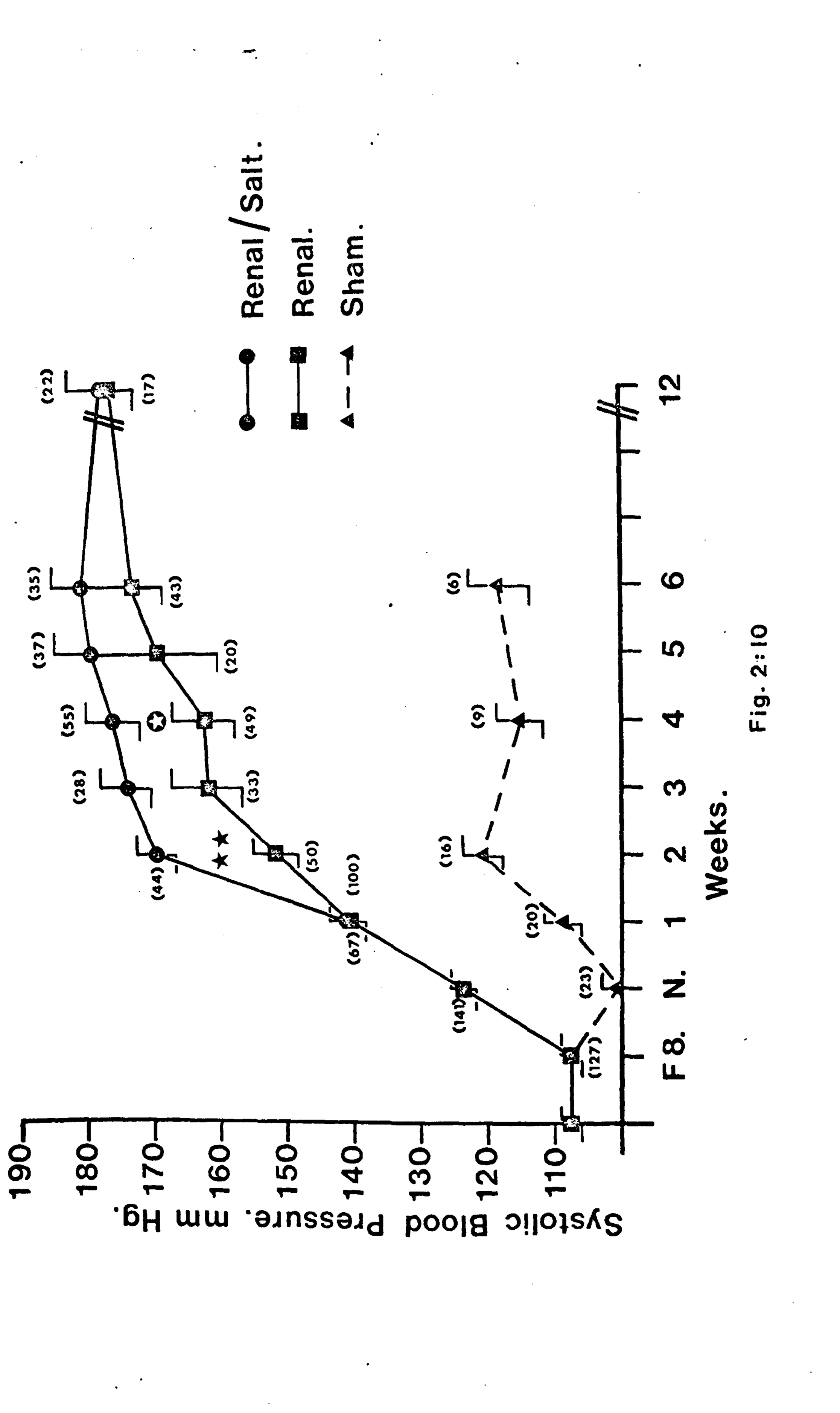

Renal/Salt.
Renal.
Sham.
(22)
(17)
(35)
(43)
(37)
(20)
(55)
(49)
(28)
(33)
(44)
(50)
(100)
(67)
(141)
(127)
(6)
(9)
(16)
(20)
(23)
12
6
5
4
3
2
1
N.
Weeks.
190
180
170
160
150
140
130
120
110
Systolic Blood Pressure. mm Hg.

Fig. 2:10

Fig. 2:10

The development of hypertension in "Grollman" hypertensive rats, numbers of animals in parentheses. F8, indicates application of the figure-of-eight ligature, N, indicates contralateral nephrectomy. The application of the figure-of-eight ligature increases the blood pressure, and the removal of the contralateral kidney further elevates the blood pressure. The blood pressure of renal/salt rats stabilizes between 170 and 180 mmHg 2 weeks after contralateral nephrectomy. Renal hypertensive rats had stable blood pressures between 160 and 170 mmHg after 3 - 4 weeks. Significant differences between the mean blood pressures of renal/salt and renal hypertensive rats are indicated by stars.

The blood pressures of the sham operated control animals fell after the first operation but then stabilized around 120 mmHg.

From 2 to 6 weeks following contralateral nephrectomy, the blood pressures of the renal/salt rats were higher than those for the renal rats, although this difference was not always statistically significant. Twelve weeks after contralateral nephrectomy there was no difference in mean blood pressure between the renal/salt and the renal hypertensive rats, (Fig. 2:10).

The mean blood pressure of the sham-operated rats decreased after the first but not after the second operation.

Salt-loading caused a greater mortality rate (10%) than in renal hypertensive rats (4-5%). Animals whose blood pressure exceeded 200 mmHg often died.

d) . DISCUSSION

A simple, effective method for inducing renal hypertension in rats has been described. The blood pressures of the operated rats rose rapidly during the 2 (renal/salt) or 4 (renal) weeks following nephrectomy and remained at hypertensive levels for the duration of the study (12 weeks).

The maximum blood pressures achieved by the hypertensive rats were similar to those reported by Grollman and Harrison (1945) and by Laramore and Grollman (1950). However, Vapaatalo, Lahovaara and Hackman (1970) and Handler and Bernheim (1950) have reported lower blood pressure levels for this method. The variation in the maximum blood pressure produced by this method is probably due to the different tensions with which the various investigators applied the "figure-of-eight" ligature.

Salt-loading accelerated the development of hypertension and increased the mortality rate, but did not influence the final peak blood pressure of the rats. Salt-loaded renal hypertensive rats were found to

have more consistent blood pressures than the renal hypertensive animals and were therefore a more useful model for the study of vascular reactivity.

A high salt intake has previously been shown to exacerbate this type of renal hypertension and to increase the mortality rate (Handler and Bernheim, 1950., Vapaatalo, Lahovaara and Hackman, 1970). Grollman and Harrison (1945) have demonstrated that drastic salt restriction lowers the blood pressure and prolongs the life of this type of hypertensive rat. Salt-loading also has a hypertensive effect in dogs which have had 70% of their renal mass removed (Douglas, Guyton, Langston and Bishop, 1964) and accelerates the development of hypertension with increased mortality in the Japanese S.H.R. (Dahl and Tuthill, 1974). However, renal hypertension induced by a "Goldblatt" clip on the renal artery, is unaffected by salt-loading (Redleaf and Tobian, 1958b., Miksche, Miksche and Gross, 1970). The absence of salt-sensitivity in "Goldblatt" renal hypertension is probably because kidney function is not impaired, but operates at a higher systemic blood pressure. Renal hypertension induced by a reduced kidney mass or a "figure-of-eight" ligature, in which there is variable damage to the nephrons is salt-sensitive because the increased load tends to overstress the remaining kidney tissue and elevates the blood pressure.

The haemodynamic events leading to hypertension after the application of the "figure-of-eight" ligature and contralateral nephrectomy are unknown. However, it is reasonable to assume that the haemodynamic changes correspond to some degree with the sequence outlined in chapter 1.

CHAPTER 3

VASCULAR REACTIVITY TO NORADRENALINE, POTASSIUM CHLORIDE AND ANGIOTENSIN II IN THE RAT PERFUSED MESENTERIC VASCULATURE PREPARATION, DURING THE DEVELOPMENT OF RENAL/SALT HYPERTENSION

a) INTRODUCTION

Considerable evidence has been amassed from human studies demonstrating an increase in cardiovascular reactivity to various physiological and pharmacological stimuli in hypertensive patients. The possibility of a link between increased vascular reactivity and elevated peripheral resistance in hypertension has led to many investigations of vascular reactivity in a variety of experimental hypertensive states.

Two major explanations have been proposed for this increase in vascular reactivity. 1. A true supersensitivity occurs when the same sub-maximal dose of agonist evokes a greater than normal degree of shortening in the vascular smooth muscle cell. In isolated arterial tissues, true supersensitivity is evidenced by a displacement of the dose-response curve to the left of the control with a lower vasoconstrictor threshold (Haeusler and Finch, 1972a). 2. An apparent hyperreactivity, occurs when the degree of smooth muscle cell shortening is normal, but, owing to an increased wall/lumen ratio, works at a greater mechanical advantage than in the normal vessel. In perfused arterial tissues, this is evidenced by a steeper dose-response curve with an elevated maximum response (Folkow, Hallbäck, Lundgren and Weiss, 1970c).

Previous investigations on reactivity in perfused vascular preparations from renal hypertensive rats have not confirmed either of these explanations, since full dose-response data at a variety of time points during the development of hypertension have not been obtained (McQueen, 1956., 1957., 1961., Tripod and Bein, 1960.,

Laverty, 1961., Nolla-Panades, 1963., Demura, Fukuchi, Takahashi and Goto, 1965., Oono, 1966., Baum and Shropshire, 1967a., McGregor and Smirk, 1968., Haeusler and Haefely, 1970., Armstrong, 1972., Haeusler and Finch, 1972b., Lundgren, 1974 a, b).

This chapter describes the changes in vascular reactivity to noradrenaline, potassium chloride and angiotensin which accompany the rise of blood pressure in experimental renal/salt hypertension. The interaction of angiotensin and noradrenaline in tissues from normotensive and hypertensive rats was also investigated, in an attempt to elucidate the cause of true vascular supersensitivity to noradrenaline.

Since the completion of the present study, Lundgren, Hallbäck, Weiss and Folkow (1974) have published a similar investigation, using the perfused hind-quarters preparation from the renal hypertensive rat.

b) METHODS

The induction of renal/salt hypertension, the perfused mesenteric vasculature preparation and the measurement of systolic blood pressure, have been described (Chap. 2).

i) Selection of hypertensive animals

Hypertensive rats with blood pressures close to the mean values given in Fig. 2:10 were used in the experiments. Rats whose blood pressures had exceeded 200 mm Hg were evaluated separately since they were judged to be in the malignant phase of hypertension, with muscular weakness, labile blood pressure, and lesions in the mesenteric vasculature typical of periarteritis nodosa.

ii) Experimental Procedure

Renal/salt hypertensive and sham operated rats were sacrificed and their mesenteric vasculatures perfused at 1,2,4 and 6 weeks post-operatively for the determination of reactivity to noradrenaline and angiotensin. Another group of hypertensive rats were sacrificed at 2,4 and 6 weeks after contralateral nephrectomy for the determination of reactivity to potassium chloride. Normotensive, unoperated rats were used to provide the control potassium chloride dose-response curve. Three hypertensive rats were sacrificed 12 weeks after contralateral nephrectomy and their mesenteric vasculatures stimulated with all three agonists.

In some experiments, the potentiating actions of angiotensin II amide on noradrenaline- and potassium chloride- evoked responses were investigated. After the initial agonist dose-response curve, the tissue was perfused with angiotensin II amide Krebs solution. An equilibration period of 15 min was allowed, during which the direct constrictor response to angiotensin occurred and subsequently tachyphylaxed. The noradrenaline or potassium chloride dose-response curve was repeated in the presence of angiotensin II amide.

The results of experiments designed to investigate the interaction of angiotensin II amide and noradrenaline in tissues from renal/salt hypertensive rats (1-2 weeks after contralateral nephrectomy), indicated that early supersensitivity to noradrenaline could have been caused by supranormal levels of endogenous angiotensin II in these animals. A series of dummy experiments were made, to test whether the noradrenaline potentiating effects of a high level of endogenous angiotensin would be detectable in the perfused mesenteric vasculature from renal/salt hypertensive rats, and whether this potentiation would attenuate the additional noradrenaline potentiating action of an infusion

of exogenous angiotensin.

iii) "Dummy" experiments

Dose-response curves (a) to noradrenaline were made on the perfused mesenteric vasculature preparation from normotensive rats. Angiotensin II amide (10^{-7}M) was infused as previously described and the noradrenaline dose-response curves (b) repeated. If it is assumed that the vasculature in the acute hypertensive rat is exposed to an abnormally high concentration of endogenous angiotensin II, then the perfused mesenteric vasculature preparation, when tachyphylaxed to exogenous angiotensin II amide, would be in a similar state. The tachyphylactic tissue was then perfused with normal Krebs solution for $1\frac{1}{2}$ - 2 h, simulating the equilibration and preliminary dosing time which would normally elapse before noradrenaline dose-response curves were obtained. The noradrenaline dose-response curve (c) was then repeated, angiotensin II amide (10^{-7}M) infused again and a final noradrenaline dose-response curve (d) made (See Fig. 3:10, dose-response curves a to d).

iv) Analysis of results

The mean dose-response curves to noradrenaline and potassium chloride were plotted in absolute values (Figs 3:1-4, 11-13) to evaluate changes in reactivity, and as a % of the maximum response (Figs 3:5-8, 14-16) to evaluate changes in sensitivity, independent of differences in contractility.

The following analyses of the dose-response data were made:-

The threshold dose

The threshold dose of noradrenaline for each group of preparations was taken as the mean of the smallest dose which evoked a response greater than 1 mm Hg. The threshold response on Figs 3:1-9

$$\text{Reactivity shift} = \frac{\text{Dose which evokes response amplitude x in control preparation.}}{\text{Dose which evokes response amplitude x in "hypertensive" preparation.}}$$

(each "hypertensive"preparation was compared with all the "control" preparations).

The mean 50% maximal responses (x) of the hypertensive dose-response data are given in Tables 3:3 and 3:9.

c) RESULTS

i) Vascular reactivity to noradrenaline

The mean body weights and systolic blood pressures of the renal/salt and sham operated rats are given in Table 3:1.

Temporal changes in vascular reactivity

The noradrenaline dose-response curves in preparations from renal/salt hypertensive rats were shifted to the left of the control at all the time points investigated (Fig. 3:1-4). At the 1 and 2 week stages, the threshold dose was decreased with no change in the maximum response. At the 4 and 6 week stages, the maximum responses were increased with no significant reduction in threshold dose (Table 3:2). The regression line gradients were greater at the 4 and 6 week stages than at the 1 and 2 week stages. However, the 4 and 6 week regression line gradients were not significantly different from the gradients of the control regression lines (Table 3:2).

The reactivity shifts of the "hypertensive" dose-response curves were calculated as described and are given in Table 3:3.

Temporal changes in vascular sensitivity

Figs 3:5-8, show the noradrenaline dose-response curve data expressed as a % of the maximum response. This negates the

Table 3:1

Weeks after second operation	Sham Control			Renal/Salt Hypertensive		
	Body Wt. g	B.P. mmHg	n	Body Wt. g	B.P. mmHg	n
1	210.6 ± 5.7	111.4 ± 5.2	5	168.0 ± 6.4*	142.3 ± 2.3*	5
2	218.4 ± 4.0	117.0 ± 6.2	5	194.5 ±18.1	182.6 ± 7.8*	6
4	263.0 ± 8.1	114.5 ± 5.2	7	226.8 ± 9.5	182.5 ± 3.0*	6
6	263.0 ± 8.1	114.5 ± 5.2	7	238.6 ±21.3	165.1 ± 5.3*	7

The mean body weights and systolic blood pressuresof renal/salt hypertensive and sham operated rats, used for the determination of noradrenaline reactivity. (* Significantly different from sham control).

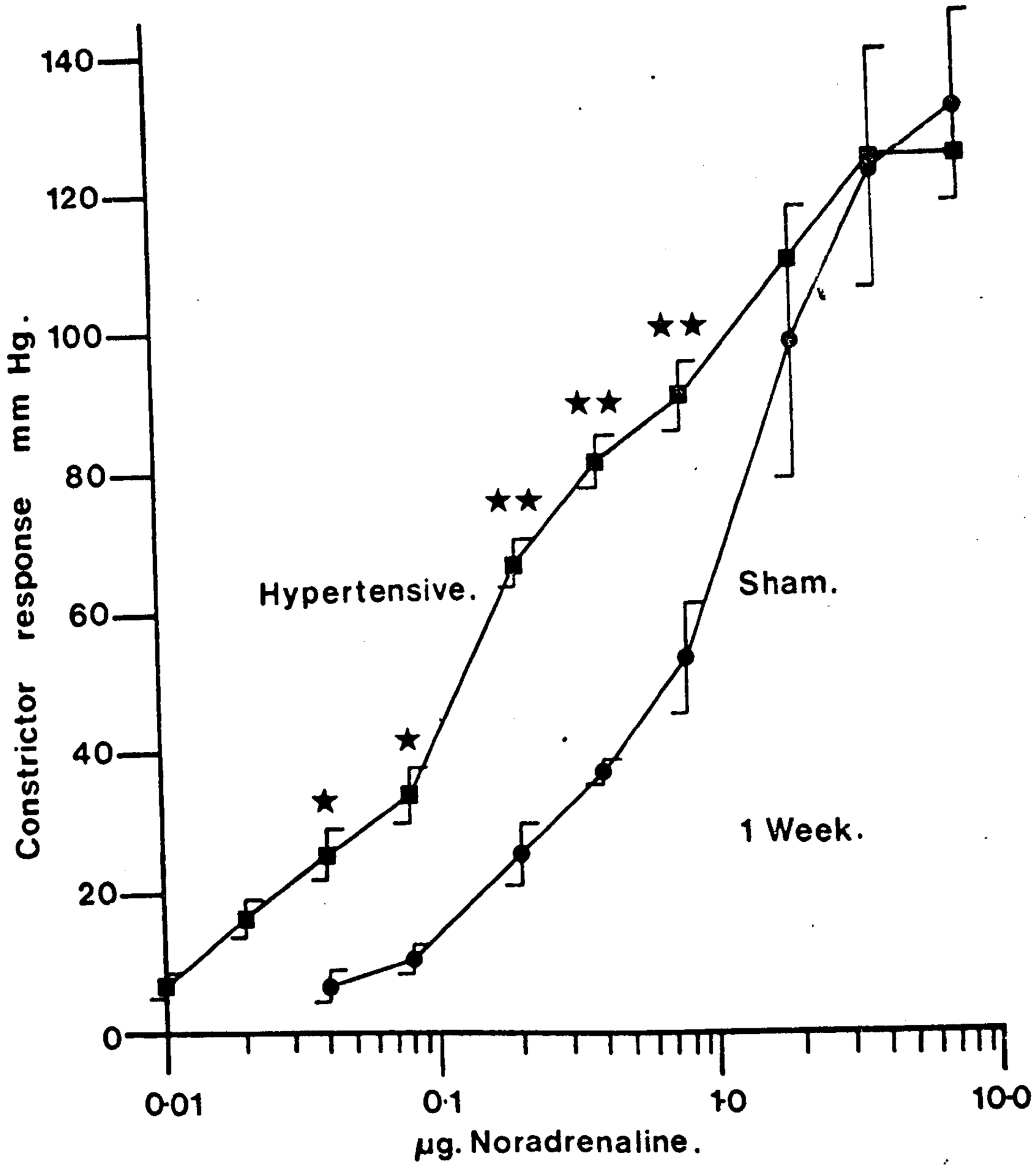


Fig. 3:1

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=5) and renal/salt hypertensive rats (n=5), 1 week post-operatively. Note the shift to the left and lower vasoconstrictor threshold of the "hypertensive" curve.

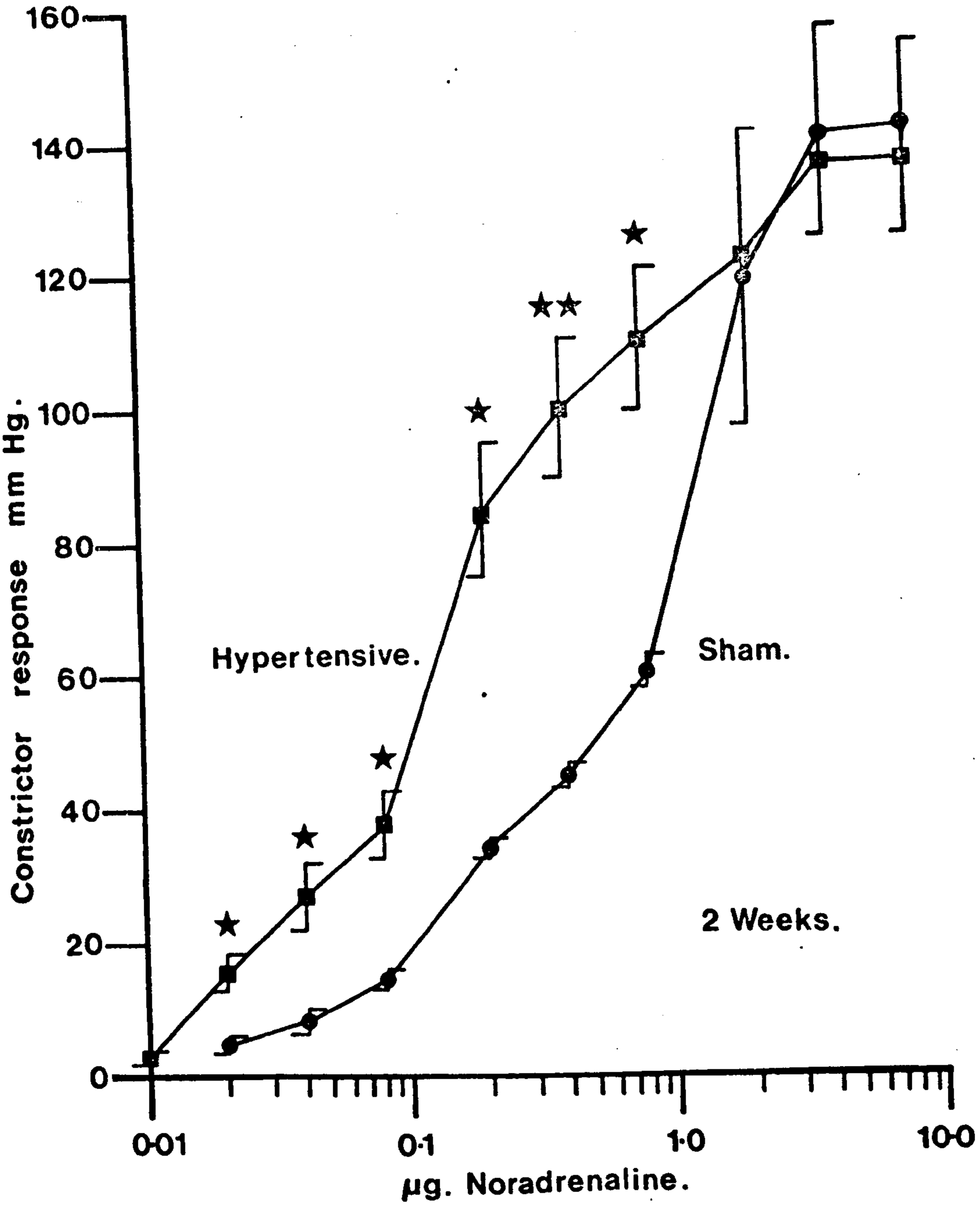


Fig. 3:2

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=5) and renal/salt hypertensive rats (n=6), 2 weeks post-operatively. Note the shift to the left and lower vasoconstrictor threshold of the

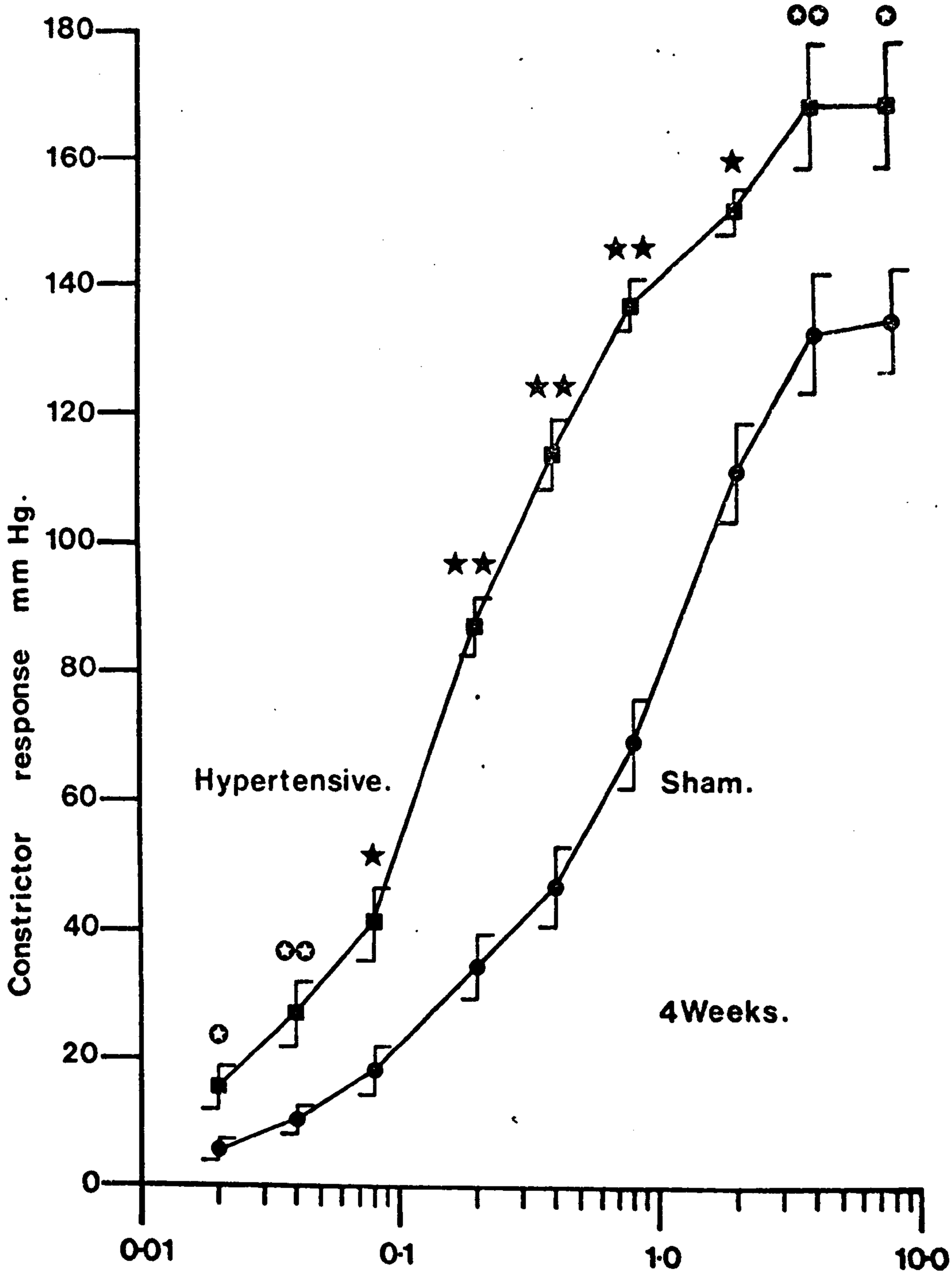


Fig. 3:3

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=7) and renal/salt hypertensive rats (n=6), 4 weeks post-operatively. Note the shift to the left and the elevated maximum of the "hypertensive"

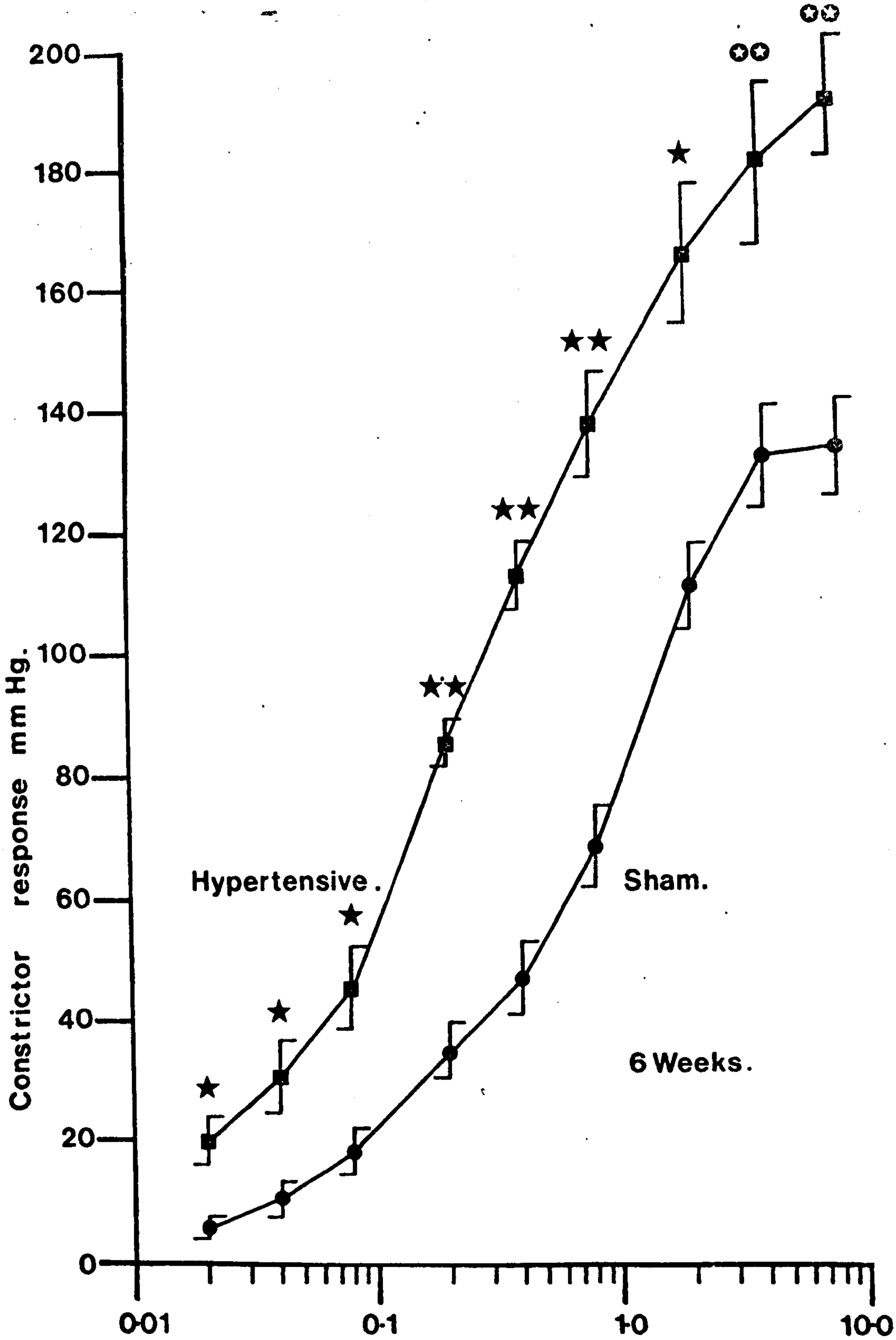


Fig. 3:4

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=7) and renal/salt hypertensive rats (n=7), 6 weeks post-operatively. Note the shift to the left and elevated maximum of the "hypertensive"

Table 3:2

Group	Regression Line Data						Threshold dose µg	Maximum Response mmHg
	Correlation Coefficient	Mean dose µg	Mean response mmHg	Gradient ± S.D.	Intercept	Degrees of freedom		
Sham								
1 week	0.83	0.696	44.1	42.4 ± 6.0	14.6	23	0.032 ± 0.005	129.4 ± 13.6
2 weeks	0.94	0.696	54.2	49.9 ± 3.8	19.5	23	0.024 ± 0.004	139.2 ± 16.0
4-6 weeks	0.89	0.696	55.8	44.9 ± 3.8	24.6	33	0.018 ± 0.002	132.7 ± 7.9
Renal/Salt								
1 week	0.79	0.696	75.8	30.4 ± 4.9	54.6	23	0.01 ± 0.0*	123.4 ± 6.6
2 weeks	0.60	0.696	90.4	31.2 ± 8.0*	68.6	28	0.012± 0.001*	136.0 ± 13.0
4 weeks	0.77	0.696	105.2	44.0 ± 6.8	74.5	28	0.015± 0.002	166.2 ± 9.4*
6 weeks	0.79	0.696	108.8	51.5 ± 6.9!	72.9	33	0.014± 0.002	189.9 ± 26.8*

Noradrenaline regression line data, threshold dose and maximum response of mesenteric vasculature preparations from renal/salt hypertensive and sham operated rats, stimulated with noradrenaline. (* Significantly different from control, ! significantly different from 1 week renal/salt gradient).

Table 3:3

Weeks after second operation	50% "hypertensive" response amplitude mmHg	Sham Mean dose µg	Renal/salt Mean dose µg	Renal/salt Reactivity Shift
1	66.7	1.49 ± 0.39	0.21 ± 0.05*	8.70 ± 1.13*
2	68.0	0.94 ± 0.04	0.15 ± 0.03*	7.99 ± 0.77*
4	83.1	1.08 ± 0.14	0.19 ± 0.02*	6.13 ± 0.37*
6	94.8	1.87 ± 0.33	0.27 ± 0.04*	7.76 ± 0.74*

The mean noradrenaline reactivity shifts of mesenteric vasculature preparations from renal/salt hypertensive rats (* Significantly different from sham control, mean dose indicates the dose to evoke a 50% "hypertensive" response amplitude in individual sham control tissues).

Table 3:4

Weeks after second operation	Sham Control $ED_{50\%}$ dose µg	Renal/salt $ED_{50\%}$ dose µg	Renal/salt Sensitivity Shift
1	1.28 ± 0.26	0.21 ± 0.05*	7.45 ± 0.82*
2	0.95 ± 0.07	0.15 ± 0.03*	7.90 ± 0.81*
4	0.74 ± 0.09	0.19 ± 0.02*	4.18 ± 0.26*
6	0.74 ± 0.09	0.27 ± 0.04*	3.10 ± 0.20*

Mean noradrenaline $ED_{50\%}$ doses for mesenteric vasculature preparations from sham operated and renal/salt hypertensive rats, and noradrenaline sensitivity shifts. (*Significantly different from sham control).

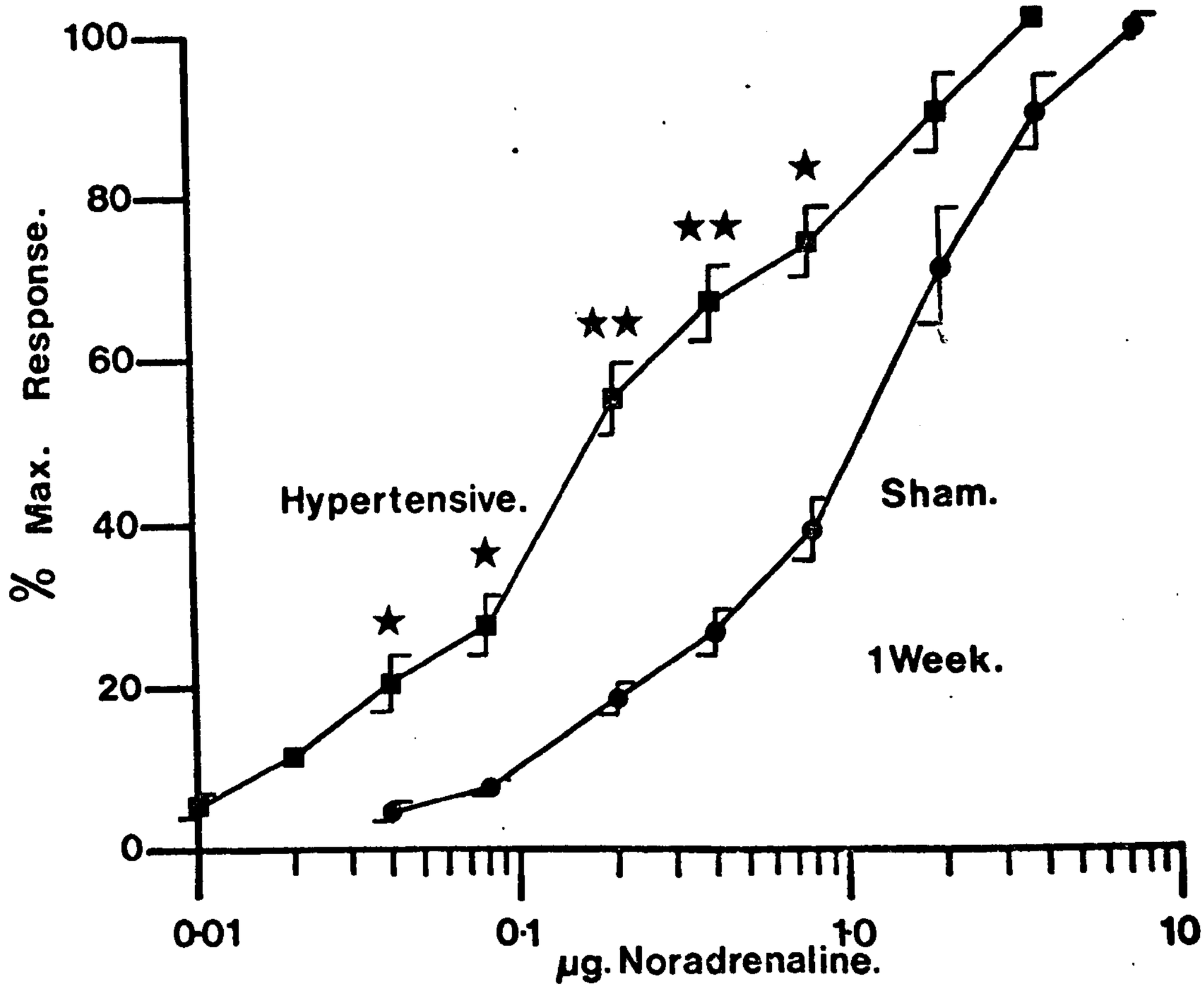


Fig. 3:5

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=5) and renal/salt hypertensive rats (n=5), one week post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is similar to the shift in Fig. 3:1.

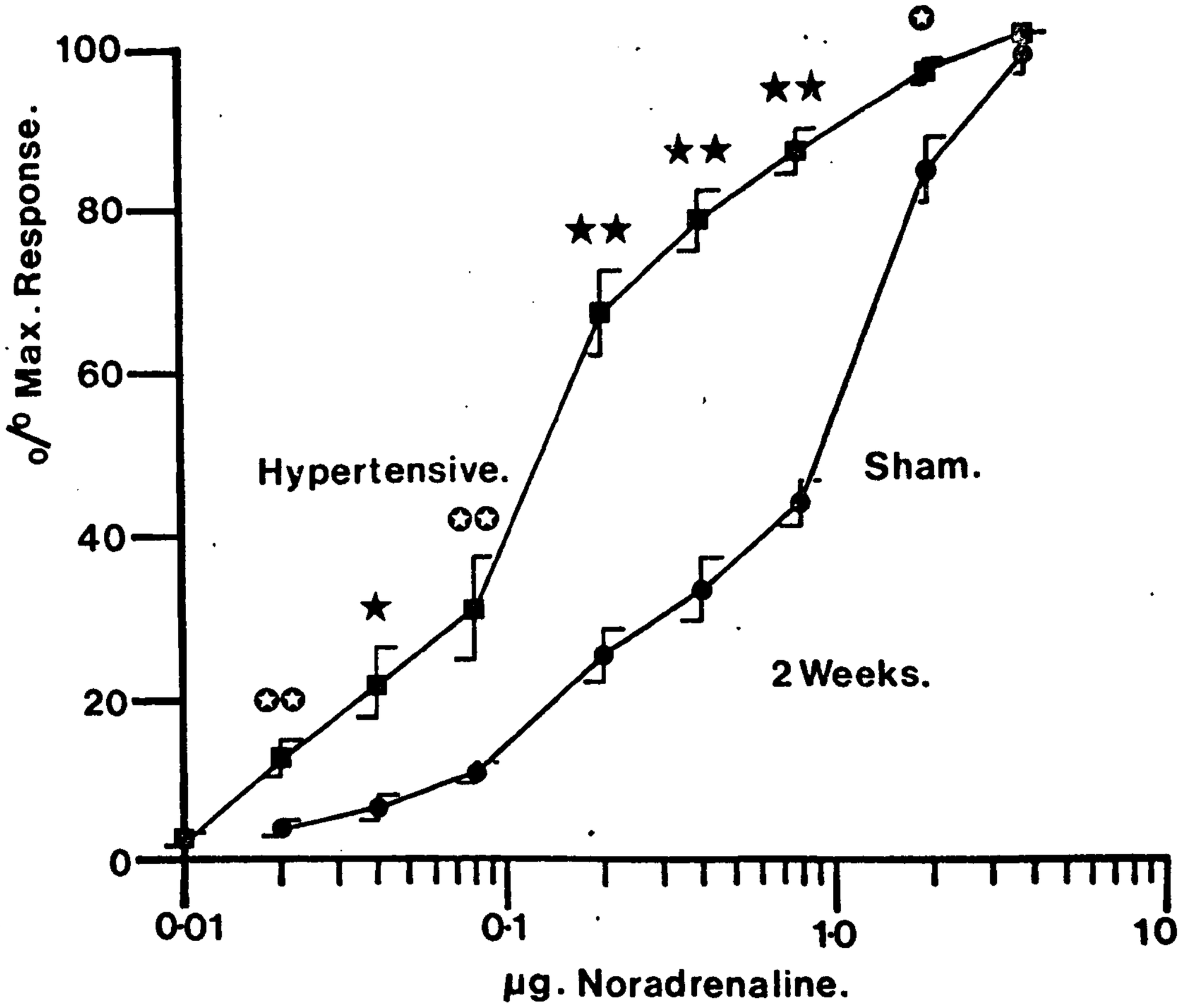


Fig. 3:6

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=5) and renal/salt hypertensive rats (n=6), 2 weeks post-operatively. Note that the shift of the "hypertensive" dose-response curve is similar to the shift in Fig. 3:2.

