Hypertensive Mechanisms Associated with Centrally Administered Aldosterone in Dogs

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SUMMARY The mechanism by which intracerebroventricularly administered aldosterone increases arterial pressure was investigated in trained, conscious dogs with cannulas chronically implanted in a lateral cerebral ventricle. In salt-replete and salt-depleted dogs, artificial cerebrospinal fluid with or without aldosterone (0.05 μg/kg/hr) was infused intracerebroventricularly for 12 days by an osmotic minipump. A similar dose of aldosterone was infused subcutaneously for 12 days. Aldosterone infused intracerebroventricularly increased blood pressure significantly in both salt-replete and salt-depleted dogs. In salt-replete animals the hypertension was associated with increased total peripheral resistance without concomitant changes in blood volume, cardiac output, or in any of the neurohumoral parameters measured. We conclude that this type of hypertension is resistance-mediated from its outset and appears to be relatively independent of salt and water retention. The mechanism by which intracerebroventricularly administered aldosterone increases vascular resistance remains to be determined. (Hypertension 11: 750-753, 1988)

KEY WORDS • mineralocorticoid hypertension • centrally administered aldosterone • hemodynamics

High affinity specific binding sites for mineralocorticoids have been described in various areas of the brain.1-4 Quantitative autoradiography with intravenously injected [3H]aldosterone has shown a wide distribution of specific binding sites in the brain, including the anterior hypothalamus, hippocampus, anterior pituitary, nucleus ventromedialis hypothalami, nucleus ambiguus, nucleus tractus solitarii, locus ceruleus, and area postrema.3 The functional importance of these binding sites was recently examined by Gomez-Sanchez in the rat.5 She found that a dose of aldosterone that was too small to produce changes in blood pressure when infused systemically produced elevations of blood pressure when infused into the lateral cerebral ventricle. The fact that the pressor effect could be blocked by the concomitant infusion of a specific aldosterone antagonist, prorenone, lent support to the concept that these mineralocorticoid binding sites in the brain are functional and that they could subserve a role in cardiovascular regulation.

We have extended these observations by confirming them in the dog and, in addition, determined the neurohumoral and hemodynamic changes that accompany this model of hypertension. We found that the predominant factor that sustained elevated arterial pressure was an increase in systemic vascular resistance. Further, there were no detectable changes in blood volume, cardiac output, or in any of the measured neurohumoral indices during the initiation and maintenance of hypertension.

Materials and Methods

Surgical Procedures and Animal Preparation

Studies were performed in 26 male mongrel dogs weighing 17 to 23 kg. Dogs were kept in temperature-controlled and moisture-controlled rooms with lights cycled on and off every 12 hours. With the dogs under morphine and pentobarbital anesthesia, an arterial catheter (15-gauge Polydaron tube) was inserted into the right iliac artery and the distal end of the catheter tip was tunneled under the skin to the back between the scapulae. Then the head was secured in a David Kopf stereotaxic unit (Tujunga, CA, USA), and a stainless steel cannula (19 gauge) was implanted into the right lateral cerebral ventricle according to the technique described by Haley and Dickinson.6 The point of entry was determined by the following stereotaxic coordinates: 17 mm anterior to the auditory meatus, 7 mm lateral and approximately 17 mm below the dura. After
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...a brain infusion cannula (Type 92-103A, Linca Lamon Instrumentation, Tel Aviv, Israel) was placed into the skull, a stainless steel cannula was inserted until it was ascertained to be present in a lateral ventricular space by spontaneous flow of cerebrospinal fluid in and out through the cannula. A polyvinyl tube was connected to the cannula, and then its distal tip was tunneled under the skin to the posterior aspect of the neck. Three stainless steel screws were placed in the skull around a cannula holder, and the entire assembly was completely embedded in dental cement. The dead space was about 0.2 ml.7

To allow continuous intracerebroventricular (i.c.v.) or subcutaneous (s.c.) infusion, a 14-day osmotic minipump (Alzet Model 2ML2, Alza, Palo Alto, CA, USA) was implanted subcutaneously with the dogs under light halothane (Fluothane) anesthesia in the back of the neck. The pump had a nominal reservoir capacity of 2.0 ml and a pumping rate of 5.0 L/hr.

