THE EFFECT OF ALDOSTERONE PLUS SALT ON BLOOD PRESSURE AND REACTIVITY OF TAIL VESSELS IN CONSCIOUS RATS

A THESIS

Presented to the

UNIVERSITY OF MANITOBA

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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JULY, 1974

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A C K N O W L E D G E M E N T S

It is indeed a pleasure to thank my supervisor, Dr. Gaskell, for the opportunity he provided me to work with him and for his dynamic direction in my research project. To Dr. Andrew Darke, I am indebted in many ways. Apart from the useful discussions relating to cardiovascular physiology and my research work, the more than frequent philosophical diatribes have stimulated my thinking and added colour to my living experience. I would be failing in gratitude if I did not mention Dr. Ken Hughes who provided me with all possible assistance when I started here as a graduate student and has since showed a continuing interest in my progress.

I would also like to thank Miss Wendy Welch for her very proficient secretarial work.

ABSTRACT

The role of aldosterone plus salt in producing hypertension in rats was investigated by studying their effect on the constricting force of the small vessels of the tail as measured by their critical opening pressure (COP). Vascular reactivity of these tail vessels was also studied by observing the change in the constrictive force in response to infusions of angiotensin and noradrenaline. Six male rats were given daily injections of aldosterone (5 ug/ 100 g i.m.) in sesame oil and NaCl to drink for three weeks. Six control rats drank tap water and received injections of the vehicle alone. Both the systolic blood pressure (SBP) and the critical opening pressure (COP) measured by a spectroscopic technique increased progressively in the aldosterone/NaCl rats during the three week treatment period. Initially, in the pre-treatment period, the systolic blood pressure in the test rats was 133 $\pm$ 4 (S.E.M.) mm Hg and in the controls 128 $\pm$ 3 (S.E.M.) mm Hg ($P > 0.30$). The corresponding COP values were 36 $\pm$ 1 (S.E.M.) mm Hg for the test rats and 34 $\pm$ 1 (S.E.M.) mm Hg for the controls ($P > 0.10$). After ganglionic blocking the SBP was 105 $\pm$ 3 for the test rats and 100 $\pm$ 2 for the controls ($P > 0.20$). The COP values were 27 $\pm$ 2 for the test rats and 26 $\pm$ 1 for the controls ($P > 0.60$). At the third week of treatment the systolic blood pressure in the test

rats was 167 ± 3 and 138 ± 2 in the controls ($P < 0.001$). The corresponding COP values were 59 ± 3 in the test rats and 40 ± 2 in the controls ($P < 0.001$). After ganglionic blocking, the SBP in the test rats was 117 ± 4 and 101± 3 in the controls ($P < 0.01$). The corresponding COP values were 36 ± 4 in the test rats and 23 ± 2 in the controls ($P < 0.02$).

Vascular reactivity to intravenous infusions of angiotensin (2, 4 and 8 ng/kg/min) and noradrenaline (30, 60 and 120 ng/kg/min), measured after ganglionic blockade in terms of changes in COP in response to these agents was determined during the third week of the treatment. The increases in the critical opening pressure for each increment in dose of angiotensin and noradrenaline (overall increase is 15 mm Hg) were not significantly greater in the test than in the control rats ($P < 0.05$).

When the aldosterone/NaCl treatment was terminated, measurements of SBP and COP were continued in 3 of the treated rats and their 3 controls for further post-treatment studies. These rats did not receive any more injections and drank only tap water. Within four weeks both the COP and systolic blood pressure of the aldosterone/NaCl treated rats returned to control levels. In the 4th post-operative week, prior to ganglionic blockade, the SBP was 139 ± 1 (S.E.M.) mm Hg for the test rats and 136 ± 1 (S.E.M.) mm Hg for the controls

($P > 0.05$). The corresponding COP values were 38 ± 3 (test) and 37 ± 1 (controls) ($P > 0.70$). After ganglionic blocking the SBP was 105 ± 2 for the test rats and 100 ± 2 for the controls ($P > 0.10$). The COP values were 25 ± 2 (test) and 24 ± 1 (controls) ($P > 0.40$).

The results indicate that the aldosterone/NaCl rats developed both a higher blood pressure and critical opening pressure compared with control rats. The difference in SBP and COP between the treated and control rats persisted after ganglionic blockade. The increased vascular smooth muscle constricting force, measured by their critical opening pressure, was not accompanied by an increase in vascular reactivity.

The recovery of systolic blood pressure in the 3 test rats to control values with the termination of the aldosterone/NaCl treatment could be interpreted to mean that the increases in the SBP and COP induced in these rats was of a transient or reversible nature.

L I S T O F F I G U R E S

INTRODUCTION

Most hemodynamic studies on hypertensive patients have indicated a generalised increase in their peripheral resistance with normal cardiac output (Freis 1960). The increased vascular resistance could be brought about by neurogenic, humoral or anatomical mechanisms. It has been suggested that these various mechanisms are inter-related and the degree of their participation varies with the stage of the disease (Page 1949). Humoral involvement in hypertension, to a large extent, relates to the renin-angiotensin-aldosterone system.

1. ALDOSTERONE AND HYPERTENSION

Various clinical and experimental observations have aroused interest in the role of the adrenal cortex in the pathogenesis of hypertension. Among the steroids secreted by the adrenal cortex of man it has been observed that aldosterone, when secreted in excess, may participate in the pathogenesis of disorders involving potassium wastage, sodium retention and arterial hypertension. These disorders may be classified into two main groups, (a) primary aldosteronism and (b) secondary aldosteronism.

(a) Primary aldosteronism refers to the over-secretion arising from an autonomous aldosterone secreting adrenocortical adenoma and the resulting disease as first

described by Conn (1955) is characterised by potassium wastage, alkalosis, muscle weakness and mild arterial hypertension. These abnormalities are corrected by the removal of the adrenal adenoma (Conn et al 1964). In pseudoprimary aldosteronism, the adrenal overactivity is a result of adrenal hyperplasia instead of an adenoma (Baer et al 1970).

(b) Secondary aldosteronism denotes increased aldosterone secretion as a result of the stimulation of the adrenal glands by sources external to these glands. The oversecretion is the result of bilateral adrenal cortical hyperfunction even though adrenal tumors are absent (Laragh & Sealey 1973). Aldosterone secretion has not been observed to be elevated in all types of hypertension (Laragh et al 1966; Ledingham 1967) but it has been found to be significantly increased in malignant hypertension (Laragh 1960a; Cope & Pearson 1963). The hyperaldosteronism of malignant hypertension is usually accompanied by hypokalemic alkalosis which may be the result of aldosterone action on the kidney (Laragh 1960a). However, this hyperaldosteronism, by itself, does not appear to be the cause of malignant hypertension since adrenalectomy does not completely correct the condition (Laragh et al 1960). It is not clear what the stimulus for aldosterone oversecretion is in malignant hypertension

but a renal-adrenal hormonal system has been suggested as a possible mechanism. Laragh and his co-workers propose that there is a renal-adrenal interaction for the normal control of sodium balance, a derangement of which participates in the pathogenesis of malignant hypertension (Laragh et al 1960). Thus, in malignant hypertension the kidney is said to be in a critical state of damage leading to increased secretion of renin into the blood where it interacts with the renin substrate to release angiotensin I which is later converted into angiotensin II. Angiotensin II, in addition to constricting the arterioles and raising the blood pressure, also stimulates the secretion of aldosterone by the adrenal cortex. Ordinarily, this feedback loop is closed as aldosterone induces sodium retention and volume expansion and this terminates the increased renin secretion. However, in malignant hypertension, because of renal damage, complications arise. The aldosterone secreted cannot, according to Laragh, suppress the renin secretion, partly because of its inability to induce appropriate sodium retention by the damaged kidney. A vicious cycle develops whereby more renin induces more angiotensin release which in turn induces more aldosterone secretion leading to increased hypertension and more renal and vascular damage. In support of Laragh's hypothesis can be cited the work of Masson et al (1962) who showed that simultaneous

administration of large doses of angiotensin and aldosterone produces the necrotizing vasculitis characteristic of malignant hypertension. They claimed that neither agent alone could produce this effect. Angiotensinemia in human malignant hypertension has been observed (Kahn et al 1952). It would seem then, that oversecretion of aldosterone in malignant hypertension, although not necessarily related to its initiation, is a consequence of renal damage and contributes to its pathogenesis. All available evidence indicates that renin via the generation of angiotensin II acts as a powerful stimulus for increased aldosterone secretion and thus constitutes the major hormonal control system involved in regulating the secretion of aldosterone.

