Role of circulating adrenaline in the pathogenesis of hypertension

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SUMMARY

As adrenaline (AD) contributes to the high blood pressure levels associated with acute stress much attention has focused on a possible role of chronic elevations of circulating AD in the pathogenesis of hypertension. In this thesis, the relationship between circulating AD and blood pressure has been examined.

Aortic catheters were implanted in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) and genetically related (Wistar Kyoto (WKY)) and unrelated (Black-hooded Wistar (BHW) and Sprague Dawley (SD)) normotensive rats, to determine mean arterial pressure (MAP) and plasma catecholamine levels in a conscious and unrestrained state.

At 5-7 weeks of age, MAP was already elevated in the hypertensive strains compared with WKY or SD rats. Plasma AD was higher (76%) in SHR and lower in SD compared to WKY. In adult rats (7-9 months of age), MAP was higher in the hypertensive strains than in WKY. Circulating AD levels were 3-4 times higher in the hypertensive rats but did not differ between normotensive strains.

Plasma catecholamine levels were also measured in WKY hyperactive (WK-HA) and WKY hypertensive (WK-HT) strains to determine if plasma AD is related to the hyperactivity trait of SHR. Catecholamine levels did not differ between strains, indicating that the elevation of plasma AD in the hypertensive rats is not attributable to their hyperactivity.

The relationship between blood pressure and plasma AD was examined by modifying AD levels in normotensive and hypertensive rat strains. Chronic minipump AD infusion did not effect MAP in WKY, even though plasma AD levels were elevated 12 fold. Ten weeks after adrenalmedullectomy in SHRSP, plasma AD was reduced by 34% and MAP was slightly higher in these rats.

These results imply that circulating AD is not a determinant of resting blood pressure. The possibility that elevated AD levels may be a consequence of hypertension was addressed by chronically altering blood pressure levels in WKY and SHRSP. WKY were

made hypertensive by administration of a nitric oxide synthesis inhibitor (L-NAME). Blood pressure was lowered in SHRSP by chronic administration of hydralazine.

Chronic L-NAME treatment in WKY, significantly elevated MAP. This hypertension was accompanied by a significant increase in circulating AD levels. Conversely, chronic hydralazine treatment in SHRSP, significantly lowered MAP and plasma AD concentrations.

These results suggest that the elevation of circulating AD in hypertensive rats is a consequence rather than a cause of their hypertension.

DECLARATION

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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PUBLICATIONS AND ABSTRACTS

PUBLICATIONS

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ABSTRACTS

1. Australian Neuroscience Society, Adelaide, February, 1992.
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ABBREVIATIONS USED IN THE TEXT

AD	Adrenaline
AMX	Bilateral adrenalmedullectomy
ANOVA	Analysis of variance
AVP	Vasopressin
BHW	Black Hooded Wistar rats
bpm	Beats per minute
^{o}C	Degrees Celsius
g	Grams
g	Gravity
hrs	Hours
HR	Heart rate
HZ	Hydralazine
L	Litre
L-NAME	Nω-nitro-L-arginine methyl ester
m	Metre
M	Molar
MAP	Mean Arterial Pressure
min	Minute
mmHg	Millimetres of Mercury
NA	Noradrenaline

prefixes:

	p	pico (10^{-12})
	n	nano (10^{-9})
	μ	micro (10^{-6})
	m	milli (10^{-3})
	c	centi (10^{-2})
	k	kilo (10^{3})

rpm	Revolutions per minute
^{3}H-SAMe	S-adenosyl-L-(methyl-^{3}H) methionine
SD	Sprague Dawley rats
SEM	Standard error of the mean
SHR	Spontaneously hypertensive rats
SHRSP	Stroke-prone SHR
U	Units
v	Volume
w	Weight
WK-HA	Wistar Kyoto hyperactive rats
WK-HT	Wistar Kyoto hypertensive rats
wks	Weeks
WKY	Wistar Kyoto rats
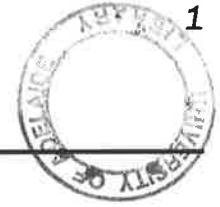
CHAPTER 1

INTRODUCTION

Stress is a difficult concept to define as it involves both physiological and psychological (emotional) reactions. Generally, a stressful situation is one in which the homeostasis of an organism is disturbed. The stress response is, therefore, a failure of the organism to adapt to that stressful stimulus. The physiological responses accompanying acute stress are mediated by general sympathetic activation and have been widely associated with the "adrenaline rush". Although the acute physiological effects of AD on blood pressure have been well documented, the long term influence of AD on the cardiovascular system remains less well defined. My overall objective, therefore, was to investigate the relationship between circulating AD and blood pressure and, in particular, to examine the role of chronically elevated plasma AD levels in genetically hypertensive rats. In pursuing this aim it is important to consider the association between circulating catecholamines, stress and the development of hypertension.

1.1   Catecholamines and Stress

The involvement of the sympathoadrenalmedullary system in the stress response was first suggested by Cannon (1929) who recognised that both physical and psychological stimuli caused a marked increase in both blood pressure and heart rate (HR). The acute elevation of sympathoadrenalmedullary activity rapidly acts to preserve the internal environment by increasing the state of arousal and preparing the system to respond quickly to potentially harmful stimuli. The cardiovascular response in this "flight or fight" reaction includes isotropic and chronotropic effects on the heart resulting in increased cardiac output, vasoconstriction of cutaneous, splanchnic and renal vascular beds, venoconstriction and a decrease in skeletal muscle resistance. Additionally, an increase in sympathoadrenal outflow increases plasma glucose by stimulating gluconeogenesis and glycogenolysis by the liver. Plasma glucagon is increased and insulin secretion is inhibited. The overall effect is an increase of blood flow and energy supply (glucose) to the skeletal muscles

and brain. Behavioural effects include anxiety, increased alertness and a reduction in muscular and psychological fatigue. Thus, these physiological changes may be viewed as an adaptive response necessary to preserve life, preparing the individual to fight or flight. The chemical mediators of these sympathoadrenal responses are believed to be AD (released from the adrenal medulla) and noradrenaline (NA, released from sympathetic nerve terminals and to a lesser extent, from the adrenal medulla).

With the development of sensitive catecholamine assays, it was found that the proportion of AD and/or NA released was dependent upon the type of stress. Early studies [von Euler & Lundberg, 1954] showed that the increase in urinary AD excretion was greater than NA excretion in situations associated with anxiety and aggression. Later studies showed that stressors associated with relatively unstressful physical activities (such as standing) elevated plasma NA more than AD. Public speaking, hypoglycaemia and strenuous exercise tend to elevate plasma AD more than NA, whereas performance of a non-distressing mental task does not appear to elevate plasma AD levels [Eisenhoffer *et al.* 1985]. Thus, it seems that the elevated NA reflects an increase in sympathetic vasoconstrictor activity which acts primarily to redistribute blood to organs mediating the stress response. On the other hand, AD release is an hormonal response to metabolic crises, situations requiring attentiveness or which lack predicability or situations in which sympathetic homeostatic mechanisms are deficient and which can be driven by behavioural change.

Humans with essential hypertension may exhibit a hyperkinetic circulation resembling a mild "flight or fight" response i.e. a high cardiac output and normal or reduced vascular resistance during the developmental phase of hypertension [Birkenhager & de Leeuw, 1984; Julius, 1990]. Consequently, much attention has focused upon the role of stress in the pathogenesis and maintenance of hypertension, since a higher level, frequency or sensitivity to stress might explain the aetiology of the high level of blood pressure.

1.2   Stress and Hypertension

Many studies have been published investigating the relationship between stress and blood pressure, although the mechanisms underlying stress induced hypertension still remain poorly understood. The "reactivity" hypothesis postulates that individuals with a heightened cardiovascular reactivity to acute stress are at increased risk of developing sustained hypertension, as repeated surges of blood pressure caused by the stress can lead to structural changes which may result in hypertension [Freeman, 1990].

Responses to psychological stressors have, therefore, been studied in relation to individuals who exhibit a "type A" behavioural pattern. People with a "type A" personality can be defined as those individuals who are competitive, ambitious, hostile and have a chronic sense of time urgency. This personality type is also recognised as a coronary-prone behaviour pattern. The absence of these characteristics defines the "type B" or non-coronary prone behaviour pattern. Thus, it is possible that "type A" individuals may have increased cardiovascular reactivity and may therefore be more susceptible to developing hypertension. A study of 39-59 year old men over an 8.5 year period showed that those with a type A personality had twice the incidence of coronary heart disease of those with a type B personality [Rosenman et al. 1975]. Other studies have also shown that individuals with a type A personality have an exaggerated increase in blood pressure, heart rate and plasma AD secretion in response to psychological stressors [Glass et al. 1980].

An association between stress and hypertension is also supported by findings that essential and borderline hypertensives may have a heightened blood pressure response to mental stress [Eliasson et al. 1983]. Furthermore, studies have indicated that normotensive subjects with hypertensive parents may have heightened cardiovascular reactivity to mental stress [Steptoe et al. 1984], although the link between stress and the development of hypertension still remains controversial [Freeman, 1990].

Thus it is possible that individuals with essential hypertension have both a personality type and a heightened cardiovascular responsiveness which, during stress, may act deleteriously to enhance the incidence or progression of coronary heart disease and possibly the hypertension.

However, several lines of evidence have failed to find a causative relationship between stress and hypertension. Prevalence of hypertension is not necessarily elevated in individuals with type A personalities [Rosenman, 1987] and a 22 year follow up study showed that type A subjects had an overall lower mortality rate than type B subjects [Ragland & Brand, 1988]. Exercise may be regarded as an acute physical stress which elevates blood pressure and plasma catecholamines in both hypertensive and normotensive individuals [Graafsma *et al.* 1989]. Yet repeated bouts of moderate to intense exercise is beneficial in lowering blood pressure in hypertensive [Kaplan, 1992] and normotensive [Kingwell & Jennings, 1993] individuals. Thus, rather than increasing blood pressure, certain forms of chronic stress may lower blood pressure.

1.2.1 Stress induced hypertension in rats

Immobilisation is considered to be a stressful procedure for the rat and has been used extensively as a model of stress. Acute immobilisation causes a decrease in adrenal AD which is accompanied by an increase in urinary catecholamine excretion [Kvetnansky & Mikulaj, 1970]. During repeated intervals of stress, however, urinary catecholamines remain elevated and both cardiac and adrenal AD levels are increased [Kvetnansky *et al.* 1984]. Accompanying the stress-induced elevation of plasma catecholamines is an elevation of blood pressure [Dobrakovova *et al.* 1984]. It seems that plasma AD is an important mediator of this blood pressure response since adrenalmedullectomy (AMX) [Dobrakovova *et al.* 1984; Majewski *et al.* 1986] or desipramine administration (which blocks neuronal uptake of AD) [Majewski *et al.* 1986] prevents the development of hypertension induced by immobilisation stress.

Yamaguchi *et al.* (1981) have shown that immobilisation stress causes desensitisation of α and β_1 receptors. However, as blood pressure levels in the immobilised rats were not measured, it is unclear whether reduced α or β_1 receptor activity would prevent the hypertension development in these rats. More importantly, Kvetnansky et al. (1979) have shown that repeated episodes of immobilisation stress actually resulted in lower chronic blood pressure levels in SHR, even though there was a persistent elevation of plasma AD and NA.

Thus, although acute physical and psychological stress can elevate blood pressure and catecholamines, the notion that chronic stressful stimuli, will result in sustained hypertension remains unresolved.

1.3   *Plasma catecholamines in hypertension*

1.3.1   Plasma catecholamines as a marker of sympathetic activity

Because the sympathetic nervous system mediates the "flight/fight" response and borderline hypertensives can display a hyperkinetic circulation which is comparable to this response, researchers have tried to identify whether or not sympathetic activity is elevated in hypertension. Attempts to assess total sympathetic outflow have commonly involved the measurement of plasma catecholamine levels, especially NA, as this is the primary neurotransmitter released from sympathetic nerves. However, much controversy exists as to whether or not measurements of plasma catecholamines per se truly reflect total sympathetic nerve outflow [Morlin *et al.* 1983; Floras *et al.* 1986]. Although a variety of stimuli which activate sympathetic outflow (such as bicycle exercise [Floras *et al.* 1986]) elevate plasma NA, other chronic factors such as environmental stressors [Buhler *et al.* 1978], sodium status [Anderson *et al.* 1989] and age [Franco-Morselli *et al.* 1977], can also influence plasma catecholamine concentrations, making the interpretation of obtained data difficult.

The concentrations of catecholamines in plasma are dependent upon a balance between catecholamine release and uptake mechanisms; thus only a small fraction of released NA diffuses into the circulation [Esler *et al.* 1985]. This spillover may also be altered in certain disease states [Goldstein *et al.* 1983]. In addition to this, the method and site of sampling will also influence the plasma catecholamine concentrations. Individuals can respond differently to the stress of arterial or venous cannulation procedures [Eliasson *et al.* 1983; Steptoe *et al.* 1984], thereby falsely elevating catecholamine concentrations. Thus, in assessing resting plasma catecholamines it is crucial to adopt a sampling procedure that minimises stress. Furthermore, because the removal of catecholamines

varies between vascular beds (arterial concentrations are usually greater than venous concentrations), plasma catecholamine concentrations will vary between sites of collection.

Despite these limitations, plasma NA concentrations can provide valuable information as to overall (not individual organ) sympathoadrenal activity, provided that the experiment is well controlled and the plasma is sampled from the same site and under uniform resting conditions.

1.3.2   Essential hypertension

A report by Goldstein and Lake (1984) summarised findings of 78 published studies between 1970 and 1984 and found plasma NA to be abnormally high in patients with essential hypertension, especially in young hypertensives (≤ 40 years). Plasma NA generally increased with age in normotensives but not in hypertensive subjects.

On the other hand, plasma AD was independent of age but was consistently elevated in a subgroup of essential hypertensives. Franco-Morselli *et al.* (1977) reported significant elevations of plasma AD (but not NA) in individuals with both sustained and labile hypertension. In that study, plasma AD levels averaged 79 pg/ml in those with labile hypertension, 84 pg/ml in sustained hypertensives and 41 pg/ml in control subjects. The values reported in the hypertensive subjects are sufficiently high to cause an elevation of heart rate, systolic blood pressure and an elevation of blood glycerol [Clutter *et al.* 1980]. Additionally, the elevated circulating AD level might also enhance α-adrenoceptor mediated vasoconstriction [Bolli *et al.* 1981]. Because of the consistent elevation of plasma AD in hypertension, it has been proposed that plasma AD might be more representative of sympathetic nerve activity than plasma NA [Franco-Morselli *et al.* 1977; Bolli *et al.* 1981]. This is because AD is secreted from chromaffin cells directly into the circulation while NA is subjected to a more complicated diffusion process from the nerve terminal to the lumen of blood vessels [Franco-Morselli *et al.* 1978].

Thus, plasma AD is generally elevated in essential hypertension and the concentration of plasma AD is sufficiently high to affect hemodynamic variables. Moreover, plasma AD responses to acute stress are exaggerated in essential hypertension.

1.3.3   Animal models of inherited hypertension

After screening hundreds of Wistar rats from the animal colony at Kyoto university, Okamoto and Aoki in 1959, developed a new inbred strain of rat with genetic hypertension. One of the male Wistar rats examined had blood pressure greater than 150 mmHg. A female Wistar rat (whose blood pressure exceeded 130 mmHg) was chosen for mating with this rat. Mating between the pair was repeated four times and the offspring of these matings which exhibited hypertension for over one month were used for brother-sister matings. Successive generations of hypertensive rats were obtained by brother-sister matings of rats selected for high blood pressure [Okamoto & Aoki, 1963]. Blood pressures of subsequent generations had plateaued by the sixth generation and by 1969, inbreeding had passed 20 generations and hence the genetic complement of these rats had become fixed [Okamoto et al. 1972]. These rats were referred to as spontaneously hypertensive rats (SHR).

Subsequently, Okamoto and Aoki developed several new substrains of the SHR. The stroke-prone SHR (SHRSP) were produced by successive brother-sister matings of offspring of SHR which had died of stroke. In this strain, all rats develop moderate to severe hypertension and 90 % of the population die of stroke. Other inbred strains include stroke-resistant SHR, spontaneous thrombogenic rats, arteriolipidosis rats and myocardial ischaemic rats [Yamori, 1982].

The normotensive control strain for the SHR strain began to be developed in 1971 in Japan. However, whereas the genetic complement of SHR had been fixed between 1959-1969, the original Wistar colony had been maintained by continued outbreeding. Thus, there were likely to be more genetic differences between SHR and WKY by the time brother-sister breeding began in 1971 [Louis & Howes, 1990]. This normotensive strain from Kyoto was known as the Wistar Kyoto (WKY) strain.

The development of blood pressure in WKY, SHR and SHRSP are shown in Figure 1 (taken from PRC Howe *et al.*, unpublished observations). SHR and SHRSP develop a higher blood pressure than WKY by 5-7 weeks of age. Blood pressure development of SHRSP is more rapid than that of the SHR and their blood pressure remains at a higher level throughout life. The blood pressure of the WKY rats remains relatively stable over time although the validity of using the WKY strain as a control for SHR and SHRSP has been questioned. The reasons why the hypertensive strains develop higher blood pressure has been extensively studied. However, no single mechanism has been found.

With the availability of these genetically hypertensive rat strains, investigators measured plasma catecholamines to try to ascertain the significance of the sympathetic nervous system in the development and maintenance of hypertension.

Early attempts to measure plasma catecholamines in SHR and WKY yielded contradictory results. After decapitation of rats, plasma NA was either not different between strains [Roizen *et al.* 1975; Nagaoka & Lovenberg, 1976], or elevated (together with plasma AD) in SHR [Grobecker *et al.* 1975]. Under these conditions, plasma dopamine β-hydroxylase and normetanephrine were elevated in SHR [Grobecker *et al.* 1975; Roizen *et al.* 1975]. During halothane anaesthesia (which does not affect sympathoadrenal outflow [Perry *et al.* 1974]) no differences in plasma catecholamines could be detected between SHR and WKY [Yamaguchi & Kopin, 1980]. Restraint of conscious rats during sampling from the tail vein, on the other hand, failed to show differences in plasma NA between the two strains but AD was significantly elevated in SHR [Vlachakis *et al.* 1980].

The methods of blood sampling in these early studies possibly explains the inconsistency in results. Because SHR react to stressful stimuli to a greater extent than WKY [McCarty & Kopin, 1978] and catecholamines are released in response to environmental stressors [Axelrod & Reisine, 1984], it seems unlikely that blood collected by these conditions (i.e. decapitation, restraint or under anaesthesia) reflect true levels of circulating catecholamines in either of these strains.

However, the refinement of methods enabling blood samples to be taken from conscious rats and the development of more sensitive assays for the low levels of catecholamines in plasma under these conditions [Peuler & Johnson, 1977] have still yielded contradictory

results as to whether or not plasma catecholamines are elevated in hypertensive rats. The findings from these studies are summarised in Table 1.

The discrepancies between the results might be explained by many factors. Firstly, the volume of blood taken from the rats might affect catecholamine measurements. It has been shown that if the volume of removed blood exceeds 0.5% of the total body weight then haemorrhagic reflexes are initiated and plasma catecholamines are falsely elevated [Pak, 1981]. Secondly, barbiturate anaesthesia inhibits sympathetic outflow and hence reduces plasma catecholamines [Howe *et al.* 1986]. On the other hand, ether anaesthesia elevates circulating catecholamines [Borkowski & Quinn, 1983a]. Thus, for blood sampling, it is important to wait as long as possible after cannulation surgery to eliminate any residual effects of anaesthesia. Finally, the levels of circulating catecholamines might be influenced by the age of the rats. One report found that plasma AD increases steadily with age in SHR between 5-20 weeks of age. There was no such correlation for plasma NA or in normotensive rats [Borkowski *et al.* 1992].

Thus, although there have been many sensitive techniques developed to determine the basal levels of plasma catecholamines in rats, there are still many discrepancies in results found in different laboratories. It is not immediately clear why there is a contradiction in findings. However, it is possible that parameters such as volume depletion, surgical procedures, age of rats and different approaches to handling rats might be vital.
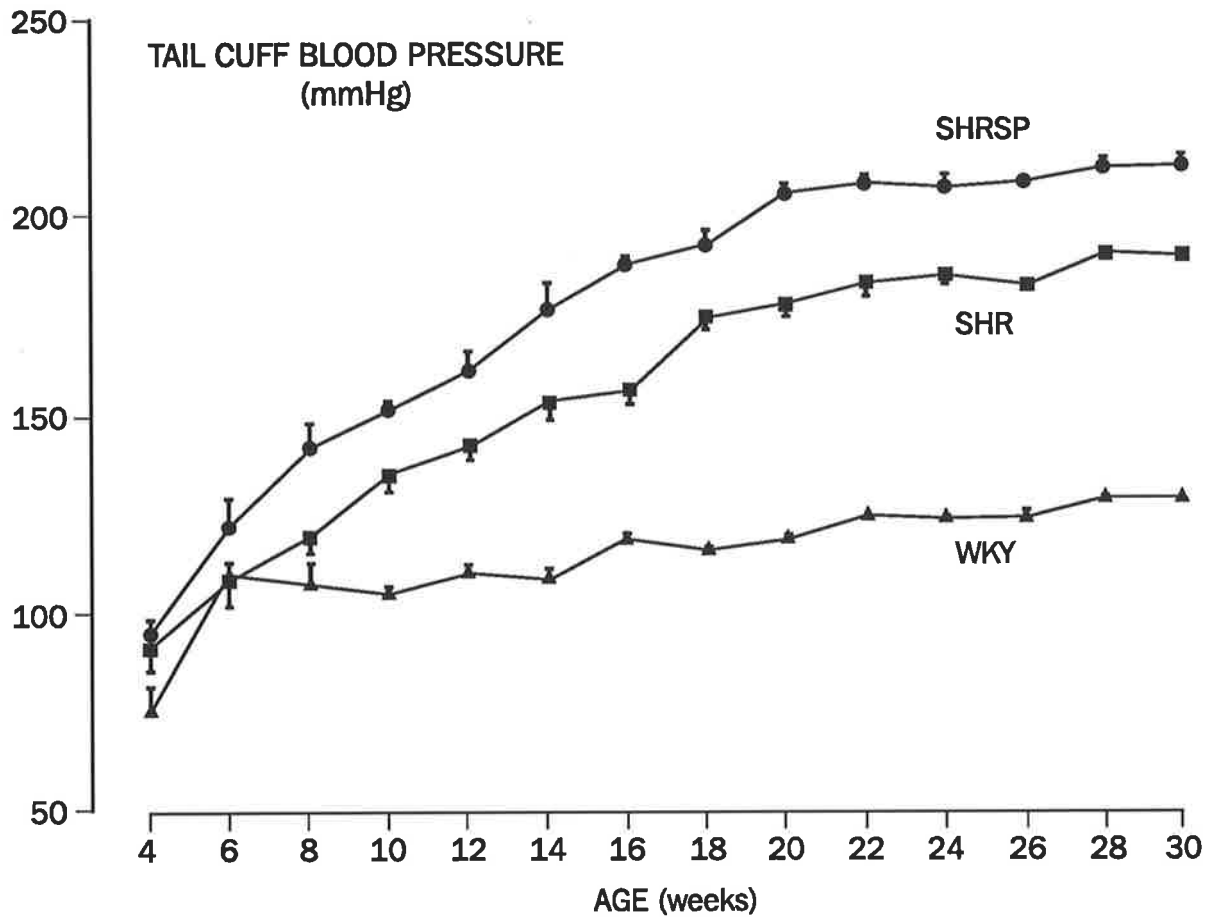
FIGURE 1 Blood pressure development in WKY, SHR and SHRSP (from PRC Howe *et al.*, unpublished observations).

TABLE 1 Summary of literature describing plasma catecholamine levels in normotensive and hypertensive rats. In all cases blood was taken from conscious and unrestrained rats.

	Catheter	Time (hrs)	Vol. (µl)	Age (wks)	Strains HT	Strains NT	Ratio (HT/NT) AD	Ratio (HT/NT) NA
[Bagdy et al. 1993]	carotid	48	600	3	SHR	WKY	1.8*	1.6*
[Borkowski & Quinn, 1983a]	carotid	48-72	1000	250-300g	SHR	WKY	1.7*†	1.2†
[Borkowski et al. 1992]	carotid	48	1000	5	SHR	WKY	1.9	2.0*
				10	SHR	WKY	0.6	1.3*
				15	SHR	WKY	1.2	2.2*
				20	SHR	WKY	1.8	4.8*
[Ferrari et al. 1981]	femoral artery	24	300-400	6	SHR	WKY	1.3	0.7
				15	SHR	WKY	0.8	1.0
[Kirby et al. 1989]	carotid	48	600	12	SHR	WKY	1.0	1.0
[McCarty & Kopin, 1978]	Tail artery	48	600	6	SHR	WKY	1.1	1.0
				8	SHR	WKY	0.9	0.9
				48	SHR	WKY	1.0	1.0
[Pacak et al. 1993]	femoral artery	24	400-500	6-7	SHR	WKY	2.1*	1.2*
				15-16	SHR	WKY	0.6	0.8
[Pak, 1981]	abdomin. aorta	few days	0.5% body wt.	5	SHR	WKY	3.1*	3.9*
				7	SHR	WKY	1.7*	2.8*
				26	SHR	WKY	1.7	1.8*
				52	SHR	WKY	1.1	1.1
[Picotti et al. 1982]	jugular	24	unknown	13-14	SHR	WKY	0.9	1.2
[Szemeredi et al. 1988]	femoral artery	unknown	500	4	SHR	WKY	2.5*	1.7*
				12			0.8†	1.0†
[Howe et al. 1986]	abdomin. aorta	48	300	16	SHR	WKY	3.3*	1.6*
[Schomig et al. 1978]	femoral artery	48	500	15	SHR	WKY	1.1†	1.6*†
[Unger et al. 1984]	femoral artery	24-48	400	32	SHR	WKY	4.9*	0.9

Explanation of table columns:

Catheter: type of catheter implanted in rats

Time: time after cannulation that blood samples were taken

Vol: volume of blood taken from rats

Age: age of rats at the time of blood sampling (weeks)

Strains: strains of rats compared : NT: normotensive strain, HT: hypertensive strain,

WT: Wistar rats.

* indicates HT significantly different (p<0.05) from NT strain

† indicates values estimated from graphed data

1.4   Possible mechanisms elevating catecholamines in hypertension

1.4.1   Increased sympathoadrenal outflow

Although several studies have indicated that plasma catecholamines might be elevated in hypertension, the mechanisms underlying this elevation are not clear. However, if plasma catecholamines are a good indicator of sympathetic nerve outflow and plasma catecholamines are elevated in hypertension, then it follows that increased sympathetic nerve outflow accompanies high blood pressure.