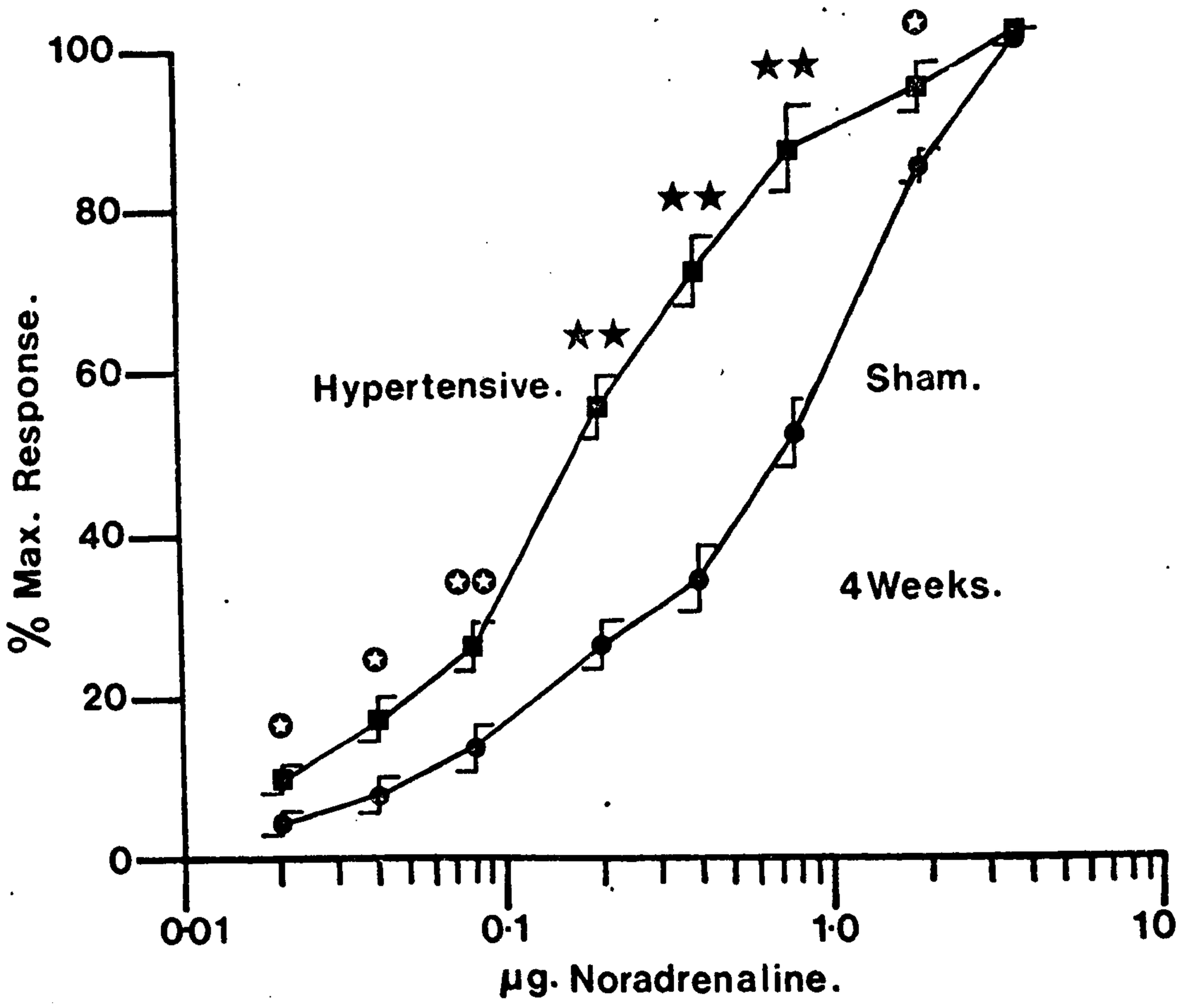


Fig. 3:7

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=7) and renal/salt hypertensive rats (n=6), 4 weeks post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is less than the shift in Fig. 3:3.

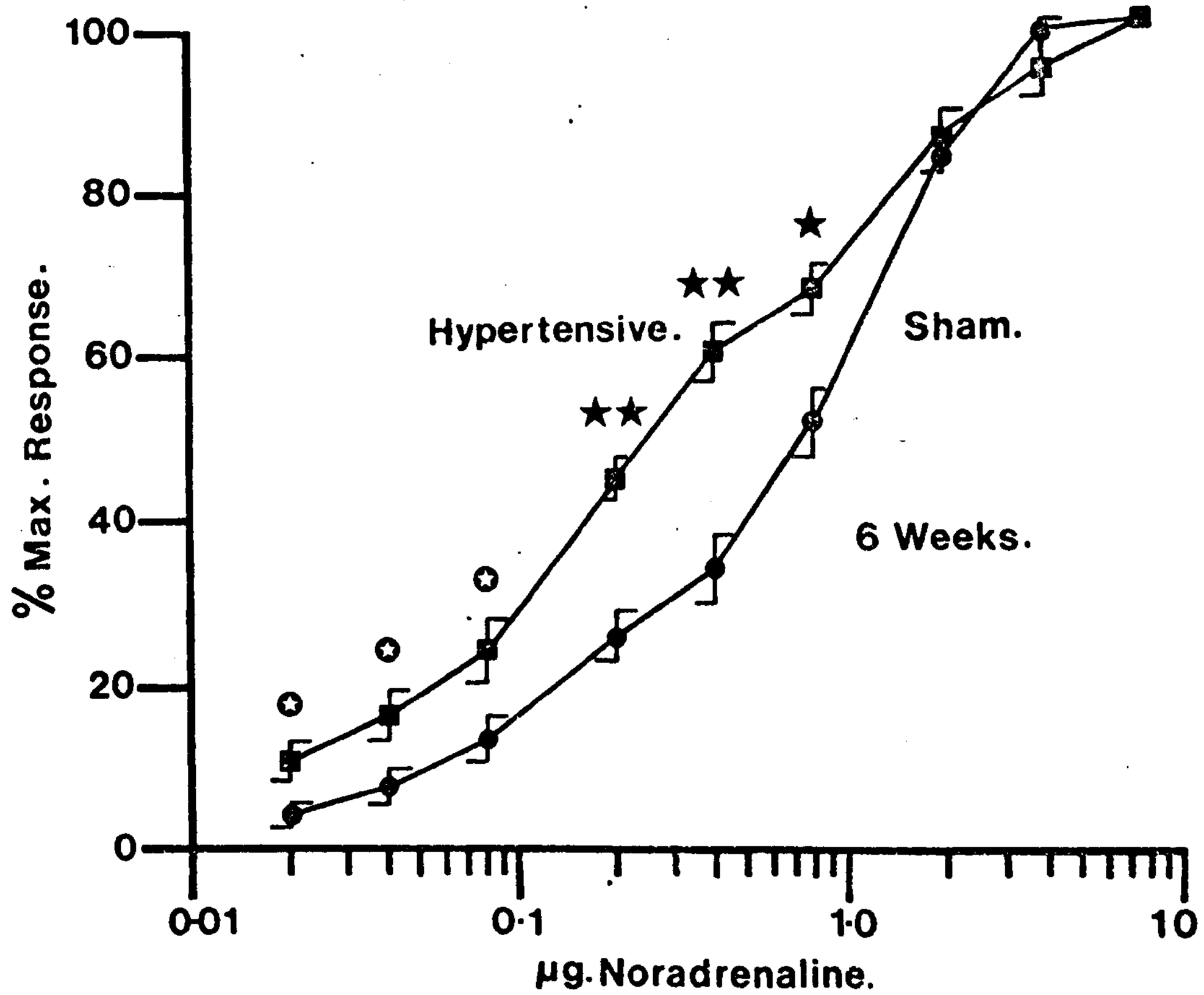


Fig. 3:8

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=7) and renal/salt hypertensive rats (n=7), 6 weeks post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is less than the shift in Fig. 3:4.

effect of an increased contractility and a consequently greater maximum response, allowing changes in the sensitivity of the blood vessels to be quantified. All the "hypertensive" dose-response curves are shifted to the left of the control, but in contrast to the reactivity shift (Figs 3:1-4, Table 3:3) the sensitivity shift decreased in the later 4-6 week stages of hypertension (Table 3:4).

ii) Vascular reactivity to angiotensin

The potentiation of noradrenaline responses by angiotensin II amide in mesenteric vasculature preparations from the normotensive rat

The noradrenaline sensitivity shifts, induced by perfusion with angiotensin II amide at 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7}M, and the angiotensin evoked constrictor response are given in Table 3:5.

The potentiation of noradrenaline responses by angiotensin was only significant at all noradrenaline dose levels at a concentration of 10^{-7}M (Fig. 3:9). Since this was also the only concentration which evoked a direct constrictor response, it was used in experiments on preparations from renal/salt hypertensive rats.

Angiotensin (10^{-7}M) caused a true noradrenaline super-sensitivity with a lower threshold dose (control threshold = 0.027 $\pm$ 0.005, angiotensin treated threshold = 0.014 $\pm$ 0.001 µg, $p<0.05$) and no elevation of the maximum response (control maximum = 107.0 $\pm$ 5.3 mmHg, angiotensin treated maximum = 109.9 $\pm$ 4.0 mmHg).

The direct angiotensin II induced constrictor response and the potentiation of noradrenaline induced responses by angiotensin II amide in mesenteric vessels from renal/salt and sham operated rats

The mean constrictor response and mean angiotensin induced

Table 3:5

Angiotensin II amide concentration	Noradrenaline sensitivity shift	Angiotensin constrictor response	n
10^{-10} M	1.26 ± 0.16	0 mmHg	6
10^{-9} M	1.82 ± 0.27*	0 mmHg	5
10^{-8} M	2.15 ± 0.25*	0 mmHg	6
10^{-7} M	2.74 ± 0.19*	14.8 ± 2.4 mmHg	11

Angiotensin induced constrictor responses and angiotensin induced noradrenaline sensitivity shifts in mesenteric vasculature preparations from normotensive rats. (* Significantly different from angiotensin untreated control).

Table 3:6

Weeks after second operation	Angiotensin constrictor response mmHg	Control noradrenaline $ED_{50\%}$ dose	Angiotensin treated noradrenaline $ED_{50\%}$ dose	Angiotensin induced noradrenaline sensitivity shift
Sham				
1	48.8 ± 8.3	1.28 ± 0.26	0.40 ± 0.09	3.34 ± 0.27
2	57.8 ± 11.1	0.95 ± 0.07	0.23 ± 0.03	4.26 ± 0.46
4-6	40.7 ± 9.5	0.74 ± 0.09	0.31 ± 0.07	2.85 ± 0.47
Renal/salt				
1	36.1 ± 8.8	0.21 ± 0.05*	0.11 ± 0.02*	2.1 ± 0.34*
2	68.2 ± 10.9	0.15 ± 0.03*	0.09 ± 0.01*	1.46 ± 0.15*
4	65.2 ± 9.6	0.19 ± 0.02*	0.08 ± 0.01*	2.58 ± 0.41
6	30.1 ± 5.4	0.27 ± 0.04*	0.14 ± 0.02*	2.30 ± 0.30

Angiotensin induced constrictor responses and angiotensin induced noradrenaline sensitivity shift in tissues from renal/salt and sham operated rats. (*Significantly different from sham control)

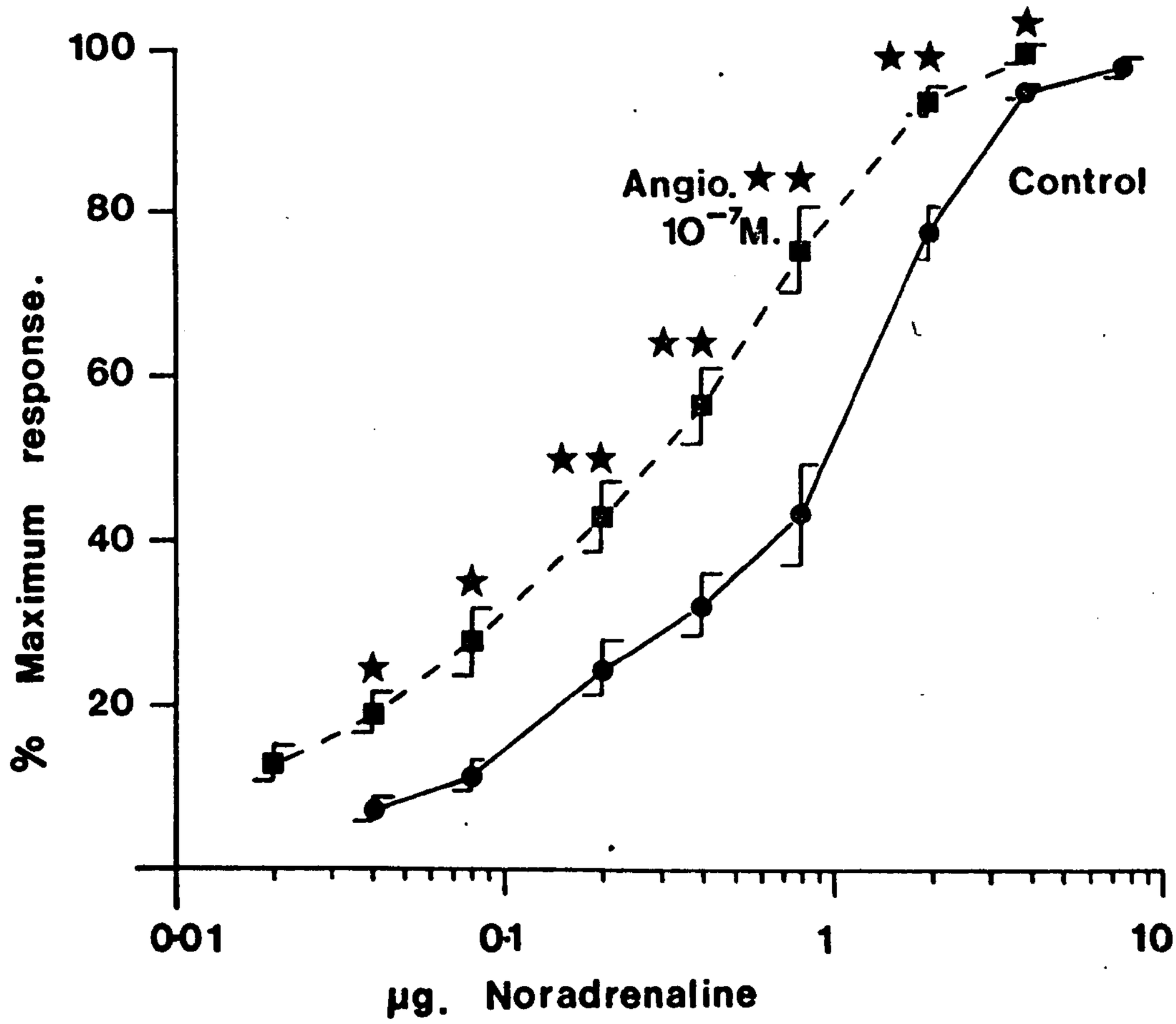


Fig. 3:9

Mean % responses (n=11) to noradrenaline, injected into the perfused isolated mesenteric vasculature of normotensive rats, in the absence (●——●) and presence (■- -■) of angiotensin II amide (10^{-7}M). Mean responses to noradrenaline were significantly greater (indicated by asterisks) in the presence of angiotensin, than in its absence.

sensitivity shift for preparations from renal/salt hypertensive and sham operated rats are given in Table 3:6.

Direct constrictor response amplitudes to angiotensin were not significantly different from the sham control in preparations from renal/salt hypertensive rats. However, the noradrenaline sensitivity shift induced by angiotensin II amide was significantly reduced in tissues from 1 and 2 week hypertensive rats whose reactivity shifts were predominantly due to increased sensitivity to noradrenaline (compare Tables 3:3 and 3:4).

"Dummy" Experiments; The duration of the potentiating action of angiotensin and its influence on a further infusion of angiotensin in tissues from normotensive rats

Mean noradrenaline dose-response curves (a), (b), (c) and (d) (n = 8, see methods) are shown in Fig. 3:10. The dose-response curves demonstrated that the noradrenaline potentiating effects of an infusion of 10^{-7}M angiotensin II amide did not wash out during $1\frac{1}{2}$ - 2 h perfusion with normal Krebs solution (compare lines b and c), and that a further exposure of the tissue to angiotensin II did not cause any further potentiation of noradrenaline induced responses (compare lines c and d). The tachyphylaxis to the direct constrictor effects of angiotensin II amide (10^{-7}M) did wash out during the $1\frac{1}{2}$ - 2 h normal Krebs perfusion. The mean constrictor response to the first angiotensin infusion was 21.3 $\pm$ 6.8 mm Hg while the second infusion evoked a mean response of 40.6 $\pm$ 2.9 mm Hg (n = 6).

iii) Vascular reactivity to potassium chloride

The mean body weights and systolic blood pressures of the renal/salt and normotensive unoperated rats are given in Table 3:7.

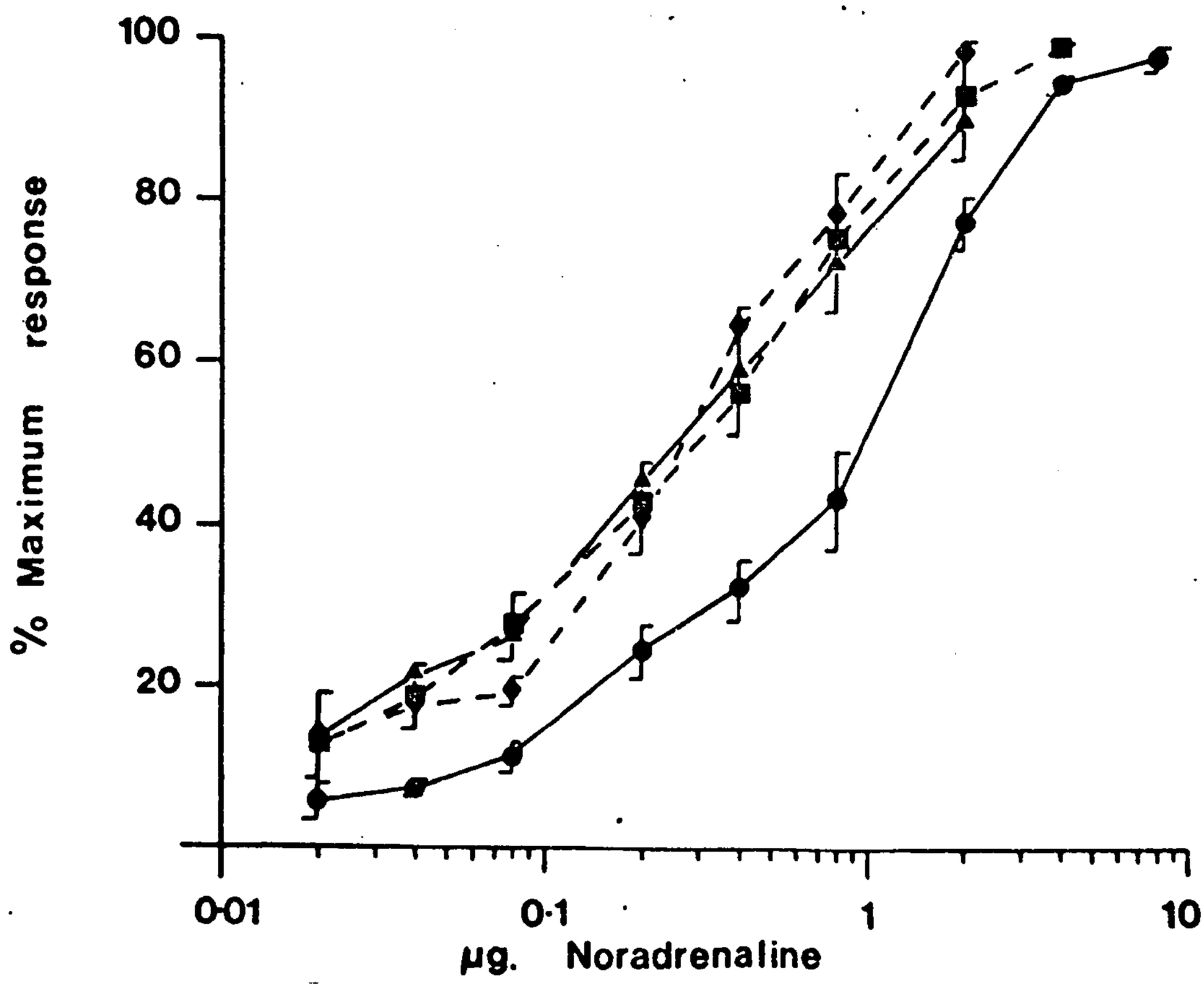


Fig. 3:10

Mean % responses to noradrenaline injected into mesenteric vasculature preparations (n=8) from normotensive rats, a) ●—● initial, b) ■—■ in the presence of 10^{-7}M angiotensin, c) ▲—▲ after perfusion for 1½-2 h with Krebs alone and d) ◆—◆ following a second angiotensin infusion.

Table 3:7

Group	Body Wt. g.	B.P. mmHg	n
Normotensive Control	238.4 ± 27.2	113.2 ± 2.3	10
Renal/salt hypertensive, Weeks after nephrectomy			
2	201.0 ± 20.0	179.5 ± 3.3*	8
4	231.7 ± 11.7	182.9 ± 8.9*	7
6	243.5 ± 25.2	158.0 ± 4.1*	5

Mean body weights and systolic blood pressures of normotensive and renal/salt hypertensive rats, used for the determination of KCl reactivity. (* Significantly different from control)

Temporal changes in vascular reactivity

In contrast to noradrenaline responses, there was no deviation in potassium chloride responses from the control, at 2 weeks after nephrectomy (Fig. 3:11). At the 4 and 6 week stages (Fig. 3:12, 13) there was a displacement of the dose-response line to the left, the maximum responses were elevated (Table 3:8) but, as with the noradrenaline data, there was no significant increase in the dose response curve gradient. The reactivity shifts of the "hypertensive" dose-response curves were calculated and are given in Table 3:9.

Temporal changes in vascular sensitivity

In contrast to the noradrenaline sensitivity changes in tissues from hypertensive rats, there was no change in sensitivity to potassium chloride (Table 3:10, Fig. 3:14-16).

The potentiation of potassium chloride responses by angiotensin II amide (10^{-7}M)

Angiotensin II amide (10^{-7}M) appeared to potentiate potassium chloride responses slightly, but this potentiation was not statistically significant at any dose level (n = 6, Fig. 3:17).

iv) Reactivity to noradrenaline, potassium chloride and angiotensin II amide in perfused mesenteric vasculature preparations from 12 week renal/salt hypertensive rats

In order to determine whether the reactivity shifts to noradrenaline and potassium chloride reached equivalence at a later stage in hypertension, three rats were studied 12 weeks post-operatively (blood pressure = 175 $\pm$ 13.4 mm Hg, body weight = 323.6 $\pm$ 8.7 g). Data from these preliminary experiments indicated that the reactivity shift to KCl was less than that to noradrenaline,

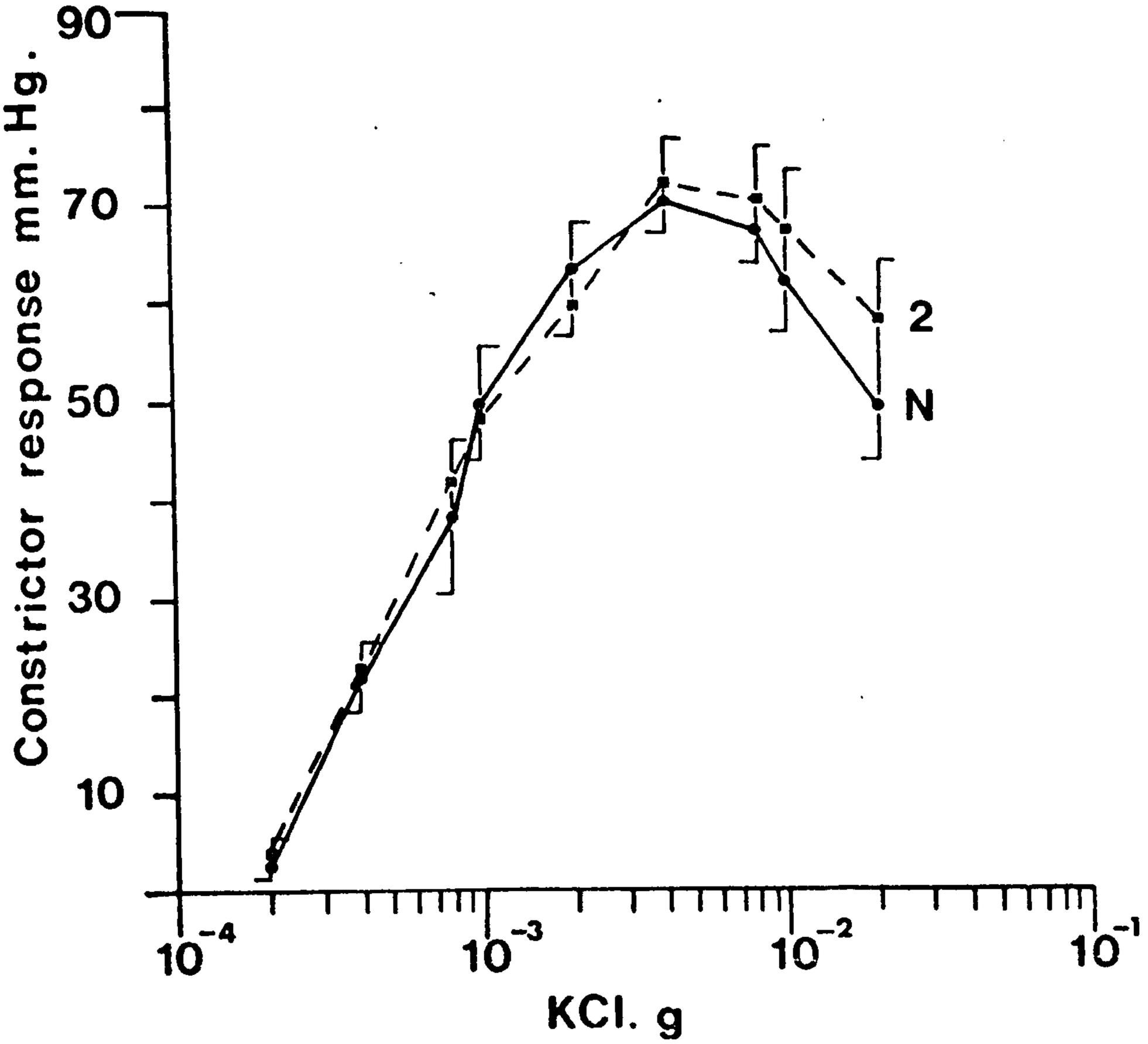


Fig. 3:11

Mean constrictor responses (mmHg) to KCl of mesenteric vasculature preparations from normotensive (N) rats (n=10) and renal/salt hypertensive (2) rats (n=8), 2 weeks post-operatively.

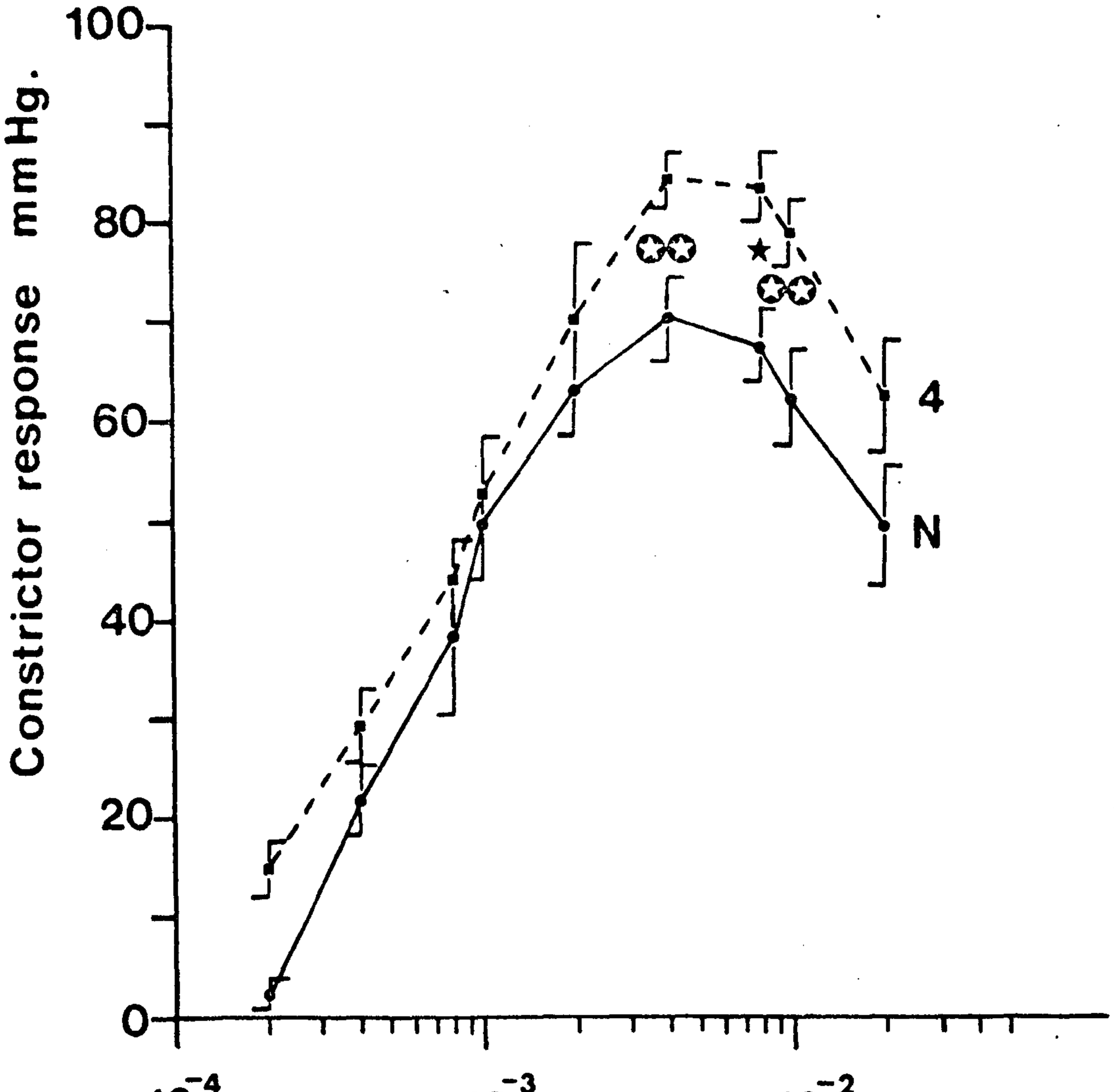


Fig. 3:12

Mean constrictor responses (mmHg) to KCl of mesenteric vasculature preparations from normotensive (N) rats (n=10) and renal/salt hypertensive (4) rats (n=7), 4 weeks post-operatively.

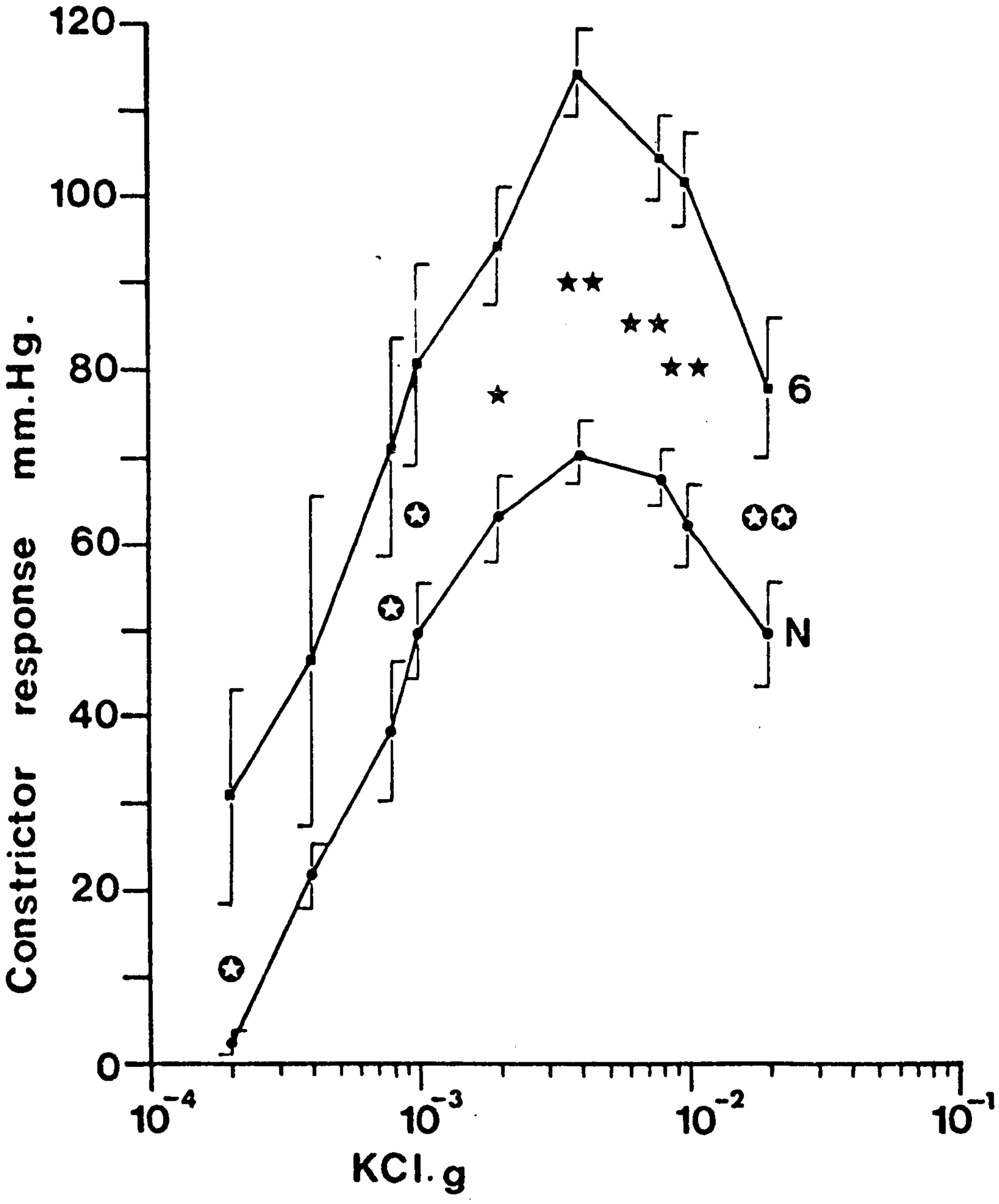


Fig. 3:13

Mean constrictor responses (mmHg) to KCl of mesenteric vasculature preparations from normotensive (N) rats (n=10) and renal/salt hypertensive (6) rats (n=5), 6 weeks post-operatively.

Table 3:8

Group	Regression Line Data						Maximum response mmHg
	Correlation Coefficient	Mean dose g x 10^{-4}	Mean response mmHg	Gradient ± S.D.	Intercept	df.	
Control	0.71	14	40.9	1.52 ± 0.20	19.6	58	70.3 ± 3.9
Renal/salt							
Weeks after nephrectomy							
2	0.79	14	40.7	1.49 ± 0.17	20.0	46	72.1 ± 4.6
4	0.81	14	48.4	1.67 ± 0.20	26.0	40	84.3 ± 2.9*
6	0.71	14	72.1	2.02 ± 0.38	43.8	28	114.6 ± 4.7*

KCl regression line data and maximum response of mesenteric vasculature preparations from renal/salt hypertensive and normotensive rats. (* Significantly different from control)

Table 3:9

Weeks after nephrectomy	50% maximal hypertensive response amplitude mmHg	Control Mean dose g x 10^{-4}	Renal/salt Mean dose g x 10^{-4}	Renal/salt Reactivity Shift
2	36.0	7.9 ± 1.3	6.8 ± 0.5	0.97 ± 0.06
4	42.0	9.7 ± 1.8	10.2 ± 1.9	1.12 ± 0.10
6	57.0	16.9 ± 4.7	6.5 ± 2.1*	3.5 ± 0.47*

The mean KCl reactivity shift of mesenteric vasculature preparations from renal/salt rats. (* Indicates significantly different from normotensive control, mean dose indicates the dose to evoke a 50% "hypertensive" response amplitude in individual sham control tissues).

Table 3:10

Control	$ED_{50\%}$ dose KCl g x 10^{-4}	Renal/salt. Weeks after nephrectomy	$ED_{50\%}$ dose KCl g x 10^{-4}
Normotensive	8.0 ± 1.1	2	6.8 ± 0.5
		4	10.2 ± 1.9
		6	6.5 ± 2.

KCl mean $ED_{50\%}$ doses for tissues from renal/salt hypertensive and normotensive rats

Table 3:11

Group	Body wt. g	B.P. mmHg	Angiotensin Response mmHg	Angiotensin induced noradrenaline sensitivity shift	n
4-6 week Sham	263.0 ± 8.1	114.5 ± 5.2	40.7 ± 9.5	2.85 ± 0.47	7
4-6 week malignant	194.0 ± 13.5*	221.5 ± 3.6*	23.5 ± 12.3	2.48 ± 0.30	6

Mean body weight, blood pressure, angiotensin response and angiotensin induced noradrenaline sensitivity shift in tissues from sham operated and malignant hypertensive rats

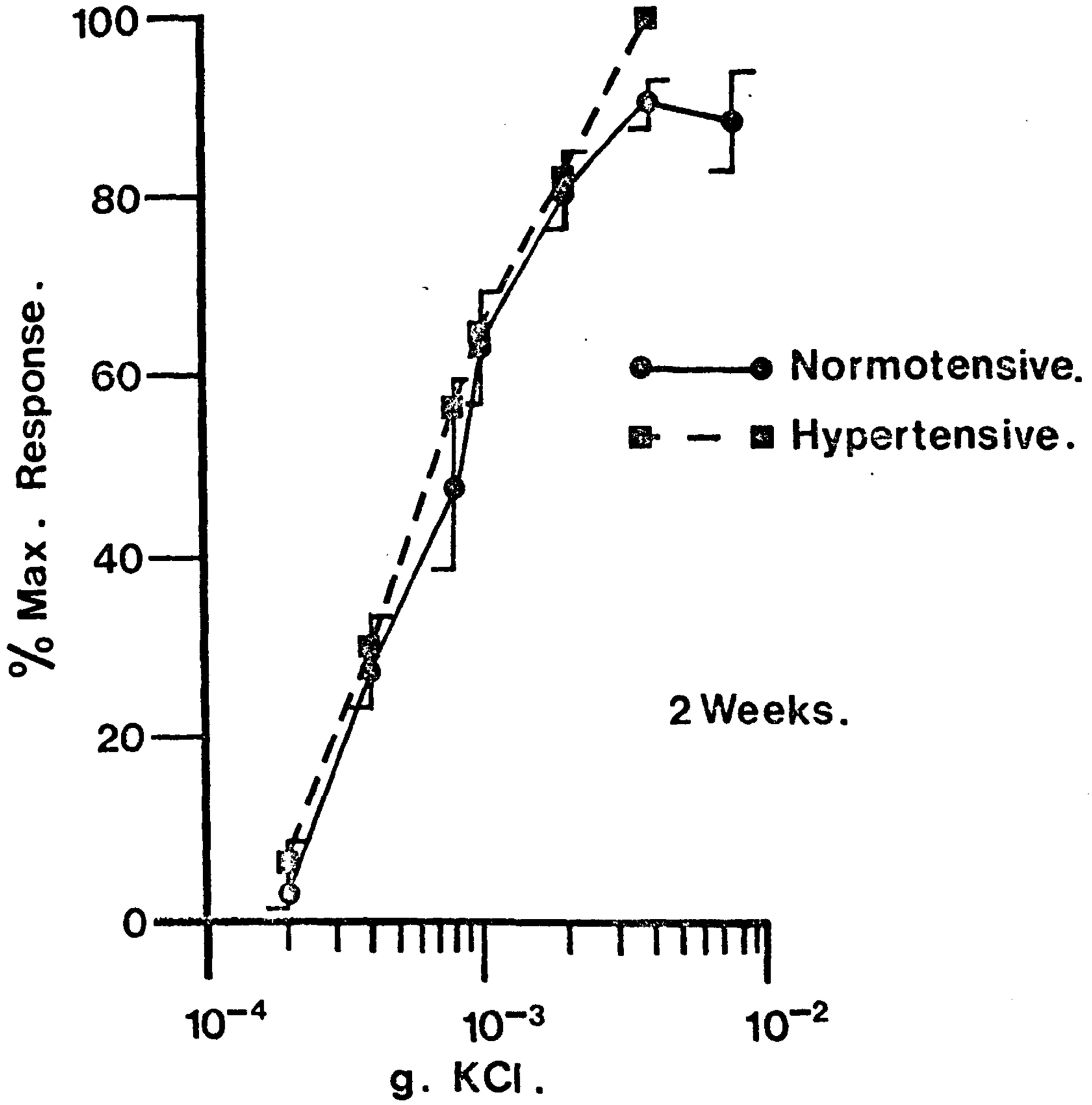


Fig. 3:14

Mean constrictor responses to KCl (% of the maximum response) of mesenteric vasculature preparations from normotensive (n=10) and renal/salt hypertensive (n=8) rats, 2 weeks post-operatively. Compare with Fig. 3:11.

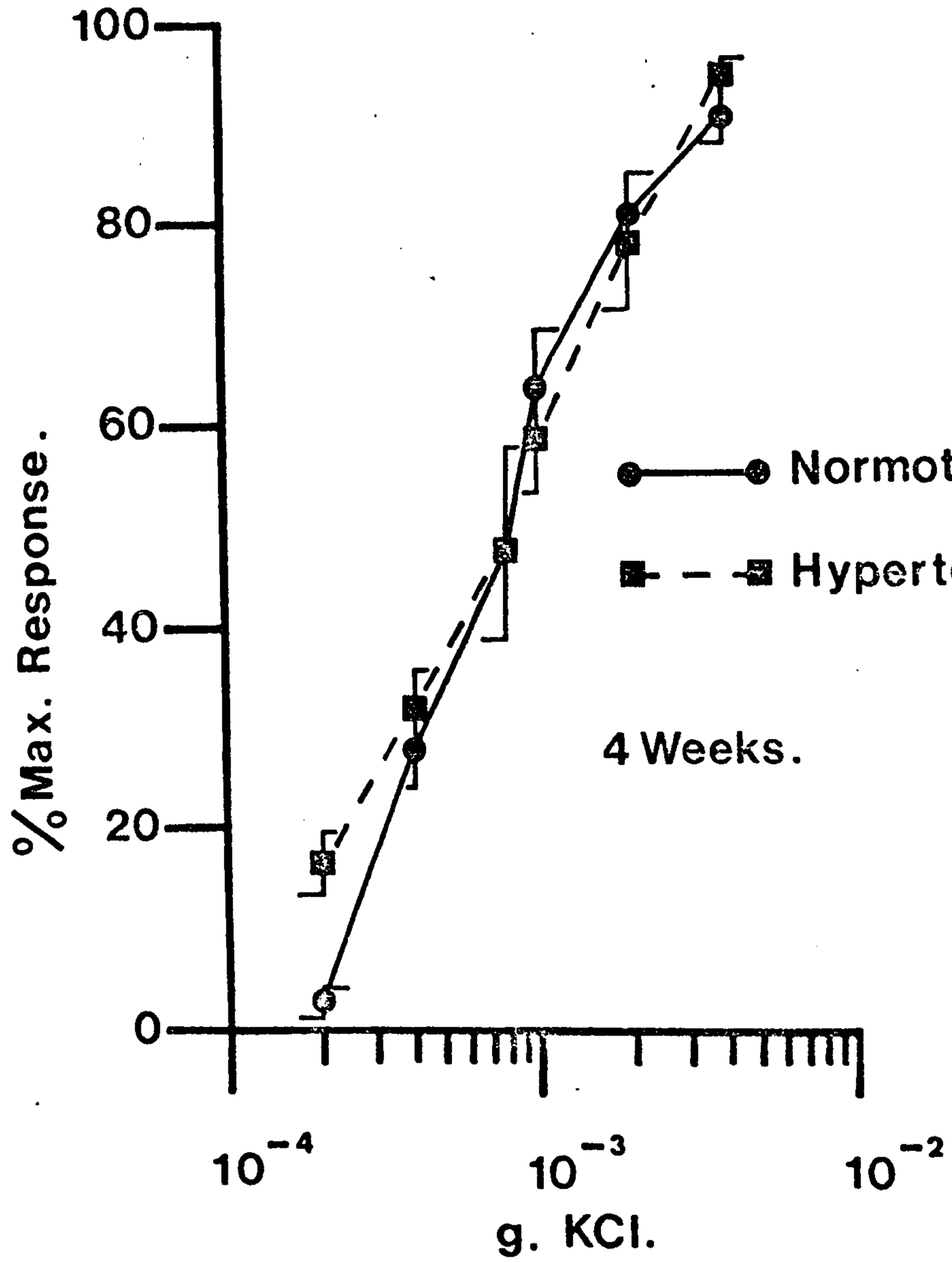


Fig. 3:15

Mean constrictor responses to KCl (% of the maximum response) of mesenteric vasculature preparations from normotensive (n=10) and renal/salt hypertensive (n=7) hypertensive rats, 4 weeks post-operatively. Compare with Fig. 3:12.