2. ROLE OF ALDOSTERONE AND ELECTROLYTE BALANCE IN HYPERTENSION

It has been recognized for some time that there is a reciprocal relationship between sodium reabsorption and potassium excretion in response to aldosterone administration. It is reported that aldosterone stimulates distal tubular reabsorption of sodium, increasing the transepithelial electrical potentials in this portion of the nephron and enhancing renal secretion of potassium (Giebisch et al 1967). The renal effect of aldosterone in increasing the secretion of potassium would appear to be indirect and secondary to the primary

action on sodium transport. However, absence of a strictly quantitative reciprocal relationship between sodium and potassium has been observed by some investigators (Mills et al 1960; Sonnenblick et al 1961). Although daily administration of aldosterone leads to an initial retention of dietary sodium with an increase in body weight, after several days sodium excretion again equals or exceeds the daily salt intake despite the continued aldosterone administration (August et al 1959; Relman et al 1952). This "escape phenomenon" could explain the absence of edema in patients with primary aldosteronism who are presumably exposed to high levels of circulating aldosterone. The administration of sodium chloride has been shown to precipitate the escape phenomenon in subjects receiving high dosages of mineralocorticoids (Laragh 1960b; Strauss et al 1959).

Patients with hyperaldosteronism, in addition to sodium retention and potassium loss have been found also to have a low plasma magnesium concentration that has been attributed to the effect of aldosterone (Mader et al 1955; Milne et al 1957). Supportive evidence of elevated magnesium clearance in primary aldosteronism has been cited (Horton et al 1962). Both aldosterone and desoxycorticosterone lowered plasma levels of magnesium in rats (Hanna et al 1960; Woodbury et al 1950), presumably by increasing renal excretion of the ion.

Among different ions mentioned above, sodium has received greater attention as the major electrolyte involved in aldosterone induced hypertension. The relationship of high sodium intake and the development of hypertension has been studied in rats and the findings have been that at least in certain strains of rats, high sodium intake lead to the development of systemic blood pressure elevation (Dahl 1961; Koletsky 1957). Increased sodium intake could contribute to blood pressure elevation by operating through at least two mechanisms:

(a) Vessel wall thickening as by intimal or total wall swelling.

(b) Changing the ionic balance of the vascular smooth muscle and enhancing vascular smooth muscle contraction.

a) The effect of increased tissue cations and water content in the vessel wall has been related to vessel wall thickening in arterial hypertension (Tobian & Binion 1952). Similarly, "water-logging" or "hypertrophy" have been shown to exist in the vascular walls of hypertensive patients (Mendlowitz & Meyer 1955; Folkow 1956). This water logging or muscular hypertrophy of arteries could decrease the lumen of the vessel and thereby increase vascular resistance. It has been recognised that the lumens of arteries and arterioles of hypertensive

subjects have a decreased "resting" diameter (Redleaf & Tobian 1958). It would seem then, that arteriolar narrowing brought about by structural changes could lead to increased vascular resistance resulting in arterial hypertension.

b) It is known that the membrane potential of many tissues can be described in terms of the distribution of K, Na and Cl ions across the cell membrane and of the relative permeability of the membrane to these ions. It is also generally believed that changes in transmembrane potential trigger the processes involved in the activation of the contractile mechanism (Bülbring 1970). The different factors responsible for the electrical properties of the membrane for excitation are the concentrations of sodium (Harris et al 1971) and potassium (Norton et al 1972) at the cell level. It is changes in the concentration of these ions that alter vascular smooth muscle contraction and relaxation. Contraction of arterial smooth muscle has been found to be associated with movement of extracellular calcium and sodium into the cell and intracellular K^+ out of the cell (Shibata et al 1972). Thus, aldosterone might alter the contractile force of the smooth muscles of the small arteriolar walls, primarly by enhancing the movement of extracellular sodium into the cell. It is believed that these

ionic imbalances could also affect vascular reactivity. Rondell and Gross (1960) were able to demonstrate that the sensitivities to angiotensin and noradrenaline of isolated sections of rabbit aorta were increased if aldosterone was added to the medium.

The exact mechanism of action of aldosterone in facilitating sodium transport across the membrane has not been satisfactorily explained so far. The hypothesis that aldosterone acts through messenger RNA synthesis and subsequent protein synthesis was put forward by Edelman et al (1963) who found that actinomycin D blocked the stimulation of sodium transport by aldosterone in toad bladder. Effects of aldosterone on RNA in kidney have been described by Castles et al (1965, 1967). Sharp and Leaf (1973) believe that the results of the work done with toad bladder and kidney indicate that the amount of mineralocorticoid specific protein synthesis stimulated by aldosterone is very small. It is possible that a similar mechanism of protein synthesis may be operating in the enhancement of sodium transport in vascular smooth muscle. How the synthesis of this new protein enhances the rate of sodium transport is not known.

STATEMENT OF THE PROBLEM

Both clinical and experimental studies have shown that increased aldosterone secretion occurs in certain types of hypertension. Among these are primary aldosteronism (Conn 1955), malignant hypertension (Laragh et al 1960), renal hypertension (Deane & Masson 1951), renovascular hypertension (Barraclough et al 1965) and perhaps uncomplicated essential hypertension (Genest et al 1956). While increased secretion of aldosterone may participate in the pathogenisis of most of the hypertensive states mentioned above, oversecretion of aldosterone has been considered to be the direct cause of hypertension in primary aldosteronism.

The question that arise is, how does aldosterone cause this increase in arterial blood pressure? Is this brought about by increasing the vascular smooth muscle contracting force? By increasing the reactivity of the blood vessels to circulating vasoactive agents? Or, is it by changing the geometry of the vessels as by vascular wall thickening? Could it possibly be by way of increasing blood volume and subsequently cardiac output and bringing about other changes in the cardiovascular system?

The present experiments are intended to study

whether aldosterone increases arterial blood pressure by enhancing vascular smooth muscle constricting activity and/or reactivity.

MATERIALS AND METHODS

A total of 12 male Long-Evans rats weighing initially about 400 gms. were used in these experiments. They were separated into pairs by matching their weight; one member of each pair becoming the test rat, to be treated with aldosterone and 1% NaCl as drinking fluid, and the other control animal, drinking tap water. Measurements were always made on both members of a pair at the same time and the overall experiment was carried out by starting with one pair and introducing the other pairs into the program in succession. Every pair followed the same protocol which was as follows.

For one week they were trained to remain in a restraining wire cage and accustomed to the experimental procedure. Measurements of systolic blood pressure (SBP) and critical opening pressure (COP) were made at this time but these preliminary measurements were discarded. In the second week definitive pre-treatment control measurements of both SBP and COP were made. In the third week, the test and control animals were cannulated with an indwelling catheter in the jugular vein. Near the end of the first week of post-operative recovery more control readings of SBP and COP were made. Since these control measurements did

not differ from the measurements made just before the cannulation, both values were included in calculating the mean of the pre-treatment measurements. The following week aldosterone/NaCl treatment was started in the test rat by daily intramuscular injections of aldosterone (5 ug/100 g) in sesame oil and the drinking water replaced with 1% NaCl. Measurements of COP and systolic blood pressure were carried out on one day near the end of the first and of the second week of treatment. On these occasions the reactivity to intravenous infusions of either angiotensin or noradrenaline was also studied to monitor possible changes of vascular reactivity with time. The reactivity studies carried out in these two weeks are not included in the 'Results' section. In the third week, the final "treatment" measurements of COP and systolic blood pressure were made on each of two days. On these days measurements of COP and SBP were followed by definitive reactivity studies with angiotensin on one day and noradrenaline on the other day. Treatment was terminated after these measurements but post-treatment measurements were made in three of the pairs of the rats for a further period of four weeks. Both COP and systolic blood pressure measurements were made every week for the four weeks but reactivity to vasoactive agents was not studied.

Cannulation of the rats was carried out under sodium pentobarbital (Nembutal 50 mg/kg i.p.) anesthesia. The jugular vein was cannulated with an indwelling catheter filled with heparinized saline (100 units/ml). The polyethylene cannula used (PE-50 tubing) for this purpose was fitted with a 2 - 3 cms. long silastic tip to prevent any possible tissue irritation (Bradham & Walsh 1965) or clotting (Reynolds et al 1965). The other end of the cannula was taken under the skin, exteriorized at the back of the neck and heat sealed.

The aldosterone solution for injection was prepared by dissolving sufficient aldosterone in 1 c.c. absolute alcohol and adding 9 c.c. sesame oil so that approximately 0.1 ml. of the final solution contained 25 ug of aldosterone. The control rat received injections of the alcohol-sesame-oil mixture only. The dose of aldosterone (5 ug/100 g) was calculated to be near the amount of aldosterone secreted by the adrenal glands of normal rats (Cade et al 1965; Eilers et al 1964; Jørgenson 1969).