Studies in man which measured sympathetic nerve activity directly from intraneural recordings (peroneal nerve) [Anderson et al. 1989] found a significant elevation in sympathetic nerve activity in borderline hypertensive subjects. However, the degree of sympathetic nerve outflow correlated with plasma NA levels only in those maintaining a low salt diet. In another study, however, measurements of peroneal nerve activity failed to show differences in sympathetic nerve activity between normotensive and hypertensive age-matched male subjects [Morlin et al. 1983]. In that study, sympathetic activity increased with age and was correlated with plasma NA concentrations but overall, there were no differences between normotensive and hypertensive subjects.

Studies in genetically hypertensive rats have also reported greater splanchnic and renal nerve activity in conscious, resting SHR [Judy et al. 1976]. In support of this, the neural component of blood pressure was significantly elevated in SHR and SHRSP [Yang et al. 1993]. Further evidence for the role of elevated sympathetic activity in the development of hypertension comes from studies in which sympathectomy attenuated the increase in blood pressure in SHR [Haeusler et al. 1972; Korner et al. 1993]. Immunosympathectomy (which does not affect the innervation of the adrenal medulla) and co-administration of an α-receptor antagonist, significantly lowered tissue NA and elevated adrenal NA [Korner et al. 1993]. This treatment completely prevented hypertension development in SHR. In another study, guanethidine induced sympathectomy decreased blood pressure and plasma NA in Lyon hypertensive and normotensive rats but did not affect plasma AD [Lo et al. 1991]. However, others have

argued against a role for the sympathetic nervous system in the development of hypertension and have evidence that the sympathetic nervous system only contributes to the maintenance of hypertension once blood pressure levels are established [Yamori, 1976].

In another study both plasma NA and AD were found to be elevated in SHRSP. After ganglion blockade however, differences in plasma NA between SHRSP and WKY were eliminated, while plasma AD levels remained 50 % higher in SHRSP [Howe *et al.* 1986]. The results of this study provide evidence that the elevated level of NA in SHRSP was attributed to a higher level of sympathetic outflow while the elevation of plasma AD can only be attributed to other non-neural mechanisms.

Certain physical and emotional states are known to increase sympathoadrenal activity both acutely (e.g. hypotension, hypoglycaemia, hypoxia, exercise, emotional stress) and chronically (e.g. uraemia, anaemia, hypothyroidism, Raynauds syndrome, congestive heart failure) [Izzo, 1984]. Thus it is possible that the presence of any of these syndromes, over a period of time, might exacerbate the progression of hypertension.

1.4.2   Increased adrenal catecholamine content

The elevated levels of plasma catecholamines in hypertension might also be attributed to elevated levels of catecholamines in the adrenal gland such that more catecholamines are released into the circulation per nerve impulse.

Several studies have reported elevated NA in the adrenal glands of 4 week old SHR [Korner *et al.* 1993] or elevated AD and NA in 4 and 12 month old SHRSP [Schober *et al.* 1989]. Furthermore, adrenal AD content is elevated in deoxycorticosterone acetate (DOCA salt) hypertensive rats [Grobecker *et al.* 1982]. Others, however, fail to find a difference in adrenal gland catecholamine contents in 15 week old SHR and WKY [Grobecker *et al.* 1982]. On the other hand, catecholamine precursor enzymes (tyrosine hydroxylase, dopamine β-hydroxylase and phenylethanolamine-N-methyl transferase) were all decreased in the adrenal glands of young SHR while in older animals, tyrosine hydroxylase was significantly elevated in SHR [Grobecker *et al.* 1982]. However, in contrast to this,

the same group find elevated precursor enzymes in the adrenal glands of young SHR [Grobecker *et al.* 1975].

Thus more catecholamines (or precursors) in the adrenal gland might lead to excess catecholamines in the circulation. This could be mediated by the sympathetic nervous system (i.e. more catecholamines released per nerve impulse) or via direct actions of certain substances (e.g. angiotensin II or substance P) on the adrenal gland to facilitate release of catecholamines.

1.4.3 Hyperresponsiveness to stress

Many studies have described the hyperresponsiveness of the SHR to various physiological and psychological stressors [Popper *et al.* 1977; McCarty & Kopin, 1978; Picotti *et al.* 1982; Kirby *et al.* 1989; Kvetnansky *et al.* 1992] which may contribute to the development of hypertension in these rats. During inescapable footshock stress, SHR showed greater responsiveness in behaviour parameters and enhanced catecholamine secretion when compared with WKY [McCarty & Kopin, 1978]. In response to novel stimuli, SHR also respond with greater increases in blood pressure and heart rate than WKY [Kirby *et al.* 1989]. Additionally, essential hypertension also appears to be associated with enhanced cardiovascular and sympathoadrenal reactivity to mental stress [Eliasson *et al.* 1983]. Thus, the heightened response to stress in hypertension may be linked to the elevated catecholamine levels observed in this condition.

Hence, the elevation of AD in hypertension can be attributed to many mechanisms ranging from an elevation of adrenal gland AD to a heightened reactivity to stress. It is now important to consider the physiological implications of elevated AD levels in the circulation.

1.5   Receptor types and physiological actions of AD

1.5.1   Presynaptic receptors

Both presynaptic α and β receptors on peripheral noradrenergic nerve terminals modulate NA release. Both AD and NA can act on these receptors although AD is more potent on β receptors while NA is more potent on α receptors.

Presynaptic α receptors were discovered when it was found that the α blocker phenoxybenzamine, increased the overflow of NA during nerve stimulation in the cat spleen [Brown & Gillespie, 1957]. The presynaptic α receptors (now known as α_2 receptors) are activated by circulating catecholamines to inhibit NA release from presynaptic nerve terminals.

Presynaptic receptors were first described by Langer [Adler-Graschinsky & Langer, 1975; Langer, 1977] in which blockade of the β receptor decreased stimulation evoked release of NA. Thus, the presynaptic β receptor (now known as the β_2 receptor) is activated by catecholamines to enhance NA release from nerve terminals.

Much attention has recently been focused on the potential physiological significance of the action of AD on the presynaptic β_2 receptor to facilitate NA release. Additionally, this system has been implicated in the development of hypertension even though studies find no differences in β adrenoceptor function between SHR and WKY [Ekas et al. 1983; Nezu et al. 1985]. Despite this, studies have shown that there may be enhanced facilitation of NA release from the isolated mesenteric bed of SHR following β receptor stimulation [Kawasaki et al. 1982], or enhanced sensitivity of the β receptor in DOCA salt hypertensive rats [Moreau et al. 1991].

AD infusion or elevation of endogenous AD levels by stress, has shown that AD significantly potentiates the release of NA from nerve terminals in many tissues including rat atria [Majewski et al. 1981a; Majewski et al. 1982b], rat kidney [Quinn et al. 1984] and dog saphenous vein [Giumares et al. 1978]. Others, however, have argued against a role for AD in the regulation of neurotransmitter release in isolated tissues [Steenberg et al. 1983; Schwartz & Eikenburg, 1988; Falckh et al. 1990; Sadeghi & Eikenburg, 1992].

Nevertheless, studies in pithed rabbits [Majewski *et al.* 1982a; Majewski *et al.* 1985; Schmidt *et al.* 1984] or rats [Majewski & Murphy, 1989] show that AD may modulate NA release *in vivo* by activating presynaptic β_2 receptors. Alternatively, several studies have shown that the potentiation of NA released by AD is not mediated via an enhancement of presynaptic β_2 receptor activity, rather, is attributed to decreased influence of presynaptic α_2 receptor inhibition [Schwartz & Eikenburg, 1988; Falckh *et al.* 1990]. Studies in humans either find potentiation of total body NA release with AD infusion [Musgrave *et al.* 1985; Kjeldsen *et al.* 1993] or no potentiation in human saphenous vein [Molderings *et al.* 1988] (see Floras (1992) for extensive review).

During AD infusion or during chronic stress, AD accumulates into presynaptic noradrenergic nerve terminals [Giumares *et al.* 1978; Majewski *et al.* 1981b; Majewski *et al.* 1982b; Schmidt *et al.* 1984; Majewski *et al.* 1986]. This uptake can be blocked by desipramine [Majewski *et al.* 1986]. During sympathetic stimulation, AD is co-released with NA into the synaptic cleft. The released AD may then act upon postsynaptic receptors directly or may alternatively act on presynaptic receptors to further enhance neurotransmitter release.

1.5.2   Postsynaptic receptors

During AD administration, both excitatory and inhibitory actions are seen due to ADs actions on both α and β receptors.

β_1 receptors are found almost exclusively on cardiac tissue which, when stimulated by AD, cause an increase in the rate (chronotropic) and force (inotropic) of cardiac contraction. Stimulation of postsynaptic β_2 receptors on the vasculature (especially in skeletal muscle) causes dilation and hence a decrease in total peripheral resistance. On the other hand, AD causes vasoconstriction when stimulating postsynaptic α_1 receptors, causing an increase in total peripheral resistance. However, because AD is more potent on β receptors, the actions caused by β receptor stimulation usually dominate during AD administration unless very high doses are administered.

1.5.3   Effects of AD on blood pressure

An acute intravenous administration of AD causes an increase in both systolic and diastolic blood pressure. An increase in pulse pressure is seen since the increase in systolic is greater than that for diastolic pressure. The increase in blood pressure is attributed to chronotropic and inotropic effects of AD on the heart and the constriction of vessels especially in the skin, mucosa and kidney. Acute AD administration also causes constriction of veins. Smaller doses of AD (0.1 µg/kg) cause blood pressure to fall since the vasodilatory actions of AD on β_2 receptors predominate. An infusion of AD (10 µg/min) causes blood pressure to increase primarily due to an increase in cardiac output. Peripheral resistance is reduced due to vasodilation in skeletal muscle. Consequently, diastolic blood pressure falls.

1.5.4   Metabolic effects of AD

Apart from the direct actions on the cardiovascular system, AD has many metabolic actions which also mediate the "fight/flight" response. These essentially act to meet the oxygen and energy requirements of the brain and skeletal muscle. AD administration can cause a 20-30% increase in oxygen consumption. This is accompanied by an increase in plasma glucose (via activation of hepatic glycogen phosphorylase and inhibition of glycogen synthase) and lactate. Insulin secretion is inhibited via actions on α receptors of β-cells of pancreatic islets and glucagon secretion is enhanced via stimulation of β receptors on pancreatic α-cells. Plasma cholesterol, phospholipids and low density lipoproteins are increased. Plasma free fatty acids are also elevated due to activation of triglyceride lipase. AD also causes rapid relaxation of bronchial smooth muscle and elevates respiratory rate and tidal volume. The resulting elevation of oxygen in the blood is an important response in the "flight/fight" reaction.

Induction of hypoglycaemia by the infusion of insulin, causes a dramatic elevation in plasma AD concentration. The elevation of plasma AD mirrors the resulting fall in the level of plasma glucose [Medbak et al. 1987]. The elevation of plasma AD is primarily neurogenic in origin; glucose sensitive pathways in the hypothalamus act via the central nervous system, to stimulate the preganglionic, cholinergic nerves that innervate the

adrenal medulla [Himsworth, 1970]. However, in the late phase of hypoglycaemia, the response may be non-neurogenic with the rate of AD release being dependent upon the level of glycaemia [Khalil *et al.* 1986]. Thus, the role of elevated plasma AD levels may be a response to hypoglycaemia which may be caused by excessive insulin secretion, exercise or impaired glucose release mechanisms.

1.6   Effects of abnormally elevated plasma AD

1.6.1   Chronic AD infusion in rat models

Henryk Majewski et al. were the first investigators to propose the "Adrenaline-mediated hypertension" hypothesis. This hypothesis proposed that the development of hypertension was due to increased release of AD from the adrenal medulla during episodes of stress [Majewski *et al.* 1981b]. It had already been suggested by Langer [Adler-Graschinsky & Langer, 1975; Langer, 1977] that AD activates presynaptic β_2 receptors at sympathetic nerve endings which result in a facilitation of NA release and hence an increase in vascular tone. Circulating AD may directly activate presynaptic receptors but might also be incorporated into noradrenergic nerve terminals and subsequently released as a co-transmitter to activate presynaptic β_2 receptors. As previously mentioned, this system has been shown to be functional in various human and animal tissues.

In an early study by Majewski, Tung and Rand, normotensive Wistar rats were given a slow release depot implantation containing AD bitartrate. The AD treated rats had elevated blood pressures from day 1 to 8 weeks after the implantation even though 8 weeks after treatment no AD from the implant could be detected in the plasma. Furthermore, the increase in blood pressure induced by AD administration was blocked by co-administration of the β blocker, Metoprolol [Majewski *et al.* 1981b].

In later studies, AD bitartrate was infused into Wistar rats via osmotic minipump [Majewski *et al.* 1982b]. Again both systolic and diastolic blood pressures were elevated by approximately 15 and 10 mmHg respectively in the AD infused rats (after 5-6 days of

treatment). HR was unaffected and again, the rise in blood pressure was attenuated by administration of Metoprolol.

These studies showed that blood pressure could be elevated by an infusion of AD and that the effect is probably mediated by activation of presynaptic β_2 receptors. The results also suggest the likelihood that AD can be stored in presynaptic nerve terminals, thus elevating blood pressure long after plasma AD levels have returned to baseline.

Studies by other groups used much higher minipump AD infusion rates in Sprague Dawley (SD) rats [Johnson et al. 1983; Schwartz & Eikenburg, 1986]. After 6 days of infusion, tail cuff blood pressure was 25 mmHg higher in AD treated rats. Even after pithing, however, MAP remained 23 mmHg higher in the treated rats suggesting a direct pressor effect of AD on the vasculature or on the heart. Despite the elevation of blood pressure, pressor responses to NA were reduced in these animals [Schwartz & Eikenburg, 1986]. Subsequent studies by the same authors failed to show a presynaptic adrenoreceptor influence by chronic AD treatment in both the perfused rat kidney [Schwartz & Eikenburg, 1988] and rat mesentery [Sadeghi & Eikenburg, 1992].

Other studies in SD rats [Johnson et al. 1983], showed that high dose AD infusion caused a 40 mmHg increase in blood pressure but once the source of AD was removed, the hypertension was not sustained and blood pressure rapidly returned to baseline levels. This contrasts with the findings of others who showed that continued elevation of plasma AD was not required to sustain hypertension after AD infusion had ceased [Majewski et al. 1981b].

Chronic intravenous (i.v.) infusion in Wistar rats showed that even though plasma AD was raised 13 times that in control rats, only a 12 mmHg increase in MAP was observed after 4 days of infusion [Zabludowski et al. 1984]. It was concluded that there is only a very small pressor effect of AD and to achieve this, large sustained increases in plasma AD are needed.

As well as effects on the cardiovascular system, AD also has metabolic actions. Daily injections of AD for 6 weeks, increased heart weight by 11.5 %. Plasma glucose, insulin and lactate were all reduced by the treatment. In this study [Fell et al. 1981] blood

pressure was not measured so it is unclear if changes in these parameters could contribute to an elevation of blood pressure with chronic AD treatment.

Thus, although an elevation of plasma AD generally elevates blood pressure in normotensive rats, the exact mechanisms underlying the hypertension remain unclear.

1.6.2   AD infusion in humans

Low dose AD infusion in humans, which elevated plasma AD levels from 100 to 1500 pg/ml, increased systolic blood pressure and decreased diastolic blood pressure such that there was no net effect on MAP [Kjeldsen *et al.* 1993]. This elevation was accompanied by an increase in HR and forearm blood flow and plasma NA. Other studies have shown that the elevation of plasma AD levels (similar to those seen during stress) can significantly amplify the blood pressure response to sympathetic stimulation [Musgrave *et al.* 1985; Blankestijn *et al.* 1991; Jern *et al.* 1991]. This enhancement has been attributed to a facilitation of NA release from the presynaptic nerve terminal as AD infusion was accompanied by an increase in plasma NA concentration both at rest [Musgrave *et al.* 1985; Jern *et al.* 1991] and during sympathetic activity [Musgrave *et al.* 1985; Blankestijn *et al.* 1991; Jern *et al.* 1991].

Studies in rats and humans have shown that only an acute increase in plasma AD (such as during mental stress) is required to sustain a long-term elevation in blood pressure. *In vitro* studies show that exogenous administration of AD causes it to accumulate into presynaptic nerve terminals. This AD can then be released up to 24 hours after cessation of its administration [Majewski *et al.* 1981b]. Upon its release AD could act upon presynaptic β_2 receptors to enhance neurotransmitter release [Quinn *et al.* 1984; Schmidt *et al.* 1984].

Eighteen hours after an AD infusion in man, a pressor effect was still evident during periods of sympathetic activity [Blankestijn *et al.* 1988; Blankestijn *et al.* 1991]. Others however, have failed to support the hypothesis that an acute increase in plasma AD causes prolonged effects on blood pressure [Jern *et al.* 1991].

1.6.3   Pheochromocytoma

Pheochromocytomas are tumours of the adrenal gland that secrete large amounts of catecholamines (primarily NA) into the circulation and induce sustained or labile hypertension.   Although pheochromocytomas are rare (occurring in 0.1 % of the hypertensive population [Pruszczyk *et al.* 1991]) they are of interest since the secondary form of hypertension which they induce is readily curable.

Even though NA is primarily secreted during pheochromocytoma, a minority of these tumours secrete predominantly AD. These are of particular interest since they may provide information as to possible mechanisms which may be involved in AD induced hypertension.

Unlike patients with NA secreting pheochromocytomas which exhibit hypertension which might be mediated via an increase in sympathetic nerve activity [Hoffman, 1991], patients with AD secreting pheochromocytomas experience episodes of both severe hypertension and hypotension, tachycardia and arrhythmia [Page *et al.* 1969; Munk *et al.* 1977; Baxter *et al.* 1992].   Hypertension can be reversed by administration of a β-blocker, however, administration of an α-blocker results in profound hypotension [Aronoff *et al.* 1980].   Due to the rarity of this condition, however, exact mechanisms underlying these responses are not known.   It is tempting to speculate that, because of the dual effects on blood pressure (i.e. hypotension and hypertension) that AD is having a direct effect on postsynaptic receptors on the heart (β_1 receptor activation elevates cardiac output) and vasculature (β_2 receptor activation results in peripheral vasodilation).   The balance between the numbers of receptors occupied at a particular time, might explain the fluctuations in blood pressure.

1.7   Effects of AD deficiency

1.7.1   Adrenalmedullectomy

If AD is important in the development or maintenance of hypertension then removal of AD should decrease blood pressure in hypertensive rats.   Several studies have shown

that AMX significantly attenuates (but does not prevent) the development of hypertension in young SHR [Borkowski & Quinn, 1983b; Borkowski & Quinn, 1985; Borkowski, 1991]. Restoration of plasma AD levels after AMX with depot implants restored hypertension development in SHR. It should be noted however, that AD infusion alone, had no significant effect on hypertension development [Borkowski, 1991]. Denervation of the adrenal medulla blocked the development of left ventricular hypertrophy after aortic coarction in the dog implicating AD as a trophic hormone [Womble *et al.* 1980].

Several studies however, have shown that bilateral AMX in adult SHR does not lower blood pressure [Aoki *et al.* 1973; Borkowski & Quinn, 1983b; Hilse & Oehme, 1987] and a blood pressure reduction can only be achieved in old rats, only if the entire adrenal gland (i.e. medulla and cortex) is removed [Aoki *et al.* 1973]. If early AMX does, in fact, prevent hypertension development in young SHR there must be a limited period of "critical sensitivity" to AD in these rats (approximately between 4-6 weeks of age) [Borkowski, 1991].

The mechanism by which AMX might lower blood pressure is unclear. Studies by Borkowski and Quinn, showed that the pressor response to neurally released NA was reduced in the mesenteric bed of adrenalmedullectomised rats which could be augmented by adding AD to the perfusate. However, there were no differences in postsynaptic responsiveness to NA or AD [Borkowski, 1991]. Thus, the adrenal medulla appears to be involved in modulating sympathetic neurogenic vasoconstriction however, the nature of this modulation is unclear. These authors also find evidence that AD has a significant prohypertensive effect that is mediated via activation of presynaptic β_2 receptors [Borkowski & Quinn, 1984; Borkowski & Quinn, 1985].

1.7.2   AD deficiency in humans

Removal of pheochromocytoma in man causes a dramatic reduction in plasma catecholamines which is usually accompanied by a rapid normalisation of blood pressure [Takeda *et al.* 1986]. However, in long-term follow up studies, permanent normalisation of blood pressure after the removal of pheochromocytoma occurred in only 62% of subjects [Pruszczyk *et al.* 1991]. It was also noted that normalisation of blood pressure

occurred more often in patients with paroxysmal hypertension than in patients with a previous history of sustained hypertension [Pruszczyk et al. 1991]. Thus it seems that a period of hypertension caused by excess catecholamine secretion (such as during pheochromocytoma) is not necessarily a pathogenic factor in the development of sustained hypertension. Furthermore, studies in bilaterally adrenalectomised patients have shown that the blood pressure elevation in response to the cold pressor test is independent of adrenal activity [Cummings et al. 1983].

Another clinical syndrome in which plasma AD and NA levels are very low is congenital dopamine β-hydroxylase deficiency. In this syndrome patients lack dopamine β-hydroxylase, the enzyme which catalyses the conversion of dopamine to NA, resulting in high levels of plasma dopamine and low levels of plasma AD and NA [Man in't Veld et al. 1988]. The clinical manifestations of congenital dopamine β-hydroxylase deficiency include orthostatic hypotension (due to NA deficiency), neurological abnormalities (due to excess dopamine) and spontaneous hypoglycaemia and hyperinsulinemia (due to adrenalmedullary failure). These patients generally have low arterial pressure although it has not been established whether this is primarily due to a deficiency in plasma AD or NA [Man in't Veld et al. 1988].

1.8   Overall objective

Both AD infusion studies and studies in which the primary source of circulating AD was removed, provide evidence implicating AD in the pathogenesis of hypertension. Although the mechanism underlying the hypertension seems to involve the presynaptic β_2 receptor, the findings from man and rat are inconsistent. Thus it seems that if AD does induce hypertension, there must be other mechanisms involved.

The present investigation was inspired by an early finding in which both plasma NA and AD concentrations were found to be elevated in SHRSP under resting conditions [Howe et al. 1986]. Subsequent preliminary studies indicated that this abnormal elevation of plasma AD may precede the development of hypertension (PRC Howe, unpublished

observations). The threefold elevation of plasma AD (compared with the 1.5 fold elevation in plasma NA) in SHRSP prompted research as to the significance of this phenomenon.

Therefore, the aim of this investigation was to examine the involvement of plasma AD in the development and maintenance of hypertension and to identify the mechanisms by which AD might be influencing blood pressure.

1.8.1   Approaches

To examine the role which plasma AD may play in the pathogenesis of hypertension, it was important firstly to confirm that plasma AD levels were abnormally elevated in spontaneously hypertensive rats under resting conditions, as several other studies have yielded contradictory results (see Table 1). Moreover, it is difficult to contrast strain differences in plasma catecholamine values obtained in independent studies, due to the different methodologies used in different laboratories. These differences may, in fact, be the cause of the inconsistencies. In the present investigation, consistent methodologies were applied so that data from different experiments could be compared.

Resting plasma catecholamine levels were measured in young and adult, genetically related and unrelated, normotensive and hypertensive rat strains. The purpose was to clarify whether plasma AD levels were abnormally elevated in SHRSP, or whether plasma AD levels were abnormally low in normotensive WKY. Furthermore, plasma catecholamines were measured in WK-HA rats (which are hyperactive but not hypertensive) and WK-HT rats (which are hypertensive but not hyperactive) to see if any differences in plasma catecholamine levels between hypertensive and normotensive rats could be attributed to the prominent hyperactivity trait of the hypertensive strains.

To examine any possible influence of AD on blood pressure, AD was chronically infused into WKY. Previous studies using this approach have used SD [Schwartz & Eikenburg, 1986] or Wistar rats [Majewski et al. 1982]. The WKY strain was used in the present study so that any effects might be more relevant to the pathogenesis of hypertension in the genetically related hypertensive strains. For comparative purposes, plasma AD levels were depleted in the hypertensive strains to determine if AD depletion could attenuate hypertension development.

Finally, to see if the elevation of plasma AD is a consequence of hypertension development, blood pressure levels were manipulated in WKY and SHRSP. WKY were made hypertensive by chronic administration of Nω-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthesis inhibitor. Similarly, blood pressure was lowered in SHRSP by chronic administration of hydralazine (HZ), a peripheral vasodilator. Plasma catecholamine levels were measured after the manipulation of blood pressure to establish if the elevated AD levels previously reported in SHRSP [Howe *et al.* 1986] were secondary to hypertension development.

1.8.2   Outline of thesis

These approaches are addressed in the following chapters.

Chapter 2 describes the methodology generally applicable to all experiments. Details of procedures specific to individual experiments are included in the relevant chapters.

Chapter 3 examines the relationship between circulating AD and blood pressure in young and adult, genetically related and unrelated, normotensive and hypertensive rat strains.

Chapter 4 extends the examination of plasma catecholamines between strains by comparing plasma AD levels in WK-HA and WK-HT rats.

Chapter 5 examines the effects of modifying plasma AD levels on blood pressure in hypertensive and normotensive rats.

Chapter 6 examines the effects of modifying blood pressure levels on plasma AD in normotensive and hypertensive rats.

The overall outcomes and their implications are discussed in chapter 7.

CHAPTER 2

METHODOLOGY

2.1 Experimental animals

SHRSP, SHR, WKY, SD and BHW rats were obtained from the CSIRO breeding colony. For consistency, only male rats were used in experiments. All experimentation was approved by the CSIRO animal ethics committee [National Health and Medical Research Council *et al.* 1990]. WK-HA and WK-HT rats were obtained from Professor Edith Hendley from the University of Vermont, USA. These rats were kept under strict quarantine conditions in accordance with Australian quarantine regulations. In all cases rats were allowed standard laboratory rat chow and drinking water *ad libitum*. After any surgical procedure, rats received Clavulox antibiotic (200 μg/ml, Beecham Veterinary Products, Victoria, Australia) in drinking water as a prophylactic measure. After surgery rats were housed in individual cages.

2.2 Arterial catheterisation procedures

The use of direct arterial blood pressure measurements in conscious, unstressed rats was crucial to provide a meaningful outcome to this study. As mentioned previously, catecholamines are sensitive to various environmental and psychological stressors, hence the need to ensure that rats are completely unstressed during blood pressure measurements and blood sampling.

Other studies which have attempted to measure plasma catecholamines in conscious rats have cannulated various blood vessels including femoral and carotid arteries (see Table 1). These procedures require indefinitely occluding these vessels and although this may lead to sufficient redistribution of blood flow to the occluded areas, it may also result

in local ischemia - an unsatisfactory condition for obtaining resting blood pressure in unstressed rats.