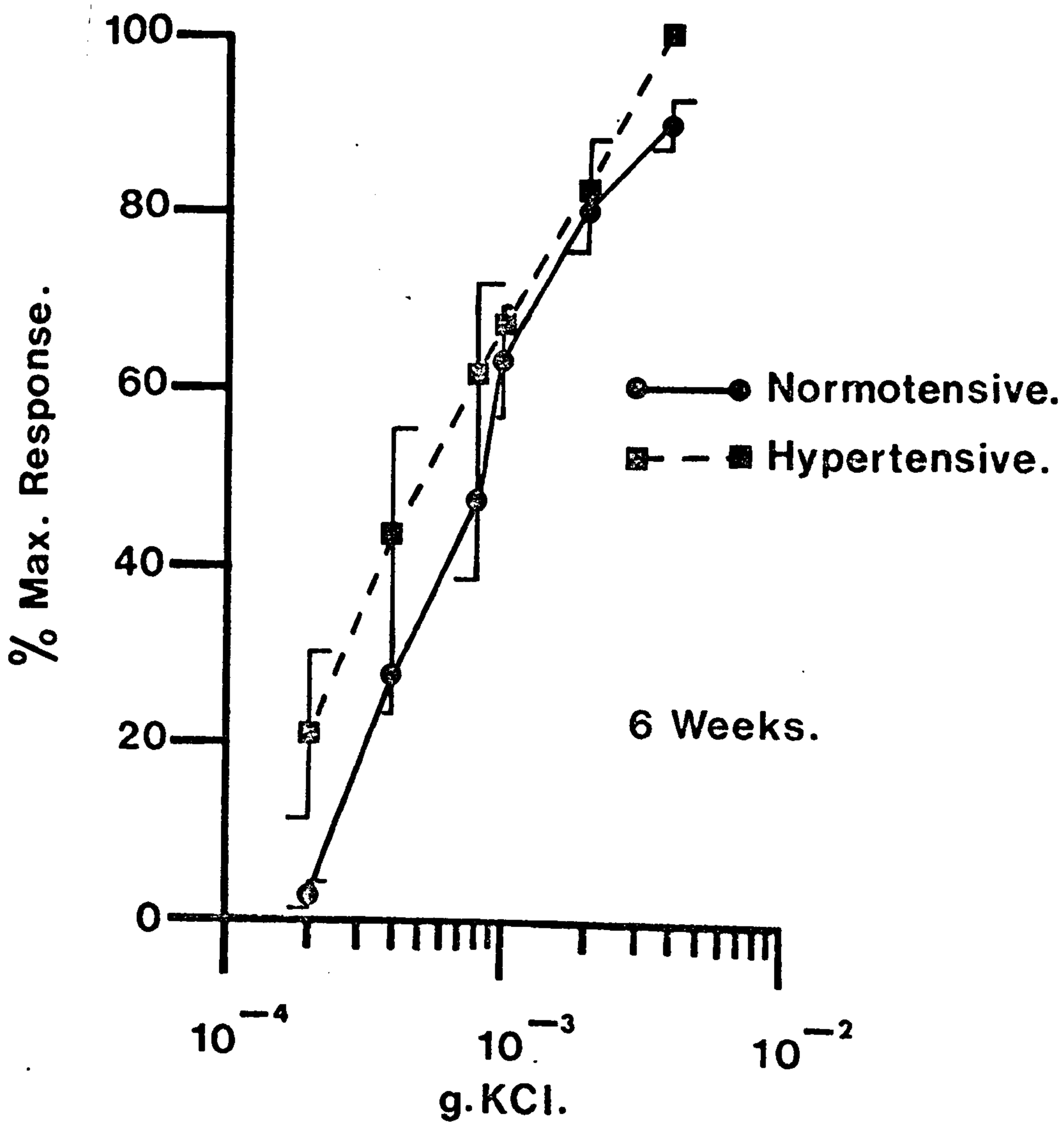


Fig. 3:16

Mean constrictor responses to KCl (expressed as % of the maximum response) of mesenteric vasculature preparations from normotensive (n=10) and renal/salt hypertensive (n=5) rats, 6 weeks post-operatively. Compare with Fig. 3:13.

while angiotensin induced responses and the angiotensin induced noradrenaline reactivity shift did not differ from the control.

v) Vascular resistance in the perfused mesenteric vasculature preparation from normotensive and "6 week" hypertensive rats

Perfusion pressure-flow characteristics of mesenteric vasculature preparations from normotensive rats (n = 6) and "6 week" hypertensive rats (n = 6, mean systolic blood pressure = 194.3 ± 7.8 mmHg) were examined. Flow rates of 2, 4 and 8 ml/min in "normotensive" tissues caused respective mean ± S.E.M. maximum pressures of 65.5 ± 0.9, 112.1 ± 1.1, 211.0 ± 2.4 mmHg, and in "hypertensive" tissues caused significantly greater pressures of 70.8 ± 1.3, 120.8 ± 3.0, and 222.5 ± 2.6 mmHg. Although an increase in vascular resistance was detected, the amplitude of this change was very small.

vi) Vascular reactivity to noradrenaline, potassium chloride and angiotensin II in mesenteric vasculature preparations from renal/salt hypertensive rats with blood pressures exceeding 200 mmHg, 4-6 weeks post-operatively.

Reactivity to noradrenaline and angiotensin

The mean systolic blood pressure, body weight, angiotensin evoked response amplitude and angiotensin induced noradrenaline sensitivity shift are given in Table 3:11. (p 109).

Both reactivity and sensitivity to noradrenaline were not significantly different from control, except that there was a significant depression of the maximum response (Fig. 3:18,19).

Reactivity to potassium chloride

Mesenteric vasculature preparations from three rats (4-6 weeks after contralateral nephrectomy, mean body weight = 201.0 ±

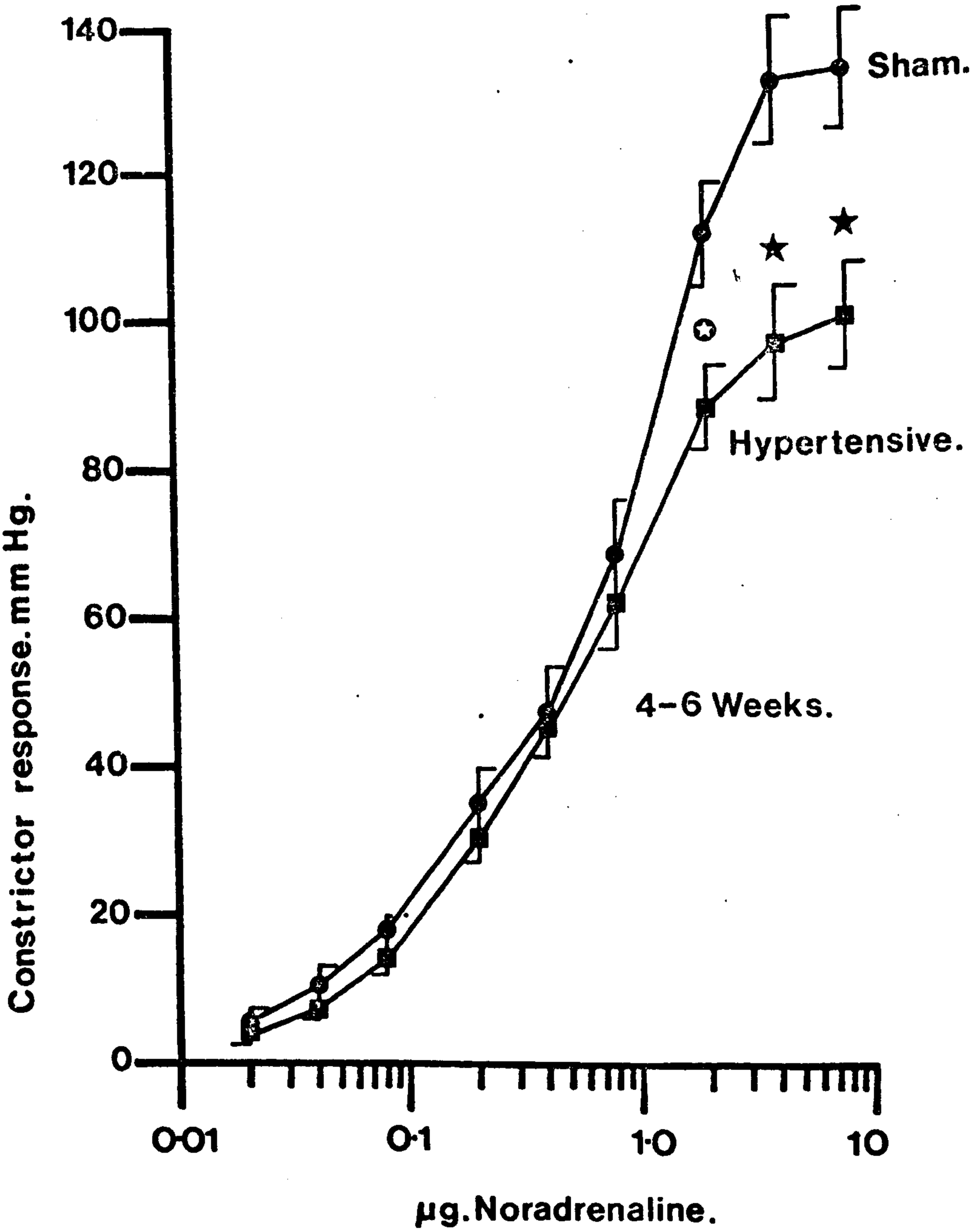


Fig. 3:18

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham operated (n=7) and renal/salt hypertensive rats with blood pressures exceeding 200 mmHg (n=6), 4-6 weeks post-operatively. Note the absence of shift of the "hypertensive" dose-response curve and the depression of the maximum response.

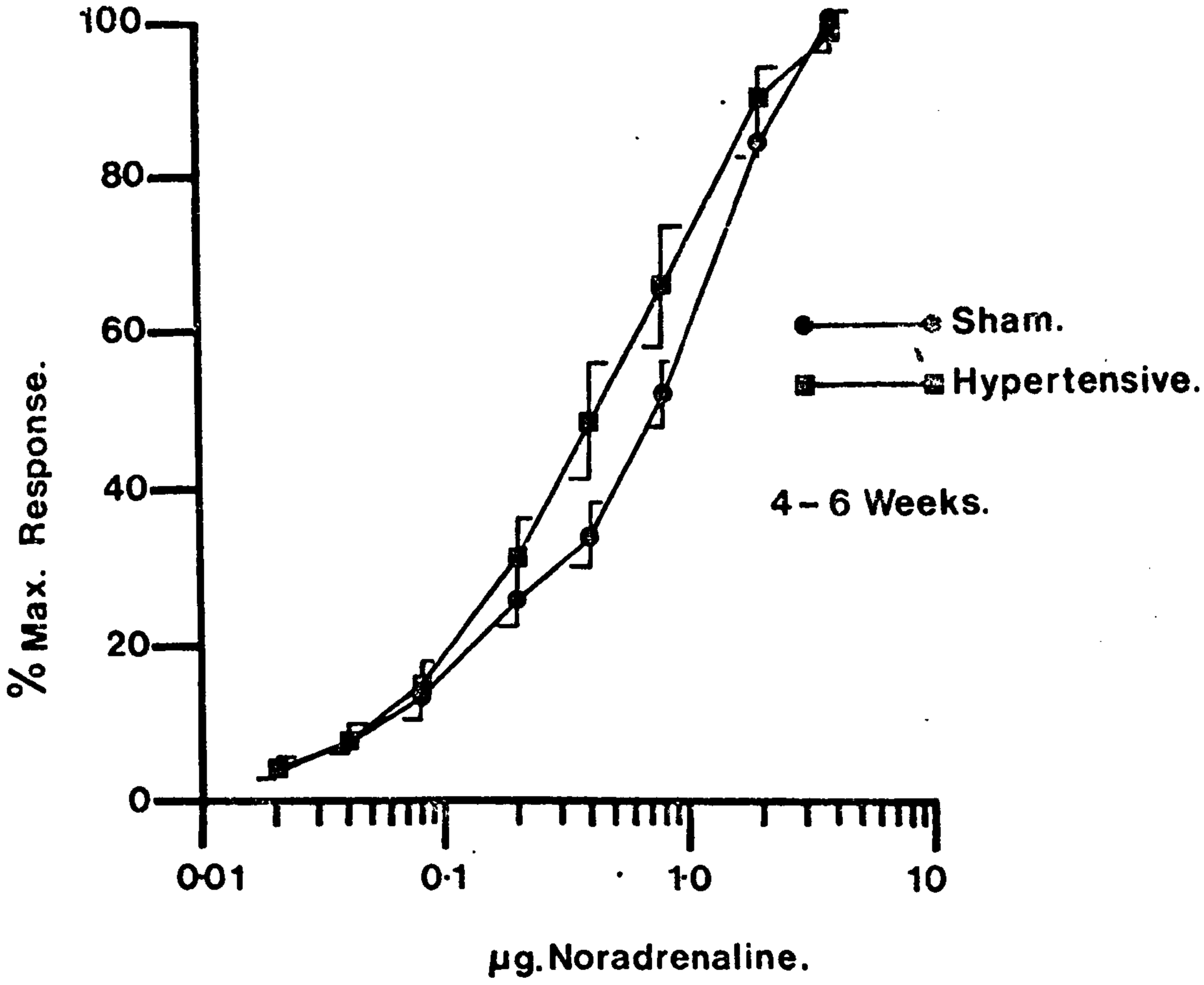


Fig. 3:19

Mean constrictor responses to noradrenaline (expressed as a % of the maximum response) of mesenteric vasculature preparations from sham operated (n=7) and renal/salt hypertensive rats whose blood pressure had exceeded 200 mmHg, 4-6 weeks post-operatively. Note the absence of a significant shift of the "hypertensive" dose-response curve.

18.7 g, mean systolic blood pressure = 211.7 ± 2.4 mmHg) were hyporeactive to potassium chloride, with depressed maximum responses (Fig. 3:20).

d) DISCUSSION

Previous investigations have demonstrated increased reactivity to noradrenaline in perfused preparations from renal hypertensive rats (Chap. 1). The results of the present study elucidate this phenomenon in detail and separate the true supersensitivity component from the increased reactivity.

During the 1 and 2 week stages of hypertension, the noradrenaline dose-response curve shift was caused by an increase in vascular sensitivity to the agonist (Fig. 3:21). The supersensitivity was characterized by a lower vasoconstrictor threshold dose with no elevation of the maximum response. Haeusler and Haefely (1970) have demonstrated a similar supersensitivity in the pharmacologically or surgically denervated mesenteric vascular bed, and the angiotensin II potentiation of noradrenaline responses, described in the present study (Fig. 3:9) also shows similar characteristics.

During the 4 to 6 week stages of hypertension the supersensitivity of the mesenteric vasculature declined and the noradrenaline dose-response curves (Fig. 3:3, 4) exhibited some of the characteristics ascribed to an increased wall/lumen ratio (Folkow, Hällback, Lundgren and Weiss, 1970c). The maximum noradrenaline evoked constrictor responses were elevated but the dose-response curves were not significantly steeper than control. The "6 week hypertensive" dose-response curve was significantly steeper than the "1 week hypertensive" curve indicating

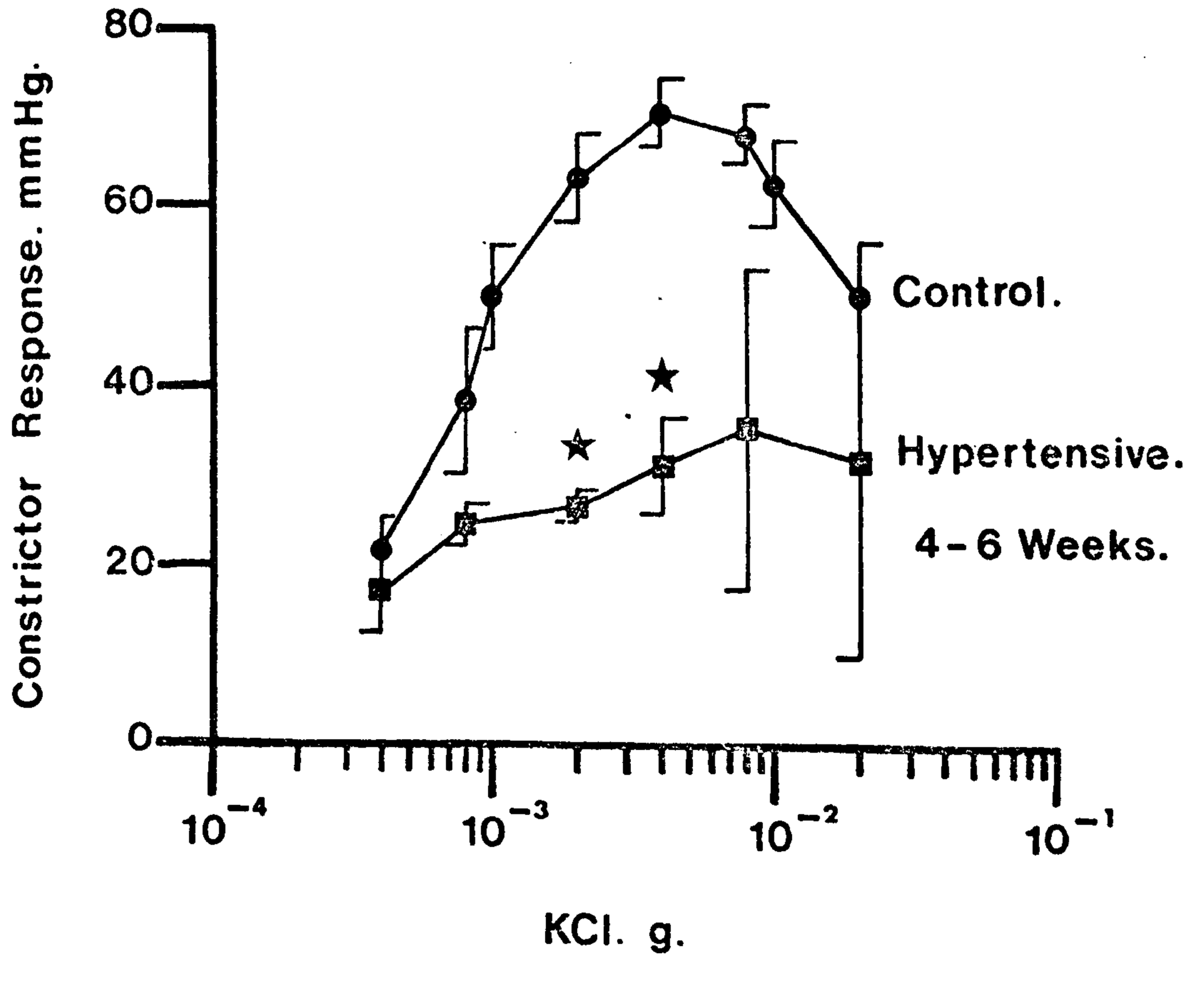


Fig. 3:20

Mean constrictor responses to KCl (mmHg) of perfused mesenteric vasculature preparations from normotensive (n=10) and renal/salt hypertensive (n=3) rats whose blood pressure had exceeded 200 mmHg, 4-6 weeks post-operatively. Note the markedly reduced maximum response of the hypertensive tissues.

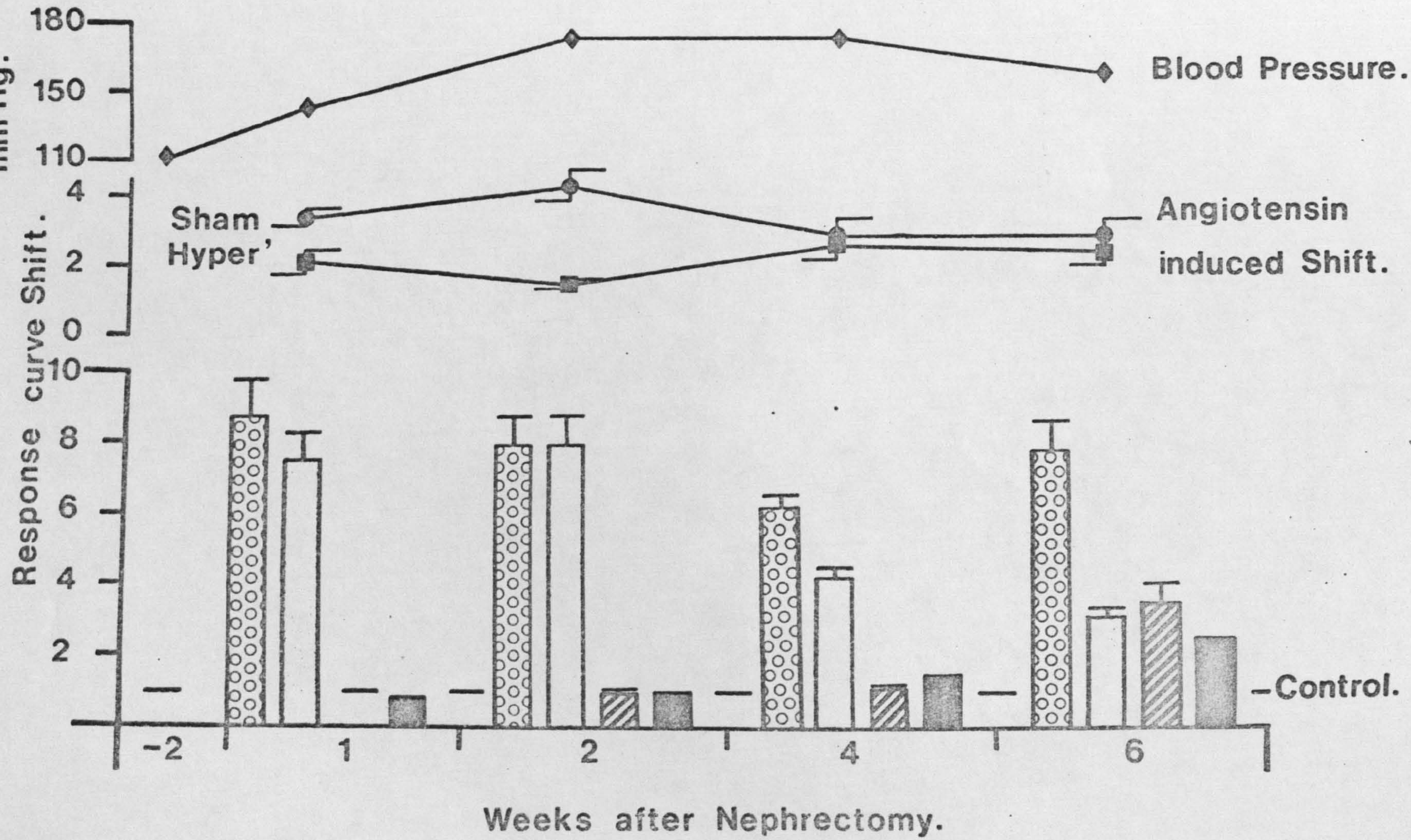

180
150
110
mm Hg.
Blood Pressure.
4
2
0
Sham
Hyper'
Angiotensin
induced Shift.
10
8
6
4
2
Response curve Shift.
-2
1
2
4
6
Control.
Weeks after Nephrectomy.

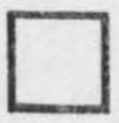
Fig. 3:21

Fig. 3:21

Summary Figure

Upper trace; mean systolic blood pressure of rats used.

Middle trace; angiotensin induced sensitivity shift of noradrenaline dose-response curves made on tissues from sham operated and renal/salt hypertensive rats.

Lower trace; histogram comparing noradrenaline reactivity shift  , noradrenaline sensitivity shift  , KCl reactivity shift  and calculated noradrenaline non-supersensitive shift remainder  .

Note 1). The reactivity shift reaches a maximum before the blood pressure.

2). In the early (1 and 2 week) stages of renal/salt hypertension the noradrenaline reactivity shift is caused by a change in sensitivity which corresponds with a reduction in the noradrenaline potentiating actions of exogenous angiotensin II.

3). Reactivity to KCl increases in the later (4 and 6 week) stages of renal/salt hypertension and corresponds to the non-supersensitivity induced noradrenaline reactivity shift remainder. The potentiating action of exogenous angiotensin II are normal in the later stages of hypertension.

that although a structural vascular change may have occurred, some degree of supersensitivity was still present. The increased resistance to flow in "6 week hypertensive" tissues was minimal, and it is possible that medial hypertrophy in this vascular bed does not involve any luminal narrowing. Other investigators of vascular reactivity in this preparation from hypertensive rats (Haeusler and Haefely, 1970., Haeusler and Finch, 1972b., McGregor and Smirk, 1970) have failed to find any increase in vascular resistance, although a small increase was demonstrated by McGregor and Smirk (1968) by using large numbers of rats.

In a recent study (Lundgren, Hallbäck, Weiss and Folkow, 1974) vascular reactivity to noradrenaline was investigated during the development of renal hypertension, using the rat perfused hind-quarters preparation. It was concluded that medial hypertrophy and an increased wall/lumen ratio occurred 2 weeks after the induction of hypertension and that there was no component of vascular super-sensitivity. Dose-response curve shift data was not reported, therefore any supersensitivity present would have been ignored in the analysis of the results. An increase in the resistance to flow, the noradrenaline dose-response curve gradient and in the maximum response at the 2 week hypertensive stage indicated apparent hyperreactivity. However, the paired "t" test used is not as rigorous as the unpaired test used in the present study. Further analysis of the results of Lundgren, Hallbäck, Weiss and Folkow (1974), using an unpaired "t" test, shows that the increased dose-response curve gradient was not significant at the 2 week hypertensive stage. The slope analysis used in this study involved measure-ment of the tangent of the angle rather than the fitting of regression lines to the dose-response data.

The results of the present study indicate that true vascular supersensitivity to noradrenaline does occur in renal hypertensive rats, and that this supersensitivity reaches a maximum before the blood pressure (Fig. 3:21). The later apparent hyperreactivity is probably a consequence of the high blood pressure since it was related to the duration of the hypertension.

Reactivity to noradrenaline in tissues from malignant hypertensive rats (blood pressure > 200 mmHg) was normal (Fig. 3:18,19) but with a reduced maximum response. The dose-response curves to potassium chloride (Fig. 3:20) also exhibited a reduced maximum, but with a hypo-reactivity over the dose-range used. The reduction in contractility may have been due to degenerative changes in the vessels (Gardner and Matthews, 1969) caused by the extremely high blood pressure and poor state of health of these animals.

The normal reactivity to potassium chloride at the 2 week hypertensive stage provides evidence that the early true noradrenaline supersensitivity was specific. Haeusler and Haefely (1970) found that denervation of the mesenteric vasculature preparation caused a pre-junctional supersensitivity to noradrenaline, but had no effect on potassium chloride induced responses.

The dose-response relations for potassium chloride at the 4 and 6 week hypertensive stages were in agreement with the noradrenaline results at these time points, i.e. an elevation of the maximum response with no significant increase in gradient over control. However the 6 week potassium chloride reactivity shift was only about 0.5 of the noradrenaline reactivity shift. An increase in wall/lumen ratio of the vessels should cause the same degree of shift to all agonists. In contrast to noradrenaline, a structural vascular change

may therefore be the sole factor involved in apparent KCl hyper-reactivity.

The inequality of the dose-response curve shifts to noradrenaline and KCl at the 12 week hypertensive stage indicates that a component of true supersensitivity to noradrenaline was still present at this stage. The shift of the noradrenaline dose-response curve due to factors other than supersensitivity was calculated by dividing the sensitivity shift into the reactivity shift (Fig. 3:21). It is interesting that the KCl apparent hyperreactivity shift at the 6 week hypertensive stage was of a similar magnitude to the non-supersensitive shift remainder for noradrenaline responses.

Direct constrictor responses to angiotensin were not significantly greater in tissues from hypertensive rats than from sham controls, but were significantly greater than responses obtained in tissues from normotensive unoperated rats. Direct constrictor responses to angiotensin II have been shown to be greater than normal in rats with adrenal regeneration hypertension (Gardner and Honoré, 1964a), renal hypertension or spontaneous hypertension (McGregor and Smirk, 1968). The results of the present study are inconclusive, since only one dose level and an infusion rather than a bolus injection of angiotensin II was used. An evaluation of reactivity to angiotensin requires full dose-response curves, but this type of study has the disadvantage that tachyphylaxis to angiotensin would make the construction of full dose-response curves very tedious.

An infusion of angiotensin II amide potentiated noradrenaline responses. This potentiation, or sensitivity shift has been previously reported, and various mechanisms have been suggested. Palaic and

Khairallah (1967) have shown that angiotensin II inhibits the neuronal uptake of noradrenaline. Pals and Fulton (1968) have suggested that a synergism exists between the contractile effects of angiotensin II and noradrenaline. Baudouin, Meyer, Fermandjain and Morgat (1972) have proposed that angiotensin II increases the amount of free intracellular Ca^{++} in the vicinity of the contractile elements in smooth muscle, by releasing or loosening membrane bound Ca^{++}. Day and Moore (1973) suggest that the enhancement of noradrenaline responses by angiotensin II may be a consequence of an inhibition of the sodium pump. The results of the present study do not indicate which of these explanations is correct. An inhibition of the sodium pump by angiotensin is, however, incompatible with the failure of angiotensin to potentiate KCl induced responses. Panisset and Bourdois (1968) studied the interaction of angiotensin and noradrenaline on the perfused cat mesenteric vasculature preparation. Their results indicated that angiotensin potentiated noradrenaline responses by a combination of re-uptake blockade and a direct action on the smooth muscle cell.

The attenuation of the noradrenaline potentiating actions of exogenous angiotensin at 1 and 2 weeks after contralateral nephrectomy could be a consequence of the "hypertensive" supersensitivity shift, which was maximal at these stages. In the early phases of renal/salt hypertension, the process causing supersensitivity could be stimulated by various factors, and exogenous angiotensin would be less effective in stimulating the already near maximally activated process. One of the factors which might stimulate the early supersensitivity could be endogenous angiotensin II. Plasma renin activity is elevated in the early stages of "Goldblatt clip" and aortic co-arctation hypertension (Brown, Davies, Olichney and Johnston, 1966., Carretero, Kuk, Piwonska, Houle and Marin-Grez, 1971). Plasma renin levels are

normal or low in "Grollman hypertensive" rats (Vapaatalo, Lahovaara and Hackman, 1970., Menard, Alexandre, Giudicelli, Auzan and Chevillard, 1973). However, these levels were measured in the later stages of hypertension, and plasma renin activities in the early stages of this form of hypertension are unknown (Chap. 5).

The "dummy" experiments using tissues from normotensive rats demonstrated that a previous exposure to a high level of angiotensin would reduce or negate the potentiating effects of a further infusion. If the circulating levels of endogenous angiotensin in the acute renal hypertensive rat were high, one might expect a depression of the noradrenaline potentiating effects of an infusion of exogenous angiotensin, and a potentiation of noradrenaline induced responses.

Potassium chloride induced responses were not significantly potentiated by 10^{-7}M angiotensin II amide. The lack of "hypertensive" supersensitivity to potassium chloride at the 2 week stage adds further evidence to the hypothesis that elevated levels of endogenous angiotensin II caused the specific noradrenaline supersensitivity in the early (1 and 2 week) stages of hypertension.

The noradrenaline dose-response curve shift, induced by 10^{-7}M angiotensin II amide, ranged from 2.6-4.3, whilst the sensitivity shift demonstrated in tissues from 1 and 2 week hypertensive rats was 7.5-8.0. This quantitative discrepancy is evidence against the hypothesis that endogenous angiotensin II caused the "hypertensive" supersensitivity. A concentration of 10^{-7}M angiotensin II would be unlikely <u>in vivo</u>, since the concentration in normal rat blood is around 2×10^{-10}M (Slack, Brown and Powell, personal communication). Angiotensin II levels in renal hypertensive rats are not available but Gocke, Gerten Sherwood and Laragh (1969) have measured levels of 10^{-10}-10^{-9}M

angiotensin II in plasma from humans with renal artery stenosis. However, endogenous 1-Asp-5-Ile angiotensin II is more potent in vivo than the synthetic 1-Asn-5-Val angiotensin II amide used in this study (Nakajima, Sakakibara, Sakuma and Sokabe, 1973) and a 15 min in vitro exposure is not quantitatively comparable with an elevation of plasma levels over a period of several days.

CHAPTER 4

STUDIES ON THE MECHANISM OF VASCULAR SUPERSENSITIVITY IN MESENTERIC VASCULATURE PREPARATIONS FROM RENAL/SALT HYPERTENSIVE RATS

INTRODUCTION

Increased vascular reactivity has been demonstrated in mesenteric vasculature preparations from renal/salt hypertensive rats (Chap. 3). The early phase of "true supersensitivity" exhibits the characteristics of an increased degree of smooth muscle shortening for a given sub-maximal dose of noradrenaline. In the later phase of increased reactivity, the vascular smooth muscle exhibits increased contractility, which might involve an increased wall/lumen ratio. In the later phases of hypertension there is a small persistent component of supersensitivity.

This chapter describes experiments designed to investigate the mechanism of this supersensitivity. Two mechanisms are considered, a) changes in cellular calcium regulation, which might cause a non-specific supersensitivity, b) changes in the characteristics of the α-adrenoceptor, which might cause a specific noradrenaline supersensitivity. An impairment of neuronal noradrenaline re-uptake might also cause a specific supersensitivity. An investigation of noradrenaline re-uptake in vessels from hypertensive animals was beyond the scope of the present study.

1) THE INVOLVEMENT OF CALCIUM IN THE INCREASED SENSITIVITY TO NORADRENALINE OF MESENTERIC VESSELS FROM RENAL/SALT HYPERTENSIVE RATS

a) INTRODUCTION

Calcium is essential for vascular smooth muscle contraction (Waugh, 1962., Bohr, 1964., Hinke, Wilson and Burnham, 1964). There is an increase in the amount of calcium or a change in the calcium dependance in vessels from renal hypertensive rats (Tobian and Chesley,

1965., Grollman and Krishnamurty, 1973). The following experiments were designed to investigate whether a re-adjustment of intracellular calcium regulation is the cause of vascular supersensitivity.

b) METHODS

The induction of renal/salt hypertension (Grollman, 1944), the measurement of systolic blood pressure (Gerold and Tschirky, 1968) and the perfused mesenteric vasculature preparation (McGregor, 1965) have been described (Chap. 2). Mesenteric vessels supplying a standard 13 cm length of intestine, from the caecal end, were perfused.

i) Perfusion solutions

The normal Krebs solution previously described (Chap. 2) with the addition of Na_2E.D.T.A. (0.026 mM) was used. When Ca^{++} - free Krebs solution was used, $CaCl_2$ and Na_2E.D.T.A. were omitted, and E.G.T.A. (0.2 mM) was added.

ii) Experimental Procedure

Mesenteric vasculature preparations from renal/salt hypertensive rats, 2 and 6 weeks after nephrectomy, and weight matched controls were perfused with normal Krebs solution (2 ml/min) for 1 h at 37°C. The following procedure was used:-

a) Krebs solution containing noradrenaline (1 µg/ml) was perfused for 5 min, during which time the evoked constrictor response stabilized.

b) Ca^{++} -free Krebs solution containing noradrenaline (1 µg/ml) was perfused for 13 min, and the decay of the constrictor response observed.

c) The tissue was perfused with normal Krebs solution for 15 min.

d) Stages a) - c) were repeated until the decay pattern stabilized.

e) Normal Krebs solution containing angiotensin II amide (10^{-7}M) was perfused for 15 min.

f) Stages a) - c) were repeated with angiotensin II amide (10^{-7}M) in all Krebs solutions.

An infusion of noradrenaline was used since preliminary experiments showed that the decay of responses to intermittent noradrenaline stimulation was >3 h in 0.5 mM E.G.T.A. Ca^{++} -free Krebs solution (n=5). A concentration of noradrenaline (1 µg/ml) which evoked a near maximum response was used.

iii) Analysis of results

The time taken for the noradrenaline evoked constrictor response to decay to 50% of the maximum response, for that tissue, was calculated and designated "decay half time". The "initial decay slope" of the noradrenaline response was evaluated by fitting regression lines to the decay pattern, plotted as a % reduction from the initial response, from 0.5 to 4.5 min in Ca^{++} -free Krebs solution.

c) RESULTS

The mean body weight, blood pressure and mesenteric vasculature noradrenaline evoked response amplitude for the hypertensive rats and their normotensive controls are given in Table 4:1.

Noradrenaline responses in tissues from all hypertensive animals and those which had been exposed to angiotensin II developed more rapidly and with a shorter latency of onset than in control tissues (Fig. 4:1).

The initial effect of exposure of the tissues to Ca^{++} -free

Table 4:1

Body weight, systolic blood pressure and mesenteric vasculature noradrenaline response amplitudes for hypertensive and normotensive rats

Parameter	Normotensive Control Rats	2 week Hypertensive Rats	Normotensive Control Rats	6 week Hypertensive Rats
Wt. (g)	206.1 ± 9.6	202.3 ± 5.5	273.0 ± 10.2	289.0 ± 7.7
B.P. (mmHg)	105.9 ± 4.4	178.6 ± 2.9*	121.4 ± 3.7	172.8 ± 8.2*
Noradrenaline response (mmHg)	153.7 ± 15.1	196.9 ± 22.1	123.4 ± 8.9	206.9 ± 16.0*
n	7	9	7	6

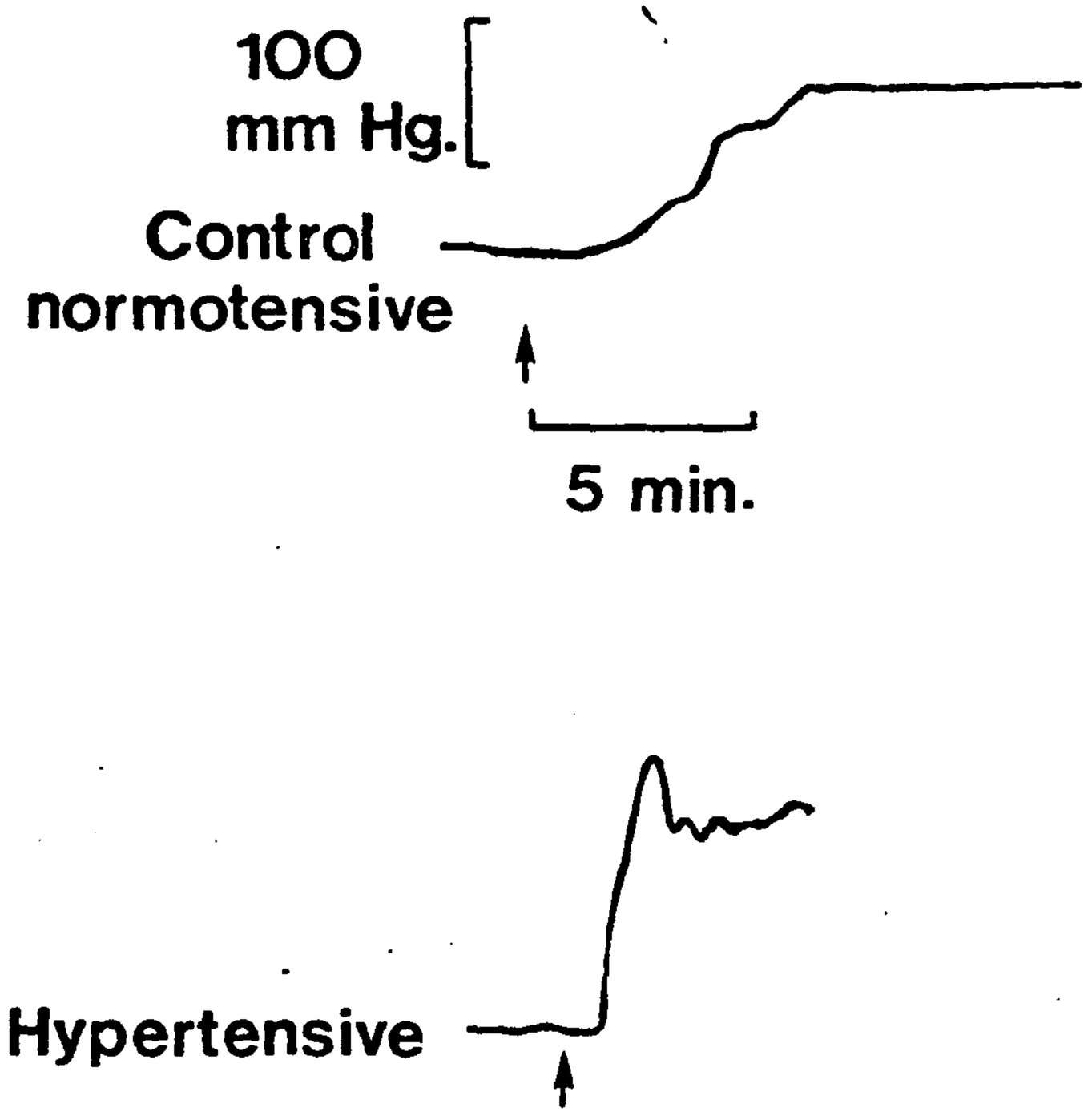
*Significantly different from weight-matched normotensive controls. The noradrenaline response amplitudes were measured after 5 min exposure to the agonist.