Measurements of systolic blood pressure and COP of small vessels in the tail were made using a modified form of the spectroscopic technique (Gaskell 1965) as described below. The rat tail was fitted with a blood pressure cuff (about 1½ inches wide) at the base and

the rest of the tail sealed inside a thin (2 mils) transparent bag. The bags were made from clear vinyl sheet (Vi-drape regular surgical film) cut and folded to size and heat sealed (clamco heat sealer) with suitable openings for the tail and for a stopper through which a connection could be made to a pressure reservoir. The bag was secured onto the tail by folding the proximal edge of the bag under itself against the skin and applying air proof, water proof, plastic adhesive tape (Elastoplast) along the edge which is to be on the inside of the bag, so that it covered both the edge of the bag and the adjacent skin. The fold of the bag around the base of the tail was then taped to the adjacent skin with additional adhesive tape to hold the bag securely to the tail. The area of the tail where this seal was to be made was painted with plastic spray solution (Vi-drape adhesive spray) to prevent perspiration from loosening the adhesive tape and also to aid the sealing process. The plastic bag containing the tail was placed within a separate rigid transparent plastic box. The plastic box prevented the vinyl bag from being over inflated and also provided a plane surface for directing light beams for spectroscopic examination. Provision was also made for the rat in its wire holder to be kept in a fairly constant warm air temperature ($32^{o}C$) and the tail to be warmed to 32^{o} - $35^{o}C$ by local warming.

The temperature of the environment of the rat and the tail temperature were monitored by thermistor probes. Both the plastic bag and the pneumatic cuff could be connected separately through stop cocks to a pressure reservoir and an aneroid manometer that had been checked previously with a mercury manometer. The air pressure in the reservoir could be increased by a hand bulb. A beam of light from a microscope illuminator with a heat filter and a green filter was directed through the plastic box to illuminate a small part of the skin. The reflected light was examined with a Zeiss hand spectroscope for the oxyhemoglobin band. The oxyhemoglobin absorption band is an index of oxygenated blood flowing through the capillaries of the skin (Ray 1946).

The procedure for actual measurements of the SBP and COP were as follows. The external pressure on the tail within the bag was increased above the systolic blood pressure by the hand bulb connected to the pressure reservoir. When the oxyhemoglobin lines had disappeared from the spectrum of reflected light the pressure was slowly decreased until the oxyhemoglobin band reappeared. The pressure within the bag at this end point was read as the extravascular pressure that would just allow the arterioles to open and let the oxygenated blood flow into the capillaries of the skin. Pressure in the bag was again increased slightly above systolic pressure to expel most

of the blood in the tail and the cuff was then also inflated to this pressure. Leaving the cuff inflated, the pressure within the bag was reduced to atmospheric pressure. When the cuff pressure was decreased slowly, the oxyhemoglobin absorption band reappeared and this end point was taken as the tail systolic blood pressure (SBP). The critical opening pressure (COP) was calculated as the difference between the systolic blood pressure at the tail and the extravascular pressure which just allowed blood flow in the vessels at the surface of the tail. On each occasion of the measurements of the COP and systolic blood pressure, four or five such measurements, taken at about three minute intervals, were used to calculate the mean values. Then, after administering a ganglionic blocking agent, chlorisondamine hydrochloride (Ecolid CIBA 2 mg/kg), intramuscularly and waiting about 20 minutes for the ganglionic blockade to become fully effective, four or five further measurements were made and the means of SBP and the means of COP again calculated.

For reactivity studies with angiotensin and noradrenaline, the seal of the cannula was broken and the jugular cannula connected to a 10 c.c. syringe filled with angiotensin (in saline) or noradrenaline (in 5% dextrose). The syringe was fitted onto a motorized pump (Harvard Apparatus Compact Infusion Pump, Model 975) and the speed

of the pump adjusted to deliver the different doses of angiotensin (2, 4 and 8 ng/kg/min) or noradrenaline (30, 60 and 120 ng/kg/min). Four or five measurements were done at each dose level after the increase in COP and systolic blood pressure for each dose had stabilised. Both the control and the test rats received the infusions at the same time and individual measurements were made alternately in the test and the control rats. Angiotensin was used as the amide (Hypertensin - CIBA) and doses are expressed in terms of the amide. Noradrenaline was used as the bitartrate (Levophed-Winthrop) and doses expressed in terms of the base.

Results in all the experiments have been expressed as the means of the four or five measurements made on each occasion such as before and after ganglionic blocking and for each dose level of the vasoactive agent infused. The significance of the differences in these results between all the control and all the aldosterone/NaCl rats have been calculated by the unpaired t-test.

RESULTS

Measurements of the critical opening pressure (COP) and systolic blood pressure (SBP), both before and after the administration of the ganglionic blocking agent (chlorisondamine), for the pre-treatment period and for weeks 1, 2 and 3 of aldosterone/NaCl treatment are represented in Figs. 1 and 2. Measurements made during the pre-treatment period indicated that there was no difference between the aldosterone/NaCl treated and control rats in either their COP or SBP both before and after ganglionic blockade. Prior to ganglionic blocking the control rats had a COP of 34 ± 1 (S.E.M.) mm Hg and the test rats 36 ± 1 (S.E.M.) mm Hg ($P > 0.10$). The corresponding SBP values were 128 ± 3 (S.E.M.) mm Hg for the controls and 133 ± 4 (S.E.M.) mm Hg ($P > 0.30$) for the test rats. After ganglionic blocking the control rats had a COP of 26 ± 1 and the test rats 27 ± 2 ($P > 0.60$). The SBP values at this time were 100 ± 2 for the controls and 105 ± 3 for the test animals ($P > 0.20$).

A slight increase in both the COP and the systolic blood pressure was observed in the aldosterone/NaCl rats after one week of treatment when measurements were made prior to ganglionic blockade. However, only the SBP was significantly greater in the test rats compared with

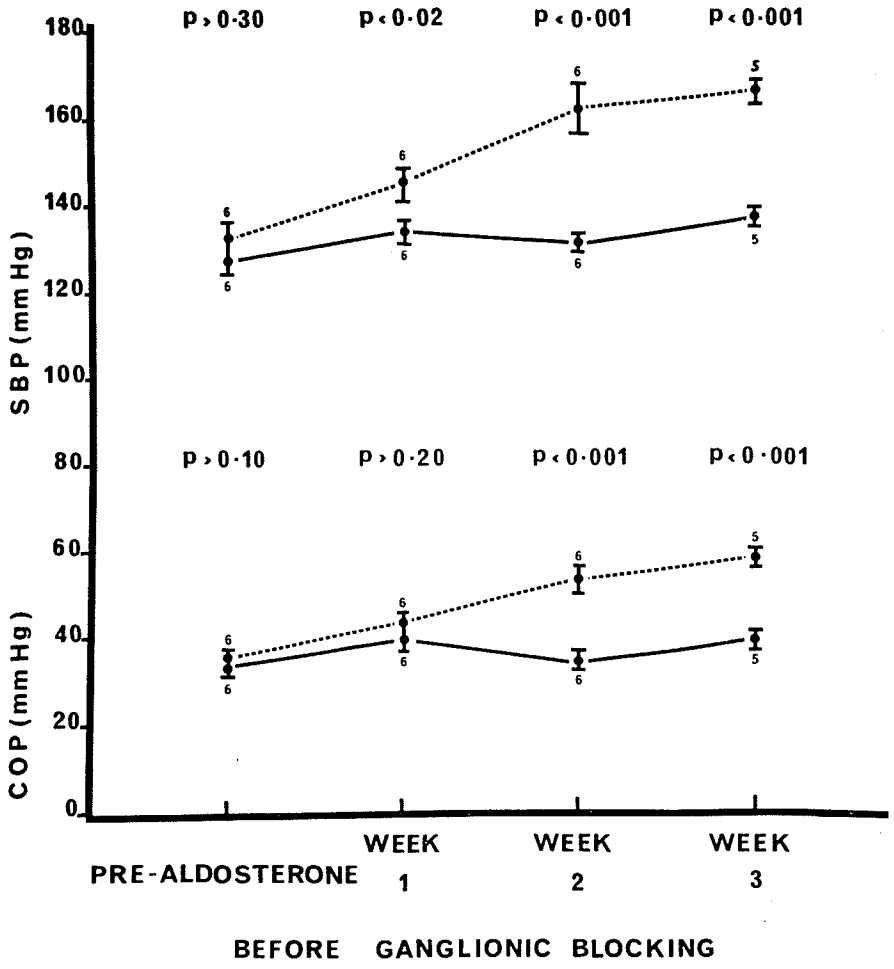

Figure 1

Critical opening pressure (COP) and systolic blood pressure (SBP) of tail vessels of rats measured before the administration of ganglionic blocking agent (chlorisondamine) and covering the pre-treatment period and three weeks of aldosterone/NaCl treatment. Measurements made on the control rats are indicated as continuous lines. The S.E.M. and number of rats in each period are also shown.