The method for measuring blood pressure in the present study involves cannulation of the abdominal aorta. The catheter used is non-occluding and only impedes blood flow minimally. In all cases blood flow to the lower limbs is ensured and rats are precluded from experiments should any ischaemia become apparent in the legs. The incidence of ischaemia is very low and, in fact, there were no such cases during the following experiments.

In these experiments the catheter was exteriorised at the back of the neck and attached to the skin with sutures. The catheter was then plugged with a fine pin. This provided minimal impedance to the animal when compared with other studies in which the catheter was attached to a metal spring [Pacak *et al.* 1993] or swivel device [Bazil *et al.* 1993].

Thus, in the following experiments every effort was made to avoid any unnecessary interference with the rats both during and after surgery.

2.2.1　Preparation of catheters

Non-occluding arterial catheters consisted of 5 mm of Teflon (internal diameter (ID) = 0.3 mm, Robell Trading Company, SA, Australia) which was attached to 25-30 cm of vinyl tubing (outer diameter (OD)=1 mm, ID=0.5 mm). All tubing (except Teflon) was obtained from Dural Plastics and Engineering (NSW, Australia). These were soaked in a 0.05% solution of benzalkonium chloride for 48 hours and then immersed in a solution of heparinised saline (30 U/ml) 24 hours before use.

2.2.2　Cannulation procedure

Rats were anaesthetised with an intraperitoneal (i.p.) injection of sodium methihexitone (40 mg/kg) and sodium pentobarbitone (30 mg/kg). A midline incision was made in the abdomen to expose the lower abdominal aorta. Fascia was cleared from the aorta between the renal and iliac arteries. This section of the aorta was briefly occluded with

artery clamps and the arterial catheter was inserted proximally. The catheter was glued to the aorta with instant glue (cyanomethacrylate-ester) and was anchored to the prevertebral muscle with sutures. Once the artery clamps had been removed and adequate blood flow had been ensured, the catheter was exteriorised intrascapularly and anchored to the skin with sutures. Approximately 100 µl of Heparin (1000 U/ml) was injected into the catheter to prevent clotting. The protruding end of the catheter was plugged with a stainless steel pin. Rats regained consciousness within 2 hours but were left 24-48 hours to fully recover from surgery. During the subsequent recording period catheters were flushed daily with heparinised saline 10 U/ml.

2.2.3   Blood pressure measurements and blood sampling

The arterial catheter was connected by vinyl tubing (OD=1.5 mm, ID=0.5 mm) to a Statham P23ID pressure transducer and Neomedix physiological recorder (Neomedix systems, NSW, Australia) for direct measurement of resting MAP and HR from conscious, undisturbed rats (Figure 2). All recordings were made between 0900 and 1600 hours. Rats were allowed to settle for approximately 1 hour. Resting values of MAP and HR were recorded when these parameters had settled to a steady minimum level. When MAP and HR had remained at a low level for at least 5 minutes, the arterial catheter was briefly disconnected from the transducer and 300-400 µl of blood allowed to flow into a chilled heparinised tube. After the collection the arterial catheter was reconnected to the transducer and the volume of blood removed was replaced with heparinised saline. Resting HR was checked after sampling to ensure that rats had not been affected by the procedure. Blood was spun at 2800 rpm for 15 minutes at $4^{\circ}C$. Plasma aliquots (150 µl) were mixed with 3 µl of a preservative solution containing 1% dithiothreitol and 1% sodium EGTA in 0.05M NaOH. Plasma was frozen at $-80^{\circ}C$ for radioenzymic catecholamine assay.

2.3 Drug administration

2.3.1 Intravenous

Rats were anaesthetised (as above) and the right jugular vein was exposed. A polyethylene venous catheter (OD=0.61 mm, ID=0.28 mm, soaked in a 0.05% solution of benzalkonium chloride for 48 hours and then immersed in a solution of heparinised saline (30 U/ml) 24 hours before use) was passed through the vein into the right atrium. The catheter was then exteriorised intrascapularly (as above) and was heat sealed. During drug administration, the venous catheter was connected to a drug filled syringe via a 20 cm length of vinyl tubing primed with heparinised saline. The chosen drug was administered intravenously (i.v.) via the syringe and was followed by 300 μl of heparinised saline (10 U/ml).

2.3.2 Intra-arterial

In rats in which intravenous catheters were not implanted, drugs were administered intra-arterially (i.a.). The arterial catheter was briefly disconnected from the transducer and was connected to the drug-filled syringe. After injection of the drug, 300 μl of heparinised saline (10 U/ml) was flushed through the catheter.

2.4 Autonomic blockade and in vivo pressor responsiveness

Rats' autonomic reflexes were blocked with pentolinium tartrate (2 mg/kg). This dosing regime has been shown to cause an optimum acute reduction of MAP [Howe et al. 1986]. When pressor responses to phenylephrine were also being tested, reflex bradycardia (which may not be completely inhibited by ganglion blockade) was prevented by administration of methyl-bromide-scopolamine (0.2 mg/kg). In some cases, the acute pressor response of vasopressin (AVP) after autonomic blockade [Jablonskis et al. 1992], was inhibited by administration of an AVP antagonist

([1-(β-mercapto-β-β-cyclopentamethylene propionic acid),2-(O-methyl)-tyrosine]) arg^8-vasopressin (30 μg/kg). Due to availability, the AVP antagonist β-mercapto β, β cyclopentamethylene propionyl$_1$ O-methyl-tyr$_2$arg^8 vasopressin (30 μg/kg) was used in experiments described in chapters 4 and 6 . Both AVP antagonists could totally block pressor responses to exogenous AVP.

When MAP had fallen to a basal level, rats were given increasing bolus doses of phenylephrine (0.05 - 4 μg, i.v.) in volumes not exceeding 200 μl. Dosing continued until an increase in dose of phenylephrine produced no greater increase in MAP. Data was analysed for each individual animal by using a sigmoidal curve fitting program (SIGMOID, Baker Medical Research Institute, Victoria, Australia). Sigmoidal curve parameters were averaged from each animal and sigmoidal curves using these averaged parameters were determined for each treatment group.

2.5   Tail cuff blood pressure measurements

The advantage of the tail-cuff method of measuring blood pressure is that it is non-invasive and can be used to monitor blood pressure over an indefinite period of time. Arterial blood pressure measurements, although more accurate, can only provide blood pressure measurements for a short period of time (e.g. 5 weeks) as catheter patency is questionable after this time.

For tail-cuff blood pressure measurements, rats were restrained in perspex tubes at room temperature and their tails were warmed with an infra-red lamp. A cuff was placed around the tail and a doppler probe (Model 841, Parks Electronics Laboratory, Oregon, USA) was placed on the tail artery so a steady pulse could be heard. The cuff was then manually inflated 10-15 mmHg higher than the pressure where the pulse was no longer audible. The cuff was slowly deflated and the pressure at which the pulse became audible again was recorded as the blood pressure measurement. At least 3 readings were taken per rat and were averaged.

2.6   Activity measurements

Spontaneous locomotor activity was measured by placing rats in a plastic box (51 x 41.5 x 37 cm) which was scored into 10-12 cm squares. Each rat was placed in the same position in the activity box and the behaviour of the rats was recorded on video camera for 10 minutes. The activity box was thoroughly cleaned between rats. Locomotor activity was scored by counting the number of times the rats' nose crossed a line.

2.7   Blood perfused mesenteric preparation

Rats were anaesthetised with an intraperitoneal injection of sodium pentobarbitone (30 mg/kg) and were tracheotomised and artificially ventilated. The carotid artery and superior mesenteric artery were cannulated and connected via an extracorporeal circuit. Heparin (1000 U) was subsequently introduced into the circuit and blood was pumped, by a Gilson peristaltic pump, into the mesenteric artery at a rate of 0.5 ml/min for approximately 30 minutes. Flow rates were then varied in the range 0.25 - 1.25 ml/min and changes in perfusion pressure were noted. To assess pressor sensitivity of the mesenteric bed, bolus doses of phenylephrine (0.25 - 4 µg) were administered via the perfusion circuit. Mesenteric pressor responses were noted and sigmoidal curves were generated by SIGMOID.

2.8   AD infusion

In all cases, AD bitartrate was administered to rats by minipump infusion. Minipumps (Alzet, Model 2002) were filled with AD bitartrate solution (made in 0.1% ascorbic saline) in accordance with the manufacturers instructions. The delivery rate of these pumps was 0.5 µl/hr. Rats were anaesthetised with sodium methihexitone (40 mg/kg, i.p.) and minipumps were placed subcutaneously in the flank region. Incisions were closed with sutures and wounds were covered with topical antibiotic powder. In experiments in which minipumps needed to be changed (after approximately 14 days), rats were

re-anaesthetised, the exhausted minipump was removed, and a freshly prepared minipump was inserted in its place.

2.9   Bilateral adrenalmedullectomy

Rats were anaesthetised with sodium methihexitone (40 mg/kg, i.p.) and sodium pentobarbitone (30 mg/kg, i.p.). Adrenal glands were exposed via flank incisions. The adrenal cortex was cut and the adrenal medulla was removed by gently squeezing the adrenal gland. Flank incisions were closed with sutures and wounds covered with topical antibiotic powder.

2.10   Catechol-O-methyl-transferase radioenzymic catecholamine assay

The assay involves the methylation of AD and NA to their respective O-methylated metanephrines (metanephrine and normetanephrine). The catechol-O-methyl transferase (COMT) enzyme is used to transfer a radioactive methyl group to a catecholamine molecule. The methyl donor used for this reaction is S-adenosyl-L-(methyl-^{3}H) methionine (^{3}H-SAMe) (see Figure 3). The tritiated metanephrines are separated from the ^{3}H-SAMe using solvent phase extraction followed by thin layer chromatography to separate the metanephrines (Howe *et al.* 1986). The standard curve of this assay was linear (see Figure 4).

2.10.1 Isolation of COMT enzyme

The COMT enzyme used for the assays in this study had been prepared in our laboratory by a procedure similar to that reported by Nikodejevic *et al.* (1970). Rats were anaesthetised and their livers perfused via the portal vein with saline. The livers were then removed and homogenised in ice-cold isotonic potassium chloride. This was filtered through cheese cloth and glass wool to remove lipids. The pH of the filtrate was adjusted to 5.3 by addition of 1 M acetic acid. After centrifugation, the supernatant was decanted,

and the pH adjusted to 6.8 with sodium sulphate (pH 7). Ammonium sulphate was added to the supernatant to 30 % saturation and the supernatant was decanted and adjusted to 55 % saturation with ammonium sulphate. The suspension was centrifuged and the supernatant discarded. The precipitate was redissolved in 1 mM Tris/HCl buffer (pH 7.4) containing 0.1 mM dithiothreitol and dialysed with 5 L of the above buffer. Aliquots of the enzyme were mixed with σ-benzylhydroxylamine hydrochloride (1 mM) prior to use to inhibit the action of DOPA decarboxylase which can be detected in COMT extracts.

2.10.2   Preparation of samples and standards

2.10.2i   Plasma

Plasma (150 μl) was thawed and precipitated with 6 μl of 5 M perchloric acid ($HClO_4$). Samples were then left on ice for at least 30 minutes to ensure maximum precipitation and were then centrifuged at 25,000 g at $4^{o}C$ for 12 minutes. Internal standards were prepared by prior addition of AD and NA to a pooled stock of rat plasma, such that 25 μl of acidified plasma supernatant contained 236 pg (free base) of each catecholamine.

2.10.2.ii   Tissues

Tissues were rapidly removed post-mortem and were snap frozen in liquid nitrogen and stored at $-80^{o}C$. Tissues were homogenised with ice cold 0.16 M $HClO_4$ (cardiac ventricles and kidneys: 2.5 % w/v, adrenals: 5 % w/v). Aliquots (200 μl) of the homogenate were centrifuged at 25,000 g at $4^{o}C$ for 12 minutes. Tissue standards consisted of sample tissue to which standard (l-epinephrine bitartrate and -Arterenol) had been added. Each internal standard tube contained 500 pg of each catecholamine as the free base. AD and NA standard was added to an appropriate volume of reaction mix (see below) prior to its addition to the internal standard tissue sample.

2.10.3 Assay procedure

Aliquots of sample supernatant (25 μl in duplicate) were added to chilled Eppendorf tubes to which reaction mix was added. Blanks consisted of 25 μl of 0.16 M $HClO_4$. The entire assay was performed on ice unless otherwise specified.

Reaction mix (per tube) consisted of the following:

- 8 μl   1 M tris buffer (to maintain pH 8-8.6) containing 1 % (w/v) EGTA (COMT is inhibited by calcium)

- 4 μl   catechol-O-methyl transferase (10 mg protein/ml)

- 1 μl   0.1 M pargyline hydrochloride (to inhibit any monoamine oxidase enzyme that might be present)

- 1 μl   1 M $MgCl_2$ (activity of COMT is dependent on the presence of magnesium)

- 0.5 μl ^{3}H-SAMe (15 Ci/mmol)

On arrival to the laboratory ^{3}H-SAMe was stored at -80°C. After the vial had been opened 250 μl of ethanol was added to prevent rapid degradation of the ^{3}H-SAMe. This was subsequently stored at -20°C. Purity of the ^{3}H-SAMe was tested regularly in test assays to ensure adequate blank values.

After addition of the reaction mix, tubes were briefly Vortexed and incubated for 1 hour at 37°C. The reaction was stopped by immersing the tubes in ice.

Tetraphenyl boron (100 μl, 1 %) in 0.125 M sodium tetraborate buffer (pH 8) was added to the tubes. Tritiated metanephrines were extracted into 700 μl of 95:5 (v/v) toluene: iso-amyl alcohol by vortexing samples for 1 min and centrifugation at 2800 rpm for 3 min at 4°C. The metanephrines were then re-extracted into an acidic phase by aliquotting 500 μl of the organic phase into tubes containing 5 μg of metanephrine and normetanephrine (cold carriers) in 25 μl of 0.1 N hydrochloric acid (HCl). Samples were Vortexed and centrifuged as above. The organic phase was aspirated and 20 μl of the acid phase was spotted onto Whatman LK6DF thin-layer chromatography plates (Whatman International, Maidstone, England) which were developed in a solvent containing 84 % chloroform, 8 % propan-2-ol and 8 % ethyl diamine. The plates were dried and the two zones containing ^{3}H-metanephrine and ^{3}H-normetanephrine (Figure

5) were scraped from the plates into vials containing 200 µl of 0.125 M sodium tetraborate and 3.5 ml of scintillant (0.4 % 2,5-diphenyloxazole, 0.004 % 1,4-bis[2-(5-phenyloxazolyl)]benzene;2,2'-p-phenylenebis(5-phenyloxazole) and 1 % v/v diethylhexylphosphate in toluene). The clear definition between bands indicates that there is very little carry-over of NA and AD into the alternate band. Previous assessment of carry-over in our laboratory showed that it was less than 1% (PRC Howe, personal communication). This final isolation step resulted in the distribution of ^{3}H-metanephrine and 3H-normetanephrine into the scintillant phase with retention of residual ^{3}H-SAMe or breakdown products (e.g. ^{3}H-Methanol) in the aqueous layer. Vials were shaken for 5 min and radioactivity was counted several hours later in a liquid scintillation counter.

The amount of radioactivity in each sample was compared with the radioactivity of the internal standard to which a known amount of catecholamine had been added (236 pg of each catecholamine for plasma, 500 pg for tissues). Protein content of tissues was measured using the method of Lowry *et al.* (1951).

The limit of sensitivity was arbitrarily defined as the pg value equivalent to twice the blank value. On average, the limit of sensitivity was 45 and 76 pg/ml for AD and NA respectively. Inter-assay variability was approximately 15 % and intra-assay variability averaged approximately 8 %.

2.11 Chemicals list

	Supplier
S-adenosyl-L-(methyl-^{3}H) methionine	Amersham, NSW, Australia
Arterenol	Sigma, Mo, USA
benzalkonium chloride	Sigma, Mo, USA
σ-benzylhydroxylamine HCl	Sigma, Mo, USA
chloroform	Ajax Chemicals, NSW, Australia

Chemicals List (continued)   Supplier

Chemical	Supplier
2,5-diphenyloxazole	Sigma, Mo, USA
DL-dithiothreitol	Sigma, Mo, USA
ethylene diamine	Sigma, Mo, USA
di-2-ethylhexylphosphate	Sigma, Mo, USA
L-epinephrine bitartrate	Sigma, Mo, USA
heparin Inj. B.P.	CSL, Vic, Australia
hydralazine hydrochloride	Sigma, Mo, USA
magnesium chloride	Aldrich Chemical Company, Wis, USA
([1-(β-mercapto-β-β-cyclopentamethylene propionic acid),2-(O-methyl)-tyrosine])	Peninsula Laboratories, Ca, USA
β-mercapto β, β cyclopentamethylene propionyl1 O-methyl-tyr$_2$arg^{8}vasopressin	Sigma, Mo, USA
DL-metanephrine HCl	Sigma, Mo, USA
methyl-bromide-scopolamine	Sigma, Mo, USA
Nω-nitro-L-arginine methyl ester	Sigma, Mo, USA
DL-normetanephrine HCl	Sigma, Mo, USA
pargyline hydrochloride	Sigma, Mo, USA
pentolinium tartrate	Sigma, Mo, USA
perchloric acid	Ajax Chemicals, NSW, Australia
L-phenylephrine HCl	Sigma, Mo, USA
1,4-bis[2-(5-phenyloxazolyl)] benzene; 2,2'-p-phenylenebis (5-phenyloxazole)	Sigma, Mo, USA
propan-2-ol	Ajax Chemicals, NSW, Australia
sodium EGTA	Sigma, Mo, USA
sodium hydroxide	ACE Chemical Company, SA, Australia
sodium methihexitone	Eli Lily, NSW, Australia

Chemicals List (continued)   **Supplier**

sodium nitroprusside   Ajax Chemicals, NSW, Australia

sodium pentobarbitone   Bomac Laboratories, NSW, Australia

di-sodium tetraborate   BDH, Vic, Australia

sodium tetraphenylboron   Sigma, Mo, USA

toluene   Ajax Chemicals, NSW, Australia

tris (hydroxymethyl) amino methane   Sigma, Mo, USA
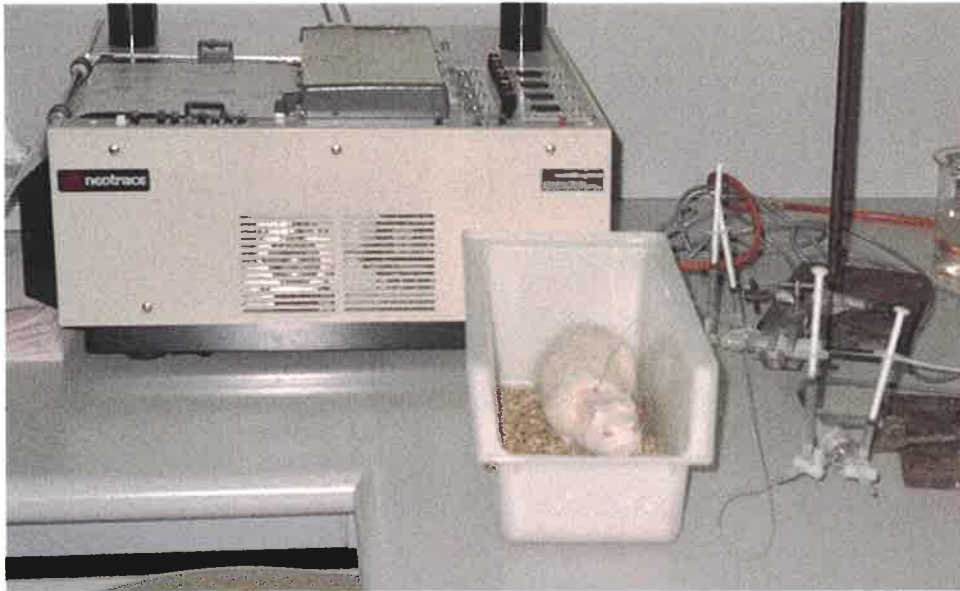
FIGURE 2 Arterial catheters were implanted in rats to monitor MAP and take blood samples under conscious and unrestrained conditions. During sampling, the rats remained undisturbed in their cages.

catecholamines

metanephrines

COMT
Mg^{2+}

^{3}H—SAMe   SAH
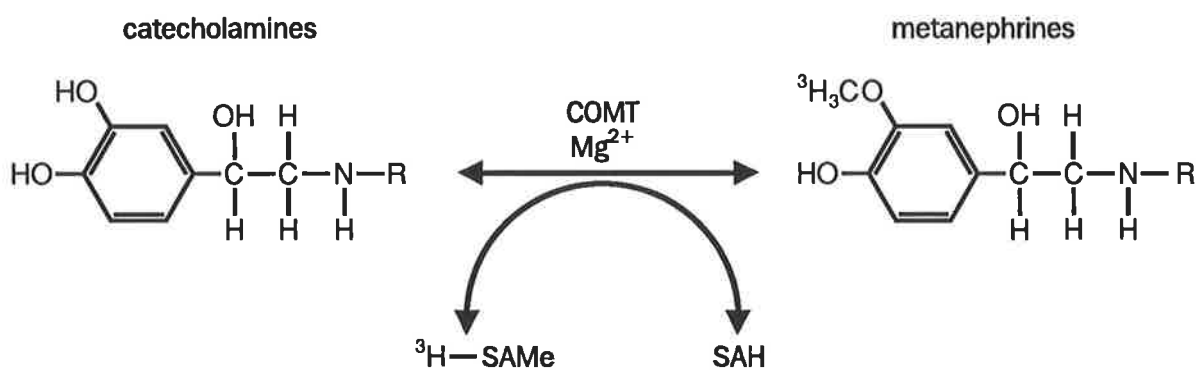
SAH = S-adenosyl-L-homocysteine
R   = CH$_3$ for AD or H for NA

Adapted from Falke & Birkenhager (1978)

FIGURE 3 Description of COMT radioenzymic catecholamine assay. The COMT enzyme is used to transfer a radioactive methyl group (methyl donor is ^{3}H-SAMe) to a catecholamine molecule.
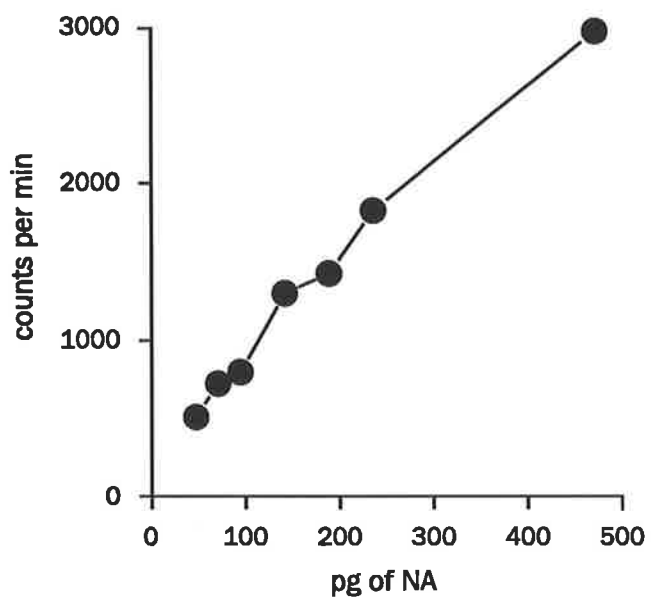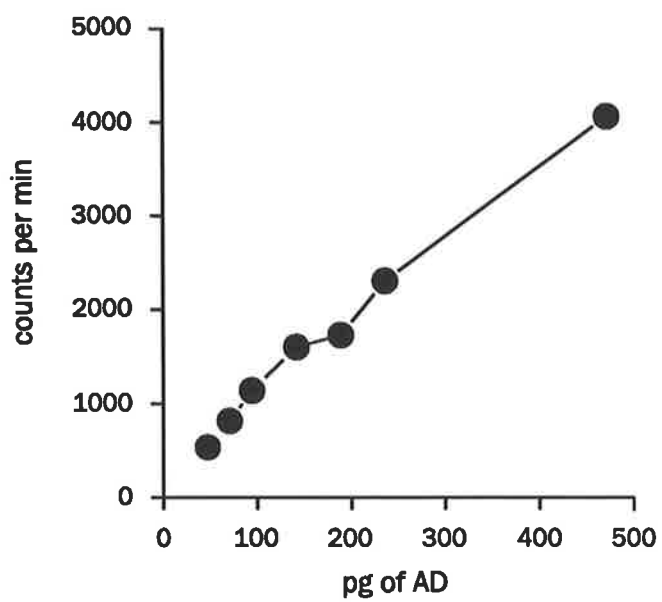
FIGURE 4 COMT catecholamine assay: examples of standard curves for AD and NA.
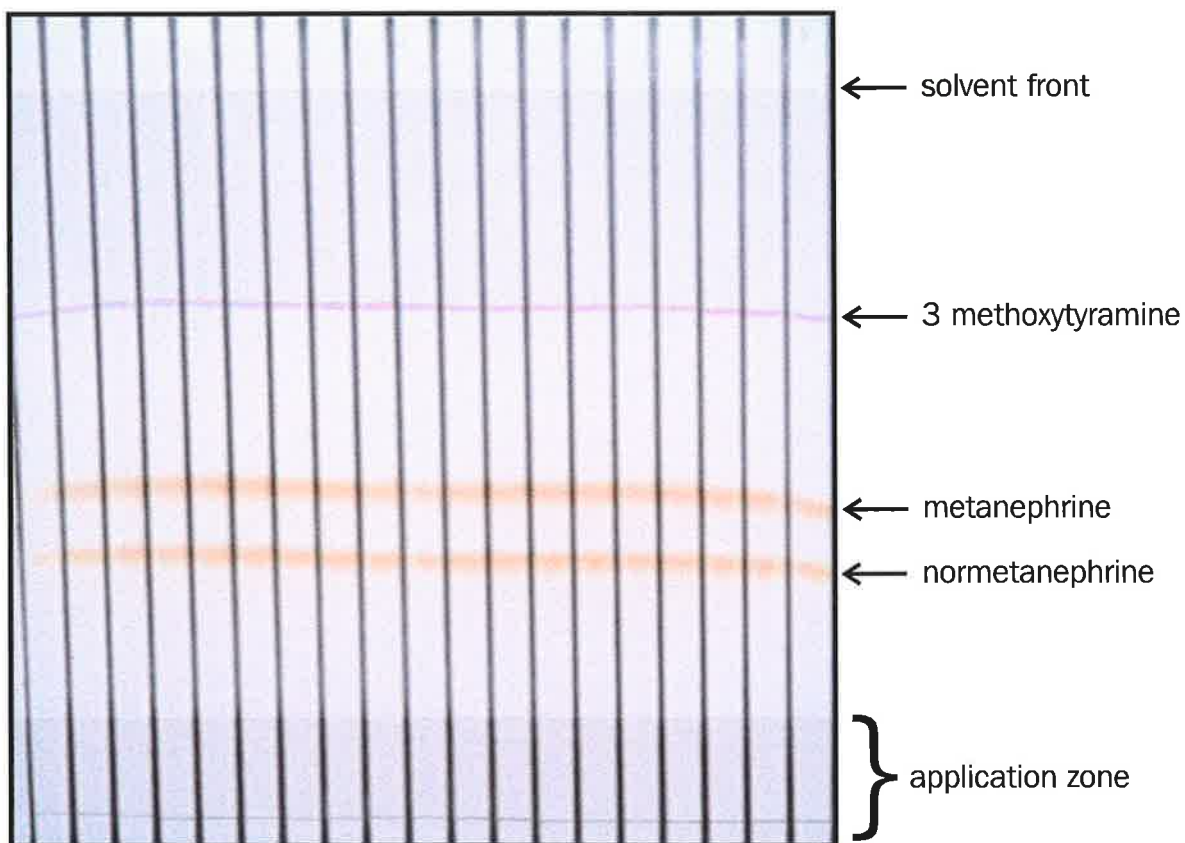
FIGURE 5 Example of a Whatman LK6DF thin layer chromatography plate showing clear separation of tritiated metanephrine bands. Samples were applied to a pre-concentrating zone in each of the 19 separate channels. Each plate contained two blanks and one internal standard.