Table 4:2

Decay half times and regression line data for tissues from 2 and 6 week renal/salt hypertensive rats and normotensive controls, with and without angiotensin II treatment

Group	Angiotensin treatment	Abbreviation	Decay $\frac{1}{2}$ time min.	Regression line data				
				$\bar{y}$	$\bar{x}$	Slope ± Sd.	c	Correl Coeff
2 weeks								
Control	-	2WC	2.94±0.22	32.0	2.5	16.6±1.4	-9.5	0.9
Control	+	2WCA	4.17±0.43*	28.3	2.5	13.7±1.2	-6.0	0.9
Hypertensive	-	2WH	4.27±0.46*	20.6	2.5	12.2±1.0*	-10.0	0.9
Hypertensive	+	2WHA	4.76±0.82*	29.5	2.5	12.9±0.9*	-2.8	0.9
6 weeks								
Control	-	6WC	4.06±0.35	17.3	2.5	13.5±1.2	-16.3	0.9
Control	+	6WCA	6.73±0.47*	18.0	2.5	9.2±0.6*	-5.0	0.9
Hypertensive	-	6WH	4.04±0.34	25.3	2.5	11.0±0.8	-2.4	0.9
Hypertensive	+	6WHA	6.49±1.0*	23.2	2.5	9.0±0.9*	0.6	0.9

*Significantly different from weight matched controls.


100
mm Hg.
Control
normotensive
5 min.
Hypertensive

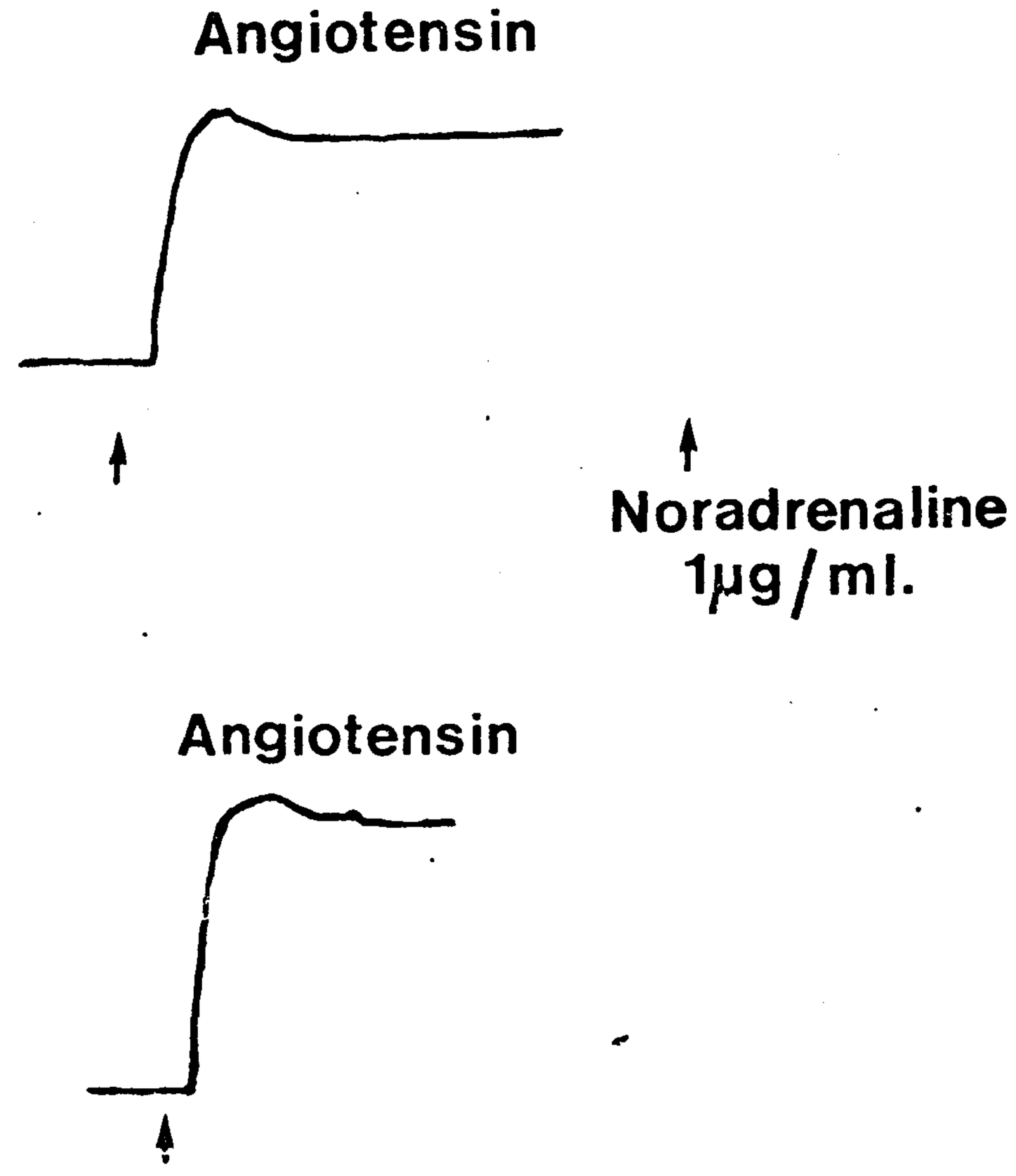

Angiotensin
Noradrenaline
1µg/ml.
Angiotensin

Fig. 4:1

Fig. 4:1

Mean pressure records of typical noradrenaline (1 µg/ml) induced constrictor responses of the perfused mesenteric vasculature preparation in the presence and absence of angiotensin II amide (10^{-7}M). After an initial 0.5 min time lag due to dead space in the apparatus, the tissue from the hypertensive rat (2 week stage) and tissues exposed to angiotensin respond immediately and the responses develop more rapidly than in control.

Krebs solution (Fig. 4:2) was often characterized by a small increase in response amplitude, probably due to the removal of membrane stabilizing calcium (Bohr, 1964). The response subsequently decayed to about 20% of its original amplitude in 12 min. A constrictor response occurred when normal Krebs perfusion was recommenced. This could have been due to residual noradrenaline, which would evoke a response as soon as activator Ca^{++} was available.

i) Analysis of the noradrenaline decay curves of tissues from renal/salt hypertensive rats 2 and 6 weeks after nephrectomy and weight matched normotensive controls

The decay patterns of tissues from 2 week control and 2 week hypertensive rats are shown in Fig. 4:3, the effects of angiotensin II on the decay pattern of 6 week control tissues is shown in Fig. 4:4. The decay half times and the regression line data computed from the noradrenaline decay curves are given in Table 4:2 (p 130).

Exposure of normal tissues (2WC, 6WC, see Table 4:2) to angiotensin (2WCA, 6WCA) caused significant prolongations of their noradrenaline decay half times. Tissues from 2 week hypertensive rats (2WH) had significantly longer decay half times than the controls (2WC), whereas decay half times, measured in tissues from 6WH rats did not differ from the controls (6WC). Exposure of tissues from hypertensive rats (2WH, 6WH) to angiotensin (2WHA, 6WHA) caused a significant prolongation of the decay half time at the 6 week but not at the 2 week hypertensive stage. The initial slopes (Table 4:2) derived from the decay of noradrenaline responses in Ca^{++} -free conditions showed an inverse relationship to decay half time. However, the initial decay slope of 6WH tissues was less than control (6WC) whereas the decay half times for the two groups were similar.

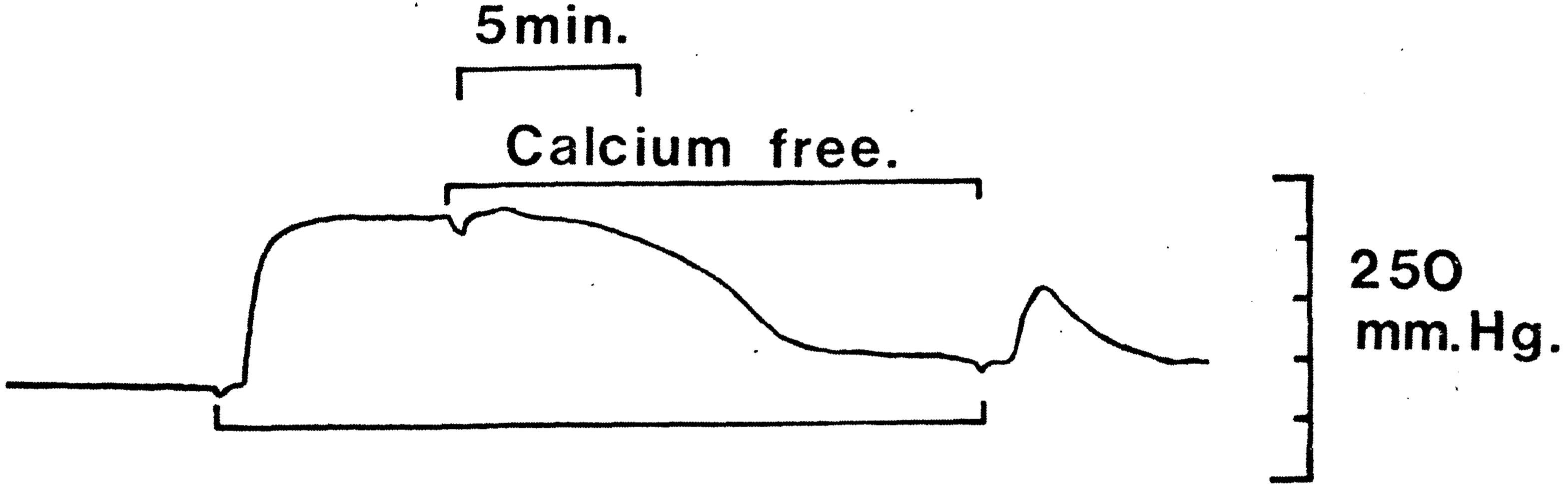


Fig. 4:2

Fig. 4:2

A typical mean pressure record of the decay of a noradrenaline induced contraction of the perfused mesenteric vasculature preparation in Ca^{++} -free conditions. An initial contraction is evoked by an infusion of 1 µg/ml of noradrenaline; transfer to Ca^{++} -free Krebs solution causes the decay of the response after an initial small potentiation. A further constrictor response occurs on recommencing normal Krebs perfusion.

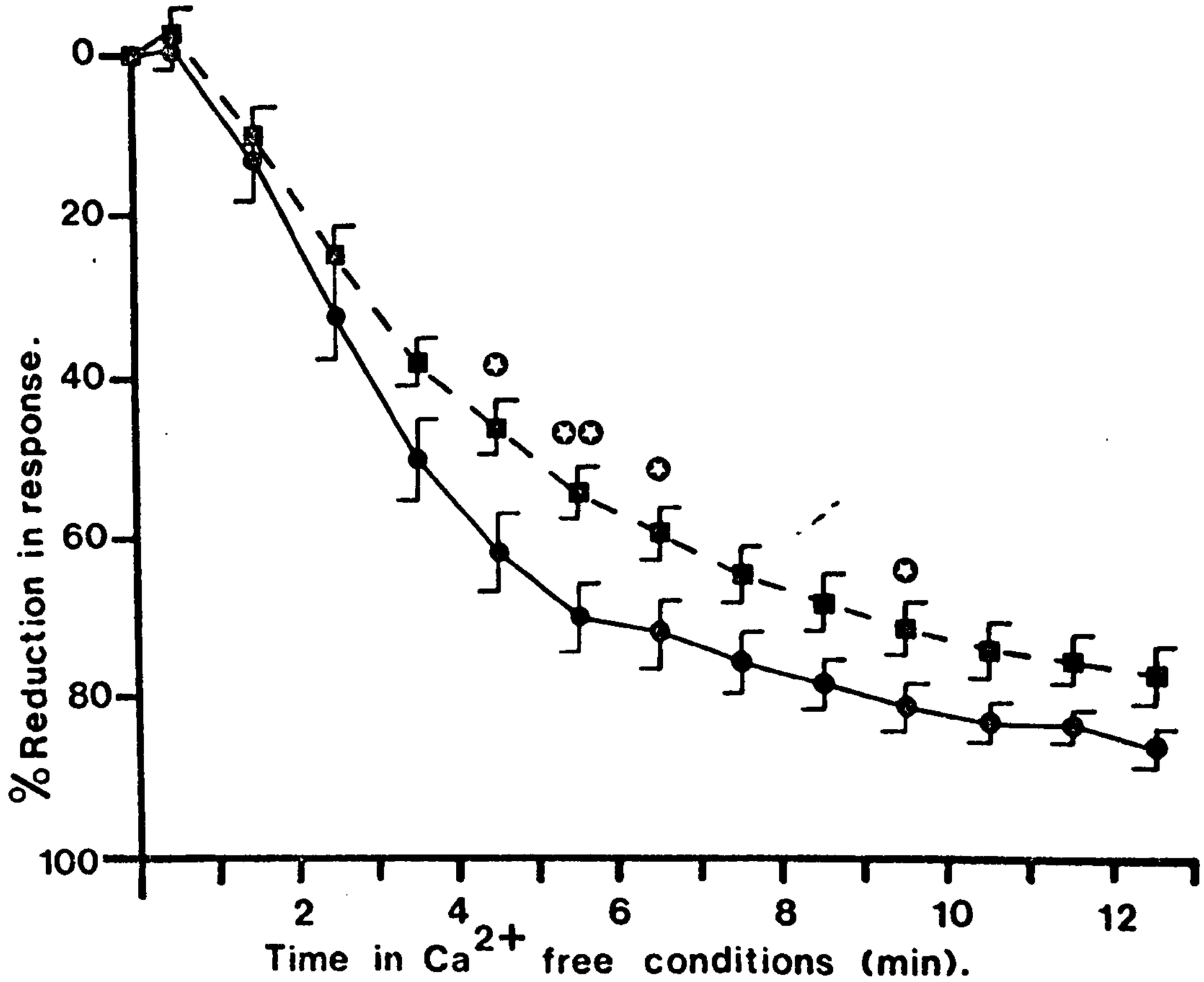


Fig. 4:3

The decay of noradrenaline (1 µg/ml) induced constrictor responses in calcium-free conditions, in mesenteric vasculature preparations from normotensive (●——●) and two week renal/salt hypertensive (■- - -■) rats. The decay is expressed as the % reduction from the original response amplitude.

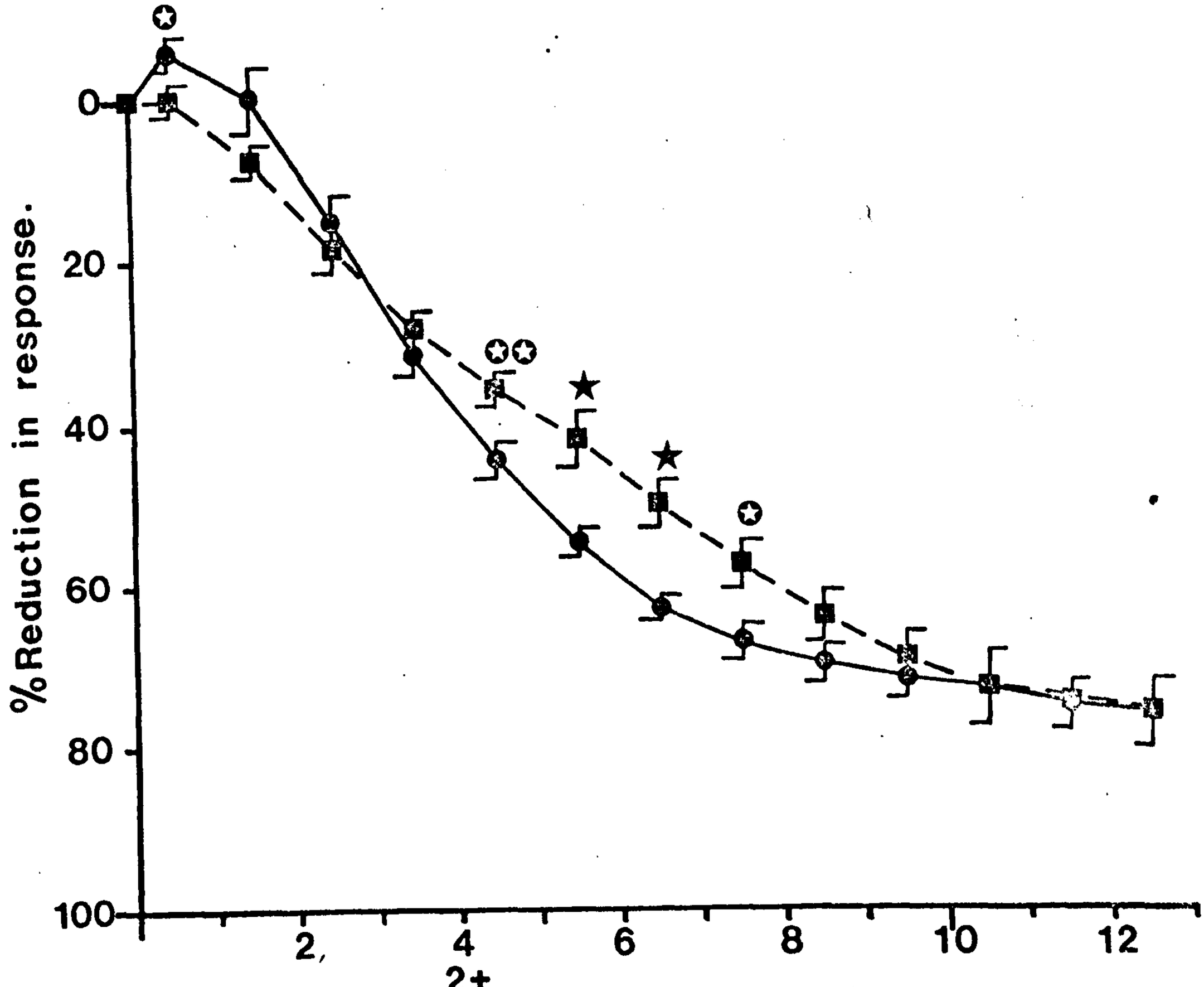


Fig. 4:4

The decay of noradrenaline (1 µg/ml) induced constrictor responses in calcium-free conditions in mesenteric vasculature preparations from normotensive rats (6 week control) in the absence (●——●) and presence (■– – –■) of angiotensin II amide 10^{-7}M. The decay is expressed as the % reduction from the original response amplitude.

d) DISCUSSION

The results of this study confirm the previous observation that there is a difference between the behaviour of mesenteric arterioles from rats at the 2 and 6 week stages of renal/salt hypertension. The near maximum noradrenaline constrictor responses evoked in 6WH tissues were significantly larger than controls (6WC), whereas the responses of 2WH and 2WC tissues were not significantly different. In contrast, the significant differences from controls of decay half time and slope in 2WH tissues were not observed in tissues from 6WH rats. Exposure of 2WH tissues to angiotensin II (2WHA) had no effect on the decay parameters, but tissues from normal (2WC, 6WC) and 6WH rats mimicked the behaviour of 2WH tissues after exposure to angiotensin II (2WCA, 6WCA, 6WHA).

This study was designed to investigate the effects of angiotensin II and hypertension on vascular calcium regulation. The decay of noradrenaline induced responses in calcium-free conditions was used as an indicator of calcium washout. The decay of noradrenaline evoked constrictor responses in Ca^{++} -free Krebs solution could have been due to distortion or disruption of the α-adrenoceptor (Tuttle and Moran, 1969). This is unlikely because complete calcium depletion was not achieved, since the contraction was not totally abolished. In addition, the noradrenaline response decay rate was mainly dependant on the intensity of stimulation rather than on the period of exposure to Ca^{++} -free Krebs solution. An inhibition of membrane bound enzyme systems was also unlikely, since vascular responses to noradrenaline and potassium chloride are known to decay at different rates in Ca^{++} -free conditions (Hinke, Wilson and Burnham, 1964). Removal of "activator" calcium from the mesenteric arterioles is the most likely explanation of the noradrenaline response decay.

A reduction in noradrenaline response decay slope or a prolongation of decay half time is therefore interpreted as indicative of an increased intracellular calcium concentration [Ca^{++}]. This could be achieved by an increased influx or a decreased efflux of the ion. The passive influx of calcium is determined by the concentration gradient and the permeability of the cell membrane (Rasmussen, 1970). Holloway and Bohr (1973), have suggested that the cell membrane of the femoral artery smooth muscle in the renal hypertensive rat is more labile than normal. This is thought to be unlikely in the early renal/salt hypertensive rat since mesenteric vasculature responses to potassium chloride have been shown to be normal (Chap. 3). An inhibition of the active efflux of Ca^{++} is the more probable explanation of the present results.

Grollman and Krishnamurty, (1973) have reported similar results using aortae from acute and chronic renal hypertensive rats. The slower rate of relaxation of strips from acute hypertensive rats was interpreted as indicating an increased amount of available calcium which could cause supersensitivity of the aorta. Aortic strips from chronic renal hypertensive rats exhibited little change in sensitivity or in the rate of relaxation. An increased intracellular [Ca^{++}] could be the cause of true vascular supersensitivity in the present study. Tissues which exhibited significantly reduced decay slopes and prolonged decay half times (2WH, 2WCA, 6WCA) have previously been shown to be super-sensitive to noradrenaline. Tissues from 6WH rats differed from controls by a small decrease in decay slope, and have a small degree of super-sensitivity (Chap. 3).

An increased intracellular [Ca^{++}] would also explain the difference in response profile between normal and hypertensive or

angiotensin-treated tissues. The early fast phase of arterial contraction to noradrenaline is thought to be due to the release of intracellular calcium, whereas the later slow phase is caused by extracellular calcium (Sitrin and Bohr, 1971). All hypertensive (2WH, 6WH) and angiotensin treated tissues (2WCA, 6WCA) responded more rapidly to noradrenaline than controls indicating a greater release of intracellular calcium. Only in 6WH tissues was the amplitude of the slow phase of the noradrenaline response significantly elevated (Table 4:1). If an adaptive structural change in 6WH tissues had occurred, as suggested by Folkow, Hallbäck, Lundgren and Weiss (1970 c), it would account for the greater amplitude of both phases of the noradrenaline response observed in the present study.

Angiotensin II prolonged the noradrenaline decay half time and reduced the decay slope. Several explanations could account for this action. Angiotensin II could stabilize the cell membrane so that Ca^{++}-free solutions would be less effective, but any stabilization would reduce reactivity. Angiotensin II might block the neuronal re-uptake of noradrenaline (Palaic and Khairallah, 1967), but this would have no effect in the present experiments since noradrenaline was in excess, and neuronal re-uptake is calcium dependant (George and Leach, 1973). Blockade of re-uptake by cocaine (Nicholas, 1970) or by desmethylimipramine at 10^{-6}M (preliminary observation, n=5) has no significant effect on the noradrenaline potentiating actions of angiotensin II. Inhibition of noradrenaline re-uptake by angiotensin II is therefore regarded as unlikely.

Daniels, Severs and Buckley (1967) have shown that angiotensin II stimulates the uptake of calcium into soft tissues. Long term infusion of angiotensin II increases the sodium and calcium content of dog arteries (Villamil, Nachev and Kleeman, 1970). Microsomal binding

of Ca^{++} is reduced by angiotensin II (Baudouin-Legros and Meyer, 1973) perhaps by reducing cyclic AMP levels (Angles D'Auriac, Baudouin and Meyer, 1972). These reports, together with the present data, support the hypothesis that angiotensin II reduces the active efflux of calcium.

The results of this study indicate that the vascular supersensitivity of renal/salt hypertension may involve an increase in available activator calcium. Angiotensin II also appears to stimulate this mechanism.

2) THE CHARACTERISTICS OF THE α-ADRENOCEPTOR IN MESENTERIC VASCULATURE PREPARATIONS FROM RENAL/SALT HYPERTENSIVE RATS 2 AND 6 WEEKS AFTER CONTRALATERAL NEPHRECTOMY

a) INTRODUCTION

There is no reported evidence of changes in the characteristics of the α-adrenoceptor in vessels from hypertensive humans or animals. The number of α-adrenoceptors, and the pA_2 and K_b values for phentolamine on these receptors, are normal in vessels from hypertensive humans and S.H.R. (Clineschmidt, Geller, Govier and Sjoerdsma, 1970., Janis and Triggle, 1972., Horwitz, Clineschmidt, Van Buren and Ommaya, 1974).

In the present study, phentolamine and indoramin were used to investigate the characteristics of the α-adrenoceptors in mesenteric vasculature preparations from renal/salt hypertensive rats. Initial studies with indoramin revealed a difference between the pA_2 value determined on the mesenteric vasculature preparation from the normotensive rat and the value previously reported for the guinea-pig aortic spiral preparation (Alps, Hill, Johnson and Wilson, 1972). This unusual action of indoramin was investigated further using the guinea-pig mesenteric vasculature preparation and the rat aortic spiral.

b) METHODS

The measurement of conscious systolic blood pressure and the induction of renal/salt hypertension have been described (Chap. 2).

i) The isolated rat mesenteric vasculature preparation

The isolated rat mesenteric vasculature preparation has been described (Chap. 2). Preparations from normotensive and renal/salt hypertensive rats 2 and 6 weeks after contralateral nephrectomy were examined.

ii) The normotensive guinea-pig isolated perfused mesenteric vasculature preparation

Male guinea-pigs (500-750 g) were used, the general technique was similar to that described for the rat mesenteric preparation. The guinea-pigs were more difficult to anaesthetize with intraperitoneal pentobarbitone sodium than rats. The technique of intra-thoracic injection of the anaesthetic (30 mg/kg) proved to be efficient. A perfusion fluid flow rate of 2.6 ml/min and a perfusion cannula with a terminal o.d. of 1.02 mm were used. The perfusion rate and cannula size were necessarily larger than those used for the rat.

iii) The normotensive rat or guinea-pig isolated aortic spiral preparation (Furchgott and Bhadrakom, 1953)

Experiments on aortic spirals were made using Krebs solution at 37° and bubbled with 5% carbon dioxide in oxygen. Organ bath volumes of 38 ml were used and ascorbic acid (10^{-4}M) was added to prevent oxidation of noradrenaline. This concentration of ascorbic acid was without effect on the contractile mechanism of the tissues used.

Contractions of the thoracic aorta were recorded by a variable inductance transducer (0.5 g load) connected to a phase discriminator and pen recorder. Cumulative dose-response curves were obtained for nor-

adrenaline, added to the bath every 5 min.

iv) Experimental Procedure

When the tissue had reached full sensitivity to noradrenaline, a control dose-response curve was made. The tissue was exposed to the antagonist drug phentolamine (5×10^{-8} - 10^{-5}M) or indoramin (5×10^{-8} - 10^{-5}M) until antagonist equilibrium was achieved i.e. when constant agonist responses were evoked. The noradrenaline dose-response curve was repeated in the presence of the antagonist. Usually, three concentrations of antagonist (in order of increasing concentration) were tested on each tissue.

v) Analysis of results

In each experiment, the mean dose-ratio was calculated from measurements taken at points approximating to $ED_{30\%}$, $ED_{50\%}$ and $ED_{70\%}$ on the log dose-response lines obtained in the presence and absence of the antagonist (Gaddum, Hameed, Hathway and Stephens, 1955). This ratio was subjected to the analysis described by Arunlakshana and Schild (1959) and the pA_2 values were determined graphically (Fig. 4:5).

The apparent dissociation constants of the antagonists from the α-adrenoceptors (K_b) were calculated from the formula:-

$$K_b = \frac{\text{Molar concentration of antagonist}}{\text{Agonist dose-ratio} - 1}$$

$$\text{or } K_b = \frac{B}{(x-1)} \text{ mol/l.}$$

The noradrenaline dose-response curves made on mesenteric vasculature preparations from renal/salt hypertensive and normotensive rats, used for pA_2 determinations, were compared and the reactivity and sensitivity shifts calculated as previously described (Chap. 3).

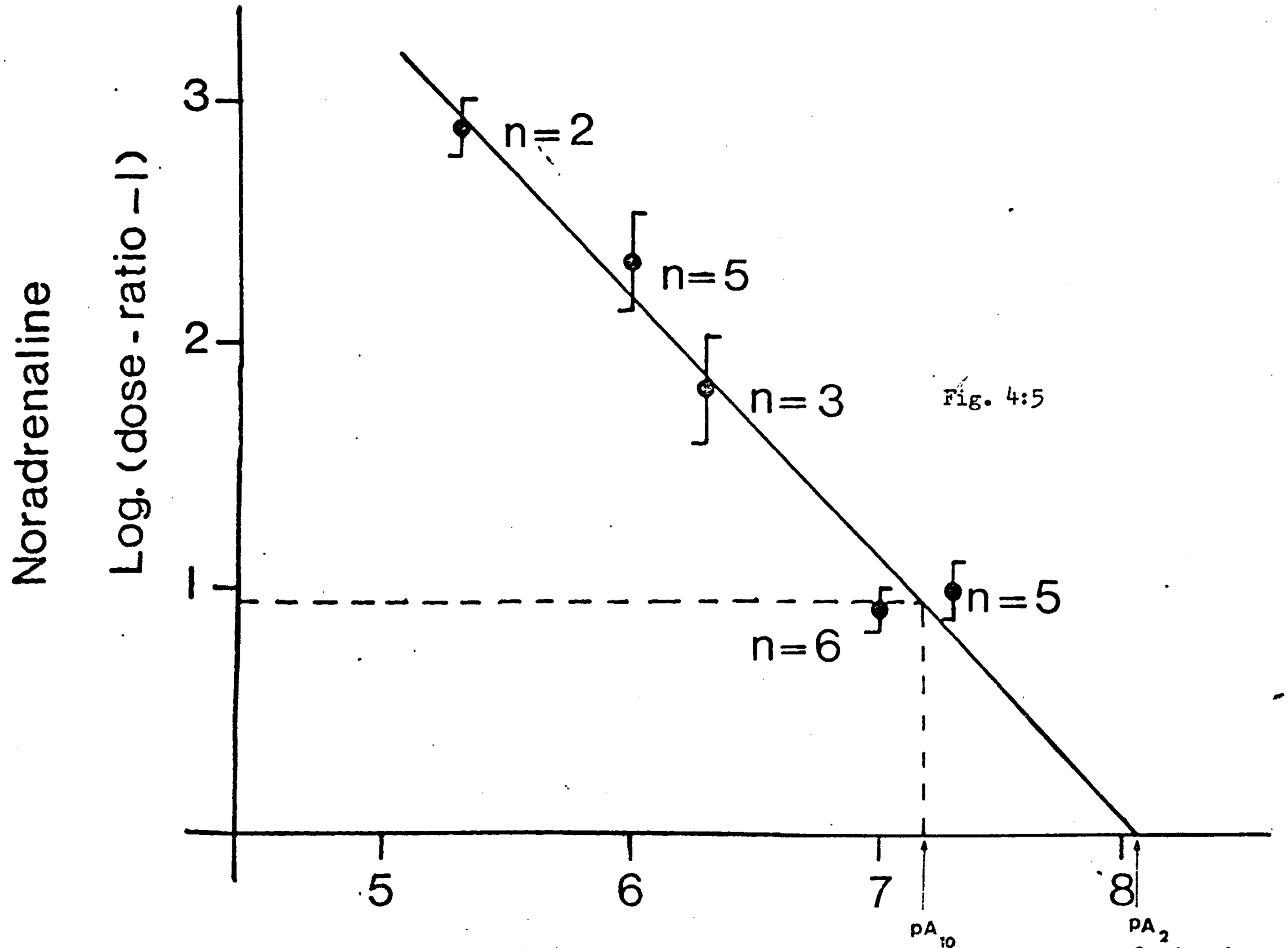

Noradrenaline
Log. (dose-ratio-1)
3
2
1
n=2
n=5
n=3
n=5
n=6
5
6
7
8
pA10
pA2
Negative log. molar concentration of indoramin.

Fig. 4:5

Fig. 4:5

The relation between the dose-ratios of noradrenaline and concentrations of indoramin hydrochloride on the mesenteric vasculature preparation of the normotensive rat, plotted by the method of Arunlakshana and Schild (1959). A calculated regression line was fitted to 21 points each of which represents a mean dose-ratio for one experiment. The slope function of the line was - 1.06, which is close to the theoretical value of unity for competitive antagonism. The line intersects the abscissa at the pA_2 value of 8.05, the pA_{10} value (where log dose-ratio -1 = 0.954) is 7.15.

c) RESULTS

Using the calculated mean dose-ratio (x) of noradrenaline from each experiment, the log. (x-1) was plotted against the negative log.of the molar concentration of the antagonist. The calculated regression line intersected the abscissa at the pA_2 value (Fig. 4:5).

i) The pA_2 values for indoramin and phentolamine on tissues from normotensive rats and guinea-pigs

The pA_2 value for indoramin of 8.05 on the normotensive rat mesenteric vasculature preparation was higher than the previously reported value on the guinea-pig aorta (7.38; Alps, Hill, Johnson and Wilson, 1972). The results of Arunlakshana and Schild (1959) analyses of phentolamine and indoramin on rat and guinea-pig mesenteric vasculature preparations and aortic spirals are given in Table 4:3.

The pA_2 results indicated that indoramin was a more potent α-adrenoceptor blocking agent on the perfused mesenteric vasculature preparation than on the aorta, irrespective of the species used. In order to determine whether this difference was statistically significant, apparent K_b values were calculated, and are given in Table 4:4.

Indoramin at 10^{-5}M was found to relax the rat aorta (n=2), but the height of the maximum noradrenaline induced contraction was unchanged. This result suggests a direct vasodilator action of indoramin at high concentration, perhaps because of its local anaesthetic activity (Alps, Hill, Johnson and Wilson, 1972). Phentolamine has been reported to have a direct action on blood vessels (Taylor, Sutherland, Mackenzie, Staunton and Donald, 1965) but in the experiments described in the present study, concentrations up to 10^{-5}M did not cause any relaxation of the rat aorta.

Table 4:3

The results of Arunlakshana and Schild analyses of the α-block pA_2 values for indoramin & phentolamine on the perfused mesenteric vessels of the rat and guinea-pig and the rat and guinea-pig isolated aortic spiral preparations

Drug	Tissue	Concentration used M.	n	slope	pA_2	pA_{10}	pA_2-pA_{10}
Indoramin	Mesenteric vasculature, rat	$5x10^{-8}$ - $5x10^{-6}$	21	-1.06	8.05	7.15	0.9
Phentolamine		$5x10^{-8}$ - 10^{-5}	14	-0.91	7.84	6.80	1.04
Indoramin	Mesenteric vasculature, guinea-pig	10^{-8} - $5x10^{-6}$	17	-1.03	8.48	7.55	0.93
Phentolamine		10^{-7} - 10^{-5}	13	-0.93	7.51	6.49	1.02
Indoramin	Aortic spiral, rat	10^{-7} - 10^{-5}	13	-0.85	7.68	6.56	1.13
Phentolamine		10^{-7} - 10^{-5}	15	-0.91	8.29	7.25	1.04
Indoramin	Aortic spiral, guinea-pig	10^{-7} - 10^{-4}	16	-1.16	7.38	6.58	0.80
Phentolamine		10^{-7} - 10^{-5}	14	-0.90	7.64	6.58	1.06

Note. The results for indoramin on the guinea-pig aorta were obtained from Alps, Hill, Johnson and Wilson (1972). The mean log (dose-ratio-1) values for each antagonist on each tissue lie on their respective regression lines.

Table 4:4

Apparent K_b values x 10^{-9} moles/l for indoramin and phentolamine on the aorta and mesenteric vessels of the rat and guinea pig. Students' t-test significance level values are given for each drug between tissues of the same species.

Drug	Guinea-pig		Rat	
	Aorta	Mesentery	Aorta	Mesentery
Indoramin K_b	40.7 ± 11.5	4.0 ± 0.9	44.2 ± 7.3	9.1 ± 1.4
Significance level	<0.01		<0.001	
Phentolamine K_b	36.4 ± 4.1	45.3 ± 7.3	9.8 ± 1.6	26.0 ± 3.5
Significance level	Not significant		<0.001	

ii) The pA_2 values for indoramin and phentolamine on mesenteric vasculature preparations from renal/salt hypertensive rats

The mean noradrenaline dose-response curves from the mesenteric vasculature preparations from renal/salt hypertensive and normotensive rats used to determine indoramin and phentolamine pA_2 values are given in Fig. 4:6. The mean $ED_{50\%}$ dose, sensitivity and reactivity shifts of the "hypertensive" preparations are given in Table 4:5. Body weights and systolic blood pressures are given in Table 4:6.

The results of Arunlakshana and Schild (1959) analyses of indoramin and phentolamine on rat mesenteric vasculature preparations from renal/salt hypertensive rats are given in Table 4:7 and K_b values in Table 4:6.

It is evident that the difference in potency between indoramin and phentolamine on tissues from normotensive animals does not occur in tissues from hypertensive rats.

d) DISCUSSION

Indoramin and phentolamine were competitive α-adrenoceptor blocking agents on all the tissues used, as judged by the slope of the log.x-1/log.B lines approximating to unity and the pA_2-pA_{10} values approximating to 0.95.

The pA_2 values determined on tissues from normotensive animals indicate that indoramin is a more potent α-adrenoceptor blocking agent on the mesenteric vasculature than on the aorta, irrespective of species. The significant differences between the apparent K_b values on the aorta and the mesenteric vasculature must reflect either a different α-adrenoceptor type in the mesenteric vasculature which has greater affinity for indoramin than for phentolamine, or that some action of

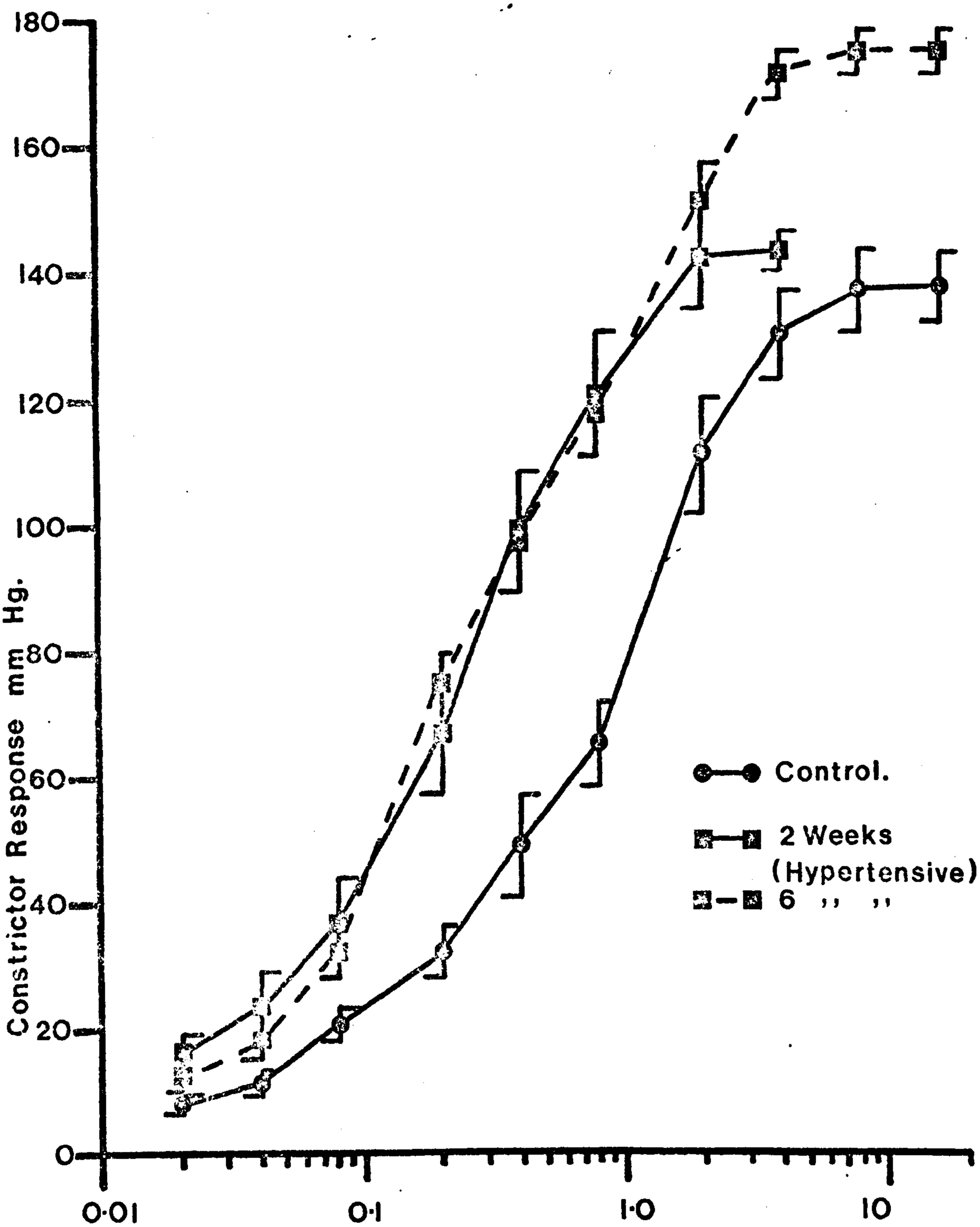


Fig. 4:6

Mean noradrenaline dose-response curves of the mesenteric vasculature preparations from normotensive (n=13), 2 week renal/salt hypertensive (n=10) and 6 week renal/salt hypertensive rats (n=10) used for the determination of α-adrenoceptor blocking pA_2 values of indoramin and phentolamine.

Table 4:5

The mean $ED_{50\%}$ dose, sensitivity and reactivity shifts for tissues from renal/salt hypertensive rats 2 and 6 weeks after contralateral nephrectomy and normotensive controls

Group	$ED_{50\%}$ dose µg	50% hypertensive response amplitude	Mean dose to evoke 50% hypertensive response amplitude in control tissues	Sensitivity Shift	Reactivity Shift
Normotensive Control	1.03 ± 0.15	-	-	-	-
2 week renal/salt	0.29 ± 0.04*	75 mmHg	1.04 ± 0.14	5.3 ± 0.8*	4.8 ± 0.4*
6 week renal/salt	0.35 ± 0.06*	87.5 mmHg	1.71 ± 0.23	3.7 ± 0.24*	6.2 ± 0.4*

* Significantly different from control.