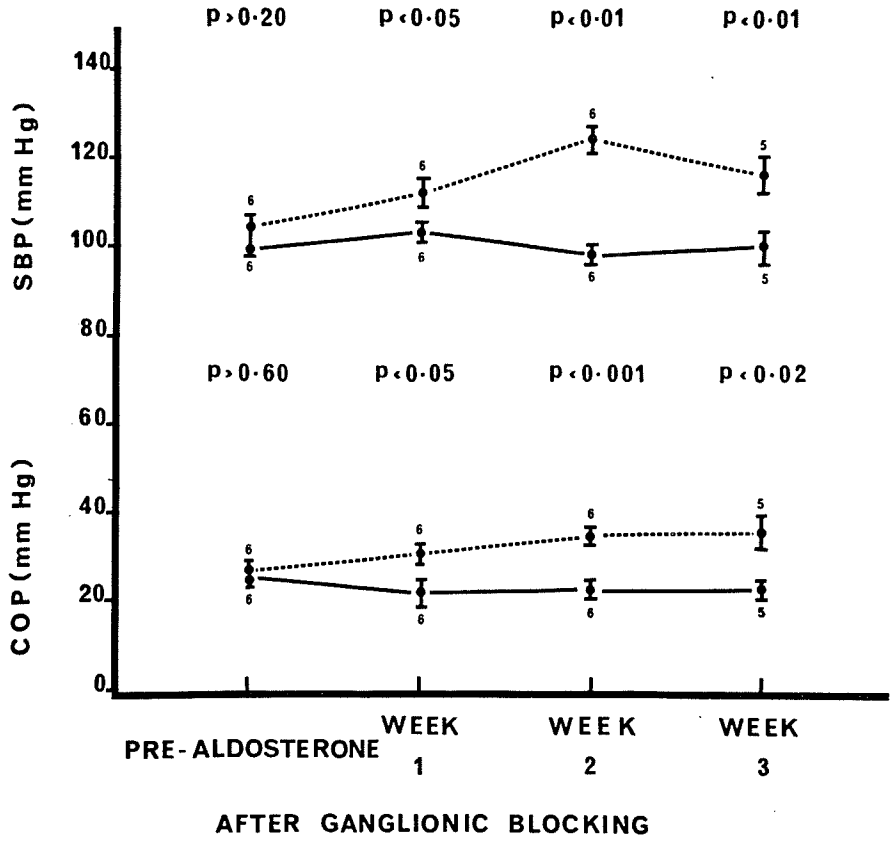

Figure 2

COP and SBP of tail vessels measured after ganglionic blockade covering the period before aldosterone/NaCl treatment, and for three weeks during the treatment. The values for control rats are represented as continuous lines and values for the treated rats as discontinuous lines. The S.E.M. and number of rats in each group are indicated in the separate periods.

the controls. The means of the COP values in the control rats was 40 ± 1.7 and in the aldosterone/NaCl rats 44 ± 2 ($P > 0.20$). The corresponding means for SBP measurements were 135 ± 1.7 in the control rats and 146 ± 3.3 for the aldosterone/NaCl rats ($P < 0.02$). After the administration of the ganglionic blocking agent there was a significant difference between the means of the values (both COP and SBP) in the control and the test rats. The mean COP in the control animals was 22 ± 2.7 and in the aldosterone/NaCl rats 31 ± 2.3 ($P < 0.05$). The mean SBP values were 104 ± 2.3 and 113 ± 3 for the controls and aldosterone/NaCl rats respectively ($P < 0.05$).

The increase in both the COP and SBP in the treated rats became much greater in the second week of aldosterone/NaCl treatment without any increase in the values in the control animals. The COP measurements before ganglionic blockade were 35 ± 1.7 for the controls and 54 ± 2.6 for the test rats ($P < 0.001$). The means of the SBP values for the control rats was 132 ± 2.4 compared with 163 ± 5.8 for the aldosterone/NaCl rats ($P < 0.001$). After ganglionic blocking the mean COP was 23 ± 1.4 for the controls and 36 ± 1.7 for the test rats ($P < 0.001$). The corresponding values of the SBP were 99 ± 2.3 (controls) and 125 ± 3.3 (aldosterone/NaCl rats) indicating a significant difference of 26 mm Hg between the two ($P < 0.001$). The higher values observed for the aldosterone/NaCl treated rats was

maintained for the third week with no further significant increase between the second and the third week either in COP or systolic blood pressure.

In the third week of treatment the COP in the control rats was 40 ± 2 and 59 ± 3 in the test animals ($P < 0.001$). The corresponding SBP values were 138 ± 2 in the controls and 167 ± 3 in the test rats ($P < 0.001$). After ganglionic blocking, the COP in the control rats was 23 ± 2 and 36 ± 4 in the test rats ($P < 0.02$). The corresponding SBP values were 101 ± 3 in the controls and 117 ± 4 in the test animals ($P < 0.01$).

The effect of the ganglionic blocking agent (chlorisondamine) on all occasions was to decrease the systolic blood pressure and the critical opening pressure (Fig. 3). It is seen that there is no difference between the decreases in the control and in the aldosterone/NaCl treated rats with the P value greater than 0.1 in all cases except for the SBP in the third week when the decrease was significantly greater in the aldosterone/NaCl treated rats ($P < 0.02$).

In the third week when the final infusions of angiotensin and noradrenaline were given to each animal and the dose response curves for COP and SBP were plotted it was found that the increases in COP and SBP produced by either angiotensin or noradrenaline were similar in

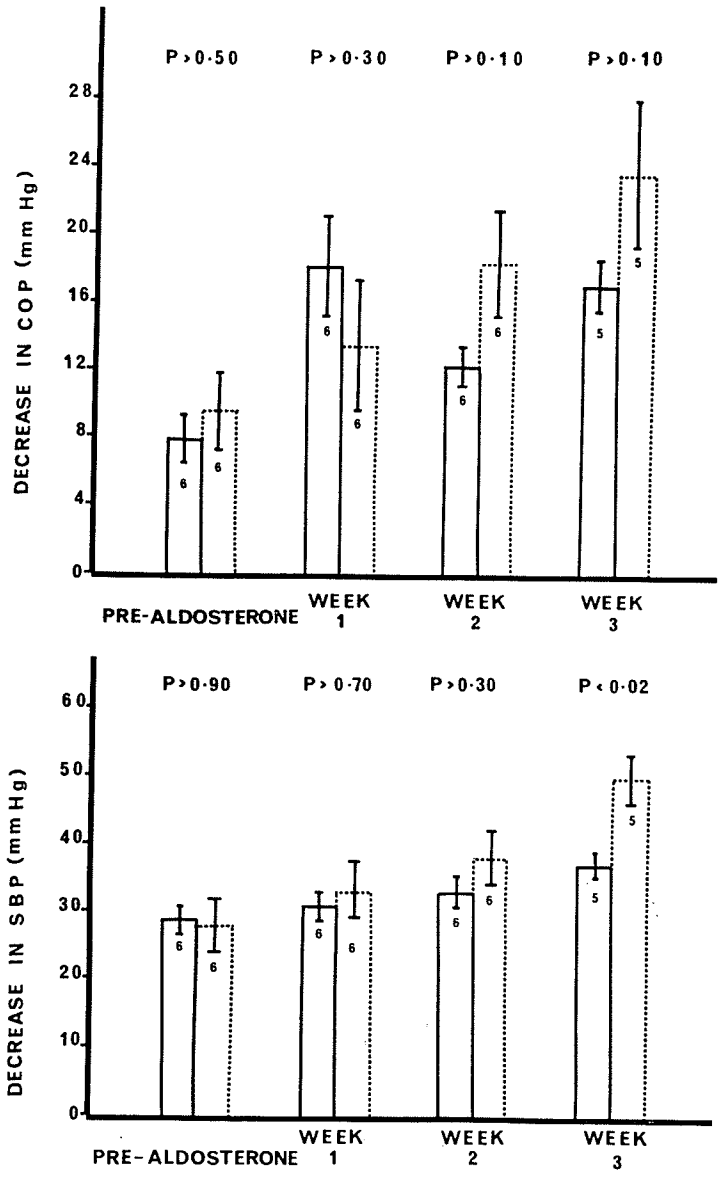

Figure 3

The mean decrease in COP and SBP occurring after the administration of the ganglionic blocking agent as calculated for the pre-aldosterone period and weeks 1, 2 and 3 of aldosterone plus 1% NaCl treatment. The decrease in both COP and SBP are represented as continuous lines for the control rats and discontinuous lines for the test rats. The S.E.M. and number of rats in each group are shown for each period.