CHAPTER 3

COMPARISON OF CARDIOVASCULAR PARAMETERS IN NORMOTENSIVE AND HYPERTENSIVE RATS

3.1 Aim

The measurement of plasma catecholamines has been used to ascertain the role of the sympathetic nervous system in the pathogenesis of essential and experimental hypertension. Although plasma NA provides a crude index of sympathetic nerve outflow (since it is released directly from sympathetic nerve terminals), it has been proposed that plasma AD (originating primarily from the adrenal gland) may provide a more accurate index of sympathetic nerve outflow [Franco-Morselli et al. 1977] since it is released directly into plasma during nerve stimulation, while NA is subjected to a more complex diffusion from the nerve terminal to the lumen of blood vessels [Franco-Morselli et al. 1978].

A previous study from our laboratory reported a marked elevation of plasma AD and NA in adult SHRSP with established hypertension [Howe et al. 1986]. The aim of the present study was to determine if the differences in plasma AD levels between SHRSP and WKY reflected an abnormality associated with the hypertensive strain or if the phenomenon could be attributed to an abnormality associated with characteristically low levels of AD in the WKY strain. For this purpose, basal plasma catecholamine levels were measured in 2 genetically related hypertensive rat strains (viz. SHR and SHRSP) and genetically related (WKY) and unrelated (BHW and SD) normotensive strains. In addition to this, studies were performed in young and adult rats to determine if the elevation of plasma AD is primary or secondary to hypertension development.

3.2   Methods

Arterial catheters were implanted into 5-6 week and 7-9 month old SD, BHW, WKY, SHR and SHRSP. Resting MAP was monitored and arterial blood samples were taken on at least 2 consecutive days and were averaged for each animal. After recording resting MAP, autonomic reflexes were blocked by administration of pentolinium (i.a.). When MAP had settled to a new basal level, an AVP antagonist was given (i.a.). Acute changes in MAP were recorded (refer to chapter 2). In a separate experiment, the AVP antagonist was administered alone to determine the relative contribution of AVP to resting MAP in adult WKY and SHR.

Data are shown as mean ± standard error of the mean (SEM). Significant differences between strains were determined using a one-way analysis of variance (ANOVA) with Dunnett's test for multiple comparisons. For these tests WKY were defined as the control strain. Paired t-tests were used for within-animal comparisons. Correlation coefficients (Pearson r) between plasma catecholamines and blood pressure across strains was determined by linear regression. These were considered significant if $p < 0.05$.

3.3   Results

3.3.1   Young rats

At 5-7 weeks of age, MAP was already 24 mmHg higher in SHR and SHRSP than in the WKY strain ($p < 0.05$) while MAP of the SD strain was similar to that of WKY. There were no differences in resting HR (beats per minute (bpm)) between SD (407 ± 4 bpm, n=10), WKY (383 ± 6 bpm, n=10), SHR (410 ± 11 bpm, n=7) and SHRSP (383 ± 5 bpm, n=10). Plasma AD tended to be elevated in both young SHR (71 %) and SHRSP (51 %) strains when compared to WKY but was significantly higher in the SHR strain only ($p < 0.05$). Plasma AD was significantly lower in the SD strain than in WKY ($p < 0.05$). Circulating NA did not differ between groups (see Figure 6a).

3.3.2   Old rats

Resting MAP was substantially elevated at 7-9 months of age in SHR (165 ± 3 mmHg, n=8) and SHRSP (182 ± 8 mmHg, n=5) compared with age-matched WKY (121 ± 1 mmHg, n=12, p<0.05). Resting MAP of the WKY strain was itself significantly higher than in the SD strain (96 ± 2 mmHg, n=5, p<0.05) but did not differ from the BHW strain (117 ± 2 mmHg, n=4). There was no difference in resting HR between SD (314 ± 6 bpm, n=5), BHW (316 ± 7 bpm, n=4), WKY (293 ± 7 bpm, n=12), SHR (326 ± 9 bpm, n=8) and SHRSP (296 ± 3 bpm, n=5). Plasma AD was 3-4 times higher in SHR and SHRSP (p<0.05) than in WKY but there were no differences in circulating AD between any of the normotensive strains. Plasma NA was 1.8 times higher in SHR than in WKY (p<0.05) but did not differ significantly in the other strains (see Figure 6b).

3.3.3   Effects of autonomic blockade

The ganglion blocking drug pentolinium was administered to rats to see whether the differences in MAP between normotensive and hypertensive rats were due to differences in sympathetic pressor tone. On the day of autonomic blockade, MAP was still elevated (p<0.05) in the adult hypertensive strains (SHR 160 ± 7 mmHg, n=4; SHRSP 185 ± 9 mmHg, n=5) when compared with age-matched WKY (120 ± 1 mmHg, n=5). MAP of the adult SD rats (92 ± 3 mmHg, n=5) was still lower (p<0.05) than in WKY rats.

With administration of pentolinium, MAP fell sharply in all strains and the difference in MAP between WKY and SD was eliminated. However, the reduction of MAP in WKY, SHR and SHRSP was similar.

Administration of an AVP antagonist to adult SHR and WKY under normal, resting conditions caused slight reductions of MAP which were not significant (3 ± 3 and 4 ± 4 mmHg in WKY n=4 and SHR n=4, respectively). After ganglion blockade, however, administration of the AVP antagonist did lower MAP significantly (p<0.05, paired t-test) in WKY, SHR and SHRSP. This fall in MAP was, however, greater (p<0.05) in both SHR (26 ± 2 mmHg, n=4) and SHRSP (38 ± 3 mmHg, n=5) strains than in WKY (16 ± 4 mmHg, n=5). The overall reduction in MAP after both pentolinium and AVP antagonist

administration appeared to be greater in SHR and SHRSP than in the WKY strain. However, the difference reached significance for SHRSP only (p<0.05). The final residual level of MAP was still significantly greater in SHR and SHRSP when compared with WKY (p<0.05, Figure 7)

At 5-7 weeks of age, resting MAP on the day of autonomic blockade, was significantly higher (p<0.05) in SHR (118 ± 5 mmHg, n=6) and SHRSP (118 ± 2 mmHg, n=7) strains when compared with WKY (95 ± 2 mmHg, n=9) or SD rats (93 ± 2 mmHg, n=9). The fall in MAP induced by ganglion blockade was similar in WKY, SHR and SHRSP. Administration of the AVP antagonist during ganglion blockade caused a further reduction of MAP in the SHR strain only (5.5 ± 1 mmHg, n=6, p<0.05, paired t-test). The total fall in MAP from the initial resting level after administration of both drugs was significantly less in the SD strain than in the WKY strain (p<0.05) but did not differ significantly between WKY and either hypertensive strain. Hence, the residual level of MAP after administration of both drugs was still significantly higher (p<0.05) in SHR and SHRSP when compared with WKY, but the difference between SD and WKY strains was eliminated.

3.3.4   Correlation coefficients

Correlation coefficients (Pearson r) of plasma AD and NA versus resting MAP, the reduction of MAP with ganglion blockade and the residual MAP in young or old rats of all strains are shown below. The relationship between plasma AD and MAP is shown in Figure 8.

If rats from all strains and ages were considered, then resting MAP correlated significantly with both plasma AD (r=0.719, n=63, p<0.01) and plasma NA (r=0.522, n=63, p<0.01).

TABLE 2 Correlation coefficients of plasma catecholamines versus various cardiovascular parameters.

	Young rats		Old rats	
	Plasma AD	Plasma NA	Plasma AD	Plasma NA
Resting MAP	0.264	0.200	0.766**	0.485*
MAP reduction	0.452**	0.000	0.742**	0.243
Residual MAP	0.180	0.224	0.732**	0.375
Plasma NA	0.180		0.673**	

Correlation coefficients were based on measurements obtained from 31-35 young rats and 28 old rats.

* indicates $p < 0.05$, ** indicates $p < 0.01$.

a) YOUNG RATS

MEAN ARTERIAL PRESSURE

PLASMA ADRENALINE

PLASMA NORADRENALINE

b) ADULT RATS

MEAN ARTERIAL PRESSURE

PLASMA ADRENALINE

PLASMA NORADRENALINE
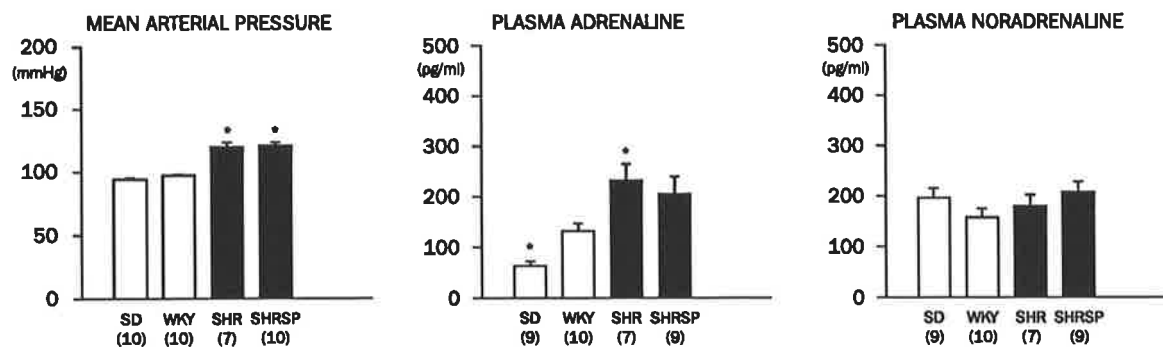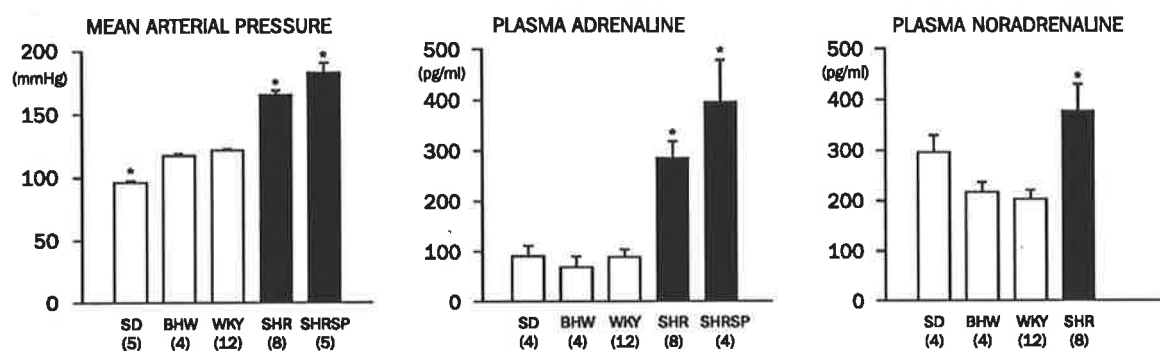
* indicates significantly different (p<0.05) from WKY

Numbers in parentheses indicate animal numbers

FIGURE 6 Blood pressure and plasma catecholamine levels in young (5-7 weeks) and adult (7 months) hypertensive (SHR and SHRSP) and genetically related (WKY) and unrelated (SD and BHW) normotensive rats.

a) Reduction of MAP (mmHg)
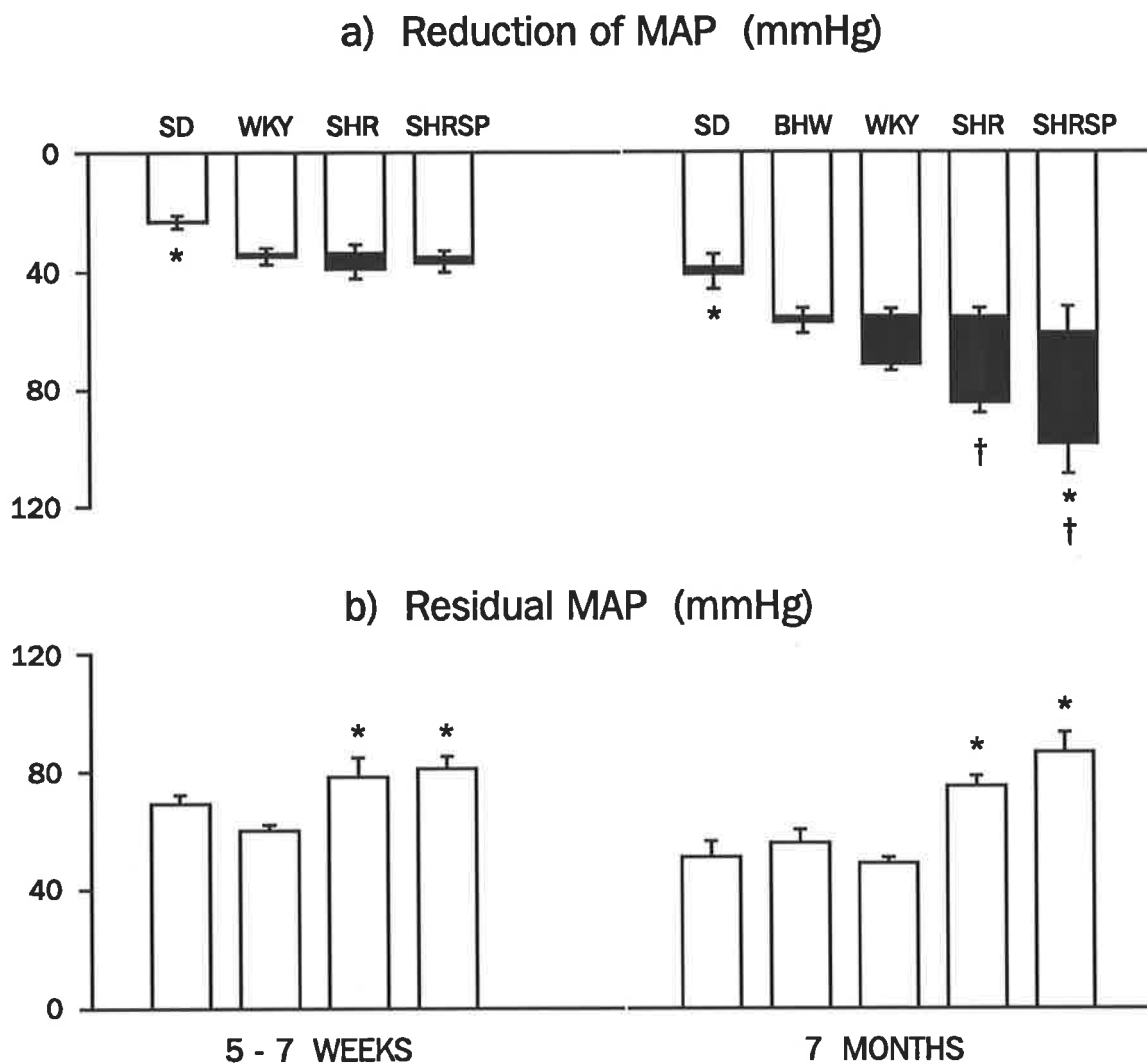
b) Residual MAP (mmHg)

* value significantly different (p<0.05) from WKY

† effect of AVP antagonist is significantly different (p<0.05) from WKY

FIGURE 7 a) Reduction in MAP induced by i.a. administration of a ganglion blocker (□) followed by an AVP antagonist (■).
b) Residual MAP after administration of both drugs.
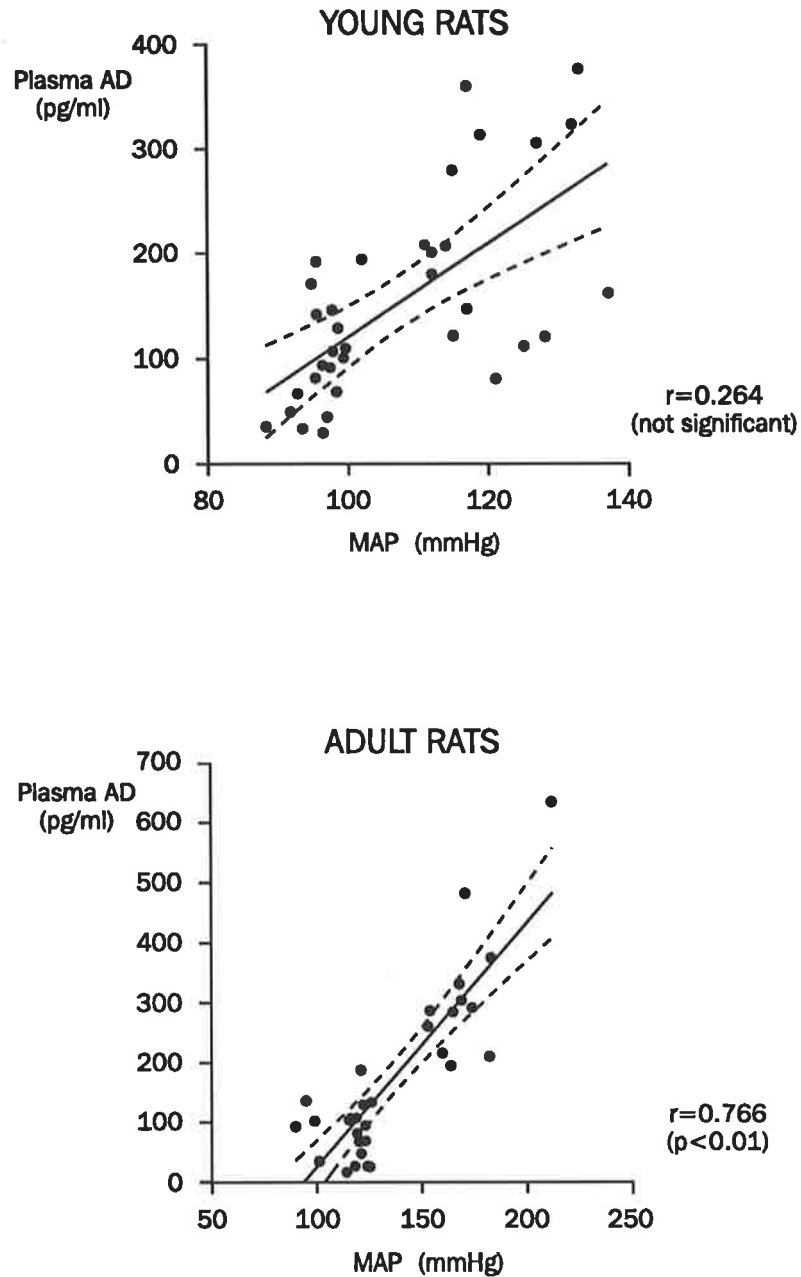
FIGURE 8 Graphs show the relationship between plasma AD and blood pressure in young and adult rats of all strains. Solid line represents linear regression; dashed lines show 95% confidence limits.
r=correlation coefficient

3.4   Discussion

This study has confirmed that there is a striking difference in plasma AD levels between WKY and SHRSP and it shows that this difference represents an abnormal elevation of AD in both spontaneously hypertensive strains (SHR and SHRSP), since plasma AD levels were uniformly lower in related (WKY) and unrelated (SD and BHW) normotensive strains. The relationship between raised plasma AD and hypertension is highlighted by a clear association between plasma AD concentrations and blood pressure across strains and in the older animals. Moreover, the selective elevation of plasma AD is evident early in the development of hypertension, even though it does not correlate with blood pressure at that stage.

These results contrast with several studies which have shown no differences in circulating AD between normotensive rats and rats with established hypertension under resting conditions [McCarty & Kopin, 1978; Schomig et al. 1978; Ferrari et al. 1981; Picotti et al. 1982; Kirby et al. 1989]. In this study, procedures refined in our laboratory were used to prevent any environmental or psychological disturbances to rats during blood sampling. Animals were left for a sufficient period of time after cannulation before any recordings were made to prevent any influence of anaesthesia or post-operative stress, which may be especially evident in the hypertensive strains. To ensure that rats were completely rested during blood sampling, samples were taken only when HR had settled to a steady minimum level. The state of rest of the animals at the time of assessment is reflected by the low HR which is at least 100 bpm lower than that usually observed, except when depressed by the residual effects of anaesthetic shortly after cannulation. Additionally, the low levels of HR in this study are similar to those recently reported in which telemetry was used to measure blood pressure and HR in conscious SHR [Bazil et al. 1993]. Furthermore, in this study, caution was taken to ensure that only small volumes of blood (300 µl) were taken so that haemorrhagic responses were not induced. Finally, repeated blood sampling on consecutive days minimised any daily fluctuations in plasma catecholamine concentrations and ensured that rats were fully acclimatised to the procedures. Thus, the high levels of plasma AD observed in the hypertensive strains cannot be attributed to environmental or surgical stimuli but are likely to reflect true resting levels of AD in the circulation.

The plasma AD levels attained in the present study for SHR and WKY rats are generally comparable to other studies which have measured plasma catecholamines in rats under conscious conditions. However, due to the large variations in catecholamine levels reported it is difficult to compare results obtained from different laboratories (for example see Borkowski & Quinn, 1983a and Pacak et al. 1993). These discrepancies could arise from differences in blood sampling techniques and methodologies. Furthermore, it could also be possible that purchase of rats from different suppliers might be a potential source of variability (see Kurtz & Morris, 1987 for review).

The mechanism by which plasma AD is elevated in the hypertensive strains is not clear. Adrenal AD content is elevated in SHRSP [Schober et al. 1989] such that they may secrete more AD per nerve impulse. Alternatively, sympathetic activity in the splanchnic nerve may be greater in SHR than in normotensive rats [Judy et al. 1976] resulting in more catecholamine release into the circulation. Under resting conditions, however, the increased release of adrenal medullary AD in the hypertensive strains is probably not due to adrenal sympathetic hyperactivity since the elevation of plasma AD persists after ganglion blockade in SHRSP [Howe et al. 1986]. However, it may be influenced by circulating hormones such as angiotensin II, since chronic administration of angiotensin converting enzyme inhibitors can significantly lower circulating AD levels in SHRSP [Howe, 1989]. There is much evidence to suggest that overactivity of the renin angiotensin system is a primary factor in the development of hypertension of the SHR [King et al. 1992]. Angiotensin II may exacerbate hypertension by a direct pressor effect, or possibly by liberation of catecholamines from the adrenal gland. If indeed the renin angiotensin system plays a crucial role in hypertension development, the elevated AD levels in the hypertensive rats could be a consequence of heightened activity of angiotensin II on the adrenal gland. Alternatively, alterations of catecholamine reuptake [Goldstein et al. 1983] or presynaptic regulatory mechanisms [Kawasaki et al. 1982; Ekas et al. 1983] in hypertension may also contribute to the overall elevation of plasma AD.

It has been hypothesised that AD may contribute to the pathogenesis of hypertension by facilitating the release of NA from sympathetic vasoconstrictor nerve terminals [Majewski et al. 1981b]. This is supported by the significant correlation between plasma AD and NA in the older animals and the higher levels of plasma NA in older SHR. On the other

hand, there was no relationship between plasma AD and NA in the young rats. Plasma NA was normal in young SHR, despite the elevation of plasma AD in this strain. However, the extent of NA spillover into plasma is dependant upon neuronal and extra neuronal uptake and metabolism. It is possible, therefore, that there may have been increased NA outflow but also increased NA uptake or metabolism. Consequently, there would be no overall change in plasma NA levels, as observed in this study.

Is the elevation of AD a primary factor in the pathogenesis of hypertension in SHR and SHRSP or is it secondary the development of hypertension? The significant correlation between plasma AD and MAP in the old rats shows a strong association between the two parameters, although this does not necessarily imply a causal relationship. However, the lack of correlation between plasma AD and MAP in the young rats makes it less likely that the development of hypertension is dependent on the elevation of plasma AD. Nevertheless, although the higher plasma AD levels may not be directly related to hypertension, they may be related to other independent factors which are characteristic of the hypertensive strains such as behavioural abnormalities (eg. hyperactivity [Hendley & Ohlsson, 1991]) or insulin resistance ([Bursztyn et al. 1992]).

3.4.1 Effects of ganglion and AVP blockade

In the present study it has been shown that circulating AVP can compensate for the acute fall in MAP in conscious rats induced by ganglion blockade, confirming a previous study [Jablonskis et al. 1992]. This fall in MAP must represent the loss of sympathetically-mediated vasoconstriction since it is not accompanied by a change in cardiac output (PRC Howe et al., unpublished observations). Administration of a specific antagonist of the pressor response to AVP alone did not significantly affect resting MAP. However, administration of the antagonist to ganglion-blocked rats caused a further reduction of MAP. Moreover, the compensation by AVP for the loss of sympathetically mediated vasoconstrictor tone appeared to be related to age and to the degree of hypertension.

Previous studies using Brattleboro and Long Evans rats have shown that the fall in blood pressure after ganglion blockade is significantly greater in the AVP deficient rats [Hiwatari

et al. 1985; Williams & Johnson, 1986]. Some have interpreted these results as suggesting that sympathetic nerve activity is greater in these rats [Williams & Johnson, 1986]. However, studies which measured plasma AVP after total autonomic blockade show that this treatment causes a 30 fold increase in plasma AVP which falsely elevates blood pressure in the Long Evans strain [Hiwatari *et al.* 1985]. Others, have also demonstrated a 60 fold increase in AVP pressor sensitivity with ganglion blockade [Osborn *et al.* 1987]. Hence, in this study it is unclear whether an increase in AVP secretion or an increase in pressor sensitivity to AVP is responsible for the elevated MAP level after ganglion blockade.