Table 4:6

Mean body weight and systolic blood pressure of normotensive and renal/salt hypertensive rats, 2 and 6 weeks after nephrectomy. Apparent K_b values x 10^{-9} moles/l for indoramin and phentolamine on the mesenteric vasculature preparations from these animals are also given

Group	Body Wt. g.	Blood pressure mmHg	K_b Indoramin	K_b Phentolamine	n
Control	262.0 ± 4.5	120.8 ± 3.3	9.1 ± 1.4	26.0 ± 3.5	13
2 week renal/salt hypertensive	217.2 ± 6.7*	163.6 ± 2.6*	37.4 ± 11.1* (<0.02)	20.6 ± 2.3	10
6 week renal/salt hypertensive	275.1 ± 11.3	174.1 ± 5.1*	23.4 ± 4.6* (<0.01)	20.4 ± 3.1	10

* Significantly different from normotensive control

Table 4:7

The results of Arunlakshana and Schild analyses of the α block pA_2 values for indoramin and phentolamine on the mesenteric vasculature preparation from renal/salt hypertensive rats 2 and 6 weeks after nephrectomy

Drug	Tissue	Concentration M	n	slope	pA_2	pA_{10}	pA_2-pA_{10}
Indoramin	2 week renal/salt	$5x10^{-8}$ - $5x10^{-6}$	17	-1.06	7.58	6.68	0.9
Phentolamine		$5x10^{-8}$ - $5x10^{-6}$	12	-1.02	7.68	6.74	0.94
Indoramin	6 week renal/salt	$5x10^{-8}$ - $5x10^{-6}$	13	-1.07	7.75	6.86	0.89
Phentolamine		$5x10^{-8}$ - $5x10^{-6}$	15	-0.99	7.76	6.80	0.96

indoramin, unrelated to α-adrenoceptor blockade, renders it more potent on resistance vessels.

With phentolamine, there was no significant difference between apparent K_b values on the guinea-pig aorta and mesenteric vasculature preparation. The apparent K_b value for phentolamine on the rat aorta was significantly different from the value on the rat mesentery. Phentolamine therefore appears to be more potent on the aorta than on the mesenteric vasculature from the rat.

On mesenteric vasculature preparations from 2 week renal/salt hypertensive rats which were supersensitive (sensitivity shift ≡ reactivity shift) the pA_2 value of indoramin was similar to its value on aortic preparations. At the 6 week hypertensive stage (sensitivity shift < reactivity shift) the significant potency difference from the normotensive result was still evident but was reduced. The α-adrenoceptor blocking potency of phentolamine was normal on mesenteric vasculature preparations from hypertensive rats.

Thus, indoramin appeared to reveal a change in the α-adrenoceptor of the renal/salt hypertensive rat which was not shown by phentolamine. The results could indicate that a subtle change in the α-adrenoceptor does occur in supersensitive mesenteric vascular preparations from renal/salt hypertensive rats. An alternative explanation is that the lower pA_2 value for indoramin on the mesenteric vasculature preparation from hypertensive rats is caused by the local anaesthetic activity of indoramin and the method used to measure response amplitude.

There is evidence that the cell membranes of small blood vessels are more labile than those of larger arteries (Somlyo and Somlyo, 1968). Indoramin is 3-4 times more potent than phentolamine as a local anaesthetic (preliminary observations n=5, guinea-pig weal test method,

Bulbring and Wajda, 1945). The mesenteric vasculature preparation from hypertensive rats responds in a biphasic manner when infused with noradrenaline (Fig. 4:1). When a bolus of noradrenaline is injected into the mesenteric vasculature preparation the slow and fast phases of the response cannot be separated and the peak overall response amplitude is measured. In tissues from normotensive rats the peak amplitude will probably occur in the slow phase of the response, which is thought to be due to the entry of extracellular calcium (Sitrin and Bohr, 1971). In supersensitive tissues from hypertensive rats the peak amplitude will probably occur in the fast phase of the response, (Fig. 4:1) which is thought to be caused by the release of intracellular calcium (Sitrin and Bohr, 1971). Both phases of the response are mediated by α-adrenoceptors and are susceptible to blockade. The slow phase of the response is probably determined by membrane permeability and would therefore be susceptible to local anaesthesia. Both phentolamine and indoramin are α-adrenoceptor blocking agents but indoramin is also a local anaesthetic (Alps, Hill, Johnson and Wilson, 1972). Therefore, on tissues which are susceptible to local anaesthesia and whose responses are evaluated in the slow phase (i.e. the mesenteric vasculature preparation of the normotensive rat and guinea-pig) indoramin will appear more potent than on tissues which are less susceptible to local anaesthesia (i.e. the rat and guinea-pig aorta). However, on tissues which are supersensitive, and whose responses are evaluated in the fast phase, which is resistant to local anaesthesia, (mesenteric vasculature preparations from hypertensive rats) indoramin will only exhibit its α-blocking activity and the pA_2 value will be the same as that determined in local anaesthetic resistant preparations (i.e. the aorta). This explanation is dependant on the assumption that the local anaesthetic action of indoramin exhibits competitive kinetics.

The results of this investigation on the mechanism of vascular supersensitivity suggest that an increase in available activator calcium is probably involved. Changes in the characteristics of the α-adrenoceptor are unlikely as the pA_2 value of phentolamine did not change. The differences in the pA_2 value of indoramin in tissues from hypertensive rats may reflect the unusual properties of this drug.

CHAPTER 5

FACTORS WHICH MIGHT STIMULATE VASCULAR SUPERSENSITIVITY IN MESENTERIC VASCULATURE PREPARATIONS FROM RENAL AND RENAL/SALT HYPERTENSIVE RATS

INTRODUCTION

Increased vascular sensitivity has been demonstrated in mesenteric vasculature preparations from renal/salt hypertensive rats (Chap 3). The mechanism of this supersensitivity might involve a change in cellular calcium regulation (Chap 4). This chapter describes experiments designed to investigate the factors which might stimulate the mechanism causing vascular supersensitivity.

Two factors are considered, the effects of sodium-loading and of endogenous angiotensin II. There is evidence that sodium-loading can increase vascular reactivity (Vick, Ederstrom and Vergeer, 1956., Beilin, Wade, Honour and Cole, 1970., Vapaatalo, Lahovaara and Hackman, 1970., Heistad, Abboud and Ballard, 1971., Harris and Palmer, 1972., Abboud, 1974). The renal/salt hypertensive rat is likely to be in positive sodium balance from an early stage because of the increased dietary sodium. In the renal hypertensive rat, with one remaining kidney, there is evidence of a positive sodium balance independant of dietary sodium-loading, (Tobian, Coffee and McCrea, 1969., Swales, Thurston, Queiroz and Medina, 1972., Menard, Alexandre, Guidicelli, Auzan and Chevillard, 1973).

Previous studies have demonstrated that exogenous angiotensin II can stimulate the process causing supersensitivity (Chaps 3 & 4). The aim of the present investigation was to determine whether elevated endogenous angiotensin II could have caused the supersensitivity of isolated vascular preparations from renal/salt hypertensive rats.

1) STUDIES ON REACTIVITY IN THE PERFUSED MESENTERIC VASCULATURE PREPARATION FROM RENAL HYPERTENSIVE AND UNINEPHRECTOMIZED SALT-LOADED RATS

a) INTRODUCTION

Vascular reactivity is increased in mesenteric vasculature preparations from renal/salt hypertensive rats (Chap 3). Salt-loading these animals might affect vascular reactivity by, 1) an increase in vascular sensitivity per se, 2) an inhibition of the renin-angiotensin system (Gross, Brunner and Ziegler, 1965) which may be involved in vascular reactivity (Chap 3), 3) an increase in the vascular receptor affinity for angiotensin II (Brunner, Chang, Wallach, Sealey and Laragh, 1972).

Vascular reactivity was therefore studied in renal hypertensive rats without salt-loading and in normotensive uninephrectomized rats which were salt-loaded.

b) METHODS

i) Renal hypertensive rats

Renal hypertensive and sham operated rats were prepared as described in Chap. 2.

ii) Uninephrectomized salt-loaded rats

Female Charles River rats were uninephrectomized and given 0.9% saline to drink. After 2 weeks of sodium-loading, the animals were killed and their mesenteric vasculatures perfused.

The method of recording systolic blood pressure in conscious rats and the perfused mesenteric vasculature preparation have been described (Chap 2). The experimental procedure and analysis of results were as described in Chap 3. Dose-response curves to noradrenaline and

the influence of angiotensin II on noradrenaline responses were investigated.

c) RESULTS

i) Studies of vascular reactivity to noradrenaline in mesenteric preparations from renal hypertensive rats

The mean body weights and systolic blood pressures of the renal hypertensive and sham operated rats are given in Table 5:1.

Temporal changes in vascular reactivity

The noradrenaline dose-response curves of preparations from renal hypertensive rats were shifted to the left of the control curve at all time points investigated (Fig. 5:1-4). At all the hypertensive stages (1, 2, 4 and 6 weeks after nephrectomy) the noradrenaline threshold dose was decreased, and at the 6 week stage, the maximum response was elevated (Table 5:2, Fig. 5:4). The regression line gradients of the noradrenaline dose-response curves of tissues from hypertensive rats were not different from control values (Table 5:2). The reactivity shifts of the "hypertensive" dose-response curves were calculated, and are given in Table 5:3.

Temporal changes in vascular sensitivity

The noradrenaline dose-response curves for tissues from renal hypertensive and sham operated rats, expressed as a percentage of the maximum response, are shown in Figs. 5:5-8. All the "hypertensive" dose-response curves were shifted to the left of the control, but in contrast to the reactivity shifts (Fig. 5:1-4, Table 5:3) the sensitivity shifts declined at the 6 week hypertensive stage (Table 5:4).

Table 5:1

The mean body weights and blood pressures of renal hypertensive and sham operated rats (*indicates significantly different from sham control).

Weeks after operation	Sham Control			Renal hypertensive		
	Body Wt. g.	B.P. mmHg.	n	Body Wt. g.	B.P. mmHg.	n
1	210.6±5.7	111.4± 5.2	5	183.0±9.1*	142.7± 1.5*	5
2	218.4±4.0	117.0± 6.2	5	197.0±21.8	162.6±9.7*	5
4	263.0±8.1	114.5± 5.2	7	246.8± 9.5	173.2±13.4*	5
6	263.0±8.1	114.5±5.2	7	251.2±17.7	163.4± 6.0*	5

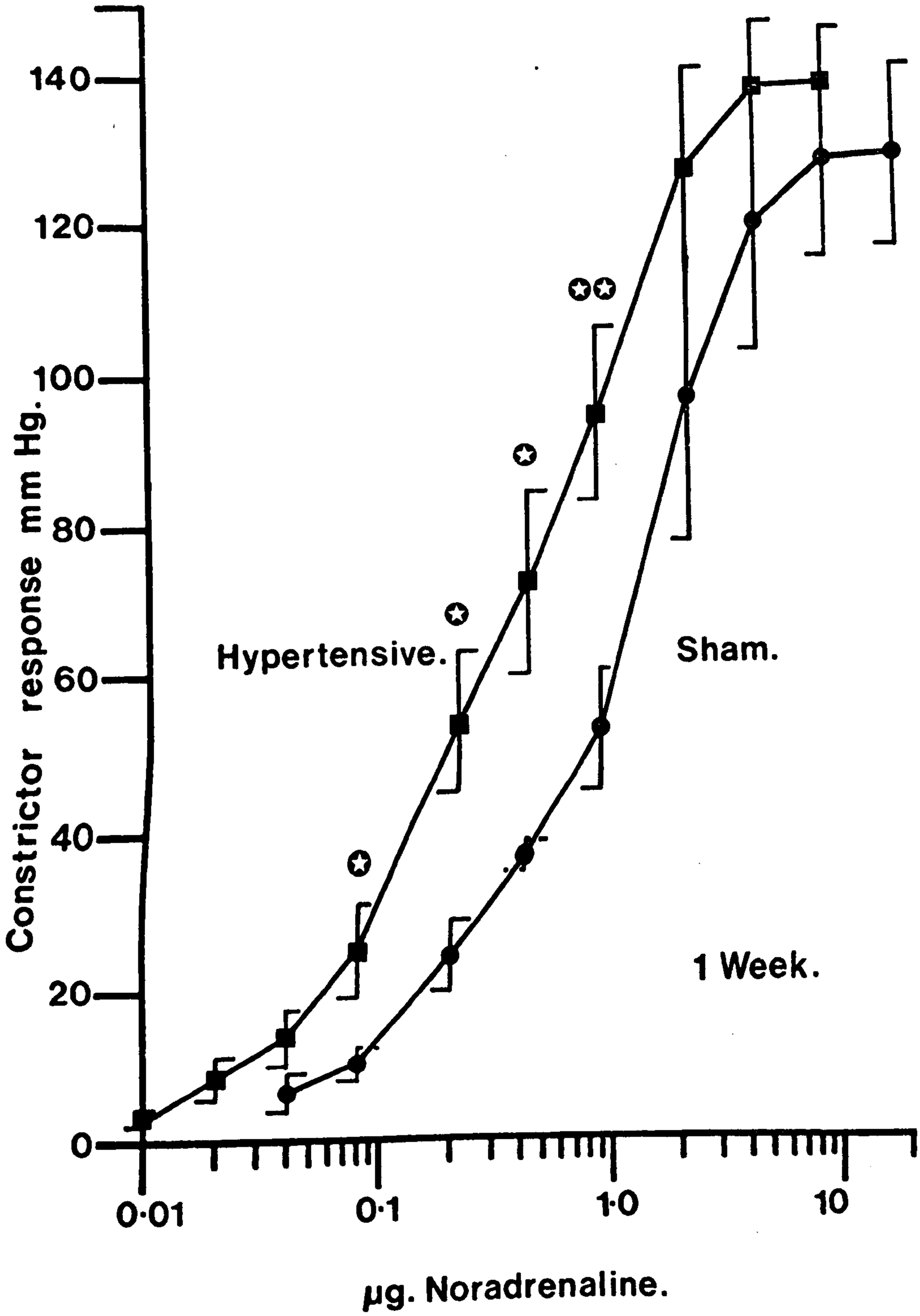


Fig. 5:1

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=5) and renal hypertensive rats (n=5) 1 week post-operatively. Note the shift to the left and lower vasoconstrictor threshold of the "hypertensive" curve.

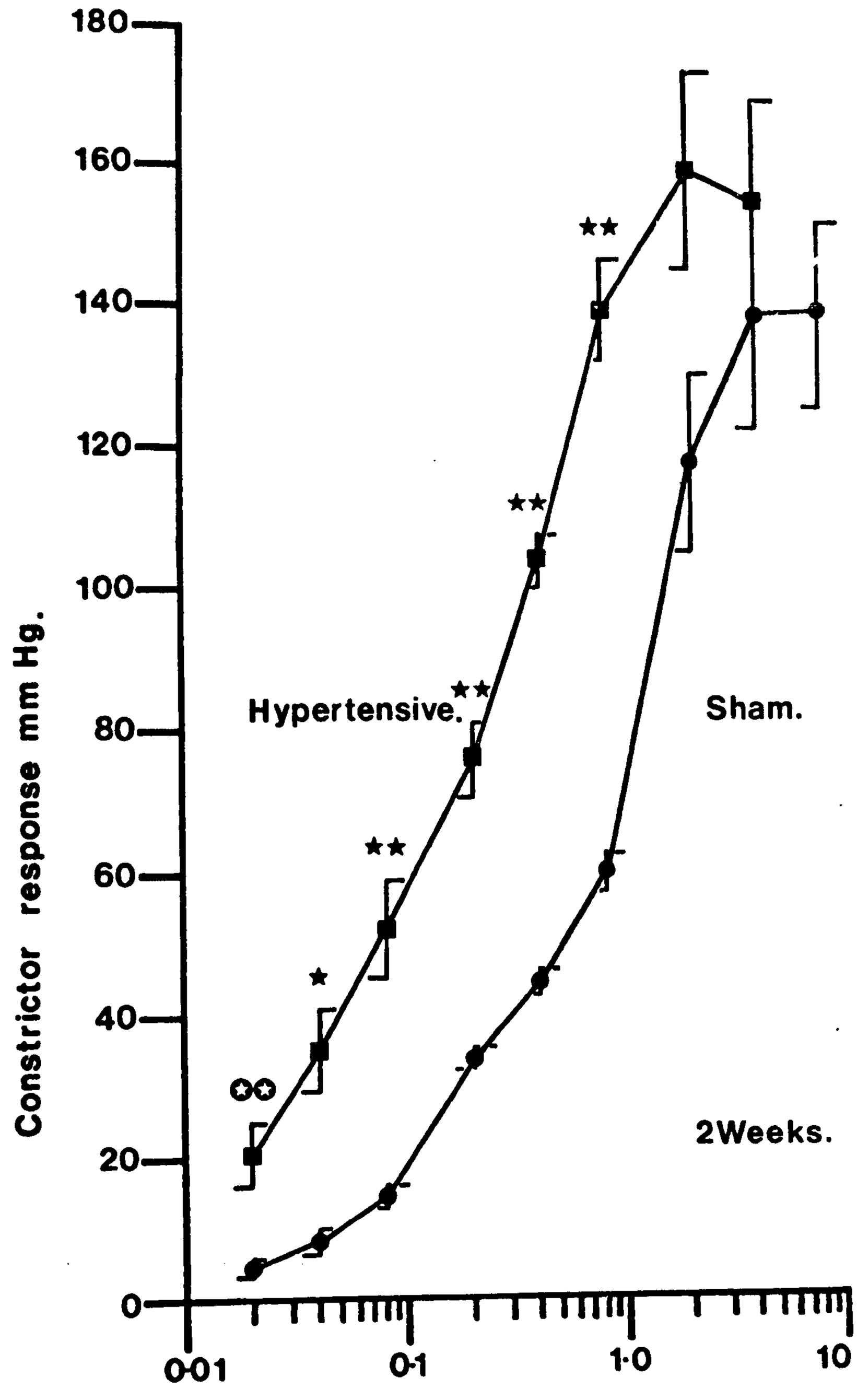


Fig. 5:2

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=5) and renal hypertensive rats (n=5) 2 weeks post-operatively. Note the shift to the left of the "hypertensive" curve.

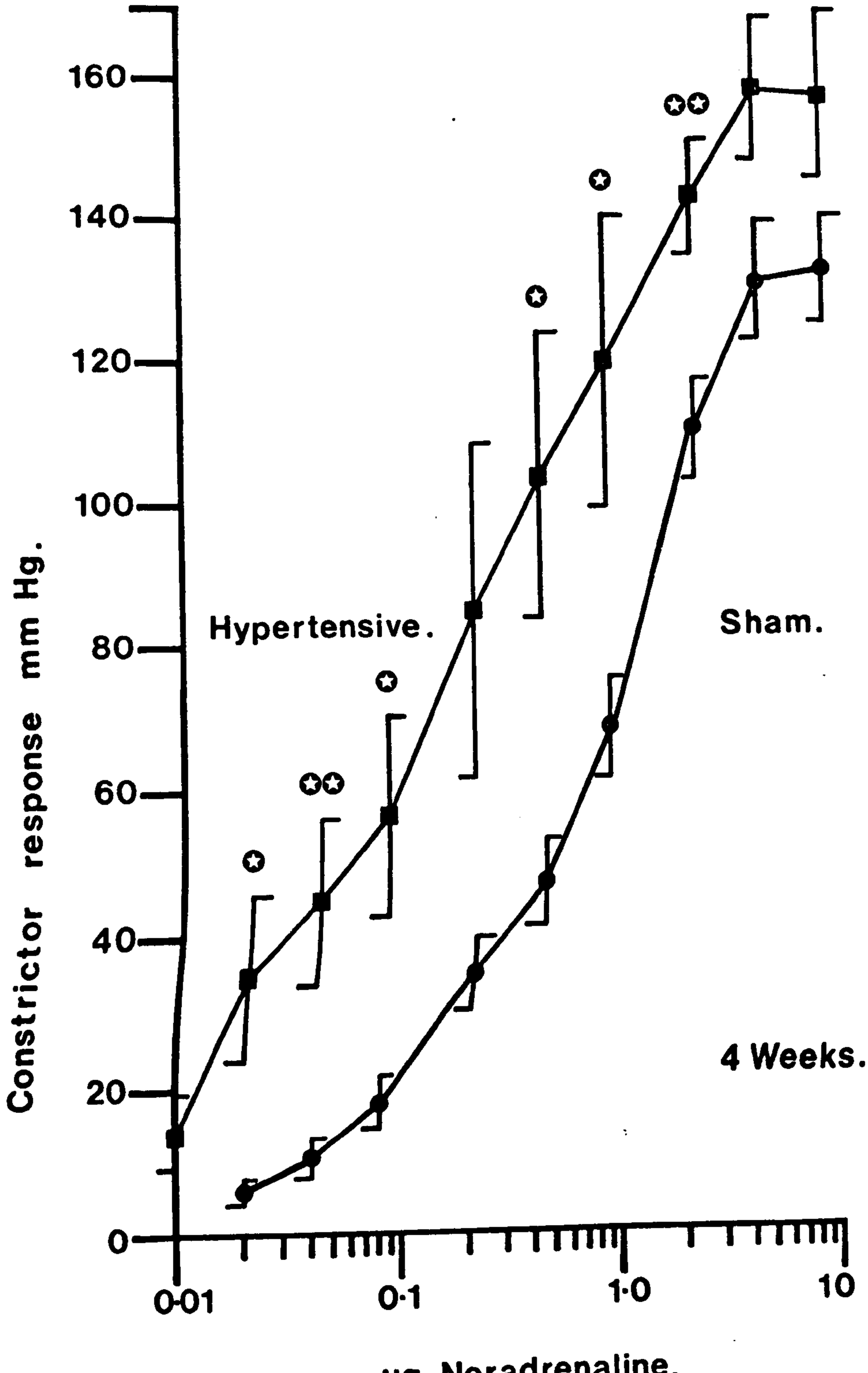


Fig. 5:3

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=7) and renal hypertensive rats (n=5), 4 weeks post-operatively. Note the shift to the left and the elevated maximum of the "hypertensive" curve.

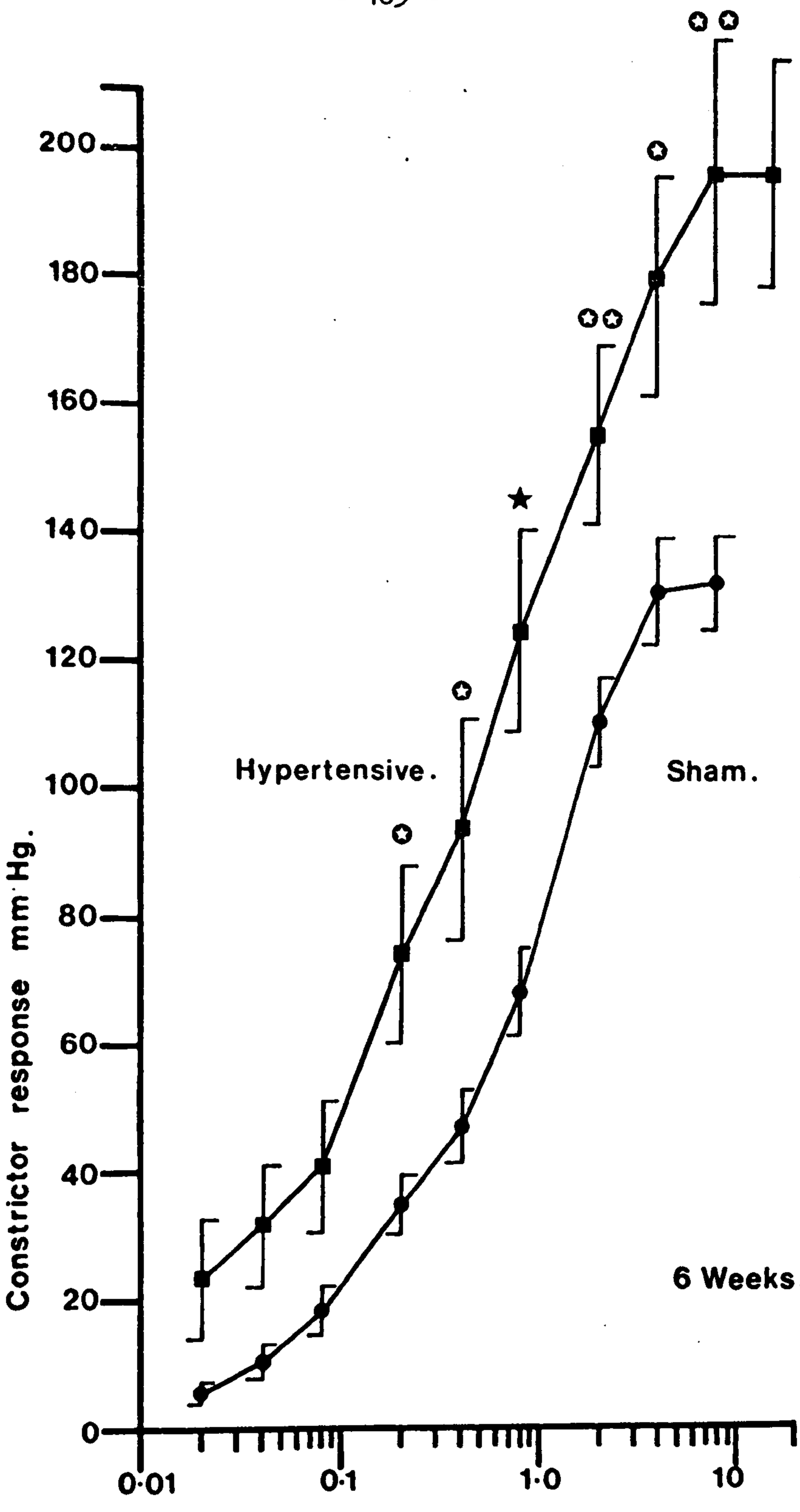


Fig. 5:4

Mean constrictor responses (mmHg) to noradrenaline of mesenteric vasculature preparations from sham control (n=7) and renal hypertensive rats (n=5), 6 weeks post-operatively. Note the shift to the left and elevated maximum of the "hypertensive" curve.

Table 5:2

Regression line data, threshold dose and maximum response of mesenteric preparations from renal hypertensive and sham operated rats. * Indicates significantly different from sham control

Group	Regression Line Data						Threshold dose µg.	Max. Response mmHg.
	Correlation Coefficient	Mean dose µg.	Mean response mm.Hg.	Gradient ± Sd.	Intercept	Degrees of freedom		
SHAM								
1 week	0.83	0.696	44.1	42.4±6.0	14.6	23	0.032±0.005	129.4±13.6
2 "	0.94	0.696	54.2	49.9±3.8	19.5	23	0.024±0.004	139.2±16.0
4-6 weeks	0.89	0.696	55.8	44.9±3.8	24.6	33	0.018±0.002	132.7± 7.9
Renal Hypertensive								
1 week	0.78	0.696	74.8	46.7±7.7	42.4	23	0.012±0.002*	139.0± 8.9
2 "	0.82	0.696	106.2	50.4±7.3	71.2	23	0.0074±0.0016*	155.2±15.0
4 "	0.61	0.696	103.7	43.3±11.8	73.5	23	0.004±0.0007*	158.8±10.4
6 "	0.74	0.696	97.9	52.0± 9.9	61.7	23	0.01 ±0.0027*	198.4±21.0*

Table 5:3

The mean noradrenaline reactivity shifts of mesenteric vasculature preparations from renal hypertensive rats. (* Indicates significantly different from sham operated control).

Weeks after operation	50% hypertensive response amplitude	Sham Mean dose µg. †	Renal hyper'. Mean dose µg. †	Reactivity Shift
1	69.5 mmHg	1.59±0.4	0.42±0.09*	5.16±0.77*
2	80.0 mmHg	1.17±0.07	0.25±0.08*	8.19±1.21*
4	79.5 mmHg	1.18±0.16	0.28±0.08*	7.19±1.31*
6	99.0 mmHg	1.80±0.31	0.42±0.08*	5.25±0.61*

Table 5:4

Mean noradrenaline $ED_{50\%}$ doses for tissues from sham operated and renal hypertensive rats and renal hypertensive sensitivity shifts (* Indicates significantly different from control), sensitivity shifts for tissues from renal/salt hypertensive rats (Chap 3) are included for comparison

Weeks after operation	Sham control $ED_{50\%}$ dose	Renal hypertensive $ED_{50\%}$ dose	Renal hypertensive sensitivity shift	Renal/salt sensitivity shift
1	1.28 ± 0.26	0.42 ± 0.09*	3.9 ± 0.6 *	7.45 ± 0.82
2	0.95 ± 0.07	0.25 ± 0.08*	6.6 ± 1.0 *	7.9 ± 0.81
4	0.74 ± 0.09	0.28 ± 0.08*	5.0 ± 0.8 *	4.18 ± 0.26
6	0.74 ± 0.09	0.42 ± 0.08*	2.2 ± 0.2 *	3.1 ± 0.20

† (Mean dose indicates the dose to evoke a 50% "hypertensive" response amplitude in individual sham control tissues).

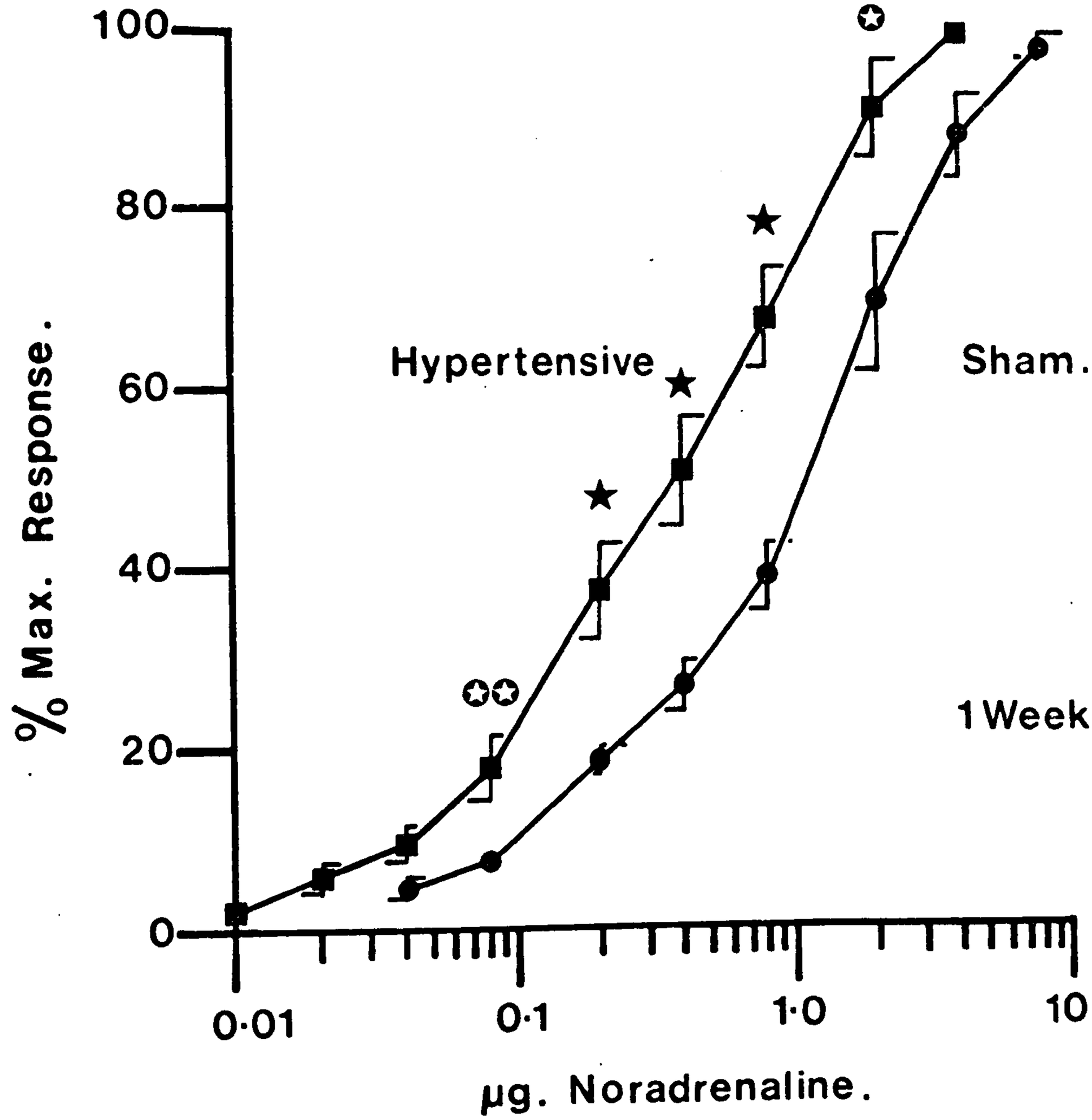


Fig. 5:5

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=5) and renal hypertensive rats (n=5) one week post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is similar to the shift in Fig. 5:1.

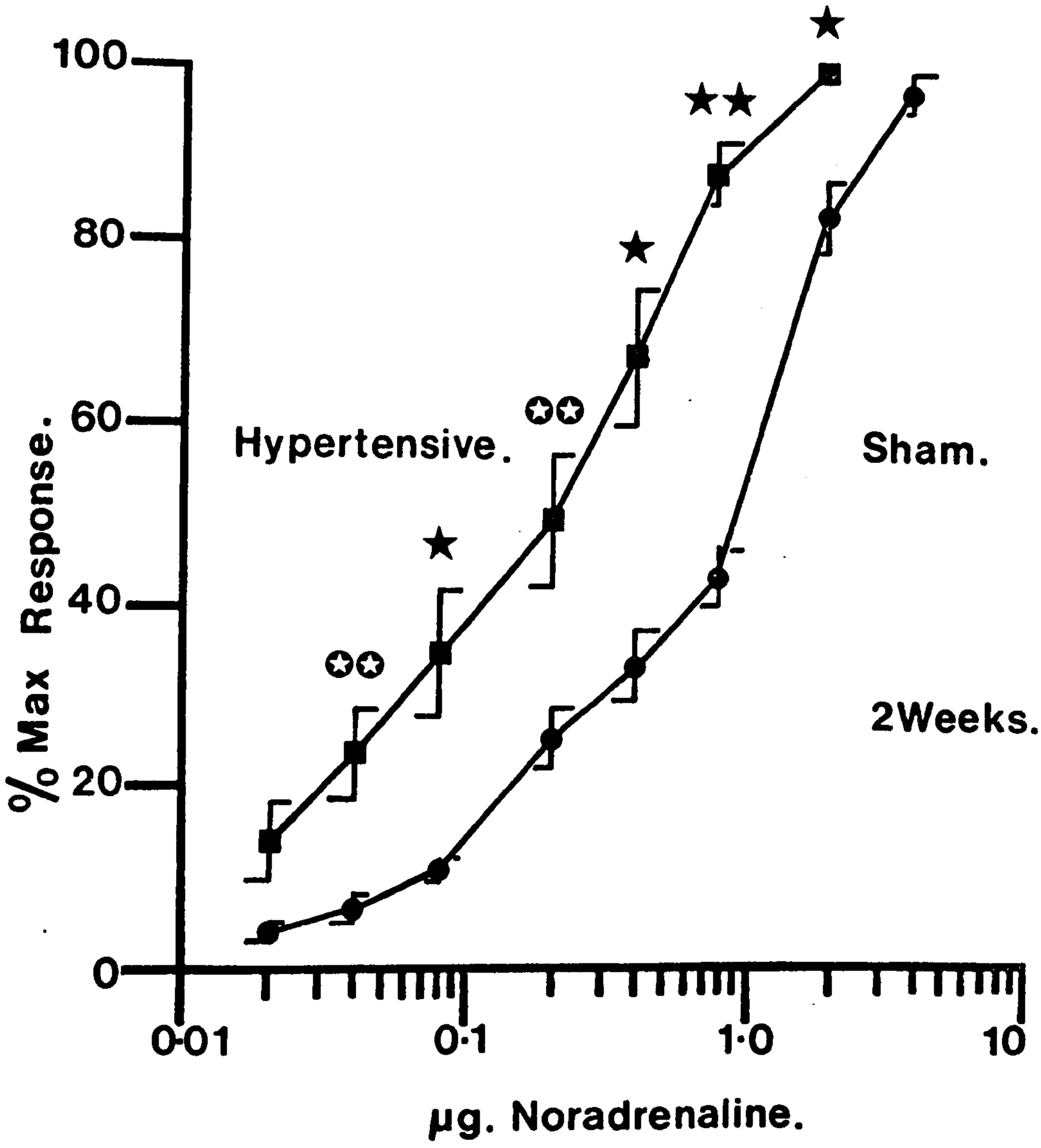


Fig. 5:6

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=5) and renal hypertensive rats (n=5), 2 weeks post-operatively. Note that the shift of the "hypertensive" dose-response curve is similar to the shift in Fig. 5:2.

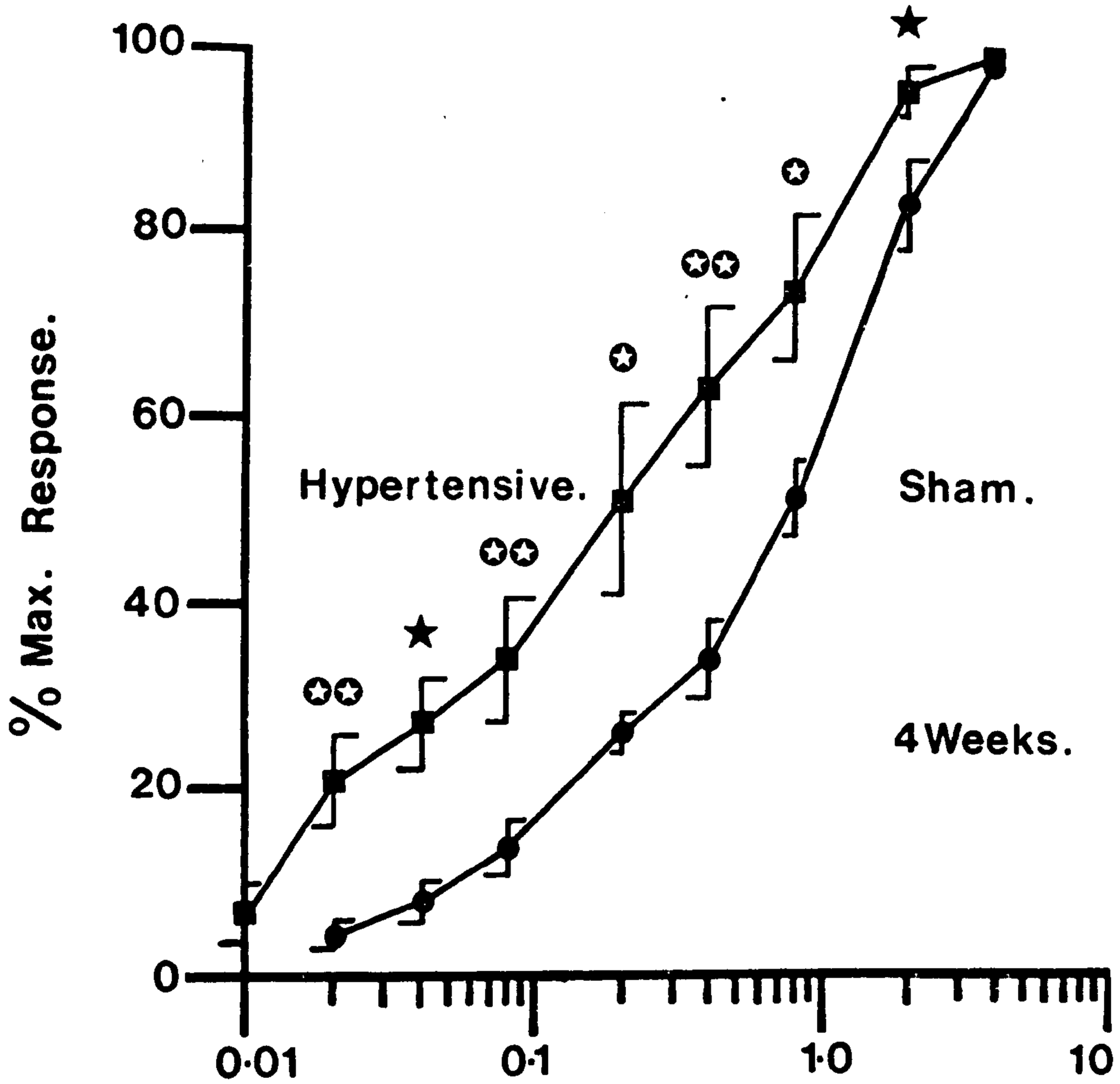


Fig. 5:7

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=7) and renal hypertensive rats (n=5), 4 weeks post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is less than the shift in Fig. 5:3.