both the control and test animals (Figs. 4 and 5). The dose response for COP changes representing reactivity of the vascular smooth muscle are seen to be essentially linear in character as has been previously found also by Darke and Gaskell (1973) and Darke (1974). The increases in COP with each increment in dose of either angiotensin or noradrenaline were similar in both control and treated rats. This is also illustrated in bar graph form in Figs. 6 and 7. However, if in spite of the usual linearity of the curves, it were to be considered that the higher initial COP measured in the treated rats might influence the magnitude of the response, then, the increase in COP in the control rats for the dose increments of 4 - 8 ng/kg/min of angiotensin or 60 - 120 ng/kg/min of noradrenaline may be compared with the increase in COP in the treated rats for the dose increments of angiotensin 0 - 4 ng/kg/min and 0 - 60 ng/kg/min of noradrenaline respectively. The results of such a comparison showed that during angiotensin infusions the mean increase in COP in the control animals for dose increase of 4 - 8 ng/kg/min was 7.0 ± 1 (S.E.M.) mm Hg and in the treated animals for dose increments of 0 - 4 ng/kg/min it was 9.0 ± 2.7 (S.E.M.) mm Hg ($P > 0.60$). Similarly in the noradrenaline infusions the mean increase in COP in the control rats for dose increase of 60 - 120

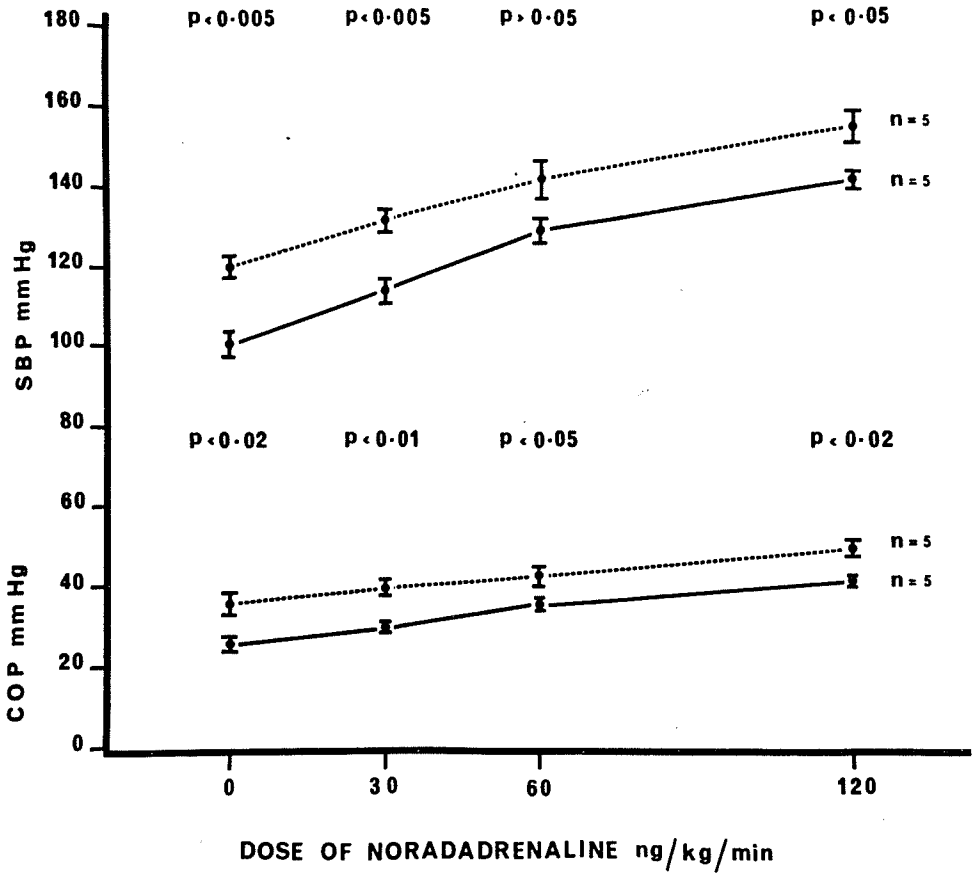

Figure 4

The response to intravenous infusion of noradrenaline of the SBP and COP of tail vessels in the aldosterone/NaCl treated and control rats after ganglionic blockade. The continuous lines represent values for the treated rats. The S.E.M. and number of rats in each group are also indicated.

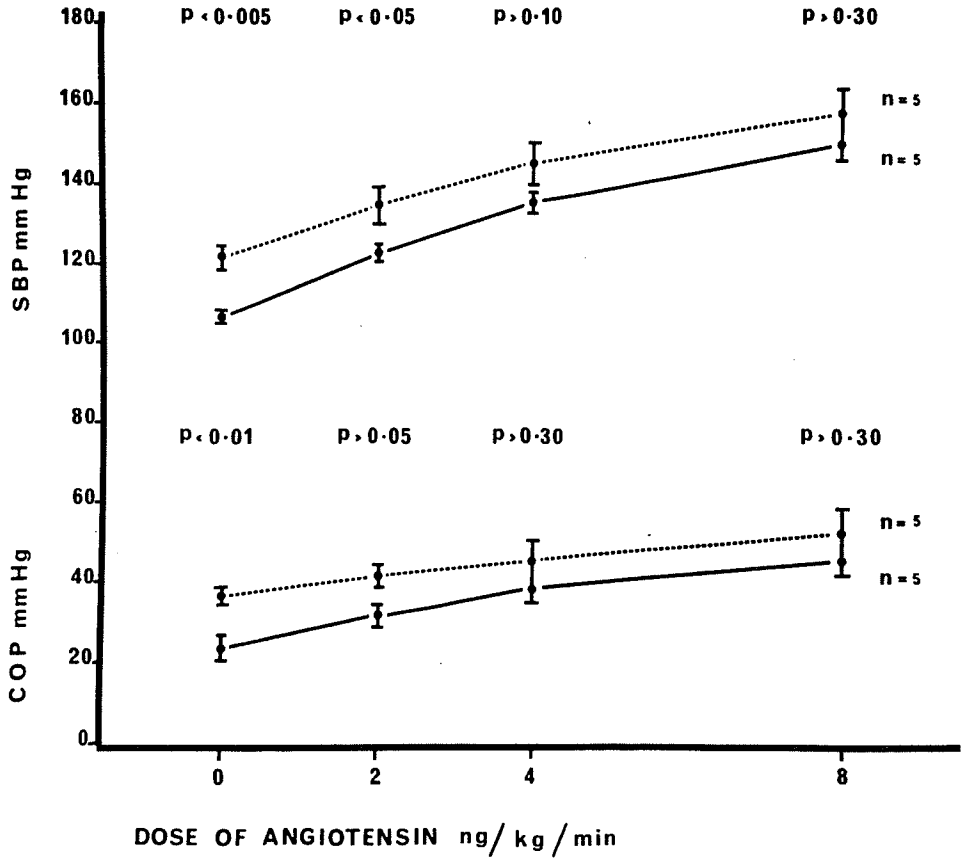

Figure 5

The response to intravenous infusion of angiotensin of the COP and SBP of tail vessels in the aldosterone/NaCl treated and control rats after ganglionic blockade. The continuous lines represent values for control rats and the discontinuous lines indicate values for the treated rats. The S.E.M. and the number of rats in each group are also indicated.

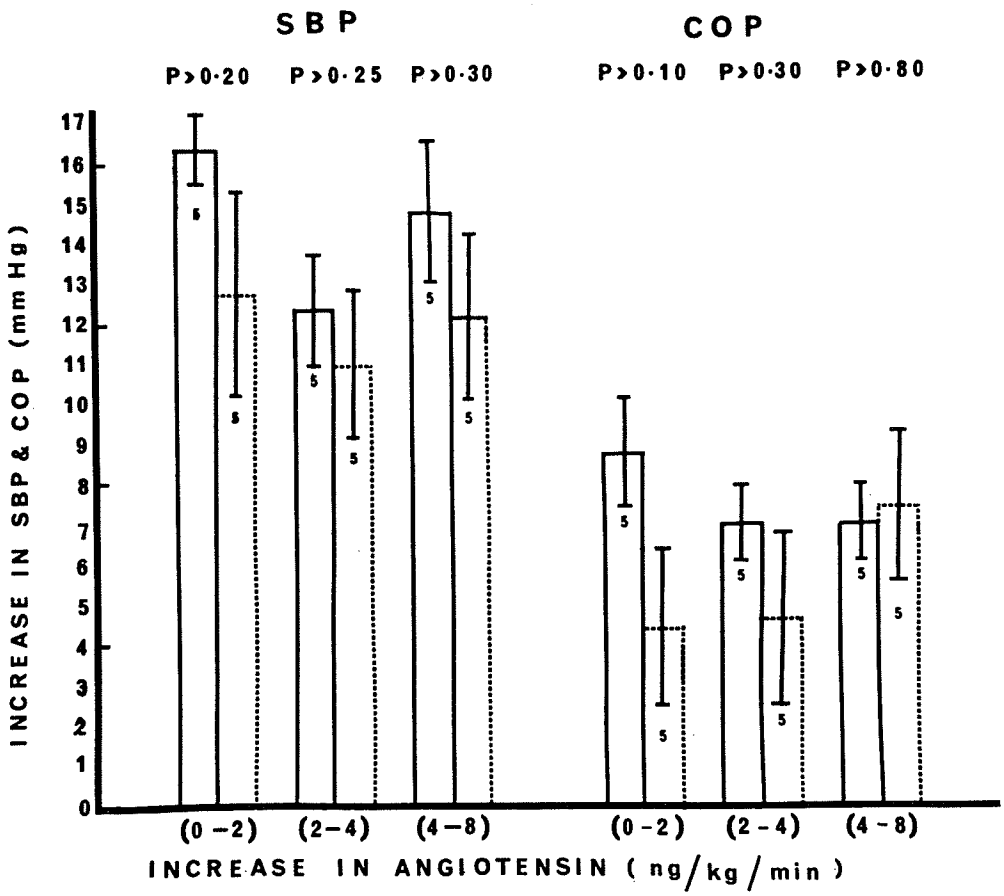

Figure 6

The mean increase in COP and SBP with each increment in dose of angiotensin in the control and aldosterone/NaCl treated rats after ganglionic blockade. The S.E.M. and number of rats in each group are indicated in each bar. Control rats are represented as continuous bars and test rats as discontinuous bars.

