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

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

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


Fig. 1 Effect of aldosterone/NaCl treatment on the critical opening pressure (COP) and systolic blood pressure (SBP) (before ganglionic blockade).

Fig. 2 Effect of aldosterone/NaCl treatment on the COP and SBP (after ganglionic blockade)

Fig. 3 Effect of ganglionic blockade on COP and SBP before and during treatment of aldosterone/ NaCl

Fig. 4 Effect on COP and SBP of control and aldosterone/NaCl treated rats of intravenous infusion of noradrenaline.

Fig. 5 Effect on COP and SBP of control and aldosterone/NaCl treated rats of infusion of angiotensin.

Fig. 6 The increase in COP and SBP for each increase in dose of angiotensin in control and aldosterone/ NaCl treated rats.

Fig. 7 The increase in COP and SBP for each increase in dose of noradrenaline in control and aldosterone/ NaCl treated rats.

Fig. 8 COP and SBP during pre-treatment, 3rd week of aldosterone/NaCl treatment and post-treatment periods (before ganglionic blockade).

Fig. 9 COP and SBP during pre-treatment, 3rd week of aldosterone/NaCl treatment and post-treatment periods (after ganglionic blockade).

## 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