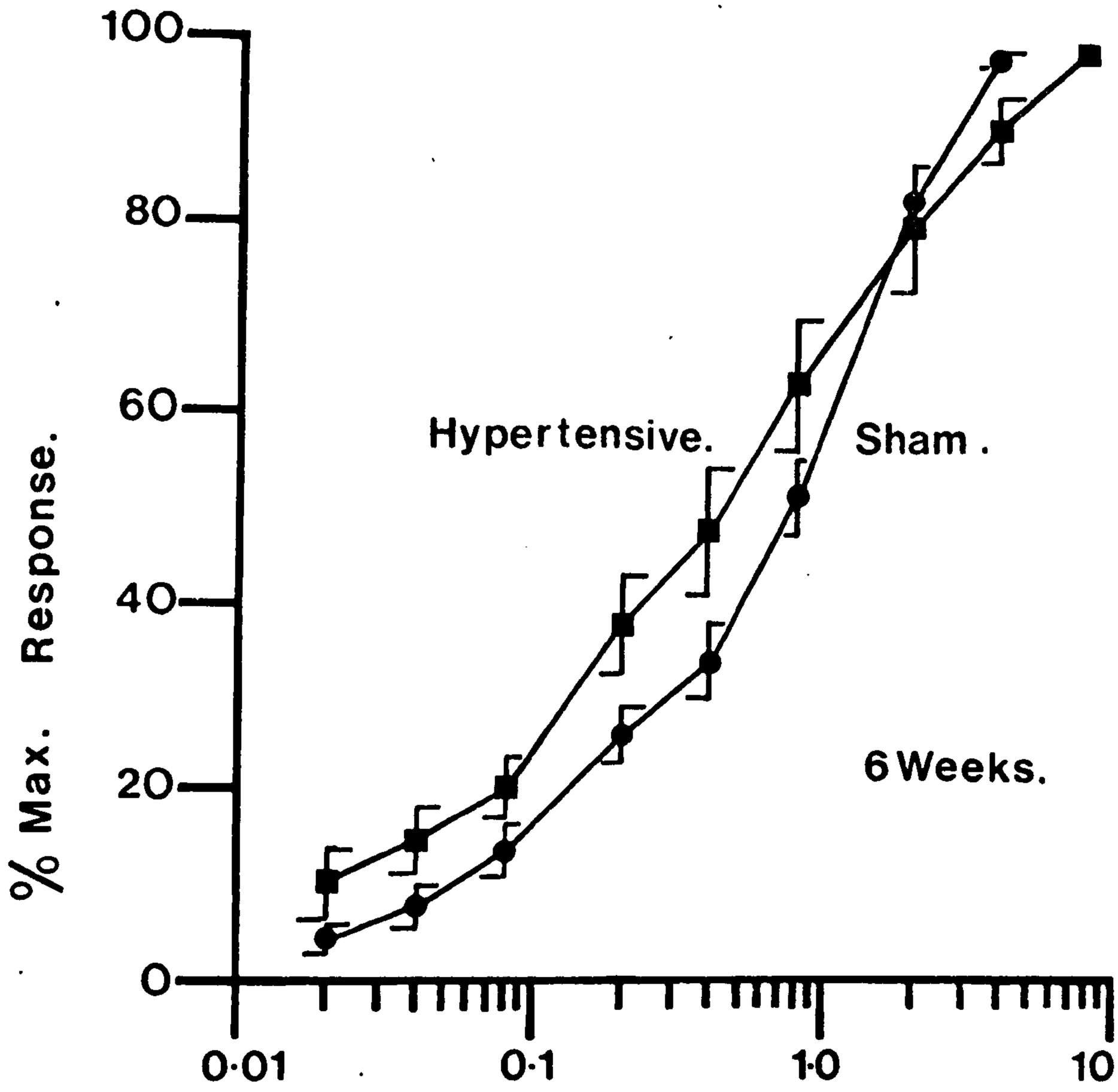


Fig. 5:8

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=7) and renal/salt hypertensive rats (n=5), 6 weeks post-operatively. Note that the shift to the left of the "hypertensive" dose-response curve is less than the shift in Fig. 5:4.

ii) Vascular reactivity to angiotensin II. The direct constrictor response and the angiotensin II induced noradrenaline sensitivity shift in mesenteric vessels from renal hypertensive and sham operated rats

The mean angiotensin II induced constrictor responses and angiotensin II induced noradrenaline sensitivity shifts for mesenteric preparations from renal hypertensive and sham control animals are given in Table 5:5. The direct constrictor responses to angiotensin were not significantly different from the sham operated control. The angiotensin induced noradrenaline sensitivity shift was significantly reduced in tissues from 1 and 2 week hypertensive rats and reduced (but not significantly) in tissues from 4 week hypertensive rats. The angiotensin induced noradrenaline sensitivity shift was reduced in tissues whose increased reactivity was predominantly due to increased vascular sensitivity (Tables 5:3 and 5:4 , p 165).

iii) Vascular reactivity to noradrenaline and angiotensin II in mesenteric preparations from uninephrectomized, salt-loaded rats

Salt-loading for two weeks in uninephrectomized rats did not significantly elevate the blood pressure (Table 5:6). Mesenteric vasculature preparations from salt-loaded rats exhibited increased reactivity to noradrenaline (Fig. 5:9, Table 5:6) with the dose-response curve shifted to the left. The increased reactivity was due to an increase in vascular sensitivity (Fig. 5:10, Table 5:6), which was markedly less than in tissues from renal and renal/salt hypertensive rats at the 2 week stage (Tables 5:4, 5:6).

Responses to angiotensin II were not significantly different in tissues from salt-loaded and sham operated rats. The angiotensin II induced noradrenaline sensitivity shift was reduced, though not to a

Table 5:5

Angiotensin induced constrictor response and angiotensin induced noradrenaline sensitivity shift in tissues from sham operated and renal hypertensive rats (*significantly different from sham control)

Weeks post operative.	Angiotensin constrictor response mmHg.	Angiotensin induced sensitivity shift.
Sham		
1	48.8 ± 8.3	3.34 ± 0.27
2	57.8 ± 11.1	4.26 ± 0.46
4	40.7 ± 9.5	2.85 ± 0.47
6	40.7 ± 9.5	2.85 ± 0.47
Renal hypertensive		
1	87.0 ± 17.9	2.23 ± 0.29*
2	80.0 ± 14.4	2.32 ± 0.4*
4	62.0 ± 20.1	1.96 ± 0.39
6	46.8 ± 15.5	2.86 ± 0.36

Table 5:6

Mean body weight and blood pressure of salt-loaded and sham operated rats, with noradrenaline threshold, maximum response, $ED_{50\%}$ dose, sensitivity and reactivity shifts, angiotensin induced response, angiotensin induced noradrenaline sensitivity shift of mesenteric vasculature preparations from these animals. (* Significantly different from sham control)

Parameter	Group 1 2 week sham control	Group 2 2 week sodium load, unilateral nephrectomy
n	5	5
Body Wt. g.	218.4 ± 4.0	224.4 ± 8.6
Blood pressure mmHg.	117.0 ± 6.2	129.6 ± 4.4
Noradrenaline threshold µg.	0.024 ± 0.004	0.016 ± 0.0024
Max. Response mmHg.	139.2 ± 16.0	127.0 ± 4.6
Noradrenaline $ED_{50\%}$ dose µg.	0.95 ± 0.07	0.52 ± 0.12*
Noradrenaline sensitivity shift.	-	2.14 ± 0.16*
Noradrenaline, 50% Group 2, dose µg.	0.9 ± 0.005	0.52 ± 0.12*
Noradrenaline reactivity shift.	-	2.02 ± 0.15*
Angiotensin response, mmHg.	57.8 ± 11.1	40.0 ± 10.6
Angiotensin induced noradrenaline sensitivity shift.	4.26 ± 0.46	3.02 ± 0.7

(Noradrenaline, 50% group 2, dose µg, indicates the dose of noradrenaline required by group 1 and 2 tissues to evoke a response of equal amplitude to 50% of the maximum response of group 2 tissues).

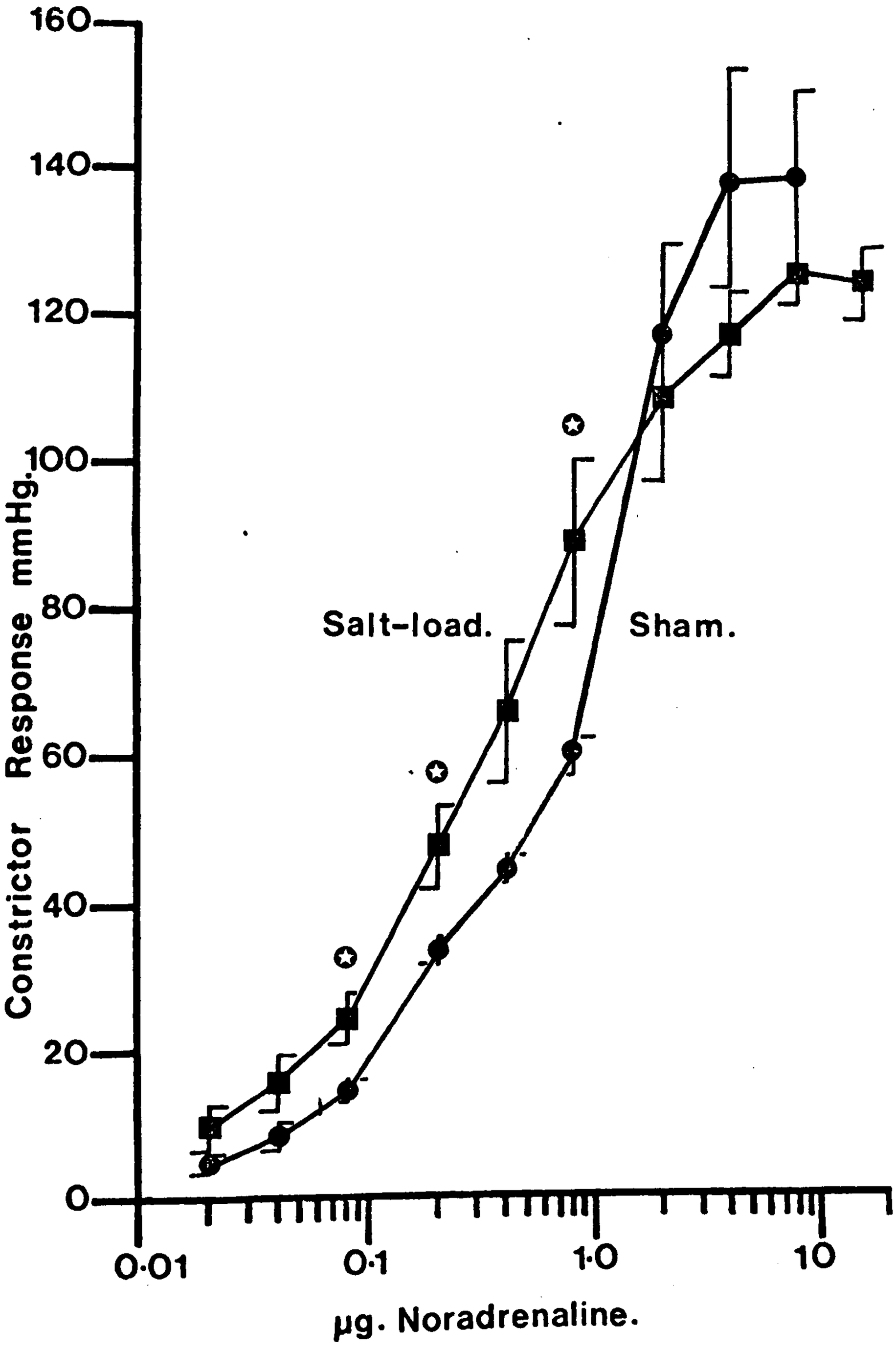


Fig. 5:9

Mean constrictor responses to noradrenaline (mmHg) of mesenteric vasculature preparations from sham control (n=5) and uninephrectomized salt-loaded rats (n=5). Note the shift to the left of the "salt-load" dose-response curve.

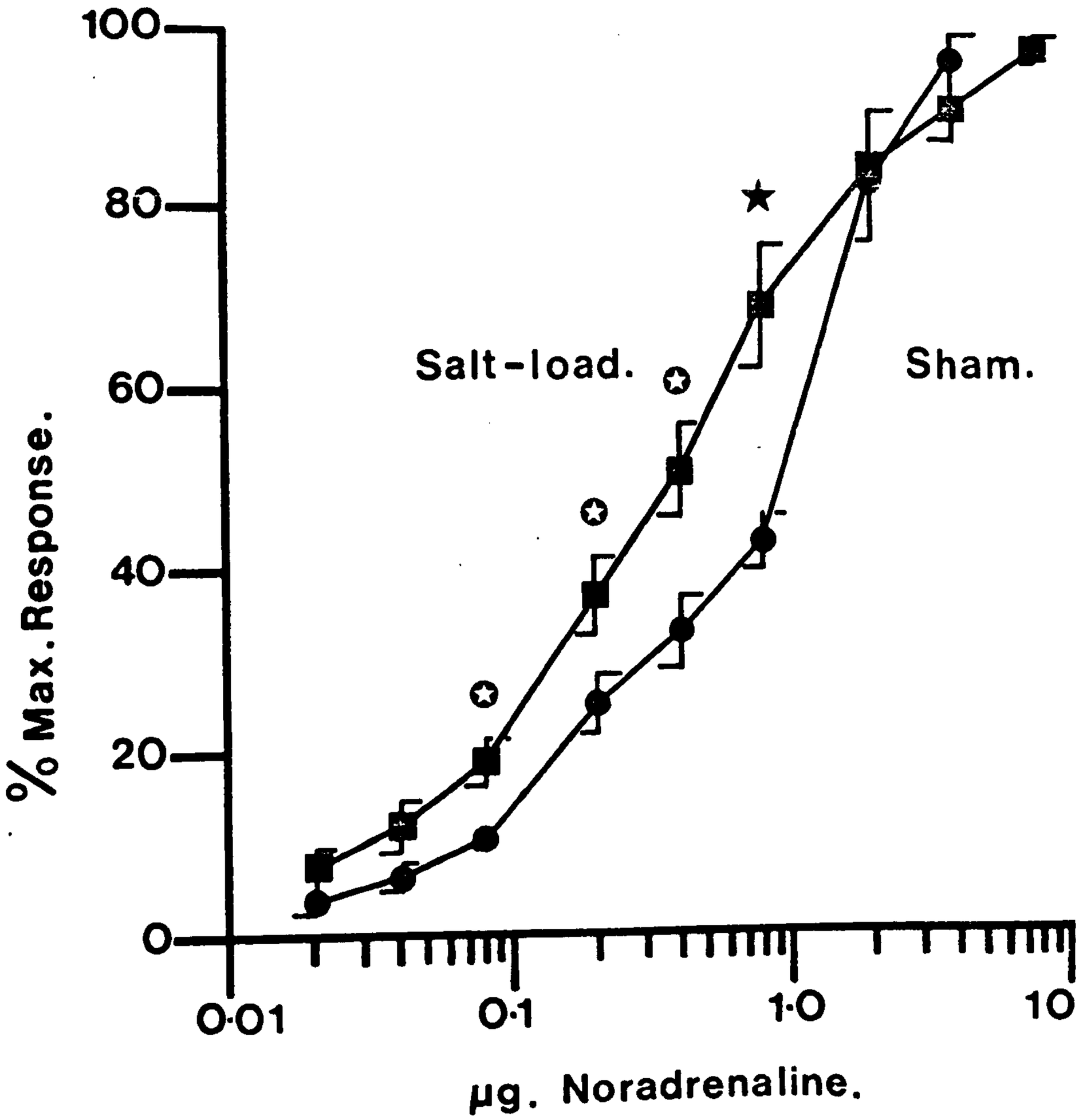


Fig. 5:10

Mean constrictor responses to noradrenaline (% of maximum response) of mesenteric vasculature preparations from sham control (n=5) and uninephrectomized salt-loaded rats (n=5). Note that the shift of the "salt-load" dose-response curve is similar to that in Fig. 5:9.

significant level, in tissues from the salt-loaded rats (Table 5:6).

d) DISCUSSION

The increase in reactivity to noradrenaline of mesenteric vasculature preparations from renal hypertensive rats was qualitatively similar to the results of experiments using tissues from renal/salt hypertensive rats (Chap 3). During the early stages of renal hypertension (1-4 weeks after nephrectomy) the increased reactivity to noradrenaline of mesenteric preparations was predominantly due to an increase in vascular sensitivity (Tables 5:3, 5:4, Figs. 5:1-5:3, 5:5-5:7). At the 6 week stage, supersensitivity was not the major factor contributing to the increased reactivity (Tables 5:3, 5:4, Figs. 5:4, 5:8). The increase in the maximum response at the 6 week hypertensive stage could be interpreted as evidence of an increased contractility and wall/lumen ratio, but, as with the renal/salt hypertensive animals, there was no significant increase in the dose-response curve gradient. The noradrenaline threshold dose was reduced at all the time points investigated, whereas it was only lower in the first two weeks of renal/salt hypertension (Chap 3). The significant reduction of the angiotensin II induced noradrenaline sensitivity shift during the early (1-2 week) but not the later (4 - 6 week) stages of renal hypertension was qualitatively similar to the reduction observed in tissues from renal/salt hypertensive rats (Chap 3).

Sodium-loading uninephrectomized rats caused an increase in vascular reactivity to noradrenaline which was due to an increased sensitivity (Table 5:6). Sodium-loading for 2 weeks did not cause an increase in the affinity of the vascular angiotensin II receptor, as responses to this peptide were not elevated, and the angiotensin II induced noradrenaline sensitivity shift was slightly depressed (Table 5:6).

The increased vascular reactivity of tissues from salt-loaded uninephrectomized rats was probably due to a direct influence of sodium on the vasculature. Arteries are rich in sodium and part of this is thought to reside in a region in or near the cell membrane, called the paracellular matrix (Friedman and Friedman, 1964., 1967). Sodium transfer into this region may be a determinant of smooth muscle tension (Friedman, Friedman and Nakashima, 1957). Evidence for this hypothesis comes from experiments on the rabbit ear artery, where enzymic depolymerization of the matrix reduces both the sodium content and the reactivity (Harris and Palmer, 1971). An increased sodium content in the arterial wall could also increase reactivity by facilitating sympathetic transmission, by increasing wall thickness or by altering calcium mobilization (Tobian and Binion, 1952., De Champlain, Krakoff and Axelrod, 1969., Sitrin and Bohr, 1971).

An increase in the sodium and water content has been demonstrated in the blood vessels from hypertensive humans and animals (Tobian and Binion, 1952., Tobian, 1956., Jones, Feigl and Peterson, 1964). In the renal and renal/salt hypertensive rat the combination of a positive sodium balance (Chap 5 pt 2) with high blood pressure would increase the amount of sodium in the arteries (Hollander, Kramsch, Farmelant and Madoff, 1968). Salt-loading in the renal/salt hypertensive rat would promote this process more rapidly than in the renal hypertensive rat.

Comparison of the present results with those from tissues of renal/salt hypertensive rats (Chap 3) reveals a marked difference in vascular sensitivity at the 1 week stage. The greater noradrenaline supersensitivity of the mesenteric vasculature preparations from the renal/salt hypertensive rat could be due to the additional influence of dietary salt-loading. In the later stages of renal hypertension a positive sodium balance is likely (Chap 5) and this would account for

the near equality of the noradrenaline sensitivity shifts in tissues from renal and renal/salt hypertensive rats (Table 5:3). The greater sensitivity shift in tissues from renal/salt hypertensive rats at the 6 week stage could also be due to the additional sodium load.

A positive sodium balance cannot cause all the early supersensitivity to noradrenaline in either model of renal hypertension. In both the renal and the renal/salt hypertensive rat, the supersensitivity declines in the later stages of hypertension. This indicates that some evanescent sensitizing factor is present in the early stages of hypertension. The possibility that endogenous angiotensin II could be this factor is now investigated.

2) THE ACTIVITY OF THE RENIN-ANGIOTENSIN SYSTEM IN RENAL AND RENAL/SALT HYPERTENSIVE RATS, AND ITS CONTRIBUTION TO INCREASED NORADRENALINE SENSITIVITY IN MESENTERIC VASCULATURE PREPARATIONS

a) INTRODUCTION

Exogenous angiotensin II can stimulate the process causing vascular supersensitivity in isolated mesenteric preparations from renal and renal/salt hypertensive rats (Chaps 3, 4). In order to determine whether endogenous angiotensin II could stimulate this process *in vivo* the activity of the renin-angiotensin system in renal and renal/salt hypertensive rats was investigated.

Plasma renin activity was used as an indicator of circulating angiotensin II levels since the development of a specific radioimmunoassay for angiotensin II was beyond the scope of this study.

Plasma renin activity may not correlate with local vascular activities, since Rosenthal and Hollander (1973) have demonstrated a three-fold increase in aortic renin activity in D.O.C. hypertensive rats with low plasma renin activity. Local generation of angiotensin II at vascular sites may play a role in the development of renal hypertension (Swales and Thurston, 1973) and the mesenteric vasculature has a significantly greater concentration of renin than other vascular beds (Genest, Simard, Rosenthal and Boucher, 1969). The possible contribution of vascular angiotensin II to the increased vascular sensitivity to noradrenaline in mesenteric preparations from renal/salt hypertensive rats was investigated with the specific angiotensin II antagonist Sar^1 $Ileu^8$ angiotensin II.

b) METHODS

The induction of renal and renal/salt hypertension, the indirect measurement of systolic blood pressure in conscious rats, and

the perfused mesenteric vasculature preparation have been described (Chap 2).

i) Radioimmunoassay of angiotensin I to determine plasma renin activity (PRA).

The main steps of the radioimmunoassay procedure were:-

1. Collection of plasma samples.
2. Preparation of samples for the assay by generation of angiotensin I.
3. Preparation of the standard curve by dilution of known amounts of angiotensin I.
4. The assay, which involves competition of radio-active angiotensin I for the antibody, and adsorption of free angiotensin I on charcoal-dextran. The bound radioactive angiotensin I was counted.

The radioimmunoassay of angiotensin I was performed using the CEA-IRE-SORIN kit (RENK).

Procedure

1) Collection and storage of plasma samples

Normotensive, sham operated, totally nephrectomized, renal and renal/salt hypertensive rats were anaesthetized by intra-peritoneal injection of pentobarbitone sodium (60 mg/kg). The abdomen was opened and clamps applied to the renal pedicles. The mesenteric artery was cannulated (Chap 2) in most of these rats and the mesenteric vein severed to prevent dilution of the blood with Krebs solution. The thorax was opened and blood taken by left ventricular puncture. Blood was collected into chilled syringes containing 10 mg of Na_2EDTA. The blood was centrifuged (1000 g) at $4^{o}C$ for 15 min. The plasma was with-

drawn and stored in plastic vials at -20°C.

2) Preparation of plasma samples for assay

The following procedures were all performed at 2-4°C unless otherwise stated. The plasma samples were thawed and 0.5 ml added to 0.5 ml of phosphate buffer containing the angiotensin converting enzyme and angiotensinase inhibitors, 2,3-dimercaptopropanol (3.5 mM) and 8-hydroxyquinoline sulphate (2mM) (Haber, Koerner, Page, Kliman and Purnode, 1969). The final pH of the mixture was 5.6-5.8 which is the optimum for renin activity (Malvano, Zucchelli, Rosa and Salvetti, 1972). After mixing, the sample was divided into two 0.5 ml aliquots, one of which was incubated at 37°C for 1.5 h and the other at 4°C.

3) Preparation of the standard curve.

Synthetic $Ileu^5$ angiotensin I (Schwarz/Mann) was diluted with 0.025 mM Tris buffer (5.4 g Tris base, 14.92 g Na_2EDTA, 2.5 g lysozyme in 1l, pH 7.4) to give solutions of 16, 8, 4, 2, 1 and 0.5 ng/ml.

4) The assay

The assay was performed in duplicate and comprised of the samples given in Table 5:7.

The ^{125}I angiotensin I used had a specific activity of ~1.5 mCi/mg. The antiserum to angiotensin I was raised in rabbits and had a titre of 1:150,000.

After mixing, all samples were incubated for 24 h. After incubation, 0.1 ml of bovine serum was added to the tubes for the standard curve and 0.1 ml Tris buffer to all other tubes. Dextran charcoal (10:1 of charcoal, Norit, 211, F.Q.P. and Dextran T70) in Tris buffer was added to all tubes except those for total activity. When 10 min had elapsed all tubes were centrifuged at 1500-2000 g for 10 min. 0.5 ml

Table 5:7

The composition of samples prepared for the radioimmunoassay of angiotensin I

Description	Code	Buffer ml	Angiotensin I standard ml	Angiotensin I antiserum ml	^{125}I-Angiotensin ml	Unknown sample ml
Total activity	T	1.4	-	-	0.1	-
Zero standard	0	0.8	-	0.1	0.1	-
Standards	A	0.7	0.1	0.1	0.1	-
	A_1	0.7	0.1	0.1	0.1	-
	A_2	0.7	0.1	0.1	0.1	-
	A_3	0.7	0.1	0.1	0.1	-
	A_4	0.7	0.1	0.1	0.1	-
	A_5	0.7	0.1	0.1	0.1	-
Samples	Sx	0.7	-	0.1	0.1	0.1

of supernatant was pipetted into counting vials and 10 ml of NE260 scintillant (Nuclear Enterprises) added. Radioactivity was counted on a Packard Tricarb liquid scintillation spectrometer, model 3380 using the ^{14}C setting (counting efficiency = 32%).

Samples were counted for 10 min or up to 10,000 disintegrations and the mean d.p.m. recorded.

Analysis of results

For each pair of duplicate tubes the mean net d.p.m. was calculated. The binding ability of the antibody was calculated from:-

$$B/_{To} = \frac{\text{Zero standard mean d.p.m.}}{\text{Total activity mean d.p.m.}} \times 100\%$$

For the standard and unknown samples:-

$$B/_{Bo} = \frac{\text{Standard or sample mean d.p.m.}}{\text{Zero standard mean d.p.m.}} \times 100\%$$

Plotting B/Bo against pg of standard angiotensin I added on a logit-log. scale produces a straight line (Fig. 5:11). B/Bo of the unknown samples can be read off as pg of angiotensin I using this graph. In order to determine the plasma renin activity (P.R.A.) the angiotensin I in the non-incubated (4^{o} C) plasma was subtracted from the angiotensin I in the incubated (37^{o}C) sample:-

$$\text{P.R.A.} = \frac{(\text{Angiotensin I incubated} - \text{Angiotensin I non-incubated}) \times 20}{1.5}$$

$$= \text{pg/ml/h.}$$

ii) Studies using the angiotensin II antagonist Sar^1 $Ileu^8$ angiotensin II

Mesenteric vasculature preparations from normotensive rats

In order to establish whether Sar^1 $Ileu^8$ angiotensin II would

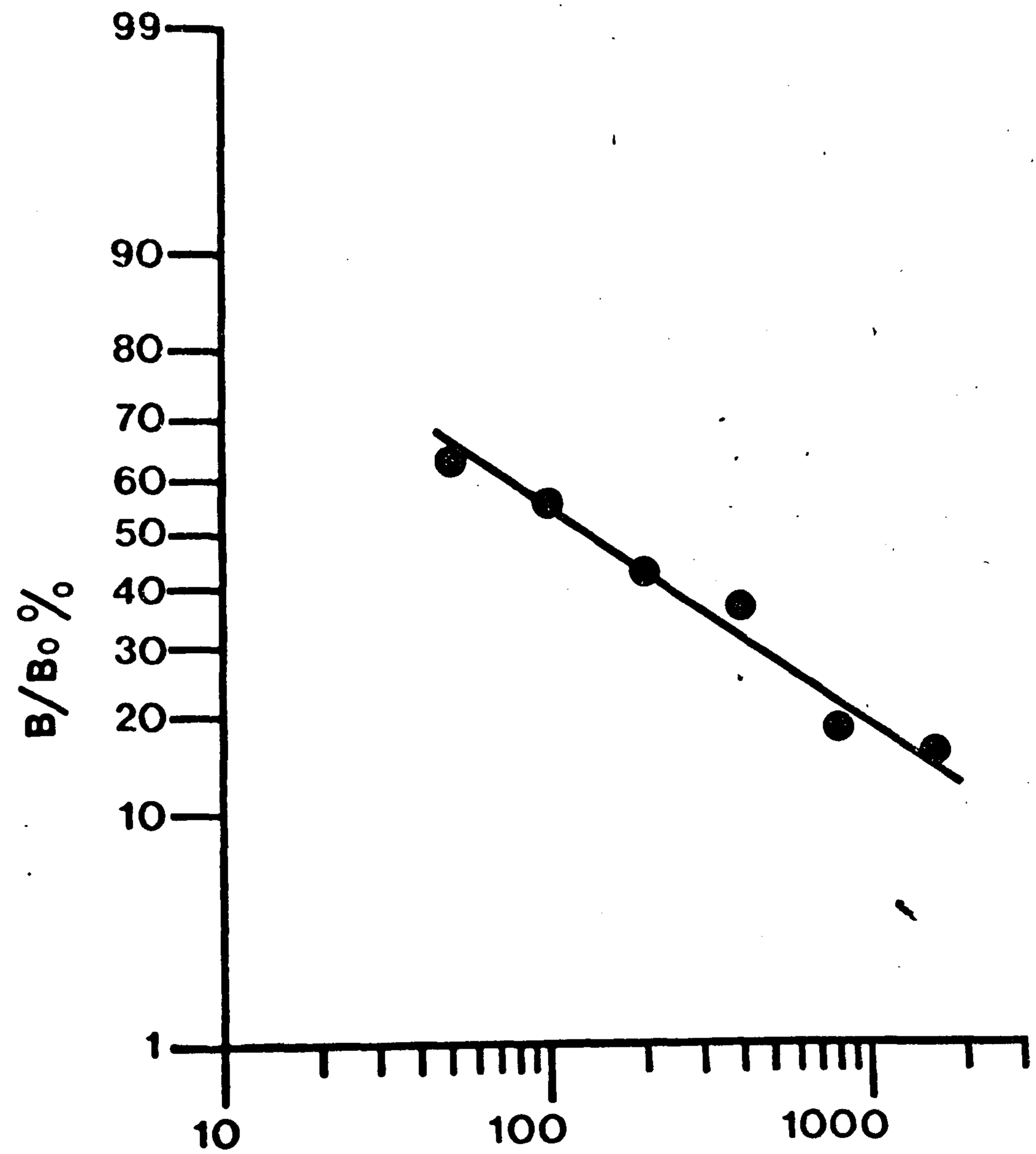


Fig. 5:11

Typical standard line for radioimmunoassay of angiotensin I. B/Bo (see text) is plotted on a logit scale and pg of standard angiotensin I added, on a log scale. Angiotensin I (pg) in an unknown sample can be read from where B/Bo lies on the calibration line.

reverse the noradrenaline potentiating action of angiotensin II amide, the following experiment was designed.

Procedure

1. Control noradrenaline dose-response curves were made in mesenteric vasculature preparations from normotensive rats.

2. Perfusion with angiotensin II amide 10^{-7}M for 15 min.

3. The noradrenaline dose-response curve was repeated in the presence of angiotensin II amide.

4. Perfusion with Sar^1 $Ileu^8$ angiotensin II (5×10^{-7}M) for 1 h. Previous studies (Chap 3) have shown that 1½-2 h perfusion with normal Krebs solution does not reverse the noradrenaline potentiation induced by angiotensin II amide.

5. The noradrenaline dose-response curve was repeated.

In order to determine whether the angiotensin II antagonist would reverse the noradrenaline supersensitivity of mesenteric preparations from 1 and 2 week renal/salt hypertensive rats, the procedure was repeated, omitting stages 2 and 3.

c) RESULTS

i) Radioimmunoassay of angiotensin I

The binding ability of the antiserum used was 61.5 ± 2.0% (six separate assays). The typical variation between test sample duplicates was 2.4 ± 0.8% (n=6). Plasma from two rats which had been totally nephrectomized 24 h previously had a low renin activity (Table 5:8) indicating that the major component of the activity in plasma from normal rats (Table 5:8) was of renal origin.

Table 5:8

Mean plasma renin activity of normotensive, sham operated, totally nephrectomized, figure-of-eight ligature, renal and renal/salt hypertensive rats

Group	Plasma Renin Activity ng/ml/h			
	1 week	2 weeks	4 weeks	6 weeks
Sham control	12.5 ± 1.2**	12.5 ± 0.7**	14.6 ± 1.0**	14.6 ± 1.0**
n	5	5	7	7
Renal/salt hypertensive	3.5 ± 1.5*	3.2 ± 0.9*	-	0.6 ± 0.2*
n	5	7	-	5
Renal hypertensive	9.0 ± 2.9	4.2 ± 0.6*	2.6 ± 0.6*	2.6 ± 0.8*
n	5	5	5	5
Normotensive control	8.6 ± 1.2*			
n	5			
5 days post Figure-of-eight ligature	14.2 ± 1.2**			
n	5			
1 day post total nephrectomy	1.3 ± 0.1**			
n	2			

* = Significantly different from sham control.

** = Significantly different from normotensive control.

The mean systolic blood pressures and body weights of the rats which were used to obtain blood for the radioimmunoassay are given in Table 5:9. The results of the radioimmunoassay are given in Table 5:8. Figure-of-eight ligation of one kidney before contralateral nephrectomy caused a significant rise in P.R.A. (compared with normotensive control). After contralateral nephrectomy, P.R.A. declined and reached low levels within 2 weeks (Table 5:8). Renal hypertensive rats had a consistently higher P.R.A. than renal/salt hypertensive rats, particularly at the 1 week stage, but this difference was not statistically significant. The sham operations significantly increased P.R.A. (Table 5:8) probably because of the kidney manipulation involved.

ii) <u>The effects of Sar^1 $Ileu^8$ angiotensin II on the noradrenaline sensitivity shift induced by angiotensin II amide, and on the supersensitive mesenteric vasculature preparation from renal/salt hypertensive rats</u>

The mean body weights and systolic blood pressures of the rats used in this study are given in Table 5:10.

Angiotensin II amide (10^{-7}M) increased the noradrenaline sensitivity of mesenteric preparations from normotensive rats. Sar^1 $Ileu^8$ angiotensin II (5×10^{-7}M) reversed this sensitivity shift (Fig. 5:12). Mesenteric vasculature preparations from 2 week renal/salt hypertensive rats were supersensitive to noradrenaline, but Sar^1 $Ileu^8$ angiotensin II did not attenuate this. (Fig. 5:12). The angiotensin antagonist also failed to reverse the noradrenaline supersensitivity of preparations from 1 week renal/salt hypertensive rats (n=3, preliminary studies).

d) DISCUSSION

The radioimmunoassay results demonstrate that renal hyper-

Table 5:9

Mean body weights and systolic blood pressures of rats whose plasma renin activity was determined

Group	Parameter	Weeks after last operation			
		1	2	4	6
Sham control	wt.	210.6 ± 5.7	218.4 ± 4.0	263.0 ± 8.1	263.0 ± 8.1
	BP	111.4 ± 5.2	117.0 ± 6.2	114.5 ± 5.2	114.5 ± 5.2
Renal/salt hypertensive	wt.	208.8 ± 3.1	219.7 ± 7.6	-	287.4 ± 15.0
	BP	145.0 ± 2.9*	174.7 ± 3.2*	-	185.6 ± 11.5*
Renal hypertensive	wt.	183.0 ± 9.1*	197.0 ± 21.8	246.8 ± 9.5	251.2 ± 17.7
	BP	142.7 ± 1.5*	162.6 ± 9.7*	173.2 ± 13.4*	163.4 ± 6.0*
Normotensive	wt.	211.6 ± 3.7			
	BP	131.2 ± 4.4			
5 days post figure-of-eight ligation	wt.	162.0 ± 6.0			
	BP	117.0 ± 2.9			

* Significantly different from sham control.

Note: The sham operated and renal hypertensive rats used for the evaluation of P.R.A. were also used to provide mesenteric vasculature preparations (Chap 5).

Table 5:10

Mean body weight and systolic blood pressure of renal/salt hypertensive and normotensive rats used in experiments with Sar ^{1} Ileu ^{8} angiotensin II.

Group	Wt. g.	BP mmHg
Normotensive n=5	261.8 ± 11.5	117.4 ± 2.2
2 week sham control n=5	218.4 ± 4.0*	117.0 ± 6.2
2 week renal/salt n=5	181.8 ± 6.4*	160.4 ± 5.8*

* Significantly different from control.

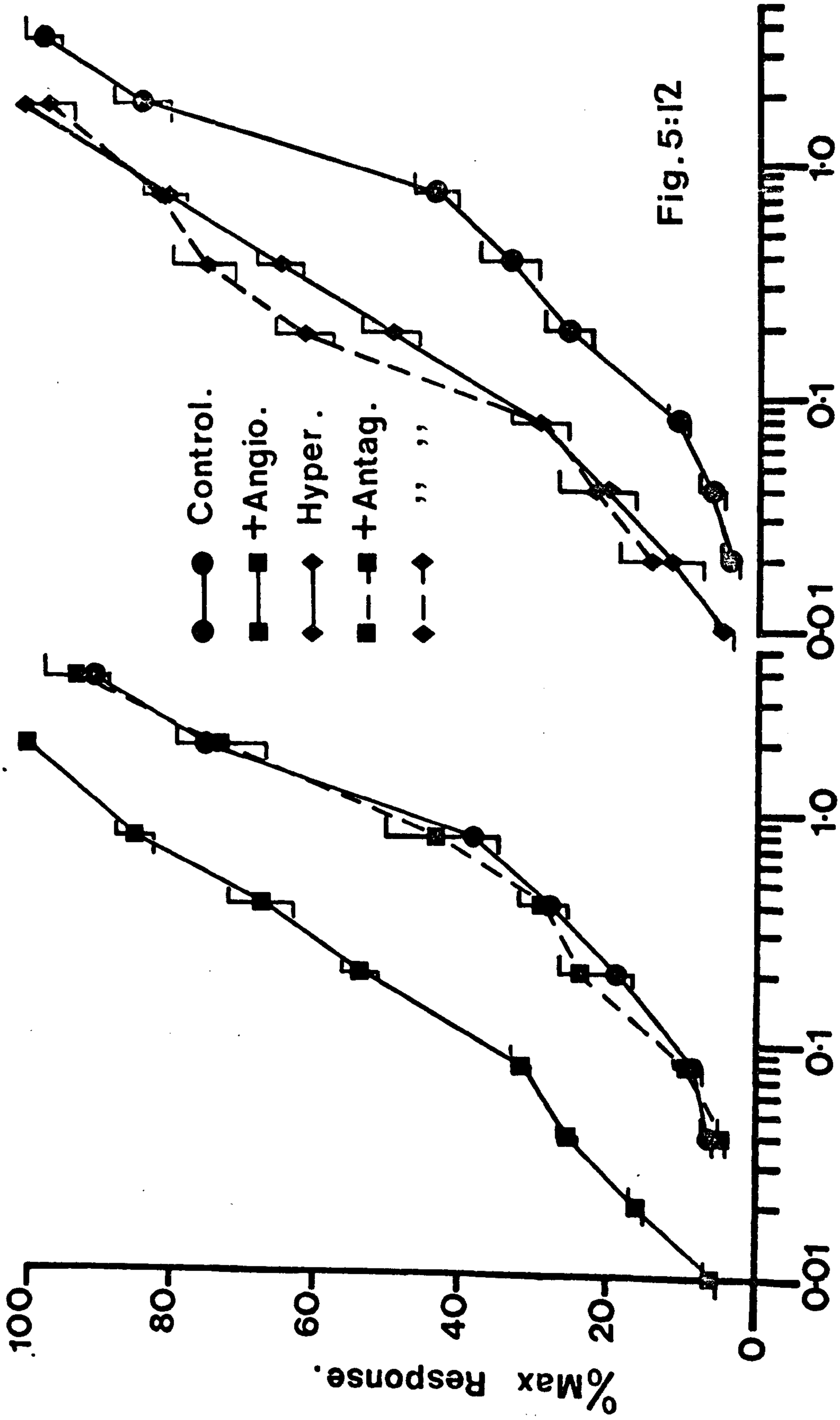

Control.
+Angio.
Hyper.
+Antag.
" "
1·0
0·1
0·01
μg l⁻¹ Noradrenaline.
100
80
60
40
20
0
0·01
0·1
1·0
% Max Response.

Fig. 5:12

Fig. 5:12

The effects of Sar^1 $Ileu^8$ angiotensin II ($5x10^{-7}M$) on the noradrenaline supersensitivity of mesenteric preparations from renal/salt hypertensive rats (n=5) 2 weeks after contralateral nephrectomy and tissues from normotensive rats (n=5) which have been treated with angiotensin II amide ($10^{-7}M$) ●——● control, for normotensive data (left side of figure) sham control for hypertensive rats (right side of figure).

■——■ Control plus angiotensin II amide.

◆——◆ Renal/salt hypertensive.

■— —■ Control, angiotensin II amide treated plus Sar^1 $Ileu^8$ angiotensin II

◆— —◆ Hypertensive plus Sar^1 $Ileu^8$ angiotensin II.

Note the angiotensin II antagonist reverses the noradrenaline supersensitivity induced by exogenous angiotensin II amide, but does not reverse the supersensitivity of tissues from renal/salt hypertensive rats.

tension induced by "figure-of-eight" ligation of one kidney and contralateral nephrectomy is associated with a low P.R.A. The results are in qualitative agreement with previously reported bio-assay studies of P.R.A. in this type of hypertension, and extend the measurements to the early stages of hypertension (Vapaatalo, Lahovaara and Hackman, 1970., Menard, Alexandre, Guidicelli, Auzan and Chevillard, 1973).

Renin secretion is mainly controlled by two intrarenal receptors, a vascular wall tension receptor and the sodium sensitive macula densa (Davis, 1973). The renal sympathetic nerves and humoral agents also exert a degree of control over renin secretion. In "Grollman" renal hypertension some of the renal arterioles will be overperfused because of the high blood pressure, resulting in an inhibition of renin release, and some will be underperfused, stimulating renin secretion. Plasma sodium is elevated in this type of hypertension (Menard, Alexandre, Guidicelli, Auzan and Chevillard, 1973) this is probably the major renin suppressing factor. The lower P.R.A. values found in renal/salt compared with those in renal hypertensive rats, particularly at the one week stage, support this suggestion. The P.R.A. of the renal hypertensive rats was markedly suppressed at the 2 week stage indicating a positive sodium balance.

The P.R.A. values determined in this study for normotensive rats were higher than some previously reported values (1-3 ng/ml/h) using similar radioimmunoassay techniques (Forman and Mulrow, 1974., Susic and Sparks, 1974). This discrepancy probably arose because both laparotomy and anaesthesia stimulate renin release (McKenzie, Ryan and Lee, 1967., Campbell, 1974). In a recent study of P.R.A. in lightly anaesthetized rats, normal values of around 20 ng/ml/h have been reported (Gavras, Brunner, Laragh, Vaughan, Koss, Cote and Gavras, 1975). P.R.A. values obviously vary with the mode of collection of blood samples. Since the

sampling technique was constant in the present study, comparative assessments are valid.

The hypothesis that elevated plasma angiotensin II levels cause part of the early increase in vascular sensitivity to noradrenaline (Chaps 3 and 5) is not supported by the P.R.A. results. When the vascular sensitivity to noradrenaline was high (1, 2 and 4 weeks after contralateral nephrectomy) the P.R.A. was low. The increase in P.R.A. after application of the figure-of-eight ligature could not have any prolonged effect on the peripheral vasculature, since similar values were found throughout the study in the sham operated rats.

Measurement of P.R.A. alone provides limited information about the levels of circulating angiotensin II. The plasma concentration of angiotensin II will also depend on the availability of renin substrate and on the rate of entry and clearance of angiotensin II from the plasma. The effects of circulating angiotensin II will depend on the affinity of the angiotensin II receptor. There is also the possibility that vascular, non-circulating, renin and angiotensin II may play a role in renal hypertension. The possibility that endogenous angiotensin II residing on the receptor sites was causing vascular supersensitivity in preparations from renal/salt hypertensive rats was investigated with a potent specific competitive angiotensin II antagonist.

Sar^1 $Ileu^8$ angiotensin II completely reversed the noradrenaline sensitizing effects of exogenous angiotensin II. Angiotensin II antibodies and angiotensin II antagonists have previously been shown to attenuate the indirect effects of angiotensin II on noradrenaline and sympathetically mediated responses (Khairallah, Davila, Papanicolaou, Glende and Meyer, 1971., Zimmerman, 1973). Previous experiments (Chap 3) have demonstrated that the noradrenaline sensitizing effects of angiotensin II amide persist

for more than 1½-2 h of washing in normal Krebs solution. Tachyphylaxis to the direct constrictor effect of angiotensin II amide washes out in this time period, which suggests that the indirect effects of angiotensin II are independant of receptor occupancy. Both actions of angiotensin II are sensitive to receptor blockade (Fig. 5:12 and Turker, Hall, Yamamoto, Sweet and Bumpus, 1972). An explanation of this apparent contradiction is that the angiotensin II receptors mediating the two effects have different cellular locations or affinities for angiotensin II.

The angiotensin II antagonist did not attenuate the noradrenaline supersensitivity of mesenteric vasculature preparations from 1 and 2 week renal/salt hypertensive rats. This is strong evidence against the hypothesis that endogenous angiotensin II, whether of circulatory or vascular origin, causes the increased noradrenaline sensitivity.