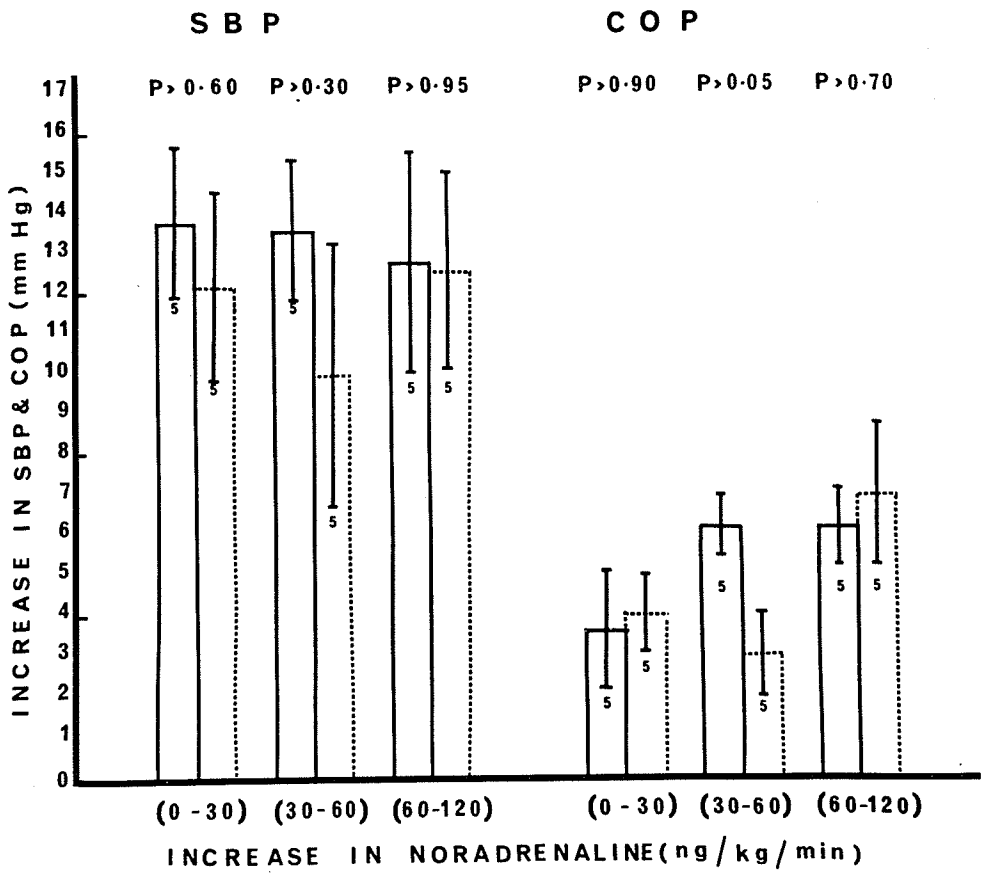

Figure 7

The mean increase in COP and SBP with each increment in dose of noradrenaline in the control and aldosterone/NaCl treated rats after ganglionic blockade. The S.E.M. and number of rats in each group are indicated in each bar. Control rats are represented as continuous bars and test rats as discontinuous bars.

ng/kg/min was 6.2 ± 1 and in treated rats for dose increments of 0 - 60 ng/kg/min it was 6.8 ± 1.7 ($P > 0.70$).

A comparison of the results of the COP and SBP of the pre-treatment, treatment with aldosterone/NaCl and post-treatment periods measured in the 3 test and the 3 control rats, in whom measurements were made in the post-treatment period, is represented in Figs. 8 and 9. Measurements made either with or without ganglionic blockade in the 4th post-treatment week indicated that there was no longer any significant difference between the control and the treated rats in terms of either their critical opening pressure or systolic blood pressure.

The body weights of all the rats were measured daily. These were scanned for evidence of increased retention of salt/water in aldosterone/NaCl rats. The mean increase in the control rats during the 3 week period was 50 ± 5 gms and in the test rats 61 ± 9 gms. The apparently greater increase in weight of the test rats was not significant ($P > 0.20$ by unpaired t-test). If this difference were in fact a reflection of increased salt and water retention, it would amount to 2 - 3% of the body weight.

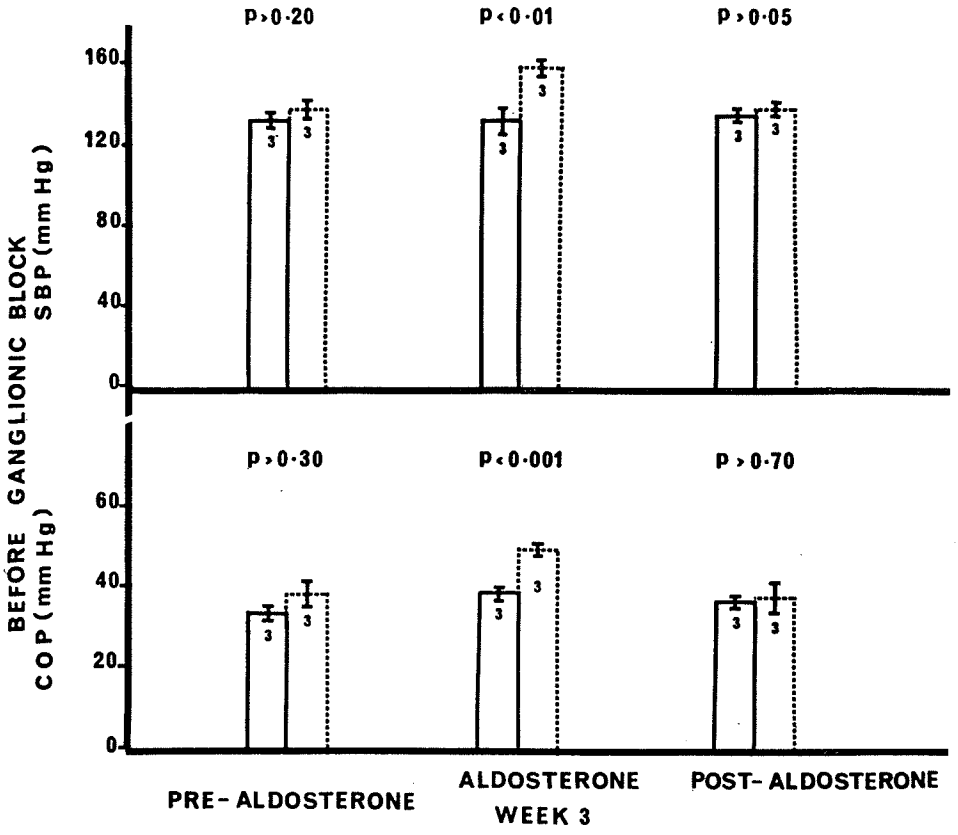

Figure 8

The means of the COP's and SBP's for the pre-treatment period, week 3 of aldosterone/NaCl treatment and the 4th post-treatment week in control and in aldosterone/NaCl treated rats. The measurements were made prior to ganglionic blockade. Control rats are represented by continuous bars and the aldosterone/NaCl treated rats by discontinuous bars. The S.E.M. and number of rats in each group are indicated in each bar.