The results of the present study also indicate that administration of ganglion blockers alone may not be an accurate method of estimating the contribution of sympathetic nerve activity to resting blood pressure since factors such as age and initial MAP may influence the degree of compensation by AVP. Both plasma AVP concentration [Terwel *et al.* 1992] and vascular sensitivity to AVP [Frolkis *et al.* 1982] increase with age, which may account for the greater degree of AVP compensation in adult animals. However, the removal of the pressor effects of AVP may also enhance secretion of or sensitivity to other circulating pressor hormones (e.g. angiotensin II [Hiwatari *et al.* 1985]). The importance of these hormones in maintaining MAP after ganglion blockade and whether or not their secretion varies according to strain or age, requires further investigation.

The results indicate that sympathetic nerve activity is elevated in adult hypertensive rats with established hypertension but not in young hypertensive rats, implying that increased sympathetic nerve activity does not contribute to the development of their hypertension. This contrasts with other evidence suggesting a pathogenic role for the sympathetic nervous system in the development but not the maintenance of hypertension [Yamori, 1976]. It is interesting to note that the final level of MAP (i.e. after administration of the ganglion blocker and the AVP antagonist) was also higher in the adult SHR and SHRSP than in WKY. This finding supports those of others [Yang *et al.* 1993] in which both neural and non-neural mechanisms were found to contribute to the hypertension of SHRSP; in SHR, however, the most important contributing factor for the hypertension was the non neural component of blood pressure. This non neural component of blood pressure may reflect structural changes in the vasculature induced by chronic elevation of sympathetic

vasoconstrictor tone. However, the residual MAP was also elevated in the young hypertensive strains even though the level of sympathetic activity was normal. The reason for this residual elevation is unclear but it may reflect higher levels of circulating pressor hormones (other than AVP) or the early existence of vascular hypertrophy, which may be a pathogenic factor in the young hypertensive rats [Folkow, 1990]. Circulating AD, however, is unlikely to be a contributing factor as it correlated significantly with residual MAP in the old rats only.

3.5   Summary

The results of this study have confirmed the finding from a previous study [Howe *et al.* 1986] that there is a marked difference in plasma AD levels between SHRSP and WKY rats. The present study extends the strain comparison to include other normotensive and hypertensive rat strains. The results show that the difference in plasma AD levels between WKY and SHRSP represents an abnormal elevation of AD in spontaneously hypertensive strain (SHRSP) rather than an irregularity associated with the WKY strain, since plasma AD levels were uniformly lower in related (WKY) and unrelated (SD and BHW) normotensive strains. The relationship between raised plasma AD and hypertension is highlighted by a clear association between plasma AD concentrations and blood pressure across strains in the old (7-9 month) animals. Moreover, the selective elevation of plasma AD is evident early in the development of hypertension (5-7 weeks of age), even though it does not correlate with blood pressure at that stage.

Compared with the normotensive strains, the acute reduction of MAP with AVP and ganglion blockade (which reflects the loss of sympathetic pressor tone) was significantly greater in the adult hypertensive strains, indicating that sympathetic nerve activity is greater in rats with established hypertension. However, elevated sympathetic nerve outflow does not appear to contribute to the early development of hypertension. The residual level of MAP (not attributed to sympathetic vasoconstriction) was already elevated in young hypertensive rats.

This study has also shown that circulating AVP can compensate for the acute fall in MAP in conscious rats, induced by ganglion blockade. Furthermore, the degree of compensation is dependant upon age and the severity of hypertension. Thus, when assessing sympathetic activity by the use of ganglion blockers, it is important to block the reflex AVP compensation.

CHAPTER 4

COMPARISON OF CARDIOVASCULAR PARAMETERS IN HYPERTENSIVE AND HYPERACTIVE RATS

4.1   Aim

In addition to having high blood pressure, SHR are also significantly more active compared to normotensive WKY rats. The aim of this study was to determine if the high level of plasma AD in the SHR is associated with their hypertensive or hyperactive phenotype. This study was made possible due to the availability of two new inbred rat strains in which the hypertensive and hyperactive phenotypes of the SHR have been dissociated;- WK-HT rats (which exhibit hypertension but not hyperactivity) and WK-HA rats (which exhibit hyperactivity but not hypertension) [Hendley & Ohlsson, 1991].

4.2   Methods

Two month old male WK-HT (F22 generation) and WK-HA (F25 generation) were obtained from the University of Vermont, Burlington, USA. When rats were 3 months of age, aortic catheters were implanted into rats for direct measurement of MAP and blood sampling under conscious, undisturbed conditions.

On day 4 of MAP recording, sympathetic outflow was blocked in rats by i.a. administration of pentolinium, an AVP blocker (see chapter 2) and the angiotensin II antagonist Losartan (10 mg/kg, Merck, Sharp and Dohme Research Laboratories, USA).

When rats were 4 months of age, tail cuff blood pressure measurements were taken on 2 separate occasions. At this time, rats' spontaneous locomotor activity was also tested.

Rats were euthanased with sodium pentobarbitone and cardiac ventricles were removed and weighed.

All data are shown as mean ± SEM and were analysed using Student's t-test.

4.3   Results

After 9 days of blood pressure recording, resting MAP of the WK-HA rats averaged 129 ± 2 mmHg (n=9) while MAP of the WK-HT rats averaged 132 ± 2 mmHg (n=11). Resting HR (averaged over 9 days) was significantly greater ($p<0.05$) in the WK-HA rats (338 ± 7 bpm, n=9 versus 306 ± 4 bpm, n=11 in the WK-HT rats). Plasma catecholamine levels did not differ between strains (Table 3).

TABLE 3 Plasma catecholamines (pg/ml)

	Adrenaline	Noradrenaline
WK-HT (n=11)	106 ± 17	167 ± 15
WK-HA (n=9)	131 ± 14	185 ± 17

Administration of pentolinium alone caused a significant reduction in MAP in all rats (44 ± 3 mmHg in WK-HT versus 34 ± 3 mmHg in WK-HA). This reduction was greater in the WK-HT strain ($p<0.05$). Successive administration of the AVP blocker and Losartan caused further significant reductions in MAP (10 - 13 mmHg after AVP blocker and a further 11 mmHg after Losartan). The reductions in MAP caused by the two blockers were similar between strains. Overall, the total reduction in MAP after pentolinium, AVP and angiotensin II blockade was greater in the WK-HT strain (Table 4).

TABLE 4 Effects of acute drug administration on MAP (mmHg)

	Resting	After ganglion blocker	After AVP blocker	After AVP & All blockers	Total reduction
WK-HT (n=11)	133 ± 3	89 ± 4	76 ± 2	66 ± 2	68 ± 3
WK-HA (n=9)	128 ± 3	94 ± 4	84 ± 4	72 ± 5	56 ± 4*

Values of MAP after administration of ganglion, AVP and angiotensin II (AII) blockers.

* significantly different from WK-HT (p<0.05)

Tail cuff blood pressure measurements taken subsequently at 4 months of age were also similar in WK-HT (141 ± 2 mmHg) and WK-HA (144 ± 3 mmHg) strains. Locomotor activity was markedly elevated (p<0.01) in WK-HA rats (269 ± 40 counts/10 minutes, n=5 versus 80 ± 13 counts/10 minutes, n=5 in WK-HT) thus confirming the hyperactivity trait in these rats.

There were no differences in ventricle weight / body weight ratios between the two strains (3.28 ± 0.04 mg/g, n=11 in WK-HT and 3.37 ± 0.2 mg/g, n=9 in WK-HA) at the time of euthanasia.

4.4 Discussion

The development of WK-HA and WK-HT strains has allowed a means of determining the association of a given attribute of the SHR with either the hypertensive or hyperactive phenotype. Already, several characteristics of the SHR previously thought to be associated with hypertension have now been shown to be related to behavioural hyperactivity e.g. hyper-responsiveness to stress [Hendley et al. 1988; Knardahl & Hendley, 1990] and the development of ventricular hypertrophy [Hendley & Ohlsson, 1991].

The hypertensive trait of the WK-HT is already evident by 4 weeks of age and persists up to 15 months of age [Hendley & Ohlsson, 1991]. Data determined from the present experimental rats at 2 months of age showed a significant elevation in tail-cuff blood pressure (measured at the University of Vermont) in the WK-HT strain (ED Hendley,

personal communication, April, 1993). However, there were no significant differences in repeated measurements of MAP obtained from 3 month old conscious, resting WK-HA and WK-HT. Even blood pressure measurements taken under stressful conditions (i.e. tail-cuff blood pressure) failed to show a difference between the two strains at 4 months of age. The level of tail-cuff blood pressure [Hendley & Ohlsson, 1991] and resting MAP [Sagvolden *et al.* 1992] quoted in this study is similar to that reported previously for the WK-HA strain but is considerably lower than that found previously for age-matched WK-HT rats. These findings contradict results found by others [Hendley *et al.* 1988; Knardahl & Hendley, 1990; Hendley & Ohlsson, 1991; Peruzzi *et al.* 1991; Sagvolden *et al.* 1992], and the reasons for the discrepancy are not apparent. When the siblings and cousins of the rats sent to Australia had their tail-cuff blood pressures measured at 3 months of age, values averaged 142 mmHg in WK-HT and 128 mmHg in WK-HA (ED Hendley, personal communication, December, 1993). These values are similar to the results obtained for 4 month old imported WK-HT. However, the values for the imported WK-HA rats are considerably higher than their American dwelling counterparts. Again, reasons for the discrepancies remain unidentified.

One possibility is that WK-HA rats (which react to a greater extent to stressful stimuli than WK-HT [Hendley *et al.* 1988; Knardahl & Hendley, 1990]) were hyperreactive to the blood pressure monitoring procedure, thereby falsely elevating their MAP and tail-cuff blood pressure. This possibility is consistent with the higher heart rates in the WK-HA strain (which have been reported previously [Hendley *et al.* 1988]) but is not consistent with the normal levels of plasma catecholamines under resting conditions and reports that WK-HA habituate to a novel stimulus quicker than WK-HT [Hendley & Ohlsson, 1991]. Another possibility is that differences in tail-cuff blood pressures between WK-HA and WK-HT rats are declining (see Appendix 1, ED Hendley, personal communication, December, 1993). The difference in tail-cuff blood pressures between 3 month old WK-HT and WK-HA was 30 mmHg in 1991. In 1993, however, this difference was only 14 mmHg. The reasons for the reductions in blood pressure in the WK-HT strain over the years is unknown.

Removal of sympathetically mediated vasoconstriction caused a greater fall of MAP in the WK-HT rats suggesting a higher level of intrinsic sympathetic vascular tone in this strain.

The level of MAP attained during blockade was, however, similar between the strains suggesting a similar level of basal vascular tone between the hyperactive and hypertensive rats. These results differ from a previous finding in which the fall in MAP induced by ganglion blockade caused a similar fall in MAP between the two strains but basal tone during blockade was slightly higher in the WK-HT strain [Knardahl & Hendley, 1990]. The finding that the WK-HA rats had a significantly lower level of sympathetic tone might also suggest that the WK-HA rats may not have been stressed by the blood pressure measuring procedure.

An interesting finding of this study was that angiotensin II can compensate for a fall in MAP induced by ganglion and AVP blockade. This is in accordance with previous studies in which autonomic blockade alone did not affect plasma angiotensin II levels in Long Evans rats but subsequent AVP blockade caused a significant elevation of plasma renin and angiotensin II [Hiwatari et al. 1985]. This indicates a compensatory activation of the renin angiotensin system after the pressor influence of AVP had been removed. Another study showed that ganglion blockade caused a two to eight fold increase in angiotensin II sensitivity but a 60 fold increase in pressor sensitivity for AVP [Osborn et al. 1987]. In the present study it was not determined if the elevated MAP after ganglion and AVP blockade was due to an increase in angiotensin II secretion or an increase in angiotensin II pressor sensitivity.

Plasma catecholamines measured under resting conditions were similar between WK-HA and WK-HT rats. Plasma AD, which is elevated in SHR and SHRSP (chapter 3) was normal in WK-HA and WK-HT. A previous study [Hendley et al. 1988], found plasma AD to be significantly lower in WK-HA and WK-HT than in WKY. In that study, plasma NA in WK-HA was slightly elevated above WK-HT levels. In the present study, there were no differences in circulating NA levels between the two strains. This might possibly reflect the low level of stress experienced by the rats with the blood pressure monitoring procedure.

The development of ventricular hypertrophy in SHR is more likely to be dependent on the hyperactive rather than the hypertensive phenotype since it is present in WK-HA rats from 6 weeks of age while ventricular enlargement occurs in WK-HT rats only once hypertension has become established [Hendley & Ohlsson, 1991]. In our study, there were no differences in the heart weight : body weight ratios between the strains.

4.5   Summary

The aim of this study was to determine if the elevated plasma AD levels in SHR were due to the hypertension or the hyperactivity seen in this strain. No differences in blood pressure could be observed between WK-HT and WK-HA strains, even though WK-HA had significantly higher activity scores and resting heart rates. WK-HT rats (while having similar MAP to WK-HA) had a higher level of sympathetic outflow as evidenced by a greater drop in MAP during ganglion, AVP and angiotensin II blockade. There were no differences in plasma catecholamine levels measured under resting conditions or in the degree of ventricular hypertrophy between the strains.

Whereas WK-HA were far more active than WK-HT, there was no difference in blood pressure between the strains. The normality of plasma AD levels in the WK-HA indicate that the high values of circulating AD found in SHR are unlikely to be related to their hyperactive phenotype.

CHAPTER 5

MODIFICATION OF CIRCULATING AD LEVELS

5.1 Aim

Evidence presented in chapter 3 shows that hypertensive rats have a higher level of circulating AD than normotensive rats. Studies with WK-HA and WK-HT rats (chapter 4) indicate that this is not a consequence of the hyperactivity trait of the SHR. It is possible therefore, that it might be related to the hypertensive trait. If the elevation of plasma AD is important in either the development or maintenance of hypertension, then an increase in plasma AD levels should increase blood pressure in otherwise normotensive rats that is assuming AD acts alone and not in concert with other predisposing abnormalities in the hypertensive strains. Alternatively, a reduction in circulating AD should decrease blood pressure in hypertensive rats. Previous studies have shown that chronic elevation of plasma AD by minipump infusion [Majewski *et al.* 1982b; Johnson *et al.* 1983; Schwartz & Eikenburg, 1986; Schwartz & Eikenburg, 1988] or depot implantation [Majewski *et al.* 1981b] can elevate blood pressure in normotensive Wistar or SD rats. Similarly, AMX of 4-6 week old SHR attenuated the development of hypertension. The progression of hypertension could be restored by subsequent infusion of β receptor agonists [Borkowski, 1991].

As yet, the effects of experimentally manipulating plasma AD levels have not been determined in either the WKY or SHRSP strains. Thus the aim of the present study was to investigate the relationship between plasma AD and the pathogenesis of hypertension in WKY and SHRSP as well as in other hypertensive and normotensive rat strains by measuring the consequences of chronically altering circulating levels of AD.

5.2   Methods

5.2.1   AD infusion studies

5.2.1.i   Preliminary study

The aim of the preliminary experiments was to determine the best route of administration and the dose of AD required to achieve a significant elevation of plasma AD. In these experiments, arterial catheters were implanted into 2-2½ month old WKY. Measurements of MAP and blood samples were taken on two consecutive days as baseline readings. Rats were subsequently given either a depot implantation or osmotic minipump containing AD bitartrate.

5.2.1.ii   Depot implantation

Rats were given a slow release depot preparation of AD by a subcutaneous (s.c.) injection in the flank region. The emulsion consisted of a homogeneous mixture (50:50 v/v) of AD bitartrate solution (made in 0.1% ascorbic saline) and incomplete Freund's adjuvant (Sigma, Mo, USA). The injection volume was 500 µl such that each rat received 20 µg AD bitartrate.

5.2.1.iii   Minipump infusion

Minipumps were filled with an AD bitartrate solution, and implanted into rats. Rats received either 3 µg/kg/hr (i.p.), 25-26 µg/kg/hr (i.p.), 25-26 µg/kg/hr (s.c.) or 250 µg/kg/hr (s.c.) of AD bitartrate.

5.2.1.iv   AD infusion in WKY, SD and SHRSP

Osmotic minipumps were implanted subcutaneously into 6 week old male WKY. Minipumps were filled with a 1.5% (w/v) solution of AD bitartrate such that rats received a continuous infusion of AD bitartrate (25-26 µg/kg/hr) for two weeks. Minipumps were replaced twice so that the infusion continued for at least 5 weeks. After 4 weeks of

infusion, arterial and venous catheters were implanted for measurement of MAP, blood sampling and drug administration.

In two month old SD rats, arterial catheters were implanted and baseline MAP measurements were taken prior to the implantation of minipumps containing AD bitartrate (as above). MAP measurements and blood sampling continued for two weeks. Minipumps for AD infusion were also implanted in 2 month old SHRSP (as above) and arterial catheters were implanted one week after the start of the infusion.

5.2.2   Bilateral adrenalmedullectomy in SHR and SHRSP

AMX was performed in 5 week old SHRSP. Nine weeks later, arterial and venous catheters were implanted. In a separate experiment, with adult (7-9 months old) SHR, arterial catheters were implanted and baseline measurements of MAP and blood samples were taken prior to AMX. MAP was monitored for a week.

5.2.3   Acute drug administration

After the basal level of MAP had been recorded and blood taken, sodium nitroprusside (10 µg/min, i.v.) was infused via a jugular catheter for a period of 10 minutes. During the eighth minute of infusion, a second blood sample was taken and assayed for catecholamines.

When MAP had returned to its resting level, autonomic reflexes were blocked in AD infused WKY and adrenalmedullectomised SHRSP. In these SHRSP, the influence of AVP on resting MAP was determined by administering an AVP antagonist immediately prior to autonomic blockade. During autonomic blockade, vasopressor activity was assessed in AD infused WKY and adrenalmedullectomised SHRSP (method described earlier). Sigmoidal curves were fitted to responses of individual animals to obtain the ED_{50} and the maximum pressor response which were averaged for rats in each treatment group.

At the end of the experiment, all rats were killed by decapitation. Adrenal glands and cardiac ventricles were removed and assayed for catecholamines.

All data are shown as mean ± SEM. Significant differences between means (p<0.05) were determined using Student's t-test for between animal and a paired t-test for within animal comparisons.

5.3   Results

5.3.1   Preliminary study

5.3.1.i   Depot implantation

Resting MAP (measured daily for one week) was unaffected by subcutaneous depot administration of AD in WKY (102 ± 1 mmHg, n=5 compared with 102 ± 1 mmHg, n=4). However, analysis of blood samples taken from these rats daily for one week after depot administration showed that this dosing regime did not significantly elevate circulating AD levels.

5.3.1.ii   Minipump infusion

The highest rate of AD bitartrate infusion (250 µg/kg/hr, s.c.) proved to be toxic and rats died within 48 hours of treatment. None of the dosing regimes affected blood pressure. Plasma AD was, however, substantially elevated by all treatments. The dosing regime 25-26 µg/kg/hr s.c. gave the most consistent elevation of plasma AD (on average a 7 fold increase) and was therefore used for subsequent experiments. The plasma AD concentrations achieved with these dosing regimes are shown in appendix 2.

5.3.1.iii   Subsequent AD infusion studies

After 5 weeks of continuous subcutaneous AD infusion in WKY, the plasma AD concentration was elevated 12 fold (see Figure 9a). There was a tendency for plasma NA to rise (41%) but this was not significant. AD infusion in WKY caused AD to accumulate in both the heart and the adrenal glands. At the same time, NA was significantly reduced in both tissues (see Table 5). However, measurements averaged over 3 consecutive days

indicated that the chronic elevation of plasma AD had no effect on either resting MAP (119 ± 9 mmHg in controls versus 118 ± 3 mmHg in AD infused rats, Figure 9a) or HR (304 ± 4 bpm in controls and 322 ± 8 bpm in AD infused rats, n=7 in each case). The cardiac ventricle weight : body weight ratio was similar in AD infused (3.34 ± 0.03 mg/g, n=7) and control WKY (3.15 ± 0.05 mg/g, n=8).

Resting MAP (averaged over 2 weeks) did not differ in AD infused SD rats (94 ± 2 mmHg, n=4) from the value in control rats (97 ± 2 mmHg, n=3), although plasma AD concentrations were 26 times higher (1225 ± 179 pg/ml versus 47 ± 9 pg/ml in controls). Plasma levels of NA (145 ± 56 pg/ml in controls, 119 ± 10 pg/ml in AD infused rats) and resting levels of HR (393 ± 18 bpm in controls, 381 ± 6 bpm in AD infused rats) were similar in both groups of SD rats.

Minipump infusion of AD for 2 weeks in SHRSP caused a 5 fold increase in the plasma AD concentration (1383 ± 90 pg/ml versus 223 ± 38 pg/ml in controls) but did not affect plasma NA (136 ± 22 pg/ml versus 186 ± 62 pg/ml in controls). There was no resultant change in either resting MAP averaged over 1 week (146 ± 3 mmHg in AD infused rats versus 145 ± 6 mmHg in controls) or HR (322 ± 6 bpm in AD infused rats versus 327 ± 9 bpm in controls, n=4 in each case).

5.3.2   Effects of Adrenalmedullectomy

Resting MAP and HR in the adult SHR averaged 165 ± 2 mmHg and 326 ± 9 bpm (n=8). After AMX, plasma AD fell within 24 hours from 286 ± 33 pg/ml to 31 ± 10 pg/ml while the plasma NA concentrations (378 ± 53 pg/ml and 273 ± 66 pg/ml after AMX) were not different from control SHRSP. Repeated daily measurements recorded in these rats showed that AMX did not affect MAP acutely (MAP during the first week after AMX averaged 159 ± 5 mmHg).

The longer term effects of AMX were examined in young SHRSP. Nine weeks after AMX, the average plasma AD concentration was still significantly lower than sham operated SHRSP (see Figure 9b). AMX in SHRSP caused a significant reduction in cardiac AD. However, cardiac NA was unaffected (Table 5). Adrenal AD and NA contents were still

67% and 71% lower respectively, than in controls. The cardiac ventricle weight : body weight ratio was unaffected by AMX (3.97 ± 0.07 mg/g, n=14 versus 3.96 ± 0.06, n=8 in controls). However, MAP (averaged over 1 week) was significantly higher in the AMX rats (167 ± 2 mmHg, n=14 and 158 ± 2 mmHg, n=8 in controls, see Figure 9b). Resting HR (334 ± 8 bpm in controls (n=8) and 331 ± 7 bpm in AMX rats (n=14)) and plasma NA levels were not affected by the treatment. It should also be noted that, at this stage, the control SHRSP were of comparable age to the normotensive control WKY but their plasma AD level was 4 times higher.

5.3.3 Effects of acute drug administration

To test the hypothesis that circulating AD can influence the stress-evoked release of intra-neuronal NA, the vasodilator nitroprusside was infused for a 10 minute period in AD-infused WKY and adrenalmedullectomised SHRSP. This caused a reduction in MAP and a reflex increase in plasma NA. However, neither AMX nor AD infusion caused any change in the magnitude of either of these responses (see Figure 10).

Administration of an AVP antagonist to SHRSP caused a small reduction in resting MAP which was similar in control (4 ± 1 mmHg) and AMX (5 ± 2 mmHg) rats. Subsequent autonomic blockade caused MAP to fall sharply in all WKY and SHRSP. This reduction was generally greater in control SHRSP (81 ± 2 mmHg, n=8) than in control WKY (49 ± 4 mmHg, n=7), however, neither of the treatments caused any change in the magnitude of these reductions (44 ± 3 mmHg, n=8 for AD infused WKY; 78 ± 3 mmHg, n=14 for SHRSP after AMX) when compared to the respective control group. The basal level of MAP attained after blockade was not affected by AD infusion in WKY (82 ± 4 mmHg versus 73 ± 4 mmHg in controls) or AMX in SHRSP (87 ± 4 mmHg versus 76 ± 2 mmHg in controls).

Administration of bolus doses of phenylephrine to autonomically blocked rats cause acute dose-dependent increases in MAP (Table 6). Analysis of the curves derived from these responses shows that there were no significant changes in either the ED_{50} or the maximum pressor response in the AD infused WKY or adrenalmedullectomised SHRSP.

TABLE 5 Catecholamine concentrations in cardiac ventricles, adrenal glands and plasma.

	Heart (ng/mg protein)		Adrenals (ng/mg protein)		Plasma (pg/ml)	
	AD	NA	AD	NA	AD	NA
WKY						
control (n=8)	0.12 ± 0.03	3.18 ± 0.2	2.09 ± 0.27	0.49 ± 0.06	71 ± 7	128 ± 11
AD infusion (n=8)	1.87 ± 0.28*	1.50 ± 0.15*	3.07 ± 0.28*	0.71 ± 0.07*	933 ± 100*	180 ± 24
SHRSP						
control (n=8)	0.08 ± 0.01	2.78 ± 0.27	3.75 ± 0.43	0.91 ± 0.1	287 ± 17	242 ± 26
AMX (n=14)	0.04 ± 0.01*	2.40 ± 0.24	1.25 ± 0.15*	0.26 ± 0.04*	178 ± 24*	222 ± 32

* indicates significantly different ($p < 0.05$) from respective control group.