CHAPTER 6

GENERAL DISCUSSION

The initial aims of this study were to determine whether an increase in vascular reactivity occurs in arteriolar vessels from renal and renal/salt hypertensive rats and whether this involved a true supersensitivity or an apparent hyperreactivity. As the study progressed, the mechanism, the endogenous stimulants and the pathogenic significance of increased vascular reactivity were investigated.

An increase in vascular reactivity to noradrenaline was demonstrated throughout the development of renal and renal/salt hypertension. During the early (1 and 2 week) stages of hypertension, increased reactivity to noradrenaline was due to a supersensitivity of the mesenteric vessels (Fig. 6:1). When the blood pressures of the rats had stabilized (4-6 weeks after contralateral nephrectomy) the increased reactivity was due to a combination of supersensitivity and another factor which involved an elevation of the maximum response. This factor, which is represented by the non-supersensitive shift remainder (Fig. 6:1), was probably a consequence of hypertension since it appeared after the blood pressure had stabilized.

The cause of the non-supersensitive shift remainder and the elevation of the maximum response might have been an increase in the wall/lumen ratio of the vessels. Evidence for such a structural change was unconvincing, possibly because the mesenteric vasculature preparation does not include the precapillary metarterioles which are most susceptible to medial hypertrophy (Folkow, Hallbäck, Lundgren, Weiss, Albrecht and Julius, 1974).

The major question about vascular reactivity in hypertension is whether the former precedes the latter, or <u>vice versa</u>. This question

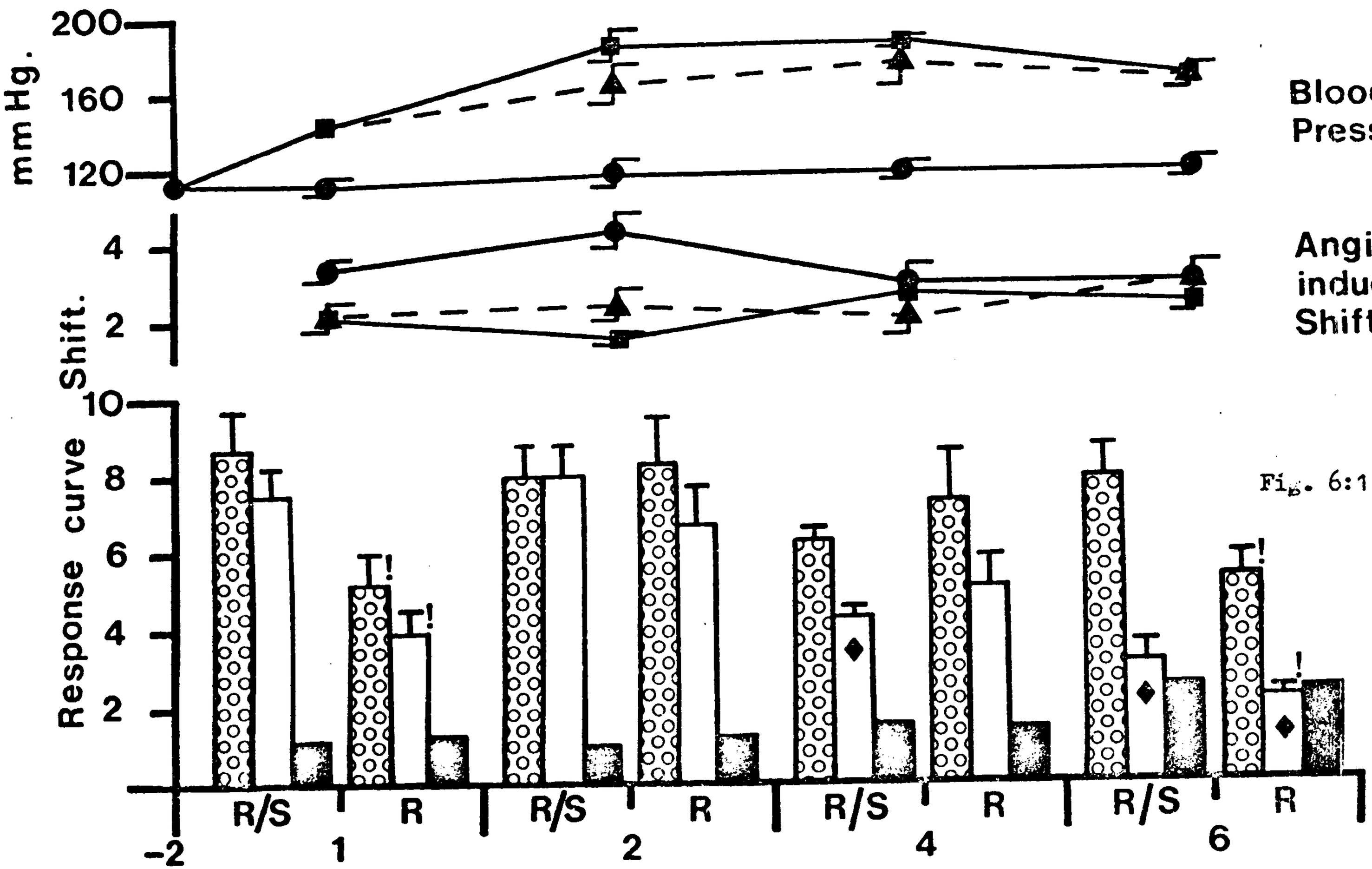

200
160
120
mm Hg.
Blood
Pressure.
4
2
Shift.
Angiotensin
induced
Shift.
10
8
6
4
2
Response curve
!
R/S
R
-2
1
2
4
6
Weeks after Nephrectomy.

Fig. 6:1

Fig. 6:1

A comparison of the noradrenaline dose-response curve sensitivity and reactivity shifts and the effects of angiotensin II amide in mesenteric vasculature preparations from renal (R) and renal/salt (R/S) hypertensive rats. Notation for the blood pressure and angiotensin induced noradrenaline sensitivity shifts traces:-

Renal/salt hypertensive.

Renal hypertensive.

Sham Control.

Notation for the histogram:-

Noradrenaline reactivity shift.

Noradrenaline sensitivity shift.

Non-supersensitive shift remainder.

Significantly different from renal/salt data.

Significantly different from reactivity shift.

Sensitivity and reactivity shifts are equivalent at 1 and 2 weeks (renal/salt)and at 1, 2 and 4 weeks (renal) after contralateral nephrectomy.

The noradrenaline potentiating effects of angiotensin II amide are attenuated at the 1 and 2 week stages (renal and renal/salt). Increased reactivity is due to a combination of increased sensitivity and some other factor at the 6 week stage (renal and renal/salt).

can only be resolved by studies of vascular reactivity in tissues from animals in a pre-hypertensive phase. The Japanese S.H.R. goes through a pre-hypertensive phase (Albrecht, 1974) and would be eminently suitable for such an investigation if a valid control animal was available. A pre-hypertensive phase in renal hypertensive rats might be induced by antihypertensive drug treatment immediately after operative procedures.

The results of the present study demonstrate that the maximum increased reactivity to noradrenaline occurred at the 1 week stage in tissues from renal/salt hypertensive rats, while the blood pressure peaked at the 2 week stage (Fig. 6:1). In the renal hypertensive rats, which had not been salt-loaded, the peak increased reactivity occurred at the 2 week stage and the maximum blood pressure at 4 weeks. These results suggest that increased reactivity to noradrenaline, caused by vascular supersensitivity precedes the increase in blood pressure. If the increase in vascular sensitivity caused an increase in peripheral resistance then the vascular supersensitivity could cause the hypertension. In order to prove this hypothesis, a direct comparison of peripheral resistance, vascular reactivity and the level of nervous and humoral activation of the vascular smooth muscle during the development of hypertension is required.

If the increased vascular sensitivity was causing the increase in blood pressure, then there should be a direct quantitative relationship between the two parameters. At the one week hypertensive stage the blood pressures of the renal and renal/salt hypertensive rats were the same, but the noradrenaline supersensitivity of mesenteric vasculature preparations from renal/salt and renal hypertensive rats were significantly different (Fig. 6:1). This quantitative discrepancy could be interpreted as evidence against a causal link between supersensitivity and blood

pressure. Plasma renin activity was suppressed in the 1 week renal/salt hypertensive rats, but similar values were found in normotensive and 1 week renal hypertensive rats. Therefore in the 1 week renal/salt hypertensive rats, increased sensitivity combined with sympathetic activation could raise the blood pressure, while in the renal hypertensive rat a smaller increase in sensitivity would be activated by both sympathetic nerves and circulating angiotensin II. Increased reactivity to angiotensin II has not been demonstrated in this study since full dose-response curves to this agonist are technically difficult.

The hypothesis that circulating angiotensin II provides the additional hypertensive stimulus in renal hypertensive rats could be tested with a specific angiotensin II antagonist. If the hypothesis is correct, renal/salt hypertensive rats should be insensitive to the antihypertensive actions of the antagonist and the renal hypertensive rats sensitive.

If it is assumed that the increased vascular sensitivity precedes the increase in blood pressure, then the vascular reactivity at week "A" will determine the blood pressure level at week "A+1". The degree of supersensitivity of mesenteric vasculature preparations from the 1 week renal/salt hypertensive rats was approximately twice that of tissues from renal hypertensive rats at the same stage (Fig. 6:1). The increase in blood pressure over the following week in the renal/salt hypertensive rats was twice that observed in the renal hypertensive rats (Fig. 6:1). Vascular reactivity in tissues from rats 1 week after application of the figure-of-eight ligature has not been investigated. If there is an increase in vascular reactivity at this stage then it would be equal in renal and renal/salt hypertensive rats, since salt-loading was commenced after contralateral nephrectomy. The blood

pressure increase during the week following contralateral nephrectomy was the same in the renal and the renal/salt hypertensive rats (Fig. 6:1). This interpretation of the results indicates a direct quantitative relationship between the increased vascular sensitivity and the increase in blood pressure during the following week. An investigation of vascular reactivity one week after application of the figure-of-eight ligature would confirm this interpretation.

In the later stages of hypertension, the only significant difference between reactivity in tissues from renal/salt and renal hypertensive rats was at the 6 week stage. The lower overall reactivity in tissues from renal hypertensive rats, at this stage, was due to a smaller degree of supersensitivity in these preparations. The difference in sensitivity of mesenteric preparations from 6 week renal and renal/salt hypertensive rats was probably due to the additional dietary salt load in the latter. More detailed studies of blood pressure and reactivity after the 6 week hypertensive stage would be necessary to determine whether this difference in reactivity has any long term effect on the blood pressure.

The uninephrectomized rats which were salt-loaded did not become hypertensive. Mesenteric preparations from these animals were supersensitive to noradrenaline. This result appears to isolate increased vascular sensitivity from the development of high blood pressure. Salt-loading of the rats would, however, suppress the renin-angiotensin system (Davis, 1973) so that the pro-hypertensive effects of increased vascular sensitivity could have been counteracted by the suppression of circulating pressor angiotensin II. It is likely that a longer period of salt-loading would cause an increase in blood pressure as salt-loading normal rats eventually causes hypertension (Meneely, Tucker, Darby and

Auerback, 1953) and this effect is exacerbated by unilateral nephrectomy (Koletsky, 1959, Koletsky and Goodsitt, 1960).

The increased vascular reactivity which may precede the increase in blood pressure was due to an increase in vascular sensitivity. The mechanism of this supersensitivity was investigated and appeared to involve an increase in the activator calcium available to the muscle myofibrils and not a change in the characteristics of the α-adrenoceptor. Whether this increased availability was due to increased levels or increased release of calcium could not be determined with the method used. For the measurement of the amount of bound intracellular calcium in vessels from normotensive and hypertensive rats, the isolated vascular tissue could be loaded with ^{45}Ca . The loosley bound extracellular ^{45}Ca could be displaced with La^{+++} (Van Breeman and McNaughton, 1970) and the residual bound calcium 45 counted. In order to determine whether a greater release of calcium occurs in vascular tissues from hypertensive rats, microsomal fractions from the vessel walls could be loaded with ^{45}Ca and the rate of efflux followed with and without agonist stimulation. Alternatively the uptake of ^{45}Ca into the microsomes could be inhibited by angiotensin or noradrenaline (Baudouin-Legros and Meyer, 1973).

A change in the characteristics of the α-adrenoceptor in mesenteric vessels from hypertensive rats is unlikely. The results obtained with indoramin probably reflected a difference in the nature of the antagonist (compared with phentolamine) rather than a change in the α-adrenoceptor. Determination of the α-adrenoceptor blocking potencies of indoramin and phentolamine on the fast and slow components of the mesenteric response to noradrenaline would confirm this. Tissues from normotensive rats which have been exposed to angiotensin II appear to mimic the behaviour of tissues from early renal/salt hypertensive rats. It would be interesting to determine whether the

α-adrenoceptor blocking potency of indoramin is reduced in angiotensin treated tissues.

An impairment of noradrenaline re-uptake into sympathetic nerves may have contributed to the increased vascular sensitivity to this agonist. This possibility could be investigated by loading mesenteric preparations with tritiated noradrenaline and following the efflux after nerve stimulation. Additional evidence could be obtained by determining vascular sensitivity to an agonist, such as angiotensin II which is not taken up into the nerve terminal.

The endogenous factors which might stimulate the early vascular supersensitivity in tissues from renal and renal/salt hypertensive rats have been investigated. Exogenous angiotensin II potentiated noradrenaline but not KCl induced responses and the attenuation of these effects in the supersensitive vascular preparations from early renal and renal/salt hypertensive rats were suggestive of an involvement of endogenous angiotensin II. The reduction in the P.R.A. of the hypertensive animals and the inability of the angiotensin antagonist to reverse the supersensitivity are strong evidence against endogenous angiotensin II stimulating vascular supersensitivity.

The effects of the prostaglandins on blood vessel tone vary with tissue and species used and whether the experiment is performed <u>in vivo</u> or <u>in vitro</u>. Prostaglandins of renal origin are unlikely to be involved in arterial supersensitivity since they are metabolised in the pulmonary circulation (McGiff, Crowshaw and Itskovitz, 1974). Local vascular prostaglandins are synthesized when sympathetic nerves are stimulated, and may blunt the noradrenergic constrictor response, <u>in vivo</u> (Brody and Kadowitz, 1974). Conversely, <u>in vitro</u>, prostaglandins

E_1 and E_2 can constrict vascular smooth muscle by augmenting responses to both noradrenaline and KCl (Greenberg and Long, 1973). The inhibition of prostaglandin synthesis by indomethacin depresses the responses of the isolated mesenteric vasculature preparation to noradrenaline (Malik and McGiff, 1975). An excess of certain prostaglandins or an imbalance between various types could produce vascular supersensitivity, although the profile of the supersensitivity caused by prostaglandin E_1 and E_2 does not mimic that found in tissues from renal/salt hypertensive rats. The involvement of local prostaglandins in vascular reactivity is a complex problem and warrants further investigation.

Nephrotensin, which is elevated in acute but not chronic renal hypertension, potentiates responses to noradrenaline and KCl (Grollman and Krishnamurty, 1973), whereas the supersensitivity of tissues from renal/salt hypertensive rats was specific for noradrenaline. Similarly the plasma vasoactive factor (Bohr and Johansson, 1966) potentiates both noradrenaline and KCl induced responses.

The early rise and later decrease in vascular sensitivity to noradrenaline could indicate that a reduction of renal mass reduces the level of a desensitizing factor whose levels rise again as renal hypertrophy occurs. If this were the case, the uninephrectomized salt/loaded rats should have exhibited a similar increase in vascular sensitivity to that found in tissues from renal/salt hypertensive rats.

The effects of a positive sodium balance, which could be measured by metabolic or total exchangeable sodium studies, (Tobian, Coffee and McCrea, 1969) could not cause all of the early vascular supersensitivity since the rats had a low P.R.A. indicating a positive sodium balance, in the later stages of hypertension.

The decline of vascular supersensitivity in the later stages of renal and renal/salt hypertension has been interpreted as indicating

that some sensitizing factor increases in the early stages but declines in the later stages of hypertension. An alternative possibility is that the sensitizing factor is elevated throughout the 6 week period but has different effects on the smooth muscle in the later stages of hypertension. If this factor affects calcium regulation, it could cause an increased availability of activator calcium in the early stages of hypertension. In the later stages the energetics of the cross-linking system of actin and myosin could change so that a greater contraction develops in response to a normal quantity of activator calcium. Such a change would shift the dose-response curve to the left of control, and elevate the maximum response to all agonists (Kalsner, 1974).

The assumption on which all studies of vascular reactivity are based is that an increase in peripheral resistance causes or maintains hypertension. Guyton, Coleman and Granger (1972) have used the systems analysis approach in their computer simulation of the control of blood pressure. This analysis revealed two very important predictions, 1) changes in peripheral resistance per se, play essentially no role in the long term regulation of arterial pressure, 2) arterial pressure cannot be changed chronically without either altering the function of the kidneys or changing the intake of water and electrolytes. This is because the renal body fluid mechanism for regulation of arterial pressure is the only control system with infinite gain (Guyton, Coleman, Cowley, Scheel, Manning and Norman, 1972). Although the peripheral resistance will maintain the high blood pressure, this can only occur in the presence of an altered kidney function. This group of investigators also suggest that pretubular mechanisms i.e. an increase in resistance to blood flow in the afferent renal artery and arterioles, are more important than tubular mechanisms, i.e. sodium retention in pressure control (Guyton, Coleman, Cowley, Manning, Norman and Ferguson, 1974). Therefore an

increase in resistance of non-renal vascular beds, perhaps caused by increased vascular reactivity, could only cause hypertension in the presence of an impaired or altered kidney function. If increased vascular reactivity also occurred in the renal afferent blood vessels then a causative link between reactivity and high blood pressure could be established. For this reason, future studies of vascular reactivity in hypertension should be directed towards the renal arterial-arteriolar vessels, an approach which has been neglected in previous studies.

REFERENCES

ABBOUD, F.M. (1974). Effects of sodium, and steroids on vascular reactivity in man. Fedn Proc. Fedn Am. Socs exp. Biol., 33, 143-149.

ALAM, G.M. and SMIRK, F.H. (1938). Blood pressure raising reflexes in health, essential hypertension and renal hypertension. Clin. Sci., 3, 259-266.

ALBRECHT, I. (1974). The haemodynamics of early stages of spontaneous hypertension in rats. Part 1: Male study, part 2: Female study. Jap. Circul. J., 38, 985-990 and 991-996.

ALPS, B.J., HILL, M., JOHNSON, E.S. and WILSON, A.B. (1972). Quantitative analysis on isolated organs of the autonomic blocking properties of indoramin hydrochloride (Wy-21901). Br. J. Pharmac., 44, 52-62.

ANGLES d'AURIAC, G., BAUDOUIN, M. and MEYER, P. (1972). Mechanism of action of angiotensin in smooth muscle cell: Biochemical changes following interaction of the hormone with its membrane receptors. Circulation Res., Suppl II. 31, 151-157.

AOKI, K., TAKIKAWA, K. and HOTTA, K. (1973). Role of the adrenal cortex and medulla in hypertension. Nature (Lond.), 214, 122-123.

ARIENS, E.J. (1964). "Molecular Pharmacology". Vol. 1, p. 5. New York: Academic Press.

ARMSTRONG, J.M. (1972). Vascular reactivity to noradrenaline and 5-hydroxytryptamine in hypertensive rats. Br. J. Pharmac., 45, 183-184P.

ARUNLAKSHANA, O. and SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. Br. J. Pharmac., 14, 48-58.

BANDICK, N.R. and SPARKS, H.V. (1970). Contractile response of vascular smooth muscle of renal hypertensive rats. Am. J. Physiol., 219, 340-344.

BÁRÁNY, F.R. (1963). Reactivity of the skin vessels to noradrenalin and angiotensin in arterial hypertension. Scand. J. clin. Lab. Invest., 15, 317-322.

BARATH, E. (1928). Arterial hypertension and physical work. Archs intern. Med., 42, 297-300.

BAUDOUIN, M., MEYER, P., FERMANDJAIN, S. and MORGAT, J.L. (1972). Calcium release induced by interaction of angiotensin with its receptors in smooth muscle cell microsomes. Nature (Lond.), 235, 336-338.

BAUDOUIN-LEGROS, M. and MEYER, P. (1973). Effects of angiotensin, catecholamines and cyclic AMP on calcium storage in aortic microsomes. Br. J. Pharmac., 47, 377-385.

BAUM, T. and SHROPSHIRE, A.T. (1967a). Vasoconstriction induced by sympathetic stimulation during development of hypertension. Am. J. Physiol., 212, 1020-1024.

BAUM, T. and SHROPSHIRE, A.T. (1967b). Sympathetic and humoral vasoconstrictor responses in deoxycorticosterone hypertension. Am. J. Physiol., 213, 499-503.

BEER, E., KING, F.H. and PRINZMETAL, M. (1937). Pheochromocytoma with demonstration of pressor substance occurring preoperatively in the blood during hypertensive crises. Ann. Surg., 106, 85-91.

BEILIN, L.J. and WADE, D.N. (1970). Vascular hyper-reactivity with sodium loading and with deoxycorticosterone induced hypertension in the rat. Nature (Lond.), 227, 1141-1142.

BEILIN, L.J., WADE, D.N., HONOUR, A.J. and COLE, T.J. (1970). Vascular hyper-reactivity with sodium loading and with deoxycorticosterone induced hypertension in the rat. Clin. Sci., 39, 793-810.

BEILIN, L.J. and ZIAKUS, G. (1971). Vascular reactivity in 'post-DOCA' hypertension. Br. J. Pharmac., 43, 427P.

BENNINGHOFF, A. (1930). Handbuch der Mikroskopischen anatomie des Menschen. Vol. 6, Pt. 1, ed. MOLLENDORFF, W.V. p.1. Berlin.

BICKERTON, R.K. and BUCKLEY, J.P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. Proc. Soc. exp. Biol. Med. 106, 834-836.

BLAIR-WEST, J.R., COGHLAN, J.P., DENTON, D.A., GODING, J.R., WINTOUR, M. and WRIGHT, R.D. (1965). Effect of variations of plasma sodium concentration on the adrenal response to angiotensin II. Circulation Res., 17, 386-393.

BOHR, D.F. (1961). Reactivity of aorta and arterioles in renal hypertension. Univ. Mich. Med. Bull., 27, 196-197.

BOHR, D.F. (1964). Contraction of vascular smooth muscle. Canad. med. Ass. J., 90, 174-179.

BOHR, D.F. (1973). Vascular smooth muscle updated. Circulation Res., 32, 665-672.

BOHR, D.F. (1974). Reactivity of vascular smooth muscle from normal and hypertensive rats: effect of several cations. Fedn. Proc. Fedn Am. Socs exp. Biol., 33, 127-132.

BOHR, D.F. and JOHANSSON, B. (1966). Contraction of vascular smooth muscle in response to plasma: comparison with response to known vaso-active agents. Circulation Res., 19, 593-601.

BOHR, D.F. and SITRIN, M.D. (1970). Regulation of vascular smooth muscle contraction: changes in experimental hypertension. Circulation Res., 27, Suppl II, 83-90.

BOLOMEY, A.A., MICHIE, A.J., MICHIE, C., BREED, E.S., SCHREINER, G.E. and LAVSON, H.D. (1949). Simultaneous measurement of effective renal blood flow and cardiac output in resting normal subjects and patients with essential hypertension. J. Clin. Invest., 28, 10-17.

BORST, J.G. and BORST-de-GEUS, A. (1963). Hypertension explained by Starlings theory of circulatory homeostasis. Lancet, 1, 677-682.

BOUCHER, R., ASSELIN, J. and GENEST, J. (1974). A new enzyme leading to the direct formation of angiotensin II. Circulation Res., 35, Suppl I, 203-209.

BOYER, J.T., FRASER, J.R. and DOYLE, A.E. (1960). The haemodynamic effects of cold immersion. Clin. Sci., 19, 539-550.

BRAUN-MENENDEZ, E., FASCIOLO, J.C., LELOIR, L.F. and MUNOZ, J.M. (1939). Hypertensin: substance causing renal hypertension. Nature (Lond.), 144, 980.

BRIGHT, R. (1836). Cases and observations illustrative of renal disease accompanied with the secretion of albuminous urine. Guy's Hosp. Rep., 1, 338-379.

BROD, J. (1960). Essential hypertension : haemodynamic observations with a bearing on its pathogenesis. Lancet, 2, 773-778.

BRODY, M.J. and KADOWITZ, P.J. (1974). Prostaglandins as modulators of the autonomic nervous system. Fedn Proc. Fedn Am. Socs exp. Biol., 33, 48-60.

BROWN, J.J., DAVIES, D.L., LEVER, A.F. and ROBERTSON, J.I.S. (1964). Variations in plasma renin concentration in several physiological and pathological states. Canad. Med. Ass. J., 90, 201-206.

BROWN, J.J., DAVIES, D.L., LEVER, A.F. and ROBERTSON, J.I.S. (1965). Plasma renin concentration in human hypertension. II : Renin in relation to aetiology. Br. Med. J., 2, 1215-1219.

BROWN, J.J., DAVIES, D.L., LEVER, A.F. and ROBERTSON, J.I.S. (1966). Plasma renin concentration in human hypertension. III : Renin in relation to complications of hypertension. Br. med. J., I, 505-508.

BROWN, J.J., FERRISS, J.B., FRASER, R., LEVER, A.F., LOVE, D.R., ROBERTSON, J.I.S. and WILSON, A. (1972). Apparently isolated excess deoxycorticosterone in hypertension. A variant of the mineralocorticoid-excess syndrome. Lancet, 2, 243-247.

BROWN, J.J., FRASER, R., LEVER, A.F., ROBERTSON, J.I.S., JAMES, V.H.T., McCUSKER, J. and WYNN, V. (1968). Renin, angiotensin, corticosteroids and electrolyte balance in Addisons disease. Q. Jl Med., 37, 97-118.

BROWN, J.J., FRASER, R., LEVER, A.F. and ROBERTSON, J.I.S. (1971). Hypertension : a review of selected topics. Abstr. World med., 45, 549-559, 633-644.

BROWN, T.C., DAVIES, J.O., OLICHNEY, M.J. and JOHNSTON, C.I. (1966). Relation of plasma renin to sodium balance and arterial pressure in experimental renal hypertension. Circulation Res., 18, 475-483.

BRUNJES, S. (1964). Catecholamine metabolism in essential hypertension. New Engl. J. Med., 271, 120-124.

BRUNNER, H.R., CHANG, P., WALLACH, R., SEALEY, J.E. and LARAGH, J.H. (1972). Angiotensin II vascular receptors : their avidity in relationship to sodium balance, the autonomic nervous system, and hypertension. J. clin, Invest., 51, 58-67.

BRUNNER, H.R., LARAGH, J.H., BAER, L., NEWTON, M.A., GOODWIN, F.T., KRAKOFF, L.R., BARD, R.H. and BÜHLER, F.R. (1972). Essential hypertension : Renin and aldosterone, heart attack and stroke. New Engl. J. Med., 286, 441-449.

BRUNNER, H.R., GAVRAS, H., LARAGH, J.H. and KEENAN, R. (1973). Hypotensive effects of angiotensin II blockade in man : Comparison with propranolol. Circulation, 48, (Suppl IV, Abstr), 72.

BÜLBRING, E. and WAJDA, I. (1945). Biological comparison of local anaesthetics. J. Pharmacol., 85, 78-84.

CAMPBELL, W.B. (1974). Renin in the spontaneously hypertensive rat. Circulation Res., 35, 961-962.

CARRETERO, O.A., ENZMANN, G., POLOMSKI, C., PIWONSKA, A., OZA, N.B. and SCHORK, A. (1973). Role of the adrenal glands in the development of severe hypertension. Circulation Res., 33, 516-520.

CARRETERO, O.A., KUK, P., PIWONSKA; S., HOULE, J.A. and MARIN-GREZ, M. (1971). Role of the renin-angiotensin system in the pathogenesis of severe hypertension in rats. Circulation Res., 29, 654-663.

CATT, K.J., CRAN, E., ZIMMET, P.Z., BEST, J.B., CAIN, M.D. and COGHLAN, J.P. (1971). Angiotensin II blood levels in human hypertension. Lancet, 1, 459-464.

CHANUTIN, A. and FERRIS, E. (1932). Experimental renal insufficiency produced by partial nephrectomy. Arch. intern. med., 49, 767-787.

CLARK, A.J. (1933). "The mode of action of drugs on cells". London : E. Arnold and Co.

CLARK, D.W.J. (1969). Effects of immunosympathectomy on the blood pressure of genetically hypertensive rats. Proc. Univ. Otago Med. Sch., 47, 42-44.

CLINESCHMIDT, B.V., GELLER, R.G., GOVIER, W.C. and SJOERDSMA, A. (1970). Reactivity to norepinephrine and nature of the alpha adrenergic receptor in vascular smooth muscle of a genetically hypertensive rat. Eur. J. Pharmacol., 10, 45-50.

CONN, J.W. (1955). Primary aldosteronism, new clinical syndrome. J. Lab. clin. Med., 45, 3-17.

CONN, J.W., COHEN, E.L. and ROVNER, D.R. (1964). Suppression of plasma renin activity in primary aldosteronism. J.A.M.A., 190, 213-221.

CONWAY, J. (1955). Behaviour of the blood pressure in normal and hypertensive rabbits in response to L-noradrenaline and to ganglion block by hexa- or pentamethonium. J. Physiol., 127, 69-80.

CONWAY, J. (1963). A vascular abnormality in hypertension. A study of blood flow in the forearm. Circulation, 27, 520-529.

COWDRY, E.V. (1938). "A textbook of histology". 2nd Ed. pp. 119-121. Philadelphia.

COWLEY, A.W., LIARD, J.F. and GUYTON, A.C. (1973). Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. Circulation Res., 32, 564-576.

CRANE, W.A.J. and DUTTA, L.P. (1963). The utilization of tritiated thymidine for deoxyribonucleic acid synthesis by the lesions of experimental hypertension in rats. J. Path. Bact., 86, 83-97.

CUSHING, H. (1932). The basophil adenomas of pituitary body and their clinical manifestations. Bull. John Hopkins Hosp., 50, 137-195.

DAHL, L.K. (1961). Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. J. exp. Med., 114, 231-236.

DAHL, L.K., HEINE, M. and TASSINARI, L. (1964). Effects of chronic excess salt ingestion : Vascular reactivity in two strains of rats with opposite genetic susceptibility to experimental hypertension. Circulation, 30, (Suppl II), 11-22.

DAHL, L.K. and TUTHILL, R. (1974). Further evidence of the toxicity of NaCl. Increased blood pressure and mortality in the spontaneously hypertensive rat. J. exp. Med., 139, 617-628.

DANIELS, A.E., SEVERS, W.B. and BUCKLEY, J.P. (1967). The effect of angiotensin II on the ^{45}Ca distribution of the rat. Life Sci., 6, 545-549.

DANIELS, E.G., HINMAN, J.W., LEACH, B.E. and MUIRHEAD, E.E. (1967). Identification of prostaglandin E_2 as the major vasodepressor lipid of rabbit renal medulla. Nature (Lond.), 215, 1298-1299.

DAVIS, E. and LANDAU, J. (1966). The influence of adrenaline on the small blood vessels in normotension and hypertension. Biblphie anat., 9, 1-6.

DAVIS, J.O. (1973). The control of renin release. Am. J. Med., 55, 333-350.

DAVIS, J.O., FREEMAN, R.H., JOHNSON, J.A. and SPIELMAN, W.S. (1974). Agents which block the action of the renin-angiotensin system. Circulation Res., 34, 279-285.

DAY, M.D. and MOORE, A.F. (1973). Potentiation by angiotensin II of noradrenaline-induced contractions of a rabbit isolated thoracic aorta. Br. J. Pharmac., 48, 338-339P.

DE CHAMPLAIN, J., KRAKOFF, L.R. and AXELROD, J. (1968). Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. Circulation Res., 23, 479-491.

DE CHAMPLAIN, J., KRAKOFF, L.R. and AXELROD, J. (1969). Interrelationships of sodium intake, hypertension, and norepinephrine storage in the rat. Circulation Res., Suppl I, 25, 75-91.

DE CHAMPLAIN, J., MUELLER, R.A. and AXELROD, J. (1969). Turnover and synthesis of norepinephrine in experimental hypertension in rats. Circulation Res., 25, 285-291.

DE JONG, W., LOVENBERG, W. and SJOERDSMA, A. (1972). Increased plasma renin activity in the spontaneously hypertensive rat. Proc. Soc. exp. Biol. Med., 139, 1213-1216.

DEMURA, H., FUKUCHI, S., TAKAHASHI, H. and GOTO, K. (1965). The vascular reactivity to vasoactive substances and the electrolyte contents in arterial walls. Tohoku J. exp. Med., 86, 366-379.

DE QUATTRO, V. and MIURA, Y. (1973). Neurogenic factors in human hypertension : mechanism or myth? Am. J. Med., 55, 362-378.

DEVINE, C.E., SOMLYO, A.V. and SOMLYO, A.P. (1972). Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell. Biol., 52, 690-718.

DICKINSON, C.J. (1965). "Neurogenic Hypertension". Oxford : Blackwell Scientific Publications.

DICKINSON, C.J. and LAWRENCE, J.R. (1963). A slowly developing pressor response to small concentrations of angiotensin : its bearing on the pathogenesis of chronic renal hypertension. Lancet, 1, 1354-1356.

DJOJOSUGITO, A.M., FOLKOW, B., OBERG, B. and WHITE, S.W. (1970). A comparison of blood viscosity measured in vitro and in a vascular bed. Acta physiol. scand., 78, 70-84.

DORR, L.D. and BRODY, M.J. (1966). Preliminary observations on the role of the sympathetic nervous system in development and maintenance of experimental renal hypertension. Proc. Soc. exp. Biol. Med., 123, 155-158.

DOUGLAS, B.H., GUYTON, A.C., LANGSTON, J.B. and BISHOP, V.S. (1964). Hypertension caused by salt loading, II : Fluid volume and tissue pressure changes. Am. J. Physiol., 207, 669-671.

DOYLE, A.E. (1968). Vascular reactivity in human hypertension. N.Z. med. J., 67, 295-303.

DOYLE, A.E. and BLACK, H. (1955). Reactivity to pressor agents in hypertension. Circulation, 12, 974-980.

DOYLE, A.E. and FRASER, J.R.E. (1961a). Essential hypertension and inheritance of vascular reactivity. Lancet, 2, 509-511.

DOYLE, A.E. and FRASER, J.R.E. (1961b). Vascular reactivity in hypertension. Circulation Res., 9, 755-758.

DOYLE, A.E., FRASER, J.R.E. and MARSHALL, R.J. (1959). Reactivity of forearm vessels to vasoconstrictor substances in hypertensive and normotensive subjects. Clin. Sci., 18, 441-454.

DOYLE, A.E. and SMIRK, F.H. (1955). Neurogenic component in hypertension. Circulation, 12, 543-552.

DUFF, R.S. (1957). Adrenaline sensitivity of peripheral blood vessels in hypertension. Br. Heart. J., 19, 45-52.

DUPONT, J. and SASSARD, J. (1974). Vascular reactivity in spontaneously hypertensive, normotensive and hypotensive rats. Br. J. Pharmac. 50, 185-188.

ENGELMAN, K. and PORTNOY, B. (1970). A sensitive double isotope derivative assay for norepinephrine and epinephrine : normal resting human plasma levels. Circulation Res., 26, 53-57.

ETTINGER, U., SEIBAL, K.L. and RIECKER, G. (1970). The reactivity of isolated small arteries for norepinephrine in essential hypertension. Int. Z. Klin. Pharmacol. Ther. Toxikol., 4, 121-122.

FERRARIO, C.M., McCUBBIN, J.W. and PAGE, I.H. (1969). Haemodynamic characteristics of chronic experimental neurogenic hypertension in unanaesthetized dogs. Circulation Res., 24, 911-922.

FERRARIO, C.M. PAGE, I.H. and McCUBBIN, J.W. (1970). Increased cardiac output as a contributory factor in experimental renal hypertension in dogs. Circulation Res., 27, 799-810.

FIELD, F.P., JANIS, R.A. and TRIGGLE, D.J. (1972). Aortic reactivity of rats with genetic and experimental renal hypertension. Can. J. Physiol. Pharmac., 50, 1072-1079.

FIELD, F.P., JANIS, R.A. and TRIGGLE, D.J. (1973). Relationship between aortic reactivity and blood pressure of renal hypertensive, hyperthyroid, and hypothyroid rats. Can. J. Physiol. Pharmac., 51, 344-353.

FINCH, L. (1971). Cardiovascular reactivity in the experimental hypertensive rat. Br. J. Pharmac., 42, 56-65.

FINCH, L. (1974). Vascular reactivity in hypertensive rats after treatment with anti-hypertensive agents. Life Sci., 15, 1827-1836.

FINCH, L. and LEACH, G.D.H. (1970). Does the adrenal medulla contribute to the maintenance of experimental hypertension. Eur. J. Pharmacol., 11, 388-391.

FISCHER, G.M. and LLAURADO, J.G. (1967). Connective tissue composition of canine arteries. Effects of renal hypertension. Archs Path., 84, 95-98.

FISCHER-FERRARO, C., NAHMOD, V.E., GOLDSTEIN, D.J. and FINKIELMAN, S. (1971). Angiotensin and renin in rat and dog brain. J. exp. Med., 133, 353-361.

FITZPATRICK, D.F., LANDON, E.J., DEBBAS, G. and HURWITZ, L. (1972). Calcium pump in vascular smooth muscle. Science, 176, 305-306.

FOLKOW, B. (1971). The haemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. Clin. Sci., 41, 1-12.

FOLKOW, B., GRIMBY, G. and THULESIUS, O. (1958). Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. Acta physiol. scand., 44, 255-272.

FOLKOW, B., GUREVICH, M., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1971). The haemodynamic consequences of regional hypotension in spontaneously hypertensive and normotensive rats. Acta physiol. scand., 83, 532-541.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y., WEISS, L., ALBRECHT, I. and JULIUS, S. (1974). Analysis of design and reactivity of series-coupled vascular sections in spontaneously hypertensive rats (SHR). Acta physiol, scand., 90, 654-656.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y., SIVERTSSON, R. and WEISS, L. (1973). Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and in spontaneously hypertensive rats. Circulation Res., 33, Suppl I, 2-13.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1970a). Structurally based increase of flow resistance in spontaneously hypertensive rats. Acta physiol. scand., 79, 373-378.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1970b). Background of increased flow resistance and vascular "reactivity" in spontaneously hypertensive rats. Acta physiol. scand., 79, 42-43A.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1970c). Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats. Acta physiol. scand., 80, 93-106.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1971). The effect of intense treatment with hypotensive drugs on structural design of the resistance vessels in spontaneously hypertensive rats. Acta physiol. scand., 83, 280-282.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1972). The effects of "immunosympathectomy" on blood pressure and vascular "reactivity" in normal and spontaneously hypertensive rats. Acta physiol. scand., 84, 512-523.

FOLKOW, B., HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1973). Time course and extent of structural adaptation of the resistance vessels in renal hypertensive rats (RHR) as compared with spontaneously hypertensive rats (SHR). Acta physiol. scand., 87, 10-11A.

FOLKOW, B. and NEIL, E. (1971). "Circulation". p269. London : Oxford University Press.

FORMAN, B.H. and MULROW, P.J. (1974). Effect of propranolol on blood pressure and plasma renin activity in the spontaneously hypertensive rat. Circulation Res., 35, 215-221.

FRASER, R., BROWN, J.J., CHINN, R.H., LEVER, A. F. and ROBERTSON, J.I.S. (1969). The control of aldosterone secretion and its' relationship to the diagnosis of hyper-aldosteronism. Scott, med. J., 14, 420-440.

FREIS, E.D. (1960). Hemodynamics of hypertension. Physiol. Rev., 40, 27-50.

FRIEDMAN, S.M. and FRIEDMAN, C.L. (1964). Ionic basis of vascular response to vasoactive substances. Can. med. Ass. J., 90, 167-173.

FRIEDMAN, S.M. and FRIEDMAN, C.L. (1967). The ionic matrix of vasoconstriction. Circulation Res., 21, Suppl II, 147-155.

FRIEDMAN, S.M., FRIEDMAN, C.L. and NAKASHIMA, M. (1957). Cationic shifts and blood pressure regulation. Circulation Res., 5, 261-267.

FROHLICH, E.D., TARAZI, R.C. and DUSTAN, H.P. (1969). Re-examination of the hemodynamics of hypertension. Am. J. Med. Sci., 257, 9-23.

FUKIYAMA, K., McCUBBIN, J.W. and PAGE, I.H. (1971). Chronic hypertension elicited by infusion of angiotensin into the vertebral arteries of unanesthetized dogs. Clin. Sci., 40, 283-291.

FURCHGOTT, R.F. and BHADROKOM, S. (1953). Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrate and other drugs. J. Pharmac. exp. Ther., 108, 129-143.

FURNESS, J.B. and MARSHALL, J.M. (1974). Correlation of the directly observed responses of mesenteric vessels of the rat to nerve stimulation and noradrenaline with the distribution of adrenergic nerves. J. Physiol., 239, 75-78.

GADDUM, J.H., HAMEED, K.A., HATHWAY, D.E. and STEPHENS, F.F. (1955). Quantitative studies of antagonists for 5-hydroxytryptamine. Q. J. exp. Physiol. 40, 49-74.

GARDNER, D.L. and HONORÉ, L.H. (1964a). Vascular response to polypeptides in adrenal-regeneration hypertension. Archs int. Pharmacodyn. Thér., 150, 492-503.

GARDNER, D.L. and HONORÉ, L.H. (1964b). Vascular response to catecholamines and to acetyl choline in adrenal-regeneration hypertension. Archs int. Pharmacodyn. Thér., 150, 504-515.

GARDNER, D.L. and MATTHEWS, V.A. (1969). Ultrastructure of the wall of small arteries in early experimental rat hypertension. J. Path. Bact., 97, 51-62.

GAVRAS, H., BRUNNER, H.R., LARAGH, J.H., VAUGHAN, E.D., KOSS, M., COTE, L.J. and GAVRAS, I. (1975). Malignant hypertension resulting from deoxycorticosterone acetate and salt excess : Role of renin and sodium in vascular changes. Circulation Res., 36, 300-309.

GENEST, J., LEMIEUX, G., DAVIGNON, A., KOIW, E., NOWACZYNSKI, W. and STEYERMARK, P. (1956). Human arterial hypertension a state of mild chronic hyperaldosteronism. Science, 123, 503-505.

GENEST, J., SIMARD, S., ROSENTHAL, J. and BOUCHER, R. (1969). Norepinephrine and renin content in arterial tissue from different vascular beds. Canad. J. Physiol. Pharmacol., 47, 87-91.