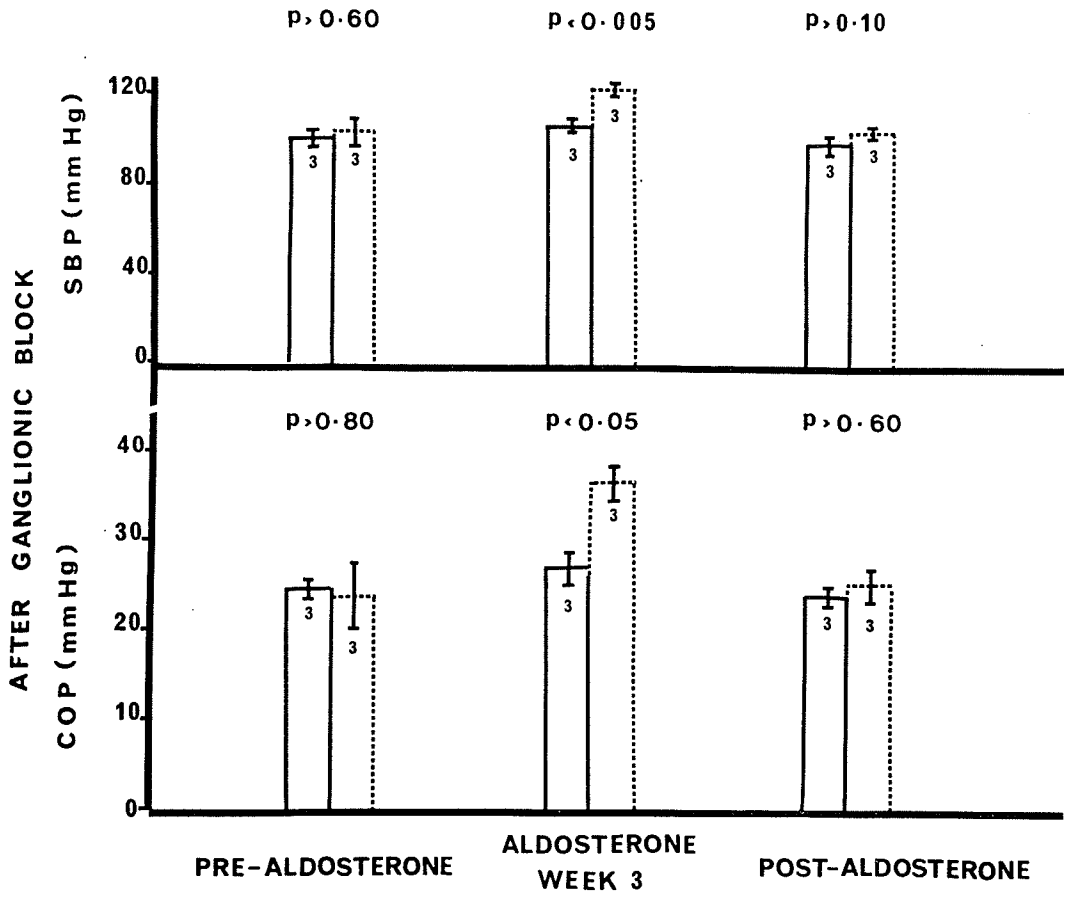

Figure 9

The means of the COP's and SBP's for the pre-treatment period, week 3 of aldosterone/NaCl treatment and the 4th week of post-treatment in the controls and in aldosterone/NaCl treated rats. The measurements were made after ganglionic blockade. Conrols rats are represented by continuous bars and the aldosterone/NaCl treated rats by discontinuous bars. The S.E.M and number of rats in each group are indicated in each bar.

DISCUSSION

Various reports have been made that mineralocorticoids like Desoxycorticosterone (DOCA) or aldosterone, together with sodium chloride, produce increased smooth muscle activity in the vascular walls. Tobian and Binion (1954) reported that both K^+ and Na^+ increased in the aorta of rats made hypertensive with DOCA/NaCl. They believed that most of the increase in the ions mentioned above had occurred intracellularly. Supportive evidence was provided by Tobian and Redleaf (1957,1958) who estimated the intra-cellular Na^+ by using measurements of chloride space. Woodbury and Koch (1957) have also found that aldosterone produces Na^+ increase intracellularly in skeletal muscles in a similar manner.

Electrophysiological studies on vascular smooth muscles have not been as extensive as on the muscle of the gut or uterus. Vascular smooth muscle cells are smaller and are embedded in a matrix of connective tissue which poses technical difficulties for intracellular recording of these cells (Bohr 1964). Almost all electrophysiological work in relation to the effect of steroids on the vascular smooth muscle and its constricting property have been carried out in aorta strips. Unfortunately, it has been found that aorta is a poor choice because

of its specialized elastic tissue content (Friedman 1963). It also behaves differently from peripheral vasculature in vivo and, in particular, shows a slower and prolonged response time (Bevan 1960; Dodd et al 1960; Furchgott et al 1953).

Studies in vivo have also created controversy about the role of aldosterone in hypertension. Increases in blood pressure resulting from chronic administration of aldosterone in combination with sodium chloride, was reported by Kumar et al (1957) and Gornall et al (1960). Contrary to these findings, Ostrovsky (1964) observed that when Wistar rats were given injections of d-aldosterone (5 ug/200 g/day) in combination with NaCl as drinking fluid for 3 months neither their basal blood pressure nor their total pressor response to either angiotensin or noradrenaline infusions were increased. Similar findings of a lack of increase in blood pressure accompanying aldosterone/NaCl administration have been made by other investigators. (Fregley et al 1960; Gaunt et al 1957; Gornall 1965).

Similarly, controversy exists as to whether aldosterone/NaCl induces increased vascular reactivity to vasoactive agents. Kaplan et al (1963) reported that it caused an increase in reactivity to infusion of angiotensin in rats. It was also found that d-aldosterone 21 acetate given for three days to hypertensive patients produced a greater response to norepinephrine infusions (Mendlowitz et al

1963). However, others have reported that aldosterone administration in combination with NaCl given as drinking fluid in rats did not cause an increased responsiveness to angiotensin infusion (Ostrovsky 1964; Katz et al 1963). The discrepancies in the various findings described above may be partly a result of the variable doses and forms of aldosterone used, differences between in vitro and in vivo preparations and the methods used for assessing the in vivo response to vasoactive agents.

It is reasonable to suppose that the discrepancy between the results and conclusions obtained by various investigators may arise out of the different sensitivities of the strains of rats used. The importance of the genetic factors has been stressed by Demanet (1967) in his work with the DOC-induced experimental hypertension of the rat. Similar differences in sensitivity have been demonstrated with respect to the hypertensive effect of sodium chloride in different hereditary strains of rats. (Dahl et al 1965).

In previous in vivo work responses to vasoactive agents have usually been measured as increases in systolic blood pressure or as changes in pressure/flow ratio. Changes in systolic blood pressure is a poor index of vascular smooth muscle activity since it encompasses any cardiac effect as well as the averaged responses of vessels in all tissues of the body.

The most common method of assessing vascular response to vasoactive stimuli has been the measurement of the changes in the pressure/flow ratio. The difficulties involved in using this method as an index of change in the vascular smooth muscle constricting force in comparing normotensive and hypertensive states are many. Some of the difficulties arise because of the following:

1. Geometrical Factors
2. Smooth muscle hypertrophy
3. Averaging effect of pressure/flow measurements.

1. Geometrical Factors:

Redleaf and Tobian (1958) showed that geometrical alterations in the vascular wall leading to a decrease in "resting" diameter of the arteries and arterioles in hypertensive states would increase the resistance of the blood vessels simply by the reduction in lumen size. They demonstrated that "water-logging" or general wall swelling occurs with sodium/water retention in the vessel walls of hypertensive rats. Other workers (Mendlowitz et al 1955; Folkow 1956; Conway 1958) on the basis of their experiments in which they tried to cause relaxation of the vessels found that the minimum resistance available in these vessels was greater in the hypertensive than in normotensive subjects.

Besides water-logging, geometrical alterations as wall thickening could be the result of increased smooth

muscle mass or true hypertrophy as well. This will have a passive effect on resistance in the same manner as thickening by water-logging. In both circumstances, the thickening of the wall will result in an enhanced increase in resistance to flow relative to the non-thickened vessels when in both the smooth muscle is made to shorten by the same percentage.

Even in a normal vessel, changes in resistance for a given vasoconstrictor stimulus depends not only on the responsiveness of the vascular smooth muscle but also on the initial radius produced in a functional way by an increased initial contraction of the smooth muscle. This will result in an enhanced increase in resistance, again on the basis of simple geometry (Redleaf and Tobian 1958). Therefore, a comparison of the flow rate or resistance change to a given stimulus in the vessels of normotensive with those of hypertensive individuals may result in a greater increase in resistance in the hypertensive subjects since the initial caliber of the vessels is likely to be smaller because of an initially greater contraction force by unit mass of muscle. This greater response in terms of increase in resistance to the given stimulus does not represent any change in actual reactivity of the vascular smooth muscle but can be, nevertheless, misleading.

2. Smooth Muscle Hypertrophy:

The problem of assessing real hyper-responsiveness becomes compounded when the vessel wall in the hypertensive subject is thickened by an increased smooth muscle mass. For a given stimulus the greater amount of muscle would provide a greater force of contraction. This would occur even if the contraction force exerted by unit mass of smooth muscle in response to a given stimulus had not increased and its reactivity was normal.