TABLE 6 Changes in MAP (mmHg) in response to i.v. phenylephrine

Dose (µg):	0.05	0.1	0.2	0.5	1	2	4	ED$_{50}$ (µg/kg)	Δmax (mmHg)
WKY									
control (n=7)	13 ± 1	23 ± 1	32 ± 2	60 ± 4	82 ± 4	96 ± 4	106 ± 6	1.5 ± 0.2	114 ± 4
AD infusion (n=6)	102	15 ± 1	31 ± 3	58 ± 4	86 ± 4	92 ± 3	107 ± 9	1.5 ± 0.1	107 ± 3
SHRSP									
control (n=8)	11 ± 1	19 ± 2	35 ± 3	62 ± 5	85 ± 2	101 ± 2	107 ± 4	1.5 ± 0.2	113 ± 3
AMX (n=13)	12 ± 1	21 ± 2	38 ± 3	62 ± 3	80 ± 3	100 ± 3	101 ± 5	1.4 ± 0.2	109 ± 6

Bolus doses of phenylephrine given to autonomically blocked rats caused acute increases in MAP.
Sigmoidal curves were derived for each animal from these responses and the computed ED50 and Δmax (maximum response) were averaged for each treatment group.

a) WKY

PLASMA ADRENALINE | PLASMA NORADRENALINE | MEAN ARTERIAL PRESSURE

b) SHRSP

PLASMA ADRENALINE | PLASMA NORADRENALINE | MEAN ARTERIAL PRESSURE
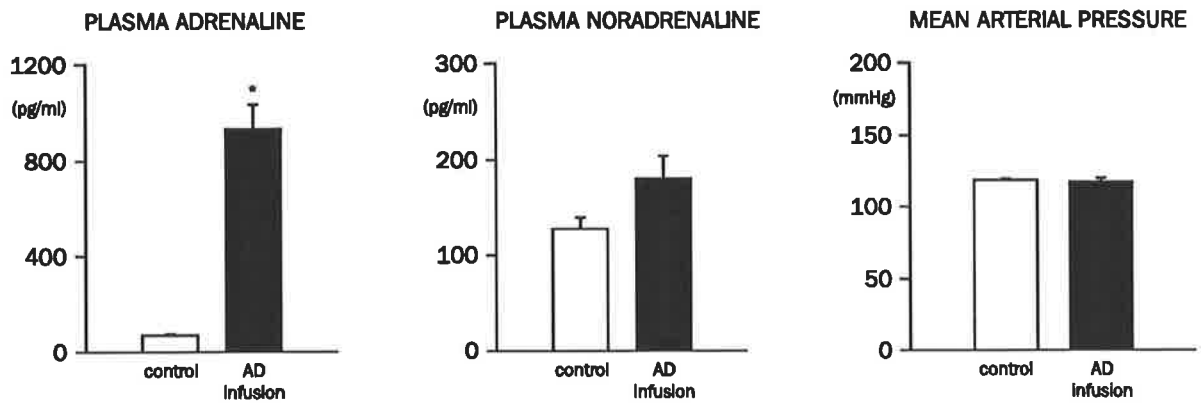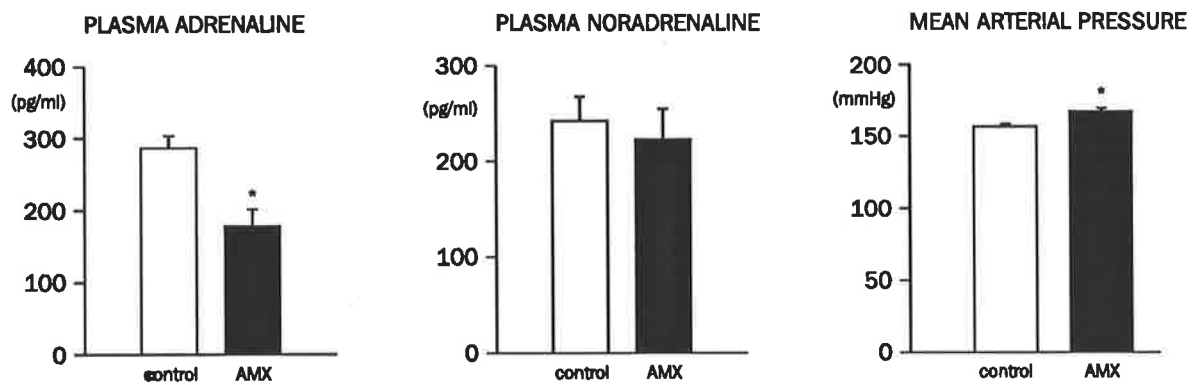
* indicates significantly different from control

FIGURE 9 Plasma AD and NA concentrations and resting levels of MAP
a) after 5 weeks of continuous AD infusion in WKY (n=7 in control and AD infused rats);
b) nine weeks after AMX in SHRSP (n=14 in AMX rats and n=8 in control SHRSP).
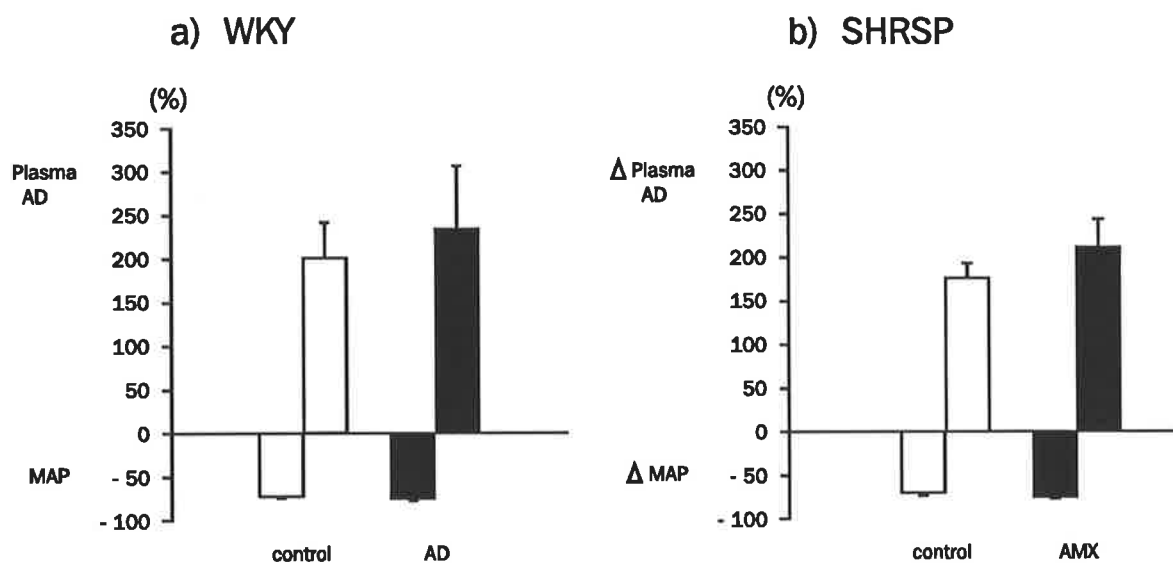
FIGURE 10 Effects of 10 minute nitroprusside infusion (10 µg/min i.v.) on resting MAP and plasma NA in
a) control and AD infused WKY (n=7 in each case)
b) control SHRSP (n=8) and after AMX (n=14).

5.4   Discussion

Resting blood pressure levels in several normotensive and genetically hypertensive rats strains were surprisingly resistant to chronic alterations of circulating AD levels. Significant increases of blood pressure in response to chronic AD infusion have been reported by Majewski et. al. in Wistar rats [Majewski et al. 1981b; Majewski et al. 1982b] and by others in SD rats [Johnson et al. 1983; Schwartz & Eikenburg, 1986], although Zabludowski et. al. (1984) found that continuous intravenous infusion of AD did not increase blood pressure in Wistar rats. The present study is the first such investigation in WKY rats. The WKY strain was used so that any observed effects of AD on blood pressure might be more relevant to the pathogenesis of hypertension in the related SHR and SHRSP strains. After 5 weeks of infusing AD bitartrate subcutaneously in WKY, resting MAP was unaffected, even though circulating AD levels were raised substantially. It is possible that the influence of circulating AD differs in WKY from other normotensive strains. However, even in normotensive SD rats, there was no increase in MAP measured under conscious, resting conditions. Even rats with a genetic predisposition to hypertension (SHRSP) failed to show a blood pressure response to further elevation of plasma AD, suggesting that circulating AD does not interact with other predisposing hypertensive factors (e.g. increased sensitivity to angiotensin II [Li & Jackson, 1989]) to raise blood pressure in this strain.

The most likely mechanism by which AD could elevate blood pressure is by activating presynaptic β_2 receptors to facilitate release of NA from vasoconstrictor nerve terminals [Adler-Graschinsky & Langer, 1975; Majewski et al. 1982a]. In this study, chronic AD infusion, caused AD to accumulate in and replace NA in cardiac tissue, such that the total catecholamine concentration was unchanged. The degree of AD accumulation in the vasculature was not measured in this study and one cannot assume that the accumulation of AD in other tissues will be similar to that observed in cardiac tissue.

AD has been shown to activate presynaptic β_2 receptors to enhance NA release in vivo [Schmidt et al. 1984; Schwartz & Eikenburg, 1986] and in vitro [Majewski et al. 1981a]. However, others have shown that presynaptic α-receptors need to be blocked before the effect can be observed [Schwartz & Eikenburg, 1988], suggesting that any potentiation of NA release which may occur during chronic AD infusion is possibly dependent on

desensitisation of presynaptic α-receptors [Falckh *et al.* 1990]. The present study assessed NA release indirectly by measuring the level of plasma NA at rest and during acute hypotensive stress. Under resting conditions, plasma NA levels were only slightly elevated by AD infusion. More importantly, however, the elevation of plasma NA in response to acute stress was similar in AD-infused and control rats suggesting that, in the intact conscious animal, NA release into plasma is not affected by chronic changes in plasma levels of AD. It must be noted however, that AD might have affected the response of the other hormones which respond to hypotensive stress (e.g. angiotensin II). The AD levels achieved in this study might have been sufficient to activate the β receptors in the kidney to potentiate angiotensin II release during hypotensive stress. If this was the case then NA release should have been potentiated as angiotensin II has been shown to enhance NA spillover [Noshiro *et al.* 1994].

Regardless of changes in plasma NA responses, the results suggest that circulating AD does not influence sympathetically mediated vasoconstriction since autonomic blockade caused similar reductions of MAP in AD infused and control WKY. The reduction of MAP during autonomic blockade represents the loss of sympathetic vasoconstrictor tone, since it is not accompanied by changes in cardiac output (PRC Howe *et. al.*, unpublished observations). The residual level of MAP after autonomic blockade (i.e. independent of sympathetic pressor mechanisms) did not differ between groups. Had AD been activating postsynaptic receptors directly, there might have been an increase in the residual level of MAP during autonomic blockade in the AD infused rats.

Subjecting rats to immobilisation stress has been shown to chronically increase plasma AD levels. This was accompanied by an elevation of blood pressure which was blocked by AMX [Dobrakovova *et al.* 1984]. On the other hand, repeated immobilisation stress decreases blood pressure in SHR although the increase of plasma AD is sustained [Kvetnansky *et al.* 1979]. Others have found that chronic stress causes either desensitisation or reduction in number of adrenoreceptors [Torda *et al.* 1981; Yamaguchi *et al.* 1981] which could have counteracted any pressor effect. However, blood pressure was not measured in these studies. Infusion of AD at much higher rates than used in the present study have been shown to cause desensitisation of post-synaptic adrenoreceptors [Schwartz & Eikenburg, 1986]. Nevertheless, blood pressure (both

conscious and after pithing) was elevated. This suggests that the elevation in blood pressure was not sympathetically mediated. In the present experiments the lack of effect of AD infusion on blood pressure in normotensive rats cannot be attributed to desensitisation of postsynaptic α-adrenoreceptors, since MAP responses to acute administration of phenylephrine in autonomically blocked rats were similar in treated and control animals.

Studies using cultured rat aortic smooth muscle cells show that AD can cause a direct dose-dependent stimulation of cell growth [Blaes & Boissel, 1983]. In addition to this, the oxidation products of catecholamines (e.g. adrenochrome and other free radicals) may also play a role in mediating various aspects of cardiac damage [Singal et al. 1982]. The involvement of AD in the induction of cardiac hypertrophy is also supported by studies in which denervation of the adrenal medulla prevented the development of cardiac hypertrophy after aortic coarctation in the dog [Womble et al. 1980]. However, the development of cardiac hypertrophy was not induced by a chronic 5 week AD infusion in WKY in this study.

An alternative approach for investigating the relationship between circulating AD and blood pressure is to reduce plasma AD levels in hypertensive rats, particularly in view of the abnormally high levels seen in SHR and SHRSP (chapter 3). Borkowski found that AMX attenuated the development of hypertension in SHR at 4-6 weeks of age but not at 14 weeks of age [Borkowski, 1991]. In the present study, AMX was performed at 5 weeks of age in the SHRSP substrain of SHR. However, resting MAP, measured 9 weeks later, was slightly higher than in control SHRSP. It is possible that SHRSP (whose blood pressure develops at a faster rate than that of SHR) have a different period of AD sensitivity. Another possibility is that plasma AD was not reduced sufficiently by AMX and may still have been able to enhance sympathetic transmission. Nine weeks after AMX, plasma and adrenal AD levels were only 38% and 67%, respectively, lower than in controls. However, these values may underestimate the acute loss of plasma AD. In adult SHR, in the first week after AMX plasma AD was reduced by 89% but resting MAP was unaffected.

Alternatively, the lack of effect of AMX on blood pressure may be due to excessive secretion of glucocorticoids during adrenal regeneration. During the early phase of regeneration

hypertension, rats are in positive sodium balance [Perrone et al. 1986] which may contribute to the hypertension. Regeneration of adrenal cortical tissue after AMX takes approximately 28-32 days and it has been reported that the development of the hypertension closely matches the time required for the adrenal regenerative process [Foulkes et al. 1987]. In the present study, however, the lack of effect on MAP is unlikely to be due to adrenal regeneration, since MAP was measured 9 weeks after AMX and any adrenal regeneration would have been completed by that time.

Despite the lack of effect of AMX on blood pressure, it is still possible that circulating AD has a pressor function, the loss of which can be compensated for by other homeostatic mechanisms. This is unlikely, however, as infusion of the vasodilator drug, nitroprusside, caused no greater fall of blood pressure after AMX than in control rats. Plasma AVP normally contributes very little to resting blood pressure but compensates very rapidly when blood pressure falls (chapter 3). Administration of an AVP antagonist to AMX and control SHRSP caused only a 4-5 mmHg fall in MAP. Thus a higher level of circulating AVP was not compensating for a decrease in plasma AD. It is still possible, although less likely, that other pressor hormones such as angiotensin II may have been compensating for the reduction in plasma AD levels.

Autonomic blockade, in the presence of the AVP antagonist, lowered MAP to the same extent in AMX and control SHRSP. Furthermore, there was no difference in either the basal level of MAP during autonomic blockade or the pressor sensitivity of postsynaptic α-receptors. These results suggest that the low level of plasma AD did not affect sympathetic nervous system function or basal vascular tone. They contrast with another study which suggested an interactive role of the adrenal medulla and the sympathetic nervous system in the pathogenesis of hypertension in SHR [Sakaguchi et al. 1983]. However, others have shown recently that the role of adrenal medullary catecholamines in blood pressure maintenance after sympathectomy is trivial [Lo et al. 1991].

The lack of effect of AMX on the development of hypertension is consistent with a previous study [Rogers et al. 1991] in which chemical means were used to deplete AD. Plasma AD was selectively reduced to only 8% of normal by administration of a combination of inhibitors of DOPA-decarboxylase (the enzyme which catalyses the formation of NA from dopamine) and phenylethanolamine-N-methyl transferase (PNMT, the enzyme which

catalyses the formation of AD from NA). Yet neither resting MAP nor centrally induced vasoconstrictor responses were affected. Similarly, chronic administration of a PNMT inhibitor which caused more than 80 % loss of adrenal AD failed to attenuate the rise of blood pressure induced by salt-loading in Dahl salt sensitive rats [Johnson & Kotchen, 1990]. Others have questioned the role of adrenal AD in hypertension development and have concluded that it is the adrenal cortex rather than the adrenal medulla that contributes to hypertension in SHR, since only complete adrenalectomy, not AMX, lowered blood pressure in their studies [Aoki *et al.* 1973; Hilse & Oehme, 1987].

In man, stepwise infusion of AD which increased plasma AD up to 15 fold, decreased forearm vascular resistance and increased both heart rate and plasma NA [Kjeldsen *et al.* 1993]. This was accompanied by a reduction in diastolic blood pressure (due to β_2 receptor mediated vasodilation) and an increase in systolic blood pressure (due to β_1 receptor effects on the heart). However, there was no net change in MAP. In another study, a 6 hour infusion of AD in man caused an initial fall followed by a post-infusion rise of blood pressure, the latter being attributed to presynaptic facilitation of NA release [Blankestijn *et al.* 1988]. Thus chronic elevation of plasma AD may be expected to result in a balance between indirect pressor and direct depressor effects. Furthermore, phaeochromocytoma patients, with elevated plasma AD but with normal plasma NA do not have sustained hypertension [Brown *et al.* 1981]. Together with the present findings, these observations throw doubt on a pathogenic role for plasma AD in hypertension. It is possible, therefore, that elevated plasma AD levels in hypertensive rats are not a cause of the hypertension, but are likely to be a consequence of the hypertension.

5.5   *Summary*

The results of the present study fail to support the hypothesis initially proposed by Majewski *et al.* (1981b), which implicated elevated levels of plasma AD as a contributory factor in the pathogenesis of hypertension. In this study, the chronic elevation of plasma AD levels by minipump infusion, failed to raise blood pressure in either WKY, SD or SHRSP strains. Conversely, AMX which caused a chronic reduction in plasma AD levels in SHRSP and SHR strains (whose plasma AD levels are already abnormally elevated) failed to lower

blood pressure. These results suggest that the elevation of plasma AD observed in hypertensive rats (chapter 3) may be a consequence rather than a cause of their high blood pressure.

Although an acute elevation of plasma AD elevates blood pressure, at low physiological doses AD also acts as a metabolic hormone. AD has potent effects on glucose mobilisation and is also a regulator of peripheral resistance by increasing blood flow (decreasing resistance) via activation of postsynaptic β_2 receptors. It is therefore possible that AD is released in response to hypertension to counteract the elevated peripheral resistance which has been reported in hypertensive rats [Pfeffer *et al.* 1973]. In addition to this, AD may act to increase plasma glucose levels to enhance substrate delivery to skeletal muscles in which blood flow has been diminished due to the elevated peripheral resistance in hypertension.

CHAPTER 6

EFFECTS OF MODIFYING BLOOD PRESSURE IN WKY AND SHRSP

6.1 Aim

In the previous chapters I have shown that plasma AD is elevated in genetically hypertensive rats but that AD infusion did not elevate blood pressure in either normotensive or hypertensive rats. Conversely, AMX in hypertensive rats which significantly lowered circulating AD levels, did not lower blood pressure. The aim of this study was to determine if the elevation of plasma AD in SHR and SHRSP is a consequence, rather than a cause, of high blood pressure. This was done by manipulating blood pressure levels in hypertensive and normotensive rats and measuring any resultant changes in plasma AD levels.

Several studies have shown that nitric oxide (an endogenous vasodilator released by endothelium) exerts a tonic vasorelaxant action in rats [Cunha et al. 1993; Johnson & Freeman, 1993]. Chronic inhibition of nitric oxide synthesis has been shown to elevate blood pressure in normotensive [Ribeiro et al. 1992; Arnal et al. 1993a; Arnal et al. 1993b; Cunha et al. 1993; Johnson & Freeman, 1993; Morton et al. 1993; Hu et al. 1994] and hypertensive [Arnal et al. 1993b] rats. Thus chronic nitric oxide inhibition has proven to be a reliable and valuable model of drug-induced hypertension. In this study, blood pressure was elevated in normotensive WKY by chronic administration of Nω-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthesis inhibitor). Similarly, blood pressure was lowered in SHRSP by chronic administration of the vasodilator drug Hydralazine (HZ). Effects of drug administration were examined to determine if changes in blood pressure, either acutely or chronically, could effect levels of plasma AD.

6.2   Methods

6.2.1   L-NAME in WKY

6.2.1.i   Experiment 1: Adult WKY study

Arterial catheters were implanted into 5 month old WKY and after three baseline blood pressure measurements and two baseline blood samples had been taken, rats received L-NAME (37 mg/kg/day) in drinking water.   MAP measurements and blood samples continued to be taken for the next 27 days.

On days 12 and 27 of treatment, rat's autonomic reflexes were blocked by administration of pentolinium, methylscopolamine and AVP blocker.   When MAP had fallen to a new plateau (after approximately 5 minutes), a 20 µg dose of phenylephrine was administered through the arterial catheter.   In preliminary experiments, this dose gave a maximum increase in MAP.   Acute changes in MAP were noted.

6.2.1.ii   Experiment 2: Chronic study in young WKY

Five week old WKY were given L-NAME in their drinking water.   The concentration was adjusted every 2 days to maintain a dose of approximately 40 mg/kg/day.   Tail cuff blood pressure measurements were taken fortnightly.   Arterial catheters were implanted in rats 10 weeks after the commencement of treatment for MAP measurements and blood sampling.   After MAP had been recorded for the fifth consecutive day and a resting blood sample had been taken, autonomic reflexes were blocked by administration of pentolinium, methylscopolamine and AVP blocker.   When MAP had reached a new plateau, a second blood sample was taken.   A dose of phenylephrine (20 µg) was subsequently administered and the elevation in blood pressure was recorded.

After the last day of blood pressure recording, rats were fasted for 14 hours and a 400 µl blood sample was taken for radio-immuno assay of insulin.   All insulin assays were performed by Dr John Oliver at the Flinders University of South Australia.   During the next week, in situ blood perfused mesenteric preparations were used to establish the pressor reactivity of the mesenteric bed (see chapter 2).

6.2.2   Experiment 3: Hydralazine (HZ) in SHRSP

Arterial catheters were implanted into 15 week old SHRSP and after blood pressure measurements and blood samples had been taken, rats received HZ (25 mg/kg/day) in drinking water.   MAP measurements and blood samples continued to be taken for the next 33 days.

On day 26 of treatment, rats autonomic reflexes were blocked by administration of pentolinium, methylscopolamine and AVP blocker.   When MAP had reached a new plateau, a dose of phenylephrine (30 µg) was administered through the arterial catheter which increased MAP to a maximum pressure. Acute changes in MAP were noted.

All data are shown as mean ± SEM.   MAP, HR and plasma catecholamine data were analysed using a split-plot ANOVA for repeated measures.   Differences between groups were analysed using Student's t-test and within animal differences were analysed using a paired t-test.   Correlation coefficients (Pearson r) were determined between plasma catecholamines and MAP and HR.   Means were considered significantly different if $p < 0.05$.

6.3   Results

6.3.1   L-NAME in WKY

6.3.1.i   Experiment 1: Adult WKY rats

Pretreatment values for MAP and HR in WKY were 126 ± 1 mmHg and 294 ± 9 bpm, n=6 respectively. After 24 hours of L-NAME treatment, MAP had risen to 148 ± 2 mmHg and HR had reflexly fallen by 23 bpm. Plasma NA was significantly ($p < 0.05$) reduced by the treatment. Plasma AD was not affected during this period of treatment. Data for control and L-NAME treated WKY are shown in Table 7.

The time course for MAP, HR and plasma catecholamines is shown in Figure 11. After 23 days of L-NAME treatment, HR had returned to control levels even though MAP was

still elevated by approximately 50 mmHg. There was a marked elevation of plasma AD (2.3 fold, p<0.01, split plot ANOVA). At this stage plasma NA was significantly higher in L-NAME rats than in controls (p<0.05, split plot ANOVA).

The effects of autonomic blockade on blood pressure are shown in Table 11. At both day 12 and day 27 of treatment, MAP was significantly higher in L-NAME rats than in controls. During autonomic blockade at day 12 of treatment, the residual blood pressure during blockade was still significantly higher in the L-NAME rats. The reduction of MAP with blockade was, however, not significantly different between control and treated groups. Subsequent administration of a maximum pressor dose of phenylephrine caused an increase in MAP which was similar in L-NAME (106 ± 3 mmHg, n=6) and control (107 ± 2 mmHg, n=6) rats.

Autonomic blockade 27 days after the commencement of treatment eliminated any differences in MAP between L-NAME and control rats and the reduction of MAP with blockade was greater in the L-NAME treated rats suggesting a higher level of sympathetic outflow in these animals (see Table 11).

At the end of the experiment, cardiac ventricles were removed and weighed. L-NAME treatment for 30 days did not significantly elevate the heart weight : body weight ratio (see Figure 12).

6.3.1.ii Experiment 2: Chronic study in young WKY

Tail cuff blood pressure measurements were taken on rats to monitor the progression of hypertension during L-NAME administration. These results are displayed in Figure 13 which shows that L-NAME administration significantly elevated blood pressure in WKY rats (p<0.01, split plot ANOVA).

After 10 weeks of treatment, rats were cannulated with arterial catheters to measure MAP in resting and unrestrained rats. Under these conditions, MAP was significantly elevated in chronically L-NAME treated rats (p<0.01) while resting HR was similar in L-NAME and control rats. Plasma AD was significantly elevated in the L-NAME rats (2.2 fold, p<0.01, split plot ANOVA) whereas plasma NA was unaffected by the treatment (see Figure 14).

Plasma insulin, on the other hand, was significantly reduced by L-NAME administration (14 ± 1 μU/ml, n=6 versus 22 ± 1 μU/ml, n=7 in controls, p<0.05).

The effects of autonomic blockade are shown in Table 11. Differences in resting MAP were eliminated by autonomic blockade suggesting a higher level of sympathetic outflow in the L-NAME rats. The increase in MAP with a maximum pressor dose of phenylephrine during blockade was significantly (p<0.05) higher in L-NAME rats (157 ± 3 mmHg, n=5) than in controls (120 ± 2 mmHg, n=7).

Ganglion blockade significantly reduced both plasma AD and NA (Table 10). Even though the values after blockade are not necessarily reliable (as they fell below the arbitrarily defined limit of sensitivity), they indicate that the initial differences in plasma AD between the L-NAME treated rats and controls are likely to be sympathetically mediated.

Blood perfused mesenteric preparations showed no differences in the slopes of the pressure/flow gradients of L-NAME (58 ± 4 mmHg/ml per min) and control rats (56 ± 4 mmHg/ml per min). The pressure/flow gradient represents a measure of vascular tone and is determined by measuring changes in mesenteric pressure in response to increased blood flow through the mesenteric bed. Pressor responses of the mesenteric bed to phenylephrine were significantly enhanced in the L-NAME treated rats. There was no change in the ED_{50} between groups (1.7 ± 0.2 versus 1.6 ± 0.6 μg/kg in L-NAME (n=3) and control rats (n=7) respectively) but the maximum pressor response attained with phenylephrine was 90 mmHg higher in the L-NAME rats (see Figure 15).

Chronic L-NAME treatment significantly elevated the heart weight : body weight ratio (see Figure 12).

6.3.2   Experiment 3: HZ in adult SHRSP

Pretreatment values for MAP and HR in SHRSP were 161 ± 6 mmHg and 318 ± 4 bpm, n=6 respectively. After 24 hours of HZ treatment, MAP had fallen to 113 ± 3 mmHg and HR had been reflexly elevated to 426 ± 11 bpm. There was a 1.5 fold increase in plasma AD (p<0.05) and a 3.7 fold increase (p<0.05,) in plasma NA within 24 hours of treatment with HZ (Table 8).

The time course for MAP, HR and plasma catecholamines is shown in Figure 15. After 19 days of treatment, resting HR and plasma NA concentrations had returned to control levels while resting MAP was still reduced by approximately 62 mmHg in the HZ treated SHRSP. Plasma AD, at this time, was also reduced by approximately 90 pg/ml by the treatment ($p<0.05$, split plot ANOVA, see Table 8 and Figure 16), but plasma NA concentrations were unaffected.