GEORGE, A.J. and LEACH, G.D.H. (1973). The effects of changes in ionic environment and modification of adrenergic function on the vascular responses to sympathomimetic amines. J. Pharm. Pharmac., 25, 521-529.

GEROLD, M. and TSCHIRKY, H. (1968). Measurement of blood pressure in unanaesthetized rats and mice. Arzneimittel-Forsch., 18, 1285-1287.

GITLOW, S.E., MENDLOWITZ, M., BERTANI, L.M., WILK, E.K. and GLABMAN, S. (1969). Tritium excretion of normotensive and hypertensive subjects after administration of tritiated norepinephrine. J. Lab. clin. Med., 73, 129-134.

GITLOW, S.E., MENDLOWITZ, M., WILK, E.K., WOLF, R.L. and NAFTCHI, N.E. (1964). Plasma clearance of dl-β-^{3}H norepinephrine in normal human subjects and patients with essential hypertension. J. clin. Invest., 43, 2009-2015.

GOCKE, D.J., GERTEN, J., SHERWOOD, L.M. and LARAGH, J.H. (1969). Physiological and pathological variations of plasma angiotensin II in man. Correlation with renin activity and sodium balance. Circulation Res., 24, Suppl I, 131-146.

GOLDBLATT, H., LYNCH, J., HANZAL, R.F. and SUMMERVILLE, W.W. (1934). Studies on experimental hypertension : I. Production of persistent elevation of systolic blood pressure by means of renal ischemia. J. exp. Med., 59, 347-379.

GOLDENBERG, M., HINES, K.L., BALDWIN, E.F., GREENE, D.G. and ROH, C.E. (1948). The hemodynamic response of man to norepinephrine and epinephrine and its relation to the problem of hypertension. Am. J. Med., 5, 792-806.

GOLDRING, W. and CHASIS, H. (1944). "Hypertension and Hypertensive Disease" New York : The Commonwealth Fund.

GOMBOS, E.A., HULET, W.H., BOPP, P., GOLDRING, W., BALDWIN, D.S. and CHASIS, H. (1962). Reactivity of renal and systemic circulations to vasoconstrictor agents in normotensive and hypertensive subjects. J. clin. Invest., 41, 203-217.

GORDON, D.B. (1962). Increased vascular responsiveness preceding renal hypertension. Fedn Proc. Fedn Am. Socs exp. Biol., (Abstr). 21, 113.

GORDON, D.B. and NOGUEIRA, A. (1962). Increased vascular reactivity in experimental hypertension. Circulation Res., 10, 269-273.

GRAWITZ, P. and ISRAEL, O. (1879). Experimentelle Untersuchung uber den Zusammenhang zwischen nierenerkrankung und herzhypertrophie. Archs Path. Anat., 77, 315-346.*

GREENBERG, S. and LONG, J.P. (1973). Enhancement of vascular smooth muscle responses to vasoactive stimuli by prostaglandin E_1 and E_2. Archs int. Pharmacodyn. Thér., 206, 94-104.

GROLLMAN, A. (1944). A simplified procedure for inducing chronic renal hypertension in the mammal. Proc. Soc. exp. Biol. Med., 57, 102-104.

GROLLMAN, A. (1970). Pressor activity of circulating blood after focal infarction of the kidney in the rat. Proc. Soc. exp. Biol. Med., 134, 1120-1122.

GROLLMAN, A. and HARRISON, T.R. (1945). Effect of rigid sodium restriction on blood pressure and survival of hypertensive rats. Proc. Soc. exp. Biol. Med., 60, 52-55.

GROLLMAN, A., HARRISON, T.R. and WILLIAMS, J.R. (1940). The effect of various sterol derivatives on the blood pressure of the rat. J. Pharmacol. exp. Ther., 69, 149-155.

GROLLMAN, A. and KRISHNAMURTY, V.S.R. (1971). A new pressor agent of renal origin : its differentiation from renin and angiotensin. Am. J. Physiol., 221, 1499-1506.

GROLLMAN, A. and KRISHNAMURTY, V.S.R. (1973). Contractile response of the aorta of the normotensive and acute and chronic hypertensive rat. Archs int. Pharmacodyn. Thér., 203, 376-387.

GROLLMAN, A. and WHITE, F.N. (1958). Induction of renal hypertension in rats and dogs by potassium or choline deficiency. Am. J. Physiol., 193, 144-146.

GROLLMAN, A., WILLIAMS, J.R. and HARRISON, T.R. (1940). Reduction of elevated blood pressure by administration of renal extract. J.A.M.A., 115, 1169-1176.

GROSS, F. (1960). Adrenocortical function and renal pressor mechanisms in experimental hypertension. In Essential Hypertension, ed. BOCK, K.D. and COTTIER, P.T. pp 92-111. Berlin : Springer-Verlag.

GROSS, F. (1971). The renin-angiotensin system and hypertension. Ann. intern. Med., 75, 777-787.

GROSS, F. and BOCK, K.D. (1962). Some contributions to the pharmacology of synthetic angiotensin. Circulation, 25, 193-199.

GROSS, F., BRUNNER, H. and ZIEGLER, M. (1965). Renin-angiotensin system, aldosterone, and sodium balance. Recent Prog. Horm. Res., 21, 119-167.

GROSS, F. and SULSER, F. (1957). Influence of the adrenals on the blood pressure increasing action of renin and pressor substances in the kidneys. Arch. exp. Path. Pharmak., 230, 274-283.

GUTKIN, M., LEVINSON, G.E., KING, A.S. and LASKER, N. (1969). Plasma renin activity in end-stage kidney disease. Circulation, 40, 563-574.

GUYTON, A.C., COLEMAN, T.G., COWLEY, A.W., MANNING, R.D., NORMAN, R.A. and FERGUSON, J.D. (1974). A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. Circulation Res., 35, 159-176.

GUYTON, A.C., COLEMAN, T.G., COWLEY, A.W., SCHEEL, K.W., MANNING, R.D. and NORMAN, R.A. (1972). Arterial pressure regulation : overriding dominance of the kidneys in long-term regulation and in hypertension. Am. J. Med., 52, 584-594.

GUYTON, A.C., COLEMAN, T.G. and GRANGER, H.J. (1972). Circulation : Overal regulation. A. Rev. Physiol., 34, 13-46.

HABER, E., KOERNER, T., PAGE, L.B., KLIMAN, B. and PURNODE, A. (1969). Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J. clin. Endocr. Metab., 29, 1349-1355.

HADDY, F.J. (1974). Local control of vascular resistance as related to hypertension. Archs intern. Med., 133, 916-931.

HADDY, F.J. and SCOTT, J.B. (1965). Effects of electrolytes and water upon resistance to blood flow through intact vascular beds. In "Electrolytes and Cardiovascular Diseases", ed. BAJUSZ, E. pp. 383-400. Baltmore : Williams and Wilkins.

HAEUSLER, G. and FINCH, L. (1972a). Vascular reactivity to 5-hydroxytryptamine and hypertension in the rat. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 272, 101-116.

HAEUSLER, G. and FINCH, L. (1972b). Vascular resistance and reactivity to various vasoconstrictor agents in hypertensive rats. In "Spontaneous Hypertension," ed. OKAMOTO, K. pp. 97-102. New York : Heidelberg.

HAEUSLER, G., FINCH, L. and THOENEN, H. (1972). Central adrenergic neurones and the initiation and development of experimental hypertension. Experientia, 28, 1200-1203.

HAEUSLER, G. and HAEFELY, W. (1970). Pre- and postjunctional supersensitivity of the mesenteric artery preparation from normotensive and hypertensive rats. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 266, 18-33.

HALLBÄCK, M. and FOLKOW, B. (1974). Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. Acta physiol. scand., 90, 684-698.

HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1971). Reactivity to noradrenaline of aortic strips and portal veins from spontaneously hypertensive and normotensive rats. Acta physiol. scand., 81, 176-181.

HALLBÄCK, M., LUNDGREN, Y. and WEISS, L. (1974). Distensibility of the resistance vessels in spontaneously hypertensive rats (SHR) as compared with normotensive control rats (NCR). Acta physiol. scand., 90, 57-68.

HAM, A.W. (1957). "Histology". p486. London : Pitman Medical Publishing Co.

HANDLER, P. and BERNHEIM, F. (1950). Importance of dietary protein, calories and salt in experimental renal hypertension. Am. J. Physiol., 160, 31-40.

HANSEN, T.R., ABRAMS, G.D. and BOHR, D.F. (1974). Role of pressure in structural and functional changes in arteries of hypertensive rats. Circulation Res., 35, Suppl I, 101-107.

HARRIS, G.S. and PALMER, W.A. (1971). The effect of enzymatic depolymerization of arterial mucopolysaccharides on sodium ion content and vessel reactivity. Clin. Sci., 40, 293-303.

HARRIS, G.S. and PALMER, W.A. (1972). Effect of increased sodium ion on arterial sodium and reactivity. Clin. Sci., 42, 301-309.

HEISTAD, D.D., ABBOUD, F.M. and BALLARD, D.R. (1971). Relationship between plasma sodium concentration and vascular reactivity in man. J. clin. Invest., 50, 2022-2032.

HELMER, O.M. (1971). Purification, standardization and assay of renin and angiotensin. In "Kidney hormones," ed. FISHER, J.W. pp 59-85. London : Academic Press.

HENNING, M. and RUBENSON, A. (1971). Evidence that the hypotensive action of methyldopa is mediated by central actions of methyl noradrenaline. J. Pharm. Pharmac., 23, 407-411.

HICKAM, J.B. and CARGILL, W.H. (1948). Effect of exercise on cardiac output and pulmonary arterial pressure in normal persons and in patients with cardiovascular disease and pulmonary emphysema. J. clin. Invest., 27, 10-23.

HINES, E.A. and BROWN, G.E. (1933). A standard test for measuring the variability of blood pressure : its significance as an index of the prehypertensive state. Ann. Intern. Med., 7, 209-217.

HINKE, J.A.M. (1965). In vitro demonstration of vascular hyper-responsiveness in experimental hypertension. Circulation Res., 17, 359-371.

HINKE, J.A.M. (1966). Effect of Ca^{++} upon contractility of small arteries from D.C.A. - hypertensive rats. Circulation Res., 19, Suppl I, 23-33.

HINKE, J.A.M., WILSON, M.L. and BURNHAM, S.C. (1964). Calcium and the contractility of arterial smooth muscle. Am. J. Physiol., 206, 211-217.

HOLLANDER, W., KRAMSCH, D.M., FARMELANT, M. and MADOFF, I.M. (1968). Arterial wall metabolism in experimental hypertension of coarctation of the aorta of short duration. J. clin. Invest., 47, 1221-1229.

HOLLOWAY, E.T. and BOHR, D.F. (1973). Reactivity of vascular smooth muscle in hypertensive rats. Circulation Res., 33, 678-685.

HOLLOWAY, E.T., SITRIN, M.D. and BOHR, D.F. (1972). Calcium dependance of vascular smooth muscle from normotensive and hypertensive rats. In "Hypertension '72". ed. GENEST, J. and KOIW, E. pp 400-408. New York : Heidelberg.

HONORÉ, L.H. and GARDNER, D.L. (1966a). Cardiovascular reactivity of rats during the development of salt hypertension. I. The response to polypeptides. Archs int. Pharmacodyn. Thér., 164, 173-182.

HONORÉ, L.H. and GARDNER, D.L. (1966b). Cardiovascular reactivity of rats during the development of salt hypertension. II. The responses to adrenaline, noradrenaline and acetyl choline. Archs int. Pharmacodyn. Thér., 164, 183-191.

HORWITZ, D., CLINESCHMIDT, B.V., VAN BUREN, J.M. and OMMAYA, A.K. (1974). Temporal arteries from hypertensive and normotensive man. Reactivity to norepinephrine and characteristics of alpha-adrenergic receptors. Circulation Res., 35, Suppl I, 109-115.

HUTCHINS, P.M. and DARNELL, A.E. (1974). Observation of a decreased number of small arterioles in spontaneously hypertensive rats. Circulation Res., 35, Suppl I, 161-165.

INNES, I.R. (1962). An action of 5-hydroxytryptamine on adrenaline receptors. Br. J. Pharmac., 19, 427-441.

IRIUCHIJIMA, J. (1973). Sympathetic discharge rate in spontaneously hypertensive rats. Jap. Heart J., 14, 350-356.

JANIS, R.A. and TRIGGLE, D.J. (1972). α-Adrenoceptors of aortae from genetically hypertensive rats : reaction with 2-halogenoethylamines. J. Pharm. Pharmac., 24, 602-608.

JOHANSSON, B. (1974). Determinants of vascular reactivity. Fedn Proc. Fedn Am. Socs exp. Biol., 33, 121-126.

JONES, A.W., FEIGL, E.O. and PETERSON, L.H. (1964). Water and electrolyte content of normal and hypertensive arteries in dogs. Circulation Res., 15, 386-392.

JUDSON, W.E., EPSTEIN, F.H. and WILKINS, R.W. (1950). The comparative effects of small intravenous doses of l-norepinephrine upon arterial pressure and pulse rate in normotensive subjects and in hypertensive patients before and after thoraco-lumbar sympathectomy. J. clin. Invest., 29, 1414-1420.

JUDSON, W.E., HOLLANDER, W. and WILKINS, R.W. (1957). Effect of exercise in the supine position on cardiovascular and renal function in hypertensive patients before and during chronic oral hydralazine therapy. J. Lab. clin. Med., 49, 672-683.

KALSNER, S. (1974). A new approach to the measurement and classification of forms of supersensitivity of autonomic effector responses. Br. J. Pharmac., 51, 427-434.

KALSNER, S., NICKERSON, M. and BOYD, G.N. (1970). Selective blockade of potassium-induced contractions of aortic strips by β-diethylaminoethyl-diphenylpropylacetate (S.K.F. 525A). J. Pharmac. exp. Ther., 174, 500-508.

KAPLAN, N.M. (1974). Adrenal causes of hypertension. Archs intern. Med., 133, 1001-1006.

KAPLAN, N.M. and SILAH, J.G. (1964). The effect of angiotensin II on the blood pressure in humans with hypertensive disease. J. clin. Invest., 43, 659-669.

KATZENSTEIN, M. (1905). Experimenteller beitrag zur erkenntris der bei nephritis auftretendon hypertrophie des linken herzens. Virchows Arch. Path. Anat., 182, 327-337.*

KHAIRALLAH, P.A., DAVILA, D., PAPANICOLAOU, N., GLENDE, N.M. and MEYER, P. (1971). Effects of angiotensin infusion on catecholamine uptake and reactivity in blood vessels. Circulation Res., 29, Suppl II, 96-104.

KOLETSKY, S. (1959). Role of salt and renal mass in experimental hypertension. Archs Path., 68, 11-22.

KOLETSKY, S. and GOODSITT, A.M. (1960). Natural history and pathogenesis of renal ablation hypertension. Archs Path., 69, 654-662.

KOLETSKY, S., SHOOK, P. and RIVERA-VELEZ, J. (1970). Lack of increased renin-angiotensin activity in rats with spontaneous hypertension. Proc. Soc.exp. Biol. Med. 134, 1187-1190.

KOLFF, W.J., NAKAMOTO, S., POUTASSE, E.F., STRAFFON, R.A. and FIGUEROA, J.E. (1964). Effect of bilateral nephrectomy and kidney transplantation on hypertension in man. Circulation, 30, Suppl II, 23-35.

KRIEGER, E.M. (1964). Neurogenic hypertension in the rat. Circulation Res., 15, 511-521.

LARAGH, J.H., SEALEY, J.E. and SOMMERS, S.C. (1966). Patterns of adrenal secretion and urinary excretion of aldosterone and plasma renin activity in normal and hypertensive subjects. Circulation Res., 18, 158-174.

LARAMORE, D.C. and GROLLMAN, A. (1950). Water and electrolyte content of tissues in normal and hypertensive rats. Am. J. Physiol., 161, 278-282.

LAUWERS, P. and GOMEZ, A. (1964). Influence of renal autografts on arterial hypertension in rats. Revue fr. Étud. clin. biol., 9, 736-741.

LAVERTY, R. (1961). Increased vascular reactivity in rats with genetic hypertension. Proc. Soc. Univ. Otago med. Sch., 39, 23-24.

LAVERTY, R., McGREGOR, D.D. and McQUEEN, E.G. (1968). Vascular reactivity in experimental hypertension. N.Z. Med. J., 67, 303-309.

LAVERTY, R. and SMIRK, F.H. (1961). Observations on the pathogenesis of spontaneous inherited hypertension and constricted renal artery hypertension in rats. Circulation Res., 9, 455-464.

LEDINGHAM, J.M. (1971). Mechanisms in renal hypertension. Proc. R. Soc. Med., 64, 409-418.

LEDINGHAM, J.M. and COHEN, R.D. (1964). Changes in extracellular fluid volume and cardiac output during the development of experimental renal hypertension. Can. med. Ass. J., 90, 292-294.

LEDINGHAM, J.M. and PELLING, D. (1967). Cardiac output and peripheral resistance in experimental renal hypertension. Circulation Res., 21, Suppl II, 187-198.

LEE, R.E. and HOLZE, E.A. (1951). Peripheral vascular hemodynamics in the bulbar conjunctiva of subjects with hypertensive vascular disease. J. clin. Invest., 30, 539-546.

LEVY, J.V. (1973). Drug studies on arterial tissue of spontaneously hypertensive rats. Fedn Proc. Fedn Am. Socs exp. Biol. 32, (Abstr) 749.

LEWIS, B.M., HOUSSAY, H.E.J., HAYNES, F.W. and DEXTER, L. (1953). Dynamics of both right and left ventricles at rest and during exercise in patients with heart failure. Circulation Res., 1, 312-320.

LEWIS, G.P. and REIT, E. (1966). Further studies on the action of peptides on the superior cervical ganglion and suprarenal medulla. Br. J. Pharmac., 26, 444-460.

LOEB, R.S., ATCHLEY, D.W., FERREBEE, J.W. and RAGAN, C. (1939). Observations on effect of desoxycorticosterone esters and progesterone in patients with Addison's disease. Trans. Ass. Am. Physns., 54, 285-296.

LOOMIS, D. (1946). Hypertension and necrotizing arteritis in the rat following renal infarction. Archs Path., 41, 231-268.

LOUIS, W.J. (1970). Turnover of catecholamines in experimental hypertension. Circulation Res., 27, Suppl II, 49-53.

LOUIS, W.J., DOYLE, A.E. and ANAVEKAR, S. (1973). Plasma norepinephrine levels in essential hypertension. New. Engl. J. Med., 288, 599-601.

LOUIS, W.J., KRAUSS, K.R., KOPIN, I.J. and SJOERDSMA, A. (1970). Catecholamine metabolism in hypertensive rats. Circulation Res., 27, 589-594.

LUNDGREN, Y. (1974a). Regression of structural cardiovascular changes after reversal of experimental renal hypertension in rats. Acta physiol. scand., 91, 275-285.

LUNDGREN, Y. (1974b). Blood pressure and vascular design in renal hypertension in rats after prolonged propranolol treatment. Acta physiol. scand., 91, 409-416.

LUNDGREN, Y., HALLBÄCK, M., WEISS, L. and FOLKOW, B. (1974). Rate and extent of adaptive cardiovascular changes in rats during experimental renal hypertension. Acta physiol. scand., 91, 103-115.

MALIK, K.U. and LING, G.M. (1969). Modification by acetylcholine of the response of rat mesenteric arteries to sympathetic stimulation. Circulation Res., 25, 1-9.

MALIK, K.U. and McGIFF, J.C. (1975). Modulation by prostaglandin E_1 of adrenergic transmission in isolated perfused rabbit and rat mesenteric arteries. Fedn Proc. Fedn Am. Socs exp. Biol. 34, (Abstr), 763.

MALLOV, S. (1959). Comparative reactivities of aortic strips from hypertensive and normotensive rats to epinephrine and levarterenol. Circulation Res., 7, 196-201.

MALLOV, S. (1961). Effect of hypertension and sodium chloride on vascular reactivity in vitro. Am. J. Cardiol., 8, 542-548.

MALVANO, R., ZUCCHELLI, G.C., ROSA, U. and SALVETTI, A. (1972). Measurement of plasma renin activity by angiotensin I radioimmunoassay : I. an assessment of some methodological aspects. J. Nucl. Biol. Med., 16, 24-31.

MARSHALL, J. (1966). Evidence upon the neurogenic theory of hypertension. Lancet, 2, 410-412.

MARSHALL, G.R., VINE, W. and NEEDLEMAN, P. (1970). Specific competitive inhibitor of angiotensin II. Proc. natn. Acad. Sci. U.S.A., 67, 1624-1630.

MASSINGHAM, R. and SHEVDE, S. (1971). Aortic reactivity and electrophysiology in normotensive rats, spontaneously hypertensive rats and rats made hypertensive with desoxycorticosterone plus salt. Br. J. Pharmac., 43, 868-870.

MASSON, G.M.C., PAGE, I.H. and CORCORAN, A.C. (1950). Vascular reactivity of rats and dogs treated with desoxycorticosterone acetate. Proc. Soc. exp. Biol. Med., 73, 434-436.

MAXIMOV, A.A. and BLOOM, W. (1957). "A textbook of histology." 7th ed. pp. 234-235. Philadelphia and London : W.B. Saunders.

McCUBBIN, J.W., GREEN, J.H. and PAGE, I.H. (1956). Baroreceptor function in chronic renal hypertension. Circulation Res., 4, 205-210.

McCUBBIN, J.W. and PAGE, I.H. (1963). Neurogenic component of chronic renal hypertension. Science, 139, 210-215.

McGIFF, J.C., CROWSHAW, K. and ITSKOVITZ, H.D. (1974). Prostaglandins and renal function. Fedn Proc. Fedn Am. Socs exp. Biol., 33, 39-47.

McGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. J. Physiol., 177, 21-30.

McGREGOR, D.D. and SMIRK, F.H. (1968). Vascular responses in mesenteric arteries from genetic and renal hypertensive rats. Am. J. Physiol., 214, 1429-1433.

McGREGOR, D.D. and SMIRK, F.H. (1970). Vascular responses to 5-hydroxy-tryptamine in genetic and renal hypertensive rats. Am. J. Physiol., 219, 687-690.

McKENZIE, J.K., RYAN, J.W. and LEE, M.R. (1967). Effect of laparotomy on plasma renin activity in the rabbit. Nature (Lond.), 215, 542-543.

McQUEEN, E.G. (1956). Vascular reactivity in experimental renal and renoprival hypertension. Clin. Sci., 15, 523-532.

McQUEEN, E.G. (1957). Increased reactivity of isolated blood vessels in experimental renal hypertension to various pressor substances and its relationship to the adrenals. Proc. Univ. Otago med. Sch., 35, 32-33.

McQUEEN, E.G. (1961). The effect of control of blood pressure on vascular reactivity in experimental renal hypertension. Clin. Sci., 21, 133-140.

MELBY, J.C., DALE, S.L. and WILSON, T.E. (1971). 18-Hydroxydeoxycorticosterone in human hypertension. Circulation Res., 28, Suppl II, 143-152.

MENARD, J., ALEXANDRE, J.M., GIUDICELLI, J.F., AUZAN, C. and CHEVILLARD, C. (1973). Lack of antihypertensive effects of chronic administration of DL-propranolol in Grollman rats. Archs int. Pharmacodyn. Thér., 202, 298-303.

MENDLOWITZ, M., NAFTCHI, N.E., WOLF, R.L. and GITLOW, S.E. (1965). Vascular responsiveness in hypertensive and hypotensive states. Geriatrics, 20, 797-807.

MENEELY, G.R., TUCKER, R.G., DARBY, W.J. and AUERBACK, S.H. (1953). Sodium chloride toxicity in the albino rat; occurrence of hypertension and of syndrome of edema and renal failure. J. exp. Med., 98, 71-80.

MIKSCHE, L.W., MIKSCHE, U. and GROSS, F. (1970). Effects of sodium restriction on renal hypertension and renin activity in the rat. Circulation Res., 27, 973-984.

MOERMAN, E.J., HERMAN, A.G., BOGAERT, M.G. and DE SCHAEPDRYVER, A.F. (1969). Noradrenergic vascular responsiveness in hypertensive dogs. Archs int. Pharmacodyn. Thér., 178, 492-493.

MOULTON, R., SPENCER, A.G. and WILLOUGHBY, D.A. (1958). Noradrenaline sensitivity of hypertension measured with a radioactive sodium technique. Br. Heart J., 20, 224-228.

MUIRHEAD, E.E., DANIELS, E.G., BOOTH, E., FREYBURGER, W.A. and HINMAN, J.W. (1965). Renomedullary vasodepression and antihypertensive function. Archs path., 80, 43-49.

MUIRHEAD, E.E., STIRMAN, J.A. and JONES, F. (1960). Renal autoexplantation and protection against renoprival hypertensive cardiovascular disease and hemolysis. J. clin. Invest., 39, 266-281.

MUIRHEAD, E.E., STIRMAN, J.A., LESCH, W. and JONES, F. (1956). The reduction of postnephrectomy hypertension by renal homotransplant. Surgery Gynec. Obstet., 103, 673-686.

NAKAJIMA, T., SAKAKIBARA, S., SAKUMA, A. and SOKABE, H. (1973). Specific pressor activity of angiotensins I and II. Jap. J. Pharmacol., 23, 591-593.

NAKAMURA, K., GEROLD, M. and THOENEN, H. (1971a). Experimental hypertension of the rat : Reciprocal changes of norepinephrine turnover in heart and brain-stem. Naunyn-Schmeidebergs Arch. exp. Path. Pharmak., 268, 125-139.

NAKAMURA, K., GEROLD, M. and THOENEN, H. (1971b). Genetically hypertensive rats : Relationship between development of hypertension and the changes in norepinephrine turnover of peripheral and central adrenergic neurons. Naunyn-Schmeidebergs Arch. exp. Path. Pharmak., 271, 157-169.

NG, K.K.F. and VANE, J.R. (1968). Fate of angiotensin I in the circulation. Nature (Lond.), 218, 144-150.

NICHOLAS, T.E. (1970). Potentiation of the effects of noradrenaline and of sympathetic stimulation of the perfused rat caudal artery by angiotensin. J. Pharm. Pharmac., 22, 37-41.

NOLLA-PANADES, J. (1963). Hypertension and increased hind-limb vascular reactivity in experimental coarctation of the aorta. Circulation Res., 12, 3-9.

OGDEN, E., BROWN, L.T. and PAGE, E.W. (1940). The increased sensitivity of arterial muscle in the prehypertensive phase of experimental renal hypertension. Am. J. Physiol., 129, 560-564.

OKAMOTO, K. and AOKI, K. (1963). Development of a strain of spontaneously hypertensive rats. Jap. Circulation. J., 27, 282-293.

OKAMOTO, K., NOSAKA, S., YAMORI, Y. and MATSUMOTO, M. (1967). Participation of neural factor in the pathogenesis of hypertension in the spontaneously hypertensive rat. Jap. Heart J., 8, 168-180.

OLMSTED, F. and PAGE, I.H. (1965). Hemodynamic changes in trained dogs during experimental renal hypertension. Circulation Res., 16, 134-139.

OONO, Y. (1966). Vascular reactivity in experimental hypertension : responses of hindquarters preparations of rats. Jap. Circulation J., 30, 267-280.

PAGE, I.H. (1939). Production of persistent arterial hypertension by cellophane perinephritis. J.A.M.A., 113, 2046-2048.

PAGE, I.H. (1974). Arterial hypertension in retrospect. Circulation Res., 34, 133-142.

PAGE, I.H., KANEKO, Y. and McCUBBIN, J.W. (1966). Cardiovascular re-activity in acute and chronic renal hypertensive dogs. Circulation Res., 18, 379-387.

PAGE, I.H. and McCUBBIN, J.W. (1966). Physiology of arterial hypertension. In "Handbook of Physiology". Section 2, Vol. 3, ed. HAMILTON, W.F. and DOW, P. pp. 2163-2208. Baltmore : Williams and Wilkins Co.

PAGE, I.H. and TAYLOR , R.D. (1949). Variations of vascular reactivity in normal and hypertensive dogs. Am. J. Physiol., 156, 412-421.

PALAIC, D. and KHAIRALLAH, P.A. (1967). Inhibition of nor-epinephrine uptake by angiotensin. J. Pharm. Pharmac., 19, 396-397.

PALS, D.T. and FULTON, R.W. (1968). Interelationship between angiotensin and vascular alpha adrenergic receptors. Am. J. Physiol., 214, 506-512.

PALS, D.T., MASUCCI, F.D., DENNING, G.S., SIPOS, F. and FESSLER, D.C. (1971). Role of the pressor actions of angiotensin II in experimental hypertension. Circulation Res., 29, 673-681.

PANISSET, J.C. and BOURDOIS, P. (1968). Effect of angiotensin on the response to noradrenaline and sympathetic nerve stimulation, and on ^{3}H-noradrenaline uptake in cat mesenteric blood vessels. Can. J. Physiol. Pharmac., 46, 125-131.

PATON, W.D.M. (1961). A theory of drug action based on the rate of drug-receptor combination. Proc. R. Soc., 154, 21-69.

PATON, W.D.M. and ZIAMIS, E.J. (1949). The pharmacological actions of polymethylene bistrimethylammonium salts. Br. J. Pharmac. Chemother., 4, 381-400.

PATTERSON, G.C., SHEPHERD, J.T. and WHELAN, R.F. (1957). The resistance to blood flow in the upper and lower limb vessels in patients with co-arctation of the aorta. Clin. Sci., 16, 627-632.

PEART, W.S., ROBERTSON, J.I. and GRAHAME-SMITH, D.G. (1961). Examination of the relationship of renin release to hypertension produced in the rabbit by renal artery constriction. Circulation Res., 9, 1171-1184.

PHELAN, E.L. (1966). Cardiovascular reactivity in rats with spontaneous inherited hypertension and constricted renal artery hypertension. Am. Heart J., 71, 50-57.

PHELAN, E.L. (1968). The New Zealand strain of rats with genetic hypertension. N.Z. med. J., 67, 334-344.

PHELAN, E.L., ERYETISHIR, I. and SMIRK, F.H. (1962). Observations on the responses of rats with spontaneous hypertension and control rats to pressor drugs and hexamethonium. Circulation Res., 10, 817-824.

PICKERING, G.W. (1936). The peripheral resistance in persistent arterial hypertension. Clin. Sci., 2, 209.

PICKERING, G.W. (1968). High blood pressure. 2nd Ed. pp. 1-5; 390-392. London : J. & A. Churchill Ltd.

PICKERING, G.W. and KISSIN, M. (1936). The effects of adrenaline and of cold on the blood pressure in human hypertension. Clin. Sci., 2, 201-207.

PLATT, R. and STANBURY, S.W. (1950). Sympathectomy in hypertension. Lancet, 1, 651-659.

POPOVIC, P., SYBERS, H. and POPOVIC, V.P. (1968). Permanent cannulation of blood vessels in mice. J. appl. Physiol., 25, 626-627.

RAAB, W., HUMPHREYS, R.J. and LEPESCHKIN, E. (1950). Potentiation of pressor effects of norepinephrine and epinephrine in man by desoxycorticosterone acetate. J. clin. Invest., 29, 1397-1404.

RASMUSSEN, H. (1970). Cell communication, calcium ion, and cyclic adenosine monophosphate. Science, 170, 404-412.

REDLEAF, P.D. and TOBIAN, L. (1958a). The question of vascular hyper-responsiveness in hypertension. Circulation Res., 6, 185-193.

REDLEAF, P.D. and TOBIAN, L. (1958b). Sodium restriction and reserpine administration in experimental renal hypertension. Circulation Res., 6, 343-351.

ROGUSKA, J., SIMON, N.M. and DEL GRECO, F. (1968). Pressor response to angiotensin II in hypertension : correlation with plasma renin activity and response to norepinephrine and metaraminol. Am. J. Cardiol., 21, 705-713.

ROSENTHAL, J. and HOLLANDER, W. (1973). Dissociation of changes in arterial and plasma renin in D.O.C.A. hypertension. Circulation, 48, Suppl IV, 43.

ROWE, G.G., CASTILLO, C.A., MAXWELL, G.M. and CRUMPTON, C.W. (1961). Haemodynamic study of hypertension including observations on coronary blood flow. Ann. intern. Med., 54, 405-412.

RUSSEK, H.I. and ZOHMAN, B.L. (1945). The influence of age upon blood pressure response to the cold pressor test. Am. Heart J., 29, 113-119.

SCROOP, G.C. and WHELAN, R.F. (1968). Vascular reactivity studies in hypertension. Aust. J. exp. Biol. med. Sci., 46, 555-561.

SEN, S., SMEBY, R.R. and BUMPUS, F.M. (1968). Antihypertensive effect of an isolated phospholipid. Am. J. Physiol., 214, 337-341.

SEN, S., SMEBY, R.R. and BUMPUS, F.M. (1972). Renin in rats with spontaneous hypertension. Circulation Res., 31, 876-880.

SEVERS, W.B., DANIELS, A.E., SMOOKLER, H.H., KINNARD, W.J. and BUCKLEY, J.P. (1966). Interrelationship between angiotensin II and the sympathetic nervous system. J. Pharmac. exp. Ther., 153, 530-537.

SHIBATA, S., KURAHASHI, K. and KUCHII, M. (1973). A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive rats. J. Pharmac. exp. Ther., 185, 406-417.

SHIBAYAMA, F., MIZOGAMI, S. and SOKABE, H. (1971). Cardiovascular reactivity in hypertensive rats. Jap. Heart J., 12, 68-78.

SHORT, D. (1966). Morphology of the intestinal arterioles in chronic human hypertension. Br. Heart J., 28, 184-192.

SINGER, B., LOSITO, C. and SALMON, S. (1963). Aldosterone and corticosterone secretion rates in rats with experimental renal hypertension. Acta endocr., 44, 505-518.

SITRIN, M.D. and BOHR, D.F. (1971). Ca and Na interaction in vascular smooth muscle contraction. Am. J. Physiol., 220, 1124-1128.

SIVERTSSON, R. (1970). The hemodynamic importance of structural vascular changes in essential hypertension. Acta physiol. scand., Suppl. 343.

SKELTON, F.R. (1955). Development of hypertension and cardiovascular-renal lesions during adrenal regeneration in the rat. Proc. Soc. exp. Biol. Med., 90, 342-346.

SMIRK, F.H. and HALL, W.H. (1958). Inherited hypertension in rats. Nature (Lond.), 182, 727-728.

SOMLYO, A.P. and SOMLYO, A.V. (1968). Vascular smooth muscle. I. Normal structure, pathology, biochemistry, and biophysics. Pharmac. Rev., 20, 197-272.

SPECTOR, S., FLEISCH, J.M., MALING, H.M. and BRODIE, B.B. (1969). Vascular smooth muscle reactivity in normotensive and hypertensive rats. Science, 166, 1300-1301.

STAQUET, M., DEMANET, J.C. and BASTÉNIE, P.A. (1965). Étude de la sensibilité vasculaire a la noradrénaline dans l'hypertension artérielle. Revue fr. Étud. clin. biol., 10, 394-399.

STURTEVANT, F.M. (1956). Studies of vascular reactivity in normotensive and metacorticoid hypertensive rats. Am. Heart. J., 51, 410-418.

SUSIC, D. and SPARKS, J.C. (1974). The renin-angiotensin system of uni-nephrectomized rats under diuretic treatment. Res. Comm. Chem. Pathol. Pharmacol., 8, 423-429.

SWALES, J.D. and THURSTON, H. (1973). Generation of angiotensin II at peripheral vascular level : studies using angiotensin II antisera. Clin. Sci., 45, 691-700.

SWALES, J.D., THURSTON, H., QUEIROZ, F.P. and MEDINA, A. (1972). Sodium balance during the development of experimental hypertension. J. Lab. clin. Med., 80, 539-547.

TARVER, J., BERKOWITZ, B. and SPECTOR, S. (1971). Alterations in tyrosine hydroxylase and monoamine oxidase activity in blood vessels. Nature (Lond.), 231, 252-253.

TAYLOR, S.H., DONALD, K.W. and BISHOP, J.M. (1957). Circulatory studies in hypertensive patients at rest and during exercise, with a note on the Starling relationship in the left ventricle in essential hypertension. Clin. Sci., 16, 351-376.

TAYLOR, S.H., SUTHERLAND, G.R., MACKENZIE, G.J., STAUNTON, H.P. and DONALD, K.W. (1965). The circulatory effects of intravenous phentolamine in man. Circulation, 31, 741-754.

TENNER, T.E. (1973). The effects of magnesium on the contractile response to calcium of spontaneously hypertensive rat aortae. Fedn Proc. Fedn Am. Socs exp. Biol. 32, 749 (Abstr.).

TIBBLIN, G., BERGENTZ, S.E., BJURE, J. and WILHELMSEN, L. (1966). Hematocrit, plasma protein, plasma volume, and viscosity in early hypertensive disease. Am. Heart J., 72, 165-176.

TIGERSTEDT, R. and BERGMAN, P.G. (1898). Niere und Kreislauf. Skand. Arch. Physiol., 8, 223-271.**

TOBIAN, L. (1956). The electrolytes of arterial wall in experimental renal hypertension. Circulation Res., 4, 671-675.

TOBIAN, L. (1960). Interrelationship of electrolytes, juxtaglomerular cells and hypertension. Physiol. Rev., 40, 280-312.

TOBIAN, L. and BINION, J.T. (1952). Tissue cations and water in arterial hypertension. Circulation, 5, 754-758.

TOBIAN, L. and BINION, J.T. (1954). Artery wall electrolytes in renal and D.C.A. hypertension. J. clin. Invest., 33, 1407-1414.

TOBIAN, L. and CHESLEY, G. (1965). Calcium content of arteriolar walls in normotensive and hypertensive rats. Proc. Soc. exp. Biol. Med., 121, 340-343.

TOBIAN, L., COFFEE, K. and McCREA, P. (1969). Contrasting exchangeable sodium in rats with different types of Goldblatt hypertension. Am. J. Physiol., 217, 458-460.

TRIPOD, J. and BEIN, H.J. (1960). Modification de la réactivité vasculaire périphérique dans l'hypertension rénale du rat. Helv. physiol. pharmac. Acta., 18, 394-403.

TURKER, R.K., HALL, M.M., YAMAMOTO, M., SWEET, C.S. and BUMPUS, F.M. (1972). A new, long-lasting competitive inhibitor of angiotensin. Science, 177, 1203-1205.

TUTTLE, R.R. and MORAN, N.C. (1969). The effect of calcium depletion on the combination of agonists and competitive antagonists with alpha adrenergic and histaminergic receptors of rabbit aorta. J. Pharmac. exp. Ther., 169, 255-263.

UEDA, H. (1968). Renin-angiotensin system and central nervous system. Proc. 5th. Eur. Congr. Cardiol., 249-251.

UEDA, H., UCHIDA, Y., UEDA, K., GONDARIA, T. and KATAYAMA, S. (1969). Centrally mediated vasopressor effect of angiotensin II in man. Jap. Heart J., 10, 243-247.

VAN BREEMAN, C. and McNAUGHTON, E. (1970). The separation of cell membrane calcium transport from extracellular calcium exchange in vascular smooth muscle. Biochem. biophys. Res. Commun., 39, 567-574.

VAPAATALO, H., HACKMAN, R., ANTTILA, P., VAINIONPÄÄ, V. and NEUVONEN, P.J. (1974). Effects of 6-hydroxydopamine on spontaneously hypertensive rats. Naunyn-Schmeidebergs Arch. exp. Path. Pharmak., 284, 1-13.

VAPAATALO, H., LAHOVAARA, S. and HACKMAN, R. (1970). Studies with renal hypertensive rats. Ann. Med. exp. Fenn., 48, 28-32.

VARNAUSKAS, E. (1955). Studies in hypertensive cardiovascular disease with special reference to cardiac functions. Scand. J. clin. Lab. Invest., 7, (Suppl. 17), 1-117.

VICK, J., EDERSTROM, H.E. and VERGEER, T. (1956). Epinephrine sensitivity of blood vessel strips from salt-fed and castrated rats. Proc. Soc. exp. Biol. Med., 93, 536-539.

VILLAMIL, M.F., NACHEV, P. and KLEEMAN, C.R. (1970). Effect of prolonged infusion of angiotensin II on ionic composition of the arterial wall. Am. J. Physiol., 218, 1281-1286.

WAUGH, W.H. (1962). Role of calcium in contractile excitation of vascular smooth muscle by epinephrine and potassium. Circulation Res., 11, 927-940.

WEEKS, J.R. and JONES, J.A. (1960). Routine direct measurement of arterial pressure in unanaesthetized rats. Proc. Soc. exp. Biol. Med., 104, 646-648.

WEISS, L. (1974). Long-term treatment with antihypertensive drugs in spontaneously hypertensive rats (SHR). Effects on blood pressure, survival rate and cardiovascular design. Acta physiol. scand., 91, 393-408.

WEISS, L. and HALLBÄCK, M. (1974). Time course and extent of structural vascular adaptation to regional hypotension in adult spontaneously hypertensive rats (SHR). Acta physiol. scand. 91, 365-373.

WERKÖ, L. and LAGERLÖF, H. (1949). Studies on the circulation in man : cardiac output and blood pressure in the right auricle, right ventricle and pulmonary artery in patients with hypertensive cardiovascular disease. Acta med. scand., 133, 427-436.

WOLFF, H.H. (1951). The mechanism and significance of the cold pressor response. Q. Jl. Med., 20, 261-273.

WOOD, A.J. and CLARK, D.W.J. (1974). Effects of chronic sympathectomy with 6-hydroxydopamine on responses of perfused mesenteric arteries to noradrenaline and 5-hydroxytryptamine in genetically hypertensive and normotensive rats. Proc. Univ. Otago Med. Sch., 52, 10-11.

YAMORI, Y., YAMABE, H., DE JONG, W., LOVENBERG, W. and SJOERDSMA, A. (1972). Effect of tissue norepinephrine depletion by 6-hydroxydopamine on the blood pressure in spontaneously hypertensive rats. Europ. J. Pharmac., 17, 135-140.

YU, R. and DICKINSON, C.J. (1965). Neurogenic effects of angiotensin. Lancet, 2, 1276-1277.

YURCHAK, P.M., HOOD, W.B., ROLETT, E.L., HICKLER, R.B. and GORLIN, R. (1964). Effect of systemic hypertension on mean left ventricular ejection rate. Am. J. Med. Sci., 247, 42-47.

ZIMMERMAN, B.G. (1973). Blockade of adrenergic potentiating effect of angiotensin by 1-Sar-8-Ala angiotensin II. J. Pharmac. exp. Ther., 185, 486-492.

* Cited in:-

GROLLMAN, A. (1973). In "Mechanisms of Hypertension." ed. SAMBHI, M.P. pp 1-8. New York: American Elsevier.

** Cited in:-

OPARIL, S. and HABER, E. (1974). The renin-angiotensin system. New Eng. J. Med., 291, 389-401.