3. Averaging Effect of Pressure/Flow Measurements:

If angiotensin or other vasoactive agents affect vascular smooth muscle in different segments of the same vessels differently, changes in rate of blood flow or of vascular resistance in response to the vasoactive agent cannot represent the reactivity of any particular vessel or set of vessels being studied. The response would then represent an average of all individual responses of the vessels.

In the present experiments the activity and reactivity of the tail vessels in rats have been studied by making measurements of their critical opening pressure (COP). The COP represents the transmural pressure required to open the blood vessels to their unstretched radius or close them from their unstretched radius. "Critical Closing Pressure" (Burton 1951) implies that the actual closure of blood vessels occurs at a positive transmural pressure. The critical opening pressure has been shown to have the same

value as the critical closing pressure and its measurement (Gaskell 1965) is based upon the same principles suggested by Burton and Yamada (1951) for measurement of the critical closing pressure (CCP). Since the COP measurement is an index of the force exerted by the smooth muscle at one particular diameter of the vessel (unstretched radius) it does not depend on a measure of change in resistance to flow. Therefore, the COP is a more direct measure of force exerted by smooth muscle at a definite degree of stretch in particular vessels. These factors confer an advantage on the measurement of COP over the measurements discussed earlier as a method for comparing reactivities of certain vessels in different individuals or in the same individual at different times. The COP measured will be the COP of the vessels with the lowest critical opening pressure among the sets of vessels in parallel and of the segment in the channel which has the highest critical opening pressure.

As with all the other measures described earlier, there are certain disadvantages with using the COP as an index of smooth muscle contraction force. When measuring COP, a short period (about 30 seconds) of reduced or absent blood flow in the part being studied is produced, and changes in the force exerted by the smooth muscle at this time would lead to an error in estimating the COP obtained at the start of the measurement. However, this

error has been found to be rather small and likely to be more or less the same for most individuals and circumstances as far as the vessels in the skin of the tissues are concerned (Gaskell & Heoppner 1967). Another disadvantage of this measurement when comparing responses in different individuals such as normotensive and hypertensive subjects, is that the estimated force exerted by the vascular smooth muscle is not expressed as force per unit mass of smooth muscle. Therefore, if hypertrophy of media had occurred in the vessels of the hypertensive subject, the increase in COP on application of a constricting stimulus would be greater on this account and would be attributed to a greater reactivity when no difference in reactivity was present. This problem exists also in all the techniques for comparing reactivities mentioned earlier. In spite of the disadvantages mentioned it is believed that the comparison of changes in COP or in CCP of small vessels in different individuals or in the same individual at different times, in response to a given stimulus, is the best and most direct in vivo method of comparing the reactivities of vascular smooth muscle of the "resistance" vessels.

The increased systolic blood pressure found in the aldosterone/NaCl treated rats could have been the result of effects on the heart, blood volume or on the blood vessels. An effect on the blood vessels might be brought about by one or more of the following mechanisms:

1. Structural alteration of the vascular walls.
2. Neurogenic influences.
3. Changes in the ionic balance of the smooth muscle cells or other direct cellular effect resulting in increased vascular smooth muscle contraction.

1. Structural Changes:

As explained earlier, evidence exists to indicate that structural changes in the vessels found in hypertensive states could result from intimal or wall swelling by retention of Na^+ and water (Tobian and Binion 1952). It is possible that such a thickening could give rise to an increase in resistance and a higher than normal blood pressure. However, measurement of COP is an index of smooth muscle constricting force and therefore, changes in lumen size by swelling of the wall will not result in an increase in COP observed in the treated rats.

On the other hand, if true hypertrophy of smooth muscle had occurred then the increased COP could have been the result of this increased smooth muscle mass contributing to greater constriction. If this had been so one would expect this increased smooth muscle to offer greater apparent reactivity to vasoactive agents than would normal vessels. Since this did not take place in the reactivity studies done with either angiotensin or noradrenaline it would seem unlikely that a proliferation

of smooth muscle did indeed occur.

2. Neurogenic Influences:

According to De Champlain et al the influence of sodium on the storage and distribution of norepinephrine in the nerve endings and its role in the pathogenesis of DOCA hypertension has been strongly suggested by the observation that deletion of sodium from the diet of the hypertensive rats results in the lowering of blood pressure to normotensive levels while simultaneously restoring to normal the endogenous storage capacity and turnover rate of norepinephrine (De Champlain et al 1969). The association of the changes in the subcellular distribution of norepinephrine with the variations of the systolic blood pressure in their animals suggested that the effect of sodium balance on the blood pressure is mediated through the sympathetic nervous system.

The effect of chlorisondamine in consistently decreasing the neurogenic influence on the vascular walls is reflected in the decrease observed in the COP of the aldosterone/NaCl treated rats and the controls (Fig. 3). There is no difference in the decrease in the COP between the test and control animals. There does seem to be a difference between the two groups of rats in the decrease of the SBP values for the third week of aldosterone/NaCl treatment. The difference between the control and test

rats is significant ($P < 0.02$) suggesting that the fall in blood pressure in the treated rats is greater. The discrepancy between the greater fall in SBP in the treated rats and the same in COP as in the control rats can be explained on a geometrical basis as discussed earlier.

3. Ionic and/or other effects in the vascular smooth muscle:

As explained earlier, evidence has been presented to show that contraction of arterial smooth muscle is associated with movement of extracellular calcium and sodium into the cell (Shibata et al 1972). Thus, it is possible that aldosterone might alter the constricting force of the smooth muscles of the arteriolar walls, primarily by accumulation of increased sodium within the wall. However, in the absence of precise measurements of ionic concentrations in the extracellular and intracellular compartments of the vascular tissue, no conclusions with reference to the direct actions of aldosterone can be drawn from these studies.

It would be expected that the treated rats would have a greater weight gain during the treatment period because of retention of salt and water. When the increase of body weights of the control and treated rats were compared over the treatment period it was found that

the aldosterone/NaCl treated rats had an increase in mean weight of 12 gms. more than in control rats. The additional weight gain was not significant ($P > 0.20$). However, the significance may have been obscured because it represented only ¼ of the total weight gain including normal growth. The variability in growth may have complicated the situation.

That changes in the vascular smooth muscle constrictive force have occurred in the aldosterone/NaCl rats is evident from the measurements of increased COP in the treated rats compared with the controls. Since the primary effect of aldosterone is retention of Na^+ and secretion of K^+ it would be logical to presume that the changes observed in the smooth muscle contractile property in the treated rats was brought about by an alteration in the balance of these ions. It may be asked whether a higher than control intake of sodium alone could cause the increase in COP which occurred in the aldosterone/NaCl treated rats. In experiments carried out in this laboratory with male Long-Evans rats kept on 1% NaCl drinking fluid for over 3 weeks no significant difference in COP or SBP was observed between such treated rats and their controls who drank tap water (unpublished observations). This suggests, therefore, that drinking 1% NaCl solution alone would not bring about the changes observed in COP

and SBP in aldosterone/NaCl treated rats.

Could aldosterone alone cause the increase in COP and SBP in a manner observed for the aldosterone/NaCl treated rats? Once again, experiments conducted in this laboratory have showed that male Long-Evans rats given desoxycorticorsterone (DOCA) alone for three weeks did not develop a higher COP or SBP when compared with their controls (unpublished observations). Since both DOCA and aldosterone, as mineralocorticoids, have similar sodium retaining and potassium secreting action one may be justified in suggesting that aldosterone given alone would not cause increases in COP and systolic blood pressure in rats so treated for 3 weeks. However, it was found that in DOCA/NaCl treated rats the increases in COP and SBP were accompanied by increased reactivity to vasoactive agents (Darke & Gaskell 1973). Therefore, it should not be taken for granted that aldosterone treatment alone will not increase COP and systolic blood pressure since the effect of aldosterone/NaCl appears to be different from that of DOCA/NaCl at least with respect to its effect on vascular reactivity.

The findings of the present experiments indicating an increased smooth muscle constricting force in the aldosterone/NaCl treated rats without an associated increase in their reactivity to vasoactive agents constitutes an interesting problem.

CONCLUSIONS

Long-Evans male rats administered d-aldosterone (5 ug/100 g) in sesame oil and given 1% NaCl fluid to drink develop a higher than normal systolic blood pressure and a higher critical opening pressure within two weeks of treatment. The increased critical opening pressure in these treated rats indicates that the constricting force of the smooth muscles of their blood vessels had increased. It is likely that the increased blood pressure was largely caused by the increase in constricting force of the smooth muscles of the arteriolar blood vessels. The increased basal contraction force of the vascular smooth muscle was not accompanied by a greater than normal reactivity of the muscle to angiotensin and noradrenaline.

The increased vasoconstriction and systolic blood pressure in the aldosterone/NaCl treated rats is reversible in character returning to control levels with discontinuance of treatment.

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