As well as lowering MAP, HZ treatment also lowered sympathetic outflow as evidenced by a smaller reduction in MAP with autonomic blockade. The residual MAP during blockade was also lower in HZ treated SHRSP (see Table 11). The increase in MAP with a maximum pressor dose of phenylephrine was less in HZ treated SHRSP (120 ± 2 mmHg, n=6) than in control rats (159 ± 3 mmHg, n=5). The HZ treatment also significantly reduced the heart weight : body weight ratio (see Figure 12).

6.3.3   Correlation coefficients

Correlation coefficients (see Table 9) were obtained from L-NAME treated and control WKY (experiment 1) and HZ treated and control SHRSP (experiment 3). In WKY, plasma catecholamines correlated significantly with both MAP and HR as well as with each other. In HZ treated SHRSP, however, plasma AD did not correlate significantly with blood pressure.

TABLE 7 Effects of L-NAME treatment in WKY

	Control WKY (n=6) pretreatment	24 hrs	23-28 days†
MAP (mmHg)	126 ± 1	126 ± 3	124 ± 2
HR (bpm)	301 ± 7	307 ± 4	314 ± 9
Plasma AD (pg/ml)	93 ± 16	115 ± 24	78 ± 12
Plasma NA (pg/ml)	215 ± 29	186 ± 26	147 ± 17
	L-NAME WKY (n=6) pretreatment	24 hrs	23-28 days†
MAP (mmHg)	125 ± 1	148 ± 2*	172 ± 7*
HR (bpm)	294 ± 11	271 ± 7*	305 ± 20
Plasma AD (pg/ml)	87 ± 13	121 ± 18	169 ± 26*
Plasma NA (pg/ml)	149 ± 14	84 ± 13*	196 ± 35*

* indicates significantly different from respective control value (p<0.05).

† values taken after 23-28 days of treatment were analysed using a split-plot ANOVA.

TABLE 8 Effects of HZ treatment in SHRSP

	Control SHRSP (n=6) pretreatment	24 hrs	19-33 days†
MAP (mmHg)	165 ± 4	166 ± 3	196 ± 5
HR (bpm)	323 ± 4	325 ± 8	318 ± 10
Plasma AD (pg/ml)	301 ± 50	245 ± 42	312 ± 56
Plasma NA (pg/ml)	209 ± 11	184 ± 14	225 ± 45
	HZ SHRSP (n=6) pretreatment	24 hrs	19-33 days†
MAP (mmHg)	162 ± 6	113 ± 3*	131 ± 2*
HR (bpm)	318 ± 5	426 ± 11*	326 ± 6
Plasma AD (pg/ml)	259 ± 25	379 ± 47*	222 ± 43*
Plasma NA (pg/ml)	326 ± 57	675 ± 92*	263 ± 31

* indicates significantly different from respective control value (p<0.05).

† values taken after 19-33 days of treatment were analysed using a split-plot ANOVA.

TABLE 9 Correlation coefficients

	L-NAME & control WKY (experiment 1)		HZ & control SHRSP (experiment 3)	
	Plasma AD	Plasma NA	Plasma AD	Plasma NA
Resting MAP	0.61**	0.25*	0.20	0.31*
Resting HR	0.30**	0.60**	0.40**	0.48**
Plasma NA	0.59**		0.43**	

Correlation coefficients (Pearson r) of plasma catecholamines and resting MAP and HR. For L-NAME treated and control WKY, n=108. For HZ treated and control SHRSP, n=55.

* indicates p<0.05, ** indicates p<0.01.

TABLE 10 Plasma catecholamine levels in L-NAME treated and control WKY, at rest and after ganglion blockade

	Plasma AD (pg/ml)		Plasma NA (pg/ml)	
	Resting	Blockade	Resting	Blockade
Controls (n=7)	56 ± 11	29 ± 4	80 ± 8	18 ± 3
L-NAME (n=4)	125 ± 21*	37 ± 8	87 ± 15	13 ± 4

Footnote: Values measured after ganglion blockade are not necessarily reliable as they fall below the arbitrarily defined limit of sensitivity of the assay.

* indicates significantly different (p<0.05) from control.

TABLE 11 Effects of autonomic blockade on blood pressure

	Resting MAP (mmHg)	MAP during blockade (mmHg)	Fall in MAP with blockade (mmHg)
L-NAME in WKY			
Adult rats (expt 1)			
DAY 12			
controls (n=6)	120 ± 3	73 ± 2	47 ± 4
L-NAME (n=6)	151 ± 4*	89 ± 4*	62 ± 6
DAY 27			
controls (n=6)	121 ± 2	66 ± 3	56 ± 4
L-NAME (n=5)	179 ± 11*	74 ± 7	105 ± 16*
Young rats (expt 2)			
controls (n=7)	116 ± 2	62 ± 2	55 ± 1
L-NAME (n=5)	196 ± 8*	63 ± 3	133 ± 8*
HZ in SHRSP			
(expt 3)			
DAY 26			
controls (n=5)	200 ± 6	86 ± 3	113 ± 6
HZ (n=6)	133 ± 3*	78 ± 1*	55 ± 3*

* indicates significantly different from control value (p<0.05).
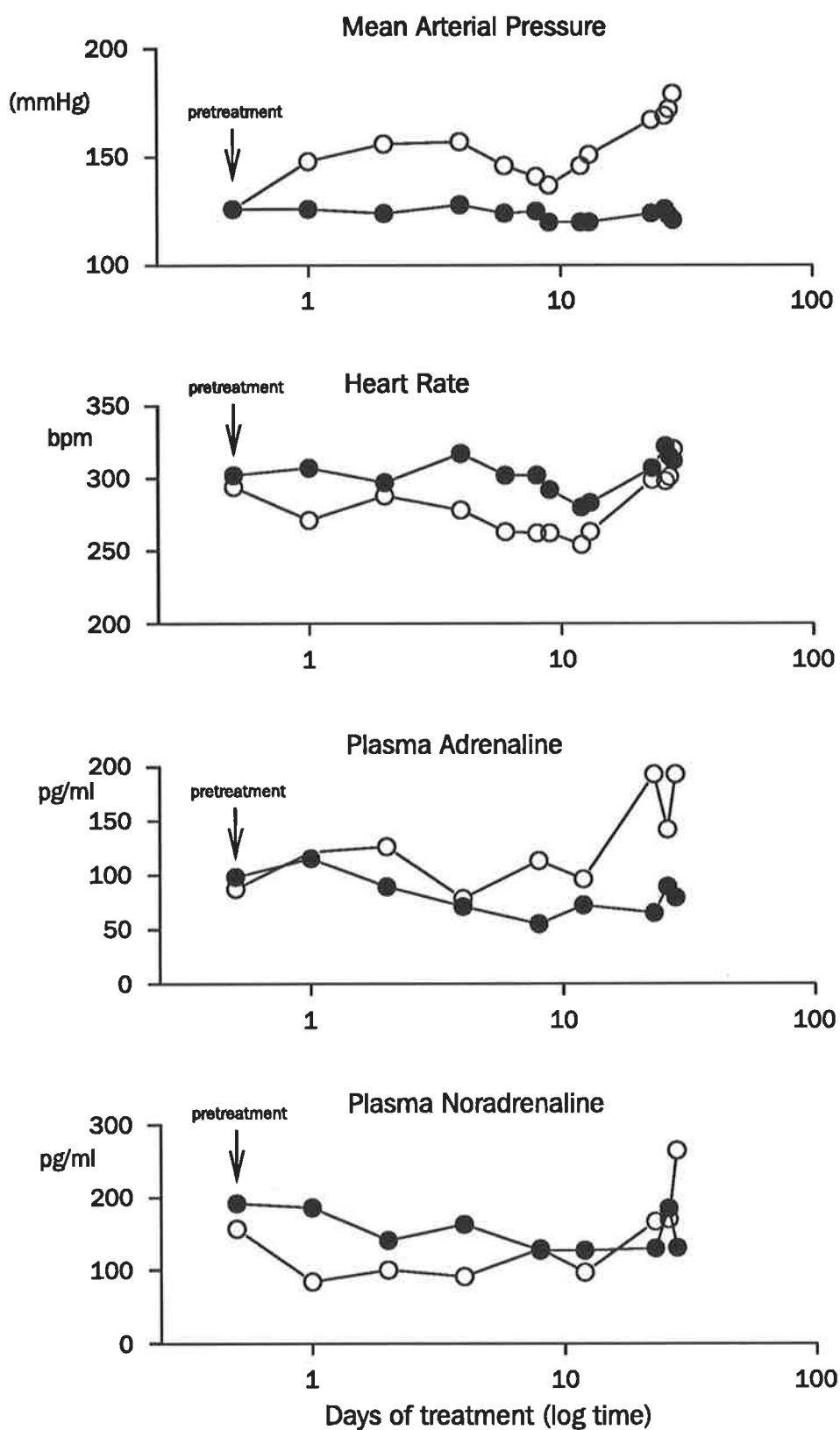
FIGURE 11 Blood pressure, heart rate and plasma catecholamine levels in adult WKY chronically treated with L-NAME. Pretreatment values are averaged from 2 readings taken prior to treatment. See text for explanation.

HEART WEIGHT : BODY WEIGHT RATIO
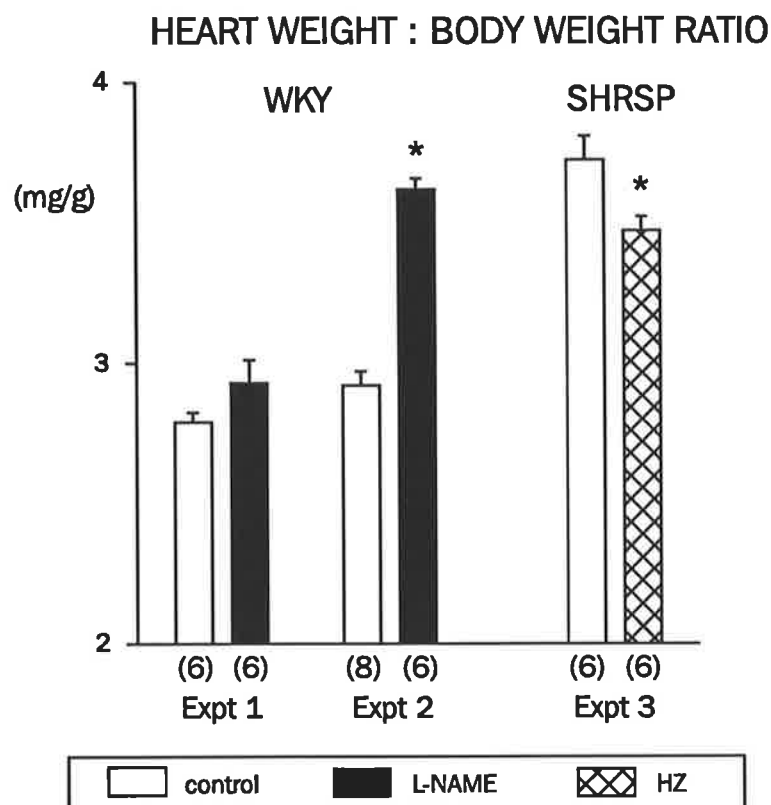
Expt 1: 30 days treatment in adult WKY

Expt 2: 10 week treatment in young WKY

Expt 3: 33 days treatment in adult SHRSP

* indicates significantly different (p<0.05) from control
Numbers in parentheses indicate animal numbers

FIGURE 12 Heart weight: body weight ratios in adult (experiment 1) and young (experiment 2) WKY chronically treated with L-NAME. Graph also shows that chronic HZ administration reversed cardiac hypertrophy in SHRSP.

TAIL-CUFF BLOOD PRESSURE
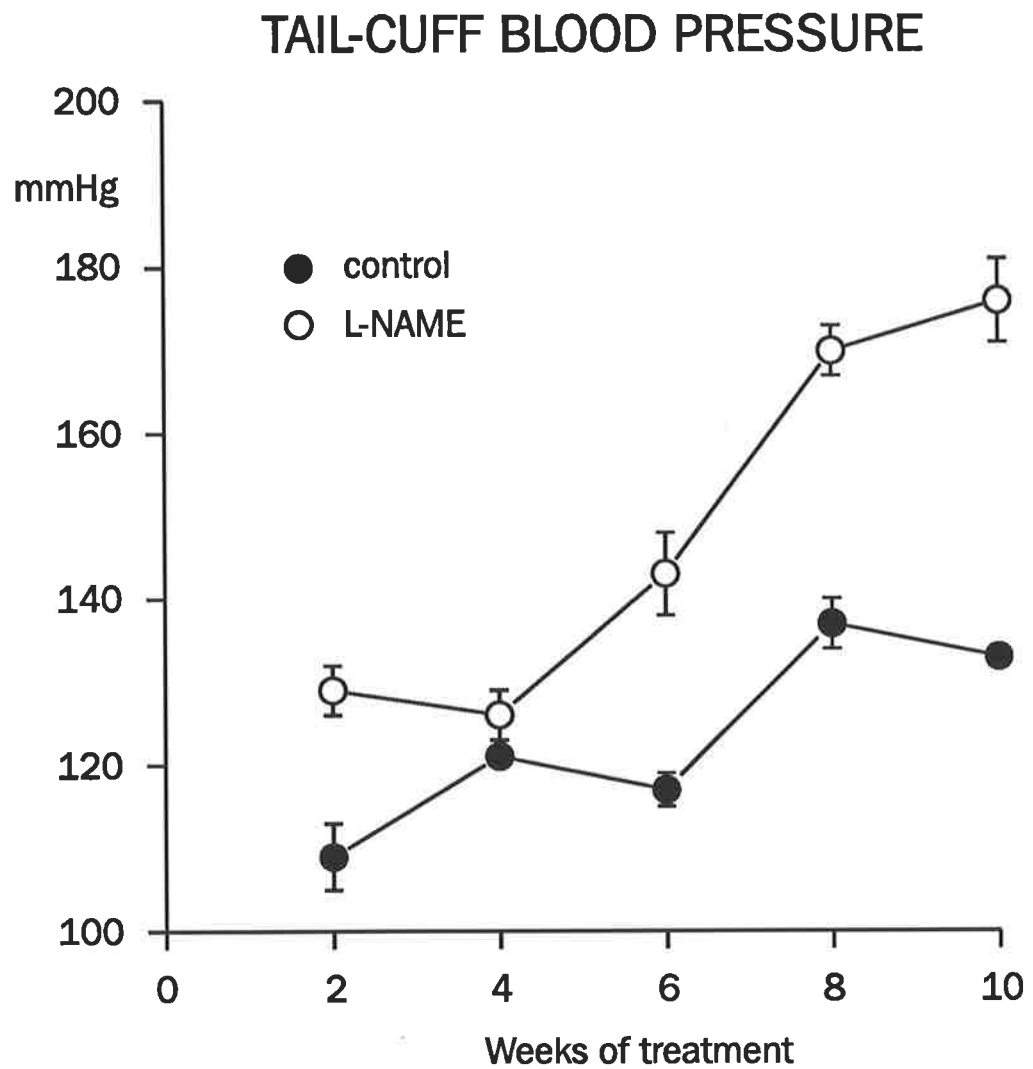
FIGURE 13 Graph shows the development of hypertension in young WKY treated with L-NAME.

Mean arterial pressure

Plasma adrenaline

Plasma noradrenaline

control	L-NAME
(n=7 in each case)	(n=6 in each case)
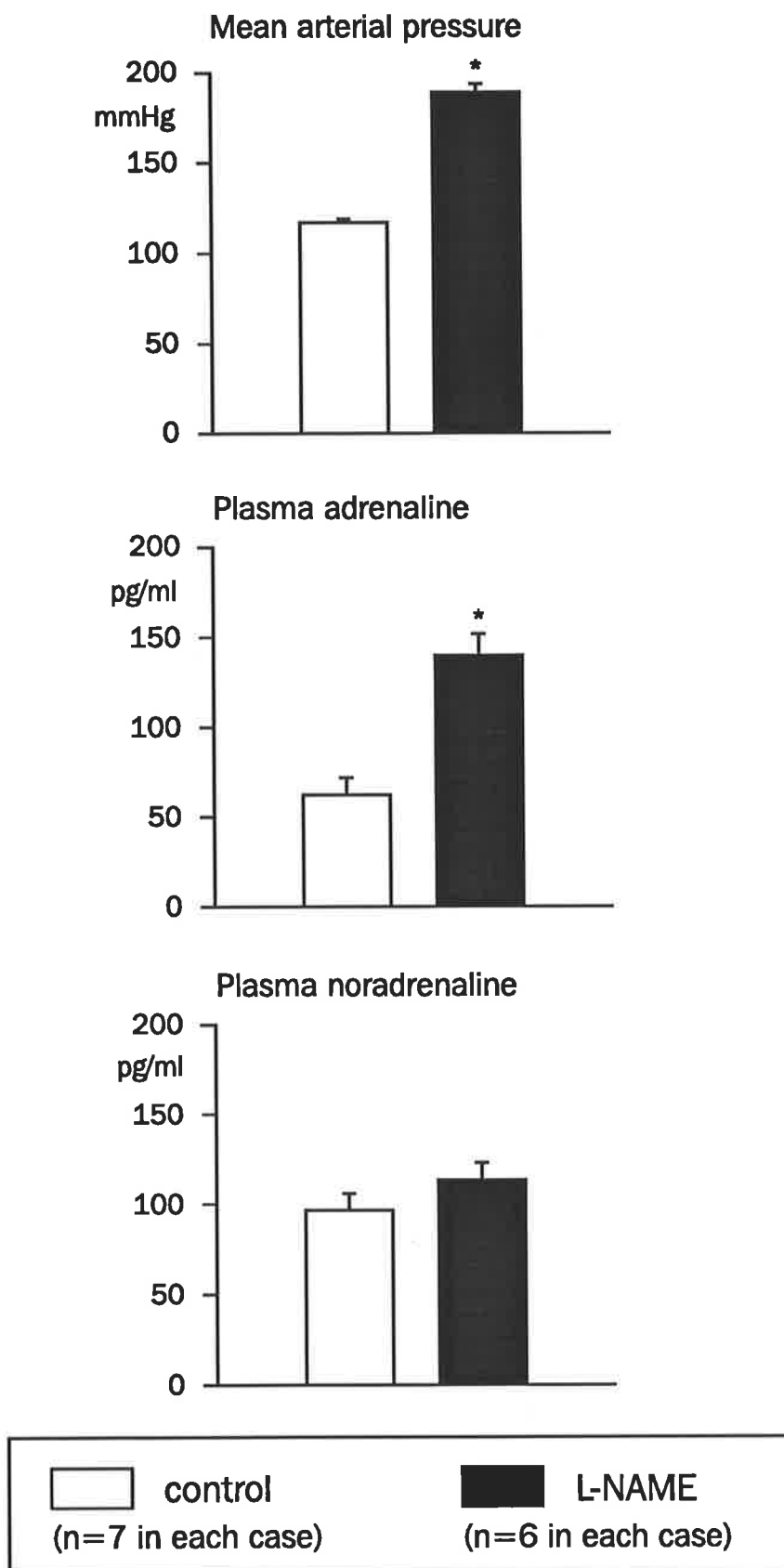
* indicates significantly different (p<0.05) from control

FIGURE 14 Blood pressure and plasma catecholamine levels in young WKY treated with L-NAME for 10 weeks. Values were obtained from an average of 4 daily readings.
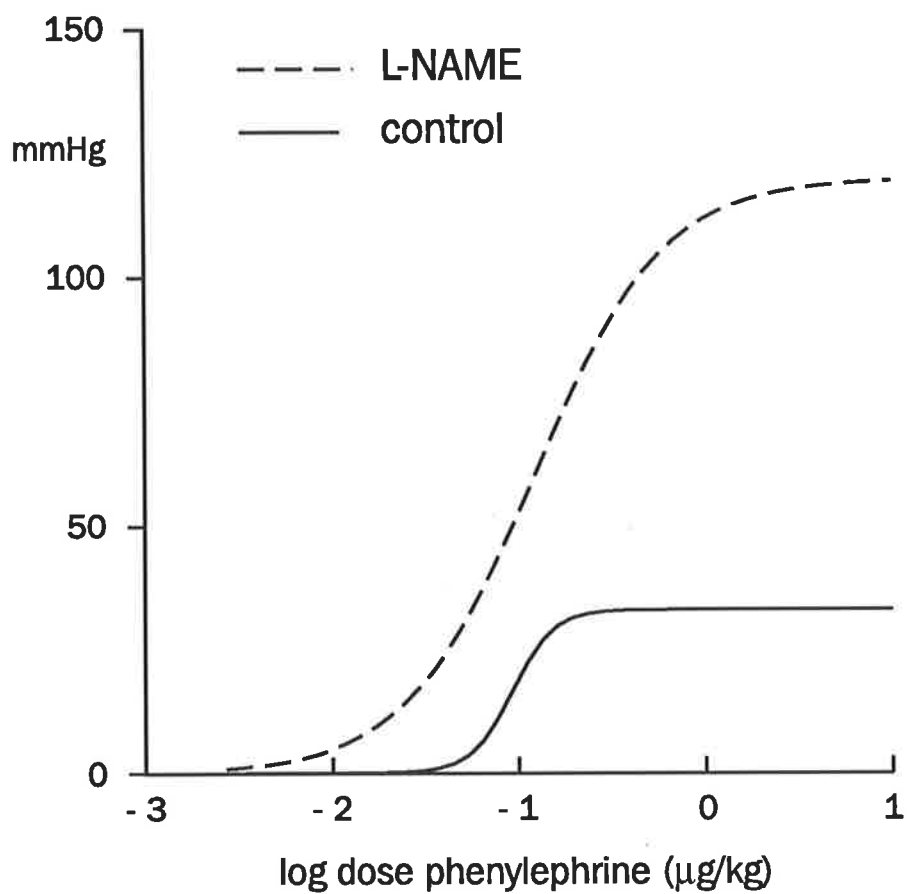
FIGURE 15 Mesenteric bed pressor responsiveness in WKY treated for 10 weeks with L-NAME. L-NAME treatment did not affect the ED_{50}, but significantly enhanced the maximum pressor response to phenylephrine.

Mean Arterial Pressure

(mmHg)

pretreatment

200

150

100

1   10   100

Heart Rate

bpm

pretreatment

500

400

300

1   10   100

Plasma Adrenaline

pg/ml

pretreatment

400

300

200

100

1   10   100

Plasma Noradrenaline

pg/ml

pretreatment

800

600

400

200

1   10   100

Days of treatment (log time)

FIGURE 16 Blood pressure, heart rate and plasma catecholamine levels in SHRSP chronically treated with HZ. Pretreatment values for MAP and HR are average values taken 2 days prior to treament. Pretreatment values for plasma AD and NA were taken 24 hours prior to treament. See text for explanation.

6.4   Discussion

Chronic nitric oxide synthesis inhibition caused severe hypertension in normotensive WKY rats which was accompanied by a significant elevation of plasma AD. This elevation was, however, abolished by ganglion blockade suggesting that the elevation of sympathoadrenal outflow in the chronically treated L-NAME rats was the cause of the elevated AD level.

In contrast, chronic reduction of blood pressure in SHRSP with established hypertension, significantly lowered plasma AD levels although plasma NA levels were normal. The chronic effects of HZ c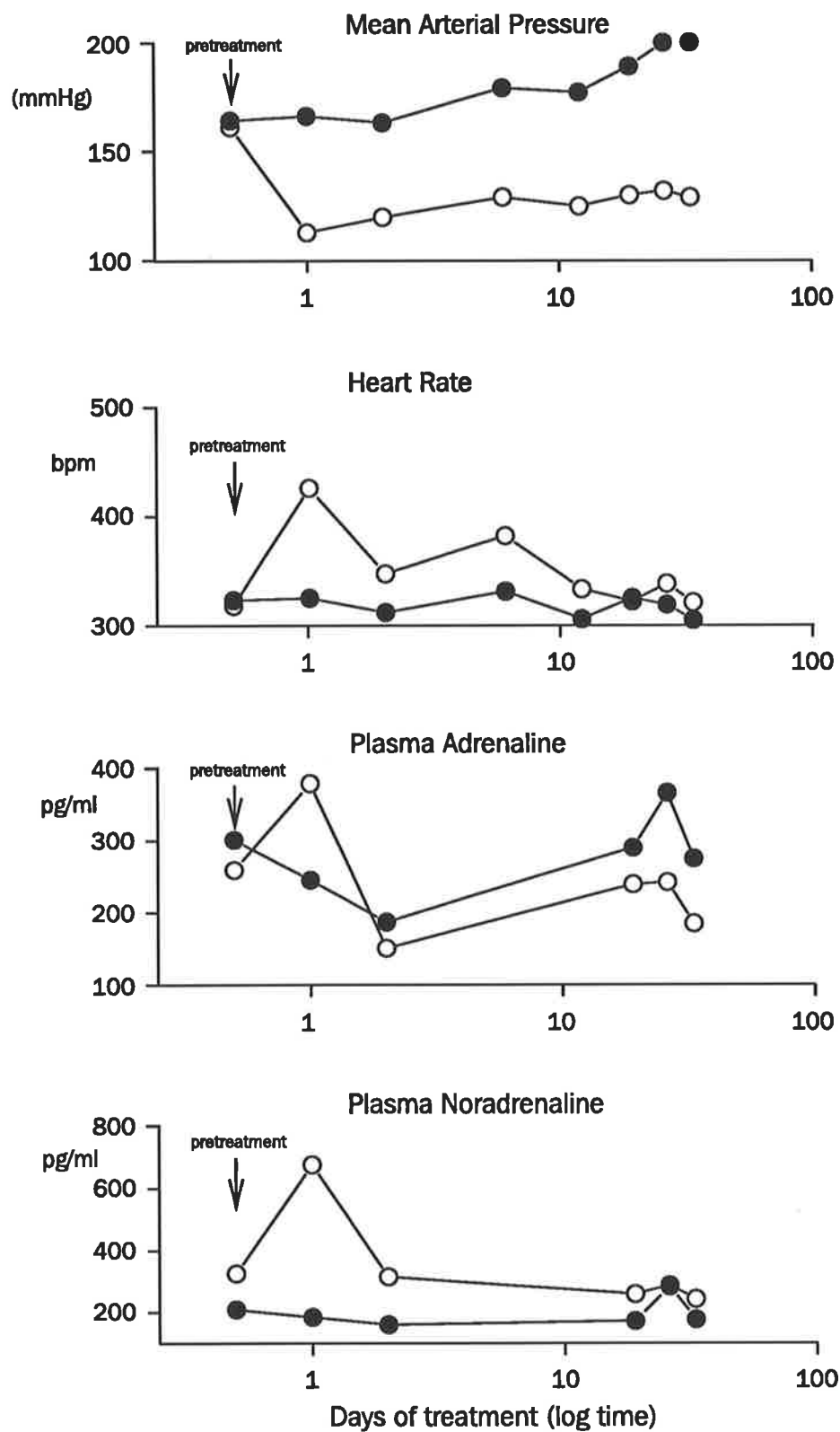ontrast with the acute effects; acute HZ treatment elevated plasma NA and AD. The elevation of plasma NA probably reflects a baroreflex response to the acute reduction of blood pressure. This is also supported by the finding that resting HR was, in the acute phase, generally higher in the HZ treated rats. After 19 days of treatment, however, resting HR had returned to control levels, suggesting that the baroreflex function had reset to operate at a lower level of blood pressure. Further evidence for this comes from the finding that sympathetic vasoconstriction, estimated by ganglion and AVP blockade, was reduced in HZ treated SHRSP after 26 days of treatment.

The elevation of sympathetic outflow during nitric oxide synthesis inhibition has been reported in other studies [Lacolley et al. 1991; Cunha et al. 1993]. Ganglion blockade, after 1 week of L-NAME treatment in Wistar rats, caused an exaggerated fall in MAP. There was however, still an elevation of the residual MAP in the L-NAME rats [Cunha et al. 1993]. In the present study, the fall in MAP after ganglion and AVP blockade was similar between groups 12 days after the commencement of treatment. The residual pressure in L-NAME treated rats was, however, still elevated. This elevation of residual MAP might be attributed to an inhibition of the tonic nitric oxide vasodilation by L-NAME. It may also be plausible to suggest that it might be attributable to an elevated level of circulating angiotensin II in the L-NAME treated rats [Hu et al. 1994]. After 27 days of treatment in adult rats and also 10 weeks of treatment of young rats, there was no elevation of residual pressure in the L-NAME rats and the hypertension could be entirely attributed to an elevation of sympathetically mediated vasoconstriction. Thus, it seems that early in the development of L-NAME hypertension, non-neural mechanisms (local vasoconstriction due to inhibition of nitric oxide synthesis) maintain the high blood

pressure. Later in L-NAME hypertension, the blood pressure seems to be maintained to a greater extent by neural mechanisms. The results suggest that it is not overactivity of sympathetic nerves that are responsible for the hypertension, but it is more likely to be attributed to an exaggerated postsynaptic pressor sensitivity to the neurotransmitter. However, caution must be taken when drawing conclusions as to the mechanisms which underlie L-NAME hypertension. Recent studies [Buxton *et al.* 1993] have shown that L-NAME and other alkyl esters of arginine are muscarinic receptor antagonists (M1, M2 and M3). If indeed L-NAME was eliminating the acetylcholine induced parasympathetic control of blood pressure, then it is probably not surprising that an elevation in sympathetic nerve activity was observed. The extent to which L-NAME antagonises the muscarinic receptor *in vivo* remains to be identified.

The elevation of MAP in the L-NAME rats was accompanied by cardiac hypertrophy but only after 10 weeks of treatment from an early age. Other studies have shown that nitric oxide synthesis inhibition will only cause hypertrophy in Wistar rats if the hypertension is very severe and is accompanied by stimulation of the renin angiotensin system [Arnal *et al.* 1993a]. Furthermore, captopril [Morton *et al.* 1993] or losartan [Ribeiro *et al.* 1992] administration attenuates L-NAME induced hypertension. In the present study the role of the renin angiotensin system in L-NAME induced hypertension was not examined but plasma renin is generally increased with nitric oxide synthesis inhibition [Hu *et al.* 1994] and therefore may be involved in the hypertensive response and might also be responsible for the elevated circulating AD levels observed in the L-NAME hypertensive rats. HZ treatment in adult SHRSP reversed the development of cardiac hypertrophy. This has been shown in several studies [Freslon & Giudicelli, 1983; Tsoporis & Leenan, 1988; King *et al.* 1992], however, the mechanism by which this occurs still remains controversial i.e. is it a cause or consequence of the blood pressure reduction.

The maximum pressor response to phenylephrine was significantly increased in the L-NAME rats but only during very long term treatment i.e. 10 weeks. The elevation of this pressor responsiveness was present both in the conscious but autonomically blocked animal and in the blood perfused mesenteric bed. Previous studies have shown either no change in sensitivity to phenylephrine in L-NAME rats [Hu *et al.* 1994] or an increase in NA pressor response after acute nitric oxide synthesis inhibition [Conrad & Whittemore,

1992]. In those studies, however, the time which rats were treated was relatively short compared with 10 weeks treatment in the present study. It is also important to note that in this study there was no change in pressor responsiveness 12 days after treatment with L-NAME.

The elevation of pressor responsiveness in the mesenteric bed of L-NAME hypertensive rats is similar to the pressor response seen in SHRSP. In the mesenteric bed, responses to phenylephrine were already elevated in 1 month old SHRSP [Rogers et al. 1994]. Thus, it seems that L-NAME hypertension provides a cardiovascular profile which is similar to that of SHRSP. The elevation of pressor responsiveness in L-NAME treated rats implies that pressor agents (such as catecholamines) in the circulation may contribute to the hypertension in these rats. On the other hand, a reduction in MAP with HZ reduced pressor responsiveness in SHRSP. In these rats there was also a reduction of plasma AD. It is therefore possible that a change in pressor responsiveness might be attributed to the level of blood pressure or possibly the circulating level of AD.

Essential hypertension is often complicated by insulin resistance, hyperinsulinemia, obesity, diabetes mellitus and hyperlipidemia. It has recently been proposed that the sympathoadrenal system may regulate the metabolic cardiovascular syndrome (Syndrome X) [Kjeldsen et al. 1992]. Julius et al. (1991) have proposed that high blood pressure (resulting from elevated peripheral resistance due to a high level of sympathetic nerve activity) may limit the availability of substrate (i.e. glucose) to skeletal muscle. The elevation of blood pressure and the resulting vascular hypertrophy and rarefaction leads to further deficiency of glucose delivery and the development of insulin resistance and later hyperinsulinemia. The chronic elevation of sympathetic activity can also increase the number of "fast-twitch" muscle fibres which are more likely to be insulin resistant [Julius et.al. 1992]. In response to the decreased availability of glucose the body's response is to increase plasma AD (part of the fight/flight response). The role of AD is then 2 fold; 1) increase plasma glucose by stimulating gluconeogenesis and glycogenolysis and inhibiting insulin secretion 2) decrease peripheral resistance to maintain adequate blood flow. The resulting elevation of plasma glucose, however, stimulates insulin secretion reinforcing hyperinsulinemia and insulin resistance. This hypothesis implicates elevated sympathetic nerve activity and elevated total peripheral

resistance in the metabolic cardiovascular syndrome associated with hypertension. In fact, antihypertensive treatment which improves peripheral resistance also lowers insulin resistance [Donnelly, 1992].

AD infusion in man has been shown to induce insulin resistance by impairing tissue sensitivity to an increase in plasma insulin [Deibert & de Fronzo, 1980]. SHR have elevated plasma AD levels (chapter 3), insulin resistance [Bursztyn et al. 1992] and hyperinsulinemia [Reaven & Chang, 1991]. Also, insulin mediated glucose uptake is impaired in SHR even in the prehypertensive stage [Reaven & Chang, 1991]. It is therefore possible that the high level of AD in SHR causes insulin resistance. Alternatively, AD secretion could be a response to elevated insulin resistance. However, both responses could be a consequence of elevated resistance levels in SHR [Yamori, 1976]. This implies that high levels of plasma AD in hypertensive rats could be a secondary response to the elevation of peripheral or insulin resistance.

In the present study, the L-NAME rats were hypoinsulinemic despite severe hypertension. These low levels of insulin may result from an inhibitory response of AD on the pancreas. Although the L-NAME rats had a high level of sympathetic outflow and plasma AD it is unknown whether or not they were insulin resistant. The proposed relationship between elevated peripheral resistance and syndrome X might explain the high levels of plasma AD in L-NAME hypertensive rats. AD could have been secreted to lower peripheral resistance or to lower plasma insulin (which may have been elevated in the early stages of L-NAME hypertension in which there could have been a large degree of local vasoconstriction). The relationship between elevated sympathetic outflow, hypertension and syndrome X is also supported by the strong correlation found between plasma AD and sympathetic outflow (chapter 3) and the strong correlation between plasma AD and blood pressure in L-NAME treated and control WKY.

The fact that peripheral resistance is reduced with HZ treatment [Struyker-Boudier et al. 1983], is also supported by the present study in which there was a significant reduction in the residual blood pressure during autonomic blockade. The reduction in peripheral resistance after chronic HZ treatment, was accompanied by a reduction in plasma AD levels in SHRSP. Unfortunately, the insulin status of these rats was not measured, although, vasodilator substances (e.g. angiotensin converting enzyme inhibitors and

calcium channel blockers) generally improve insulin resistance [Donnelly, 1992]. The results of the present study suggest that a reduction in blood pressure will lower plasma AD levels (although the two parameters were not significantly correlated). This is in accordance with another study from our laboratory in which chronic administration of enalapril significantly lowered plasma AD levels in SHRSP [Howe, 1989]. These results support the hypothesis of Julius *et.al.* (1991) i.e. a reduction in peripheral resistance (such as during HZ administration) would cause sufficient glucose delivery to working muscles, thus eliminating the need for elevated plasma AD levels normally observed in SHRSP.

6.5 *Summary*

Chronic elevation of MAP elevated plasma AD levels in WKY and chronic reduction of MAP reduced circulating AD in SHRSP.

In the early phase of L-NAME hypertension in WKY, the hypertension seems to be attributed to an elevation in local vasoconstrictor mechanisms. With longer term treatment, however, the hypertension was attributed entirely to an increase in sympathetic outflow. L-NAME hypertension (after long term treatment) was accompanied by an increase in pressor responsiveness, hypoinsulinemia and cardiac hypertrophy. Alternatively, a reduction in MAP following acute administration of HZ caused a reflex elevation of plasma NA, AD and HR. During chronic treatment, however, plasma NA and HR levels returned to normal while plasma AD was significantly reduced. Chronic HZ treatment reduced the level of sympathetic activity measured by ganglion and AVP blockade. This was accompanied by a reduction in pressor responsiveness and a reversal of cardiac hypertrophy.

The results of this study and the finding that AD infusion does not affect blood pressure in normotensive rats (chapter 5), suggest that the elevation of plasma AD levels in SHR and SHRSP are a secondary consequence of hypertension development. It is possible that the high level of plasma AD found in SHR and SHRSP is secreted in response to an elevation of peripheral or insulin resistance seen in young hypertensive rats. AD itself, may not act to affect blood pressure directly, but may be secreted to maintain sufficient glucose delivery to working muscles.

CHAPTER 7

CONCLUSIONS

7.1   Summary of experimental observations

The principal conclusion from this investigation is that the elevated plasma AD concentrations found in hypertensive rats are unlikely to contribute directly to their high blood pressure.

In the initial experiments, plasma AD levels were found to be elevated in both young (5-7 week) and adult (7-9 month) hypertensive rats. Overall, there was a strong significant correlation between plasma AD and resting MAP. The elevation of plasma AD in the hypertensive rats could not be attributed to their behavioural hyperactivity.

Despite the elevation of plasma AD in the hypertensive rats, chronic elevations of plasma AD concentrations in normotensive rats did not elevate blood pressure. Furthermore, chronic AD infusion in hypertensive rats failed to exacerbate the hypertension development. Similarly, chronic reductions in plasma AD concentrations by AMX caused blood pressure to be slightly elevated, not lowered in hypertensive rats. On the other hand, a chronic elevation of blood pressure in WKY significantly elevated plasma AD concentrations and a chronic reduction in blood pressure in SHRSP significantly reduced plasma AD concentrations.

Previous research investigating the role of plasma AD in the pathogenesis of hypertension has focused primarily upon mechanisms by which AD may directly elevate blood pressure, e.g. by enhancing peripheral neurotransmission. However, the results of the present investigation, which focused on the chronic effects of AD, suggest that the role of AD in hypertension may be a compensatory rather than a facilitatory one. Although hypertension is accompanied by an elevation of plasma AD, it is likely that, in hypertension, the metabolic effects of AD are more important than any direct effect on

systemic blood pressure. It is possible, therefore, that plasma AD does not directly contribute to the development of hypertension but instead is released to lower excessive peripheral resistance in skeletal muscle during hypertension.

7.2 Influence of plasma AD on total peripheral resistance

The early elevation of plasma AD before the establishment of hypertension, suggested that AD might have been a pathogenic factor in hypertension development. The initial increase in blood pressure in SHR has been attributed to the early increase in cardiac output [Pfeffer & Frohlich, 1973]. As the hypertension progresses, cardiac output returns to normal and there is a significant elevation in peripheral resistance. [Pfeffer & Frohlich, 1973]. Others, however, have shown that resistance may already be elevated in young SHR [Leenen et al. 1994]. The increase in total peripheral resistance may limit blood flow and hence, limit supply of oxygen and glucose to skeletal muscles. Both ischemic and hypoglycaemic stresses (both seen during exercise) are strong stimuli for AD secretion. Being a potent β_2 receptor agonist, AD can increase blood flow to skeletal muscle beds and can modulate skeletal muscle substrate utilisation by regulating plasma glucose levels. Thus, it is possible that the elevation of plasma AD is secondary to the elevation of peripheral resistance and may be acting to sustain blood flow to skeletal muscles during hypertension.

This hypothesis is supported by the present investigations. The increased residual blood pressure measured after ganglion and AVP blockade which was seen in hypertensive rats even from an early age (chapter 3), is suggestive of an early elevation of basal vascular tone. Plasma AD correlated significantly with this level of blood pressure after ganglion blockade in the adult rats. This implies a significant (although not necessarily a causal) relationship between plasma AD levels and vascular tone. Further evidence for this was the finding that chronic HZ treatment in SHRSP, which lowers peripheral resistance, also lowered plasma AD. In other studies, chronic administration of enalapril to SHRSP (which lowers resistance) also lowered plasma AD [Howe, 1989]. The reduction of plasma AD levels with HZ treatment could be attributed to a low level of blood pressure, however,

antihypertensive drugs such as β blockers which do not dilate blood vessels, do not lower plasma AD levels in rats [Sugawara *et al*. 1980].

The elevation of plasma AD in hypertensive rats seems to be therefore, related to an elevation of peripheral resistance which can be caused by either enhanced sympathetic nerve activity [Yang *et al*. 1993], increased pressor responsiveness [Leenen *et al*. 1994] or vascular hypertrophy [Folkow, 1990]. In the present study, plasma AD concentrations tended to increase with age. The measure of vascular tone in the present study (level of MAP after autonomic blockade) however, showed that it was independent of age in either normotensive or hypertensive rats (Figure 7). Other studies which have measured peripheral resistance have shown that it increases with age in WKY and SHR [Leenen *et al*. 1994]. If resistance does in fact increase with age, then it could account for the higher levels of AD in the older hypertensive rats.

7.3 Association of plasma AD with metabolic abnormalities in hypertension

The elevation of plasma AD due to increased peripheral resistance is likely to be a response to the metabolic needs of vital organs. Hypoglycaemic stress caused by insulin administration causes a dramatic elevation of plasma AD concentrations. To restore plasma glucose, AD stimulates glycogenolysis, gluconeogenesis and inhibits the action of insulin. However, an infusion of AD has been shown to impair insulin sensitivity [Deibert & de Fronzo, 1980]. Thus, although elevated plasma AD levels may be beneficial in elevating plasma glucose and reducing peripheral resistance, they may also adversely affect other homeostatic systems e.g. enhance insulin resistance and elevate plasma triglycerides.

The ultimate determinant of insulin sensitivity is the skeletal muscle. Insulin sensitivity is also related to the skeletal muscle morphology [Lillioja *et al*. 1987]; an abundance of fast twitch fibres, with a reduction in slow twitch fibres (as evident in essential hypertensives [Juhlin-Dannfelt *et al*. 1979]) results in the development of insulin resistance. Previous studies in normotensive rats, have shown that administration of β_2-receptor agonists increased the density of fast twitch fibres. Conversely, a β_2

antagonist reduced the density of fast twitch fibres [Zeman *et al.* 1988]. In addition to this, 4 week old SHR have a higher fast twitch fibre distribution than WKY [Bachir-Lamrini *et al.* 1990]. These results, indicate that AD, a potent β_2 agonist and which is elevated in the circulation of young SHR (chapter 3), may contribute to the development of insulin resistance in these rats by modulating skeletal muscle fibre composition. Furthermore, infusion of AD in man has been shown to directly impair insulin sensitivity via action on a β-adrenergic receptor.

In the present investigation, the insulin status of the young and adult hypertensive rats (chapter 3) was not measured. Previous studies, however, have shown an elevated level of plasma insulin in 6-7 week old SHR [Reaven & Chang, 1991] and that at this young age, insulin-stimulated glucose transport in adipocytes was already lower in SHR than in age-matched WKY. Thus it might be possible that the hypertensive rats used in this study were both hyperinsulinemic and insulin resistant. Whether or not, however, there is a relationship between plasma AD concentrations and the level of plasma insulin or the extent of insulin resistance is not clear. An interesting study by Morgan *et al.* (1993) showed that SHR may have an exaggerated sympathoadrenal response to hyperinsulinemia. These authors also showed that this exaggerated sympathoadrenal activation was not accompanied by a pressor response. Whether or not the elevation of plasma AD is a primary or secondary consequence of hyperinsulinemia warrants further investigation.

Results from the present investigation showed that WKY rats with L-NAME induced hypertension were hypoinsulinemic despite having elevated levels of plasma AD. This suggests that the elevation of plasma AD might precede hyperinsulinemia, at least in experimentally induced hypertension. It is uncertain if this is the case in genetic hypertension. However, it must be noted that not all forms of secondary hypertension include an insulin resistant state [Shamiss *et al.* 1992].

The frequent association of elevated sympathetic nerve activity, hyperlipidemia, hyperinsulinemia, insulin resistance, glucose intolerance and obesity with hypertension [Julius *et al.* 1991; Kjeldsen *et al.* 1992; Supiano *et al.* 1992; Lind & Lithell, 1993] has led to the hypothesis that these associated risk factors (collectively defined as the cardiovascular metabolic syndrome or syndrome X) may somehow exacerbate

hypertension development. As mentioned above, the long term effects of elevated plasma AD may be detrimental in that they may exacerbate various components of the cardiovascular metabolic syndrome such as insulin resistance and hyperlipidemia.

Even from a young age, SHR have higher levels of plasma triglycerides than normotensive WKY which persist to the established stage of hypertension [Reaven & Chang, 1991]. Several studies have reported an increase in plasma triglyceride levels during public speaking [Taggart *et al.* 1973] and racing car driving [Taggart & Carruthers, 1971]. It is possible therefore, that plasma catecholamines, via the activation of the sympathetic nervous system, can elevate blood lipids. Studies in cultured human fibroblasts, suggest that AD can directly decrease low density lipoprotein processing (uptake and degradation) [Maziere *et al.* 1985]. This process might explain the detrimental effect that elevated plasma AD levels have on the development of atherosclerosis [Chakravarti *et al.* 1977]. These findings help to explain the association between elevated plasma AD levels in hypertension and the implication of plasma AD in the elevation of plasma triglyceride levels in the cardiovascular metabolic syndrome.

7.4 Proposed role of circulating AD in the pathogenesis of hypertension

The results of the present investigation suggest that although there is a significant correlation between plasma AD levels and blood pressure, elevated plasma AD levels are unlikely to be a causative factor in the development of hypertension.

It is likely that AD is secreted in response to the elevation of peripheral resistance which accompanies hypertension. In this situation the function of AD is twofold; 1) being a peripheral vasodilator, it may act to lower peripheral resistance and thus counteract a reduction in blood flow to skeletal muscle during hypertension development, 2) AD may act to maintain energy (glucose) supply to skeletal muscles in the face of diminished blood flow by stimulating glycogenolysis and possibly gluconeogenesis and by inhibiting insulin secretion.

The resultant hyperglycaemia and increased blood flow is beneficial as it provides sufficient substrate (glucose) to skeletal muscles. However, the elevated plasma AD may ultimately prove detrimental to the organism as it can potentiate the development of insulin resistance, elevate plasma cholesterol and triglycerides and aggravate the development of cardiac hypertrophy or atherosclerosis.

Due to the multifactorial nature of hypertension, some caution needs to be displayed when addressing the origins of its development. The hyperreactivity hypothesis, which implicates high levels of plasma AD induced by stress in the development of hypertension, is not supported by this investigation. Although it can not be disputed that an elevation of plasma AD during an episode of stress can temporarily elevate blood pressure, the evidence from this investigation shows that, overall, frequent exposure to stress (resulting in chronic elevation of plasma AD) is unlikely to permanently elevate blood pressure. In fact, the present results show that elevation of plasma AD can occur secondarily to hypertension. As yet, a solitary mechanism responsible for the elevation of blood pressure in hypertension has not been discovered. It is generally accepted, however, that hypertension is the product of the interaction of multiple environmental and genetic influences. The elevated circulating AD levels in hypertension are, therefore, most likely a response to these interactions.

APPENDIX 1

From ED Hendley, unpublished data

**Systolic pressure in males; mmHg ± SEM (n)**

Year	AGE	WK-HA	WK-HT	SHR	WKY
1993	5 weeks	92 ± 4 (6)	103 ± 12 (6)		
	2 months	113 ±3 (10)	128 ± 4 (12)		
	3 months	128 ± 2 (19)	142 ± 3 (11)	155 ± 3 (28)	105 ± 3 (20)
1992	3 months	130 ± 2 (37)	151 ± 4 (42)	157 ± 2 (47)	116 ± 2 (16)
1991	2 months	121 ± 2 (24)	159 ± 2 (26)	146 ± 3 (26)	120 ± 3 (26)
	3 months	137 ± 4 (13)	167± 3 (15)	163 ± 3 (11)	117± 2 (18)

Activity scores in males; counts/15 min ± SEM (n)

Year	AGE	WK-HA	WK-HT	SHR	WKY
1993	5 weeks	601 ± 69 (6)	318 ± 33 (6)		
	2 months	664 ± 75 (10)	194 ± 26 (12)		
	3 months	709 ± 44 (19)	314 ± 28 (11)	455 ± 18 (28)	204 ± 16 (20)
1992	3 months	878 ± 50 (37)	316 ± 16 (42)	501 ± 16 (47)	238 ± 18 (16)
1991	2 months	717 ± 34 (24)	314 ± 21 (26)	549 ± 17 (26)	279 ± 18 (26)
	3 months	945 ± 91 (13)	326 ± 27 (15)	551 ± 31 (11)	264 ± 15 (18)

Tail cuff blood pressure and activity scores from WK-HA and WK-HT rats taken at the University of Vermont (1991-1993). Numbers in parentheses indicate animal numbers.

APPENDIX 2
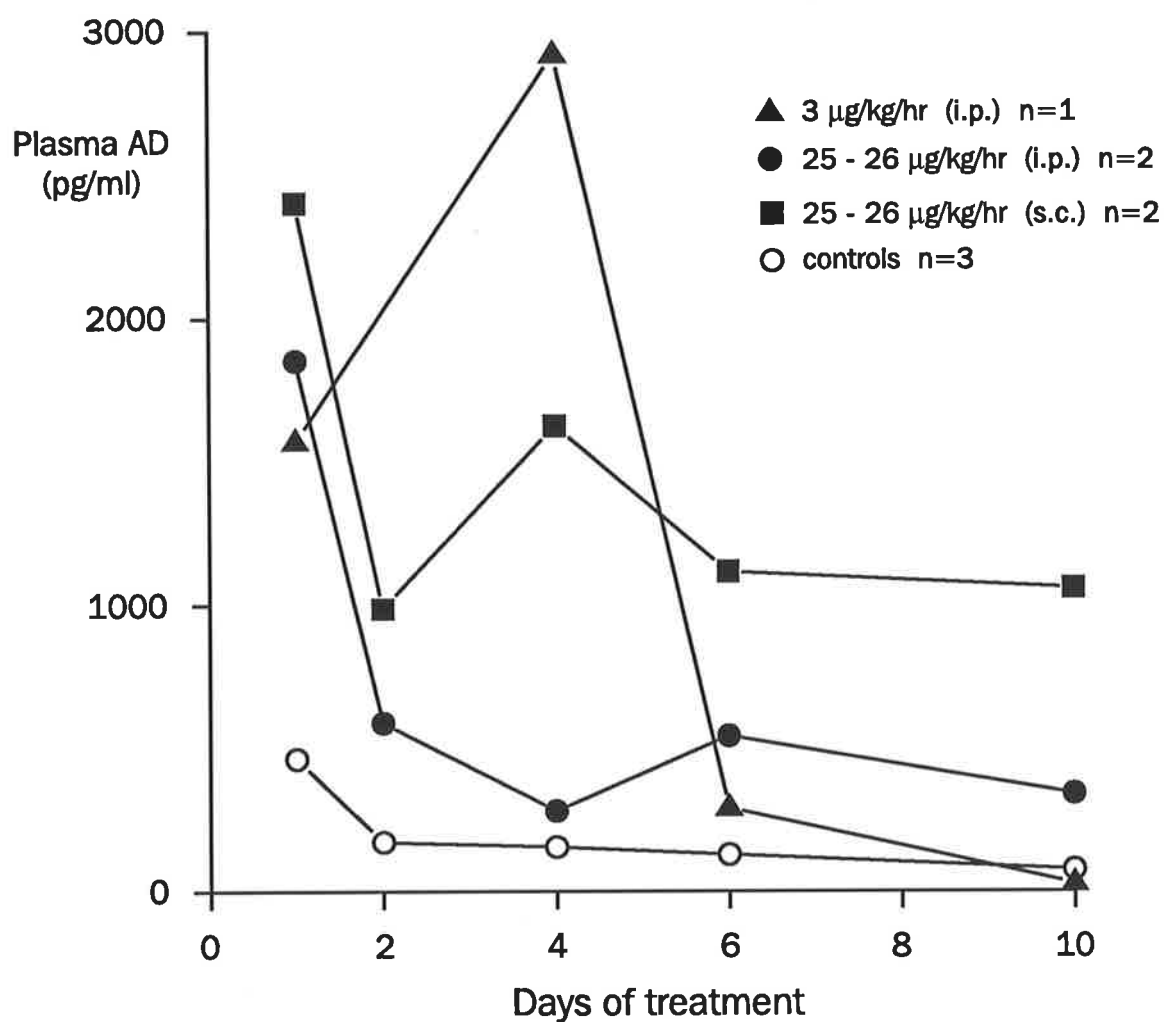
Results from a preliminary experiment showing the effects of different doses and modes of administration of AD in WKY. In all cases, AD was infused by osmotic minipump. The dosing regime 25-26 μg/kg/hr (s.c.) was used for subsequent experiments.

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