Preparation of Infusates

The composition of artificial cerebrospinal fluid (CSF) was as follows (mM): Na, 154; K, 3.3; Ca, 1.25; Mg, 1.2; Cl, 162; HPO4, 0.48; glucose, 3.4; and urea, 2.2. The pH was adjusted between 7.2 and 7.4 by aerating with 95% O2. Aldosterone (d-aldosterone, crystalline, CIBA-Geigy, Summit, NJ, USA) was rendered soluble by using an ultrasonic cleaner (Model B-22-4, Brunson, Shelton, CT, USA). It was dissolved in artificial CSF for i.c.v. infusion and in normal saline for s.c. administration.

Experimental Protocols

After recovery from the operation, all dogs underwent a training period of 3 to 6 weeks. Dogs were then divided into three groups according to treatment: 1) i.c.v. infusion of aldosterone (0.05 μg/kg/hr) in dogs on normal dietary sodium (n = 8) and in dogs on low dietary sodium (n = 6), 2) i.c.v. infusion of artificial CSF (n = 6), and 3) s.c. aldosterone (0.05 μg/kg/hr) infusion (n = 6). Normal dietary sodium consisted of 60 mEq Na, 100 mEq K (laboratory canine diet, Ralston Purina, St. Louis, MO, USA). Low dietary sodium consisted of 6 mEq Na, 100 mEq K (Prescription diet canine h/d, Hills Pet Products, Topeka, KS, USA).

All studies were performed in a quiet laboratory in the morning with the dog in the fasting state and resting comfortably on a padded laboratory bench. Studies were performed over a 12-day period. Arterial blood pressure was recorded on Days 0, 3, 6, 9, and 12. Hemodynamic studies and measurements of serum electrolytes and plasma concentrations of catecholamines, aldosterone, renin activity, and vasopressin were performed on Days 0, 6, and 12 of the study.

Hemodynamic Measurements

Arterial blood pressure was recorded directly through a femoral arterial catheter connected to a Gould Statham P23Db transducer (Saddle Brook, NJ, USA). Cardiac output was determined in triplicate using indocyanine green dye (5 mg) introduced into a plastic tube connected to a venous catheter and then flushed as rapidly as possible.8,9 Dye dilution curves were obtained by the usual method, and blood was reinflused immediately after curve inscription to avoid blood loss. Cardiac output was calculated by the Stewart-Hamilton method, and total peripheral resistance was obtained by dividing mean arterial pressure by cardiac output x 1000. Day-to-day variation in the measurement of cardiac output was 7%. Plasma volume was determined by injection of 3 ml of Evans blue dye, with the use of a 10-minute equilibration sample.9,10 Total blood volume was calculated from the diluent volume and arterial hematocrit. Variation in the measurements was 6%.

Biochemical Methods

Plasma aldosterone concentration, plasma renin activity, and plasma vasopressin were measured by radioimmunoassay techniques.7 Plasma catecholamines were measured by radioenzymatic assay.12 Serum electrolytes were measured by flame photometer.

Statistical Analysis

Data computation was accomplished using PROPHET, a national computer resource supported in part by Biotechnology Resource Program, Division of Research Resource, National Institutes of Health (Bethesda, MD, USA). Comparisons between the groups were evaluated by BMDP2V (analysis of variance and covariance including repeated measures), then the significance was obtained using unpaired t test or one-way analysis of variance followed by Newman-Keuls multiple range test between two or more groups, respectively. All values are expressed as means ± SE.

Results

Dogs that received i.c.v. aldosterone infusion had significant increases in blood pressure (Figure 1). Ele-
vations of blood pressure were evident from the third day on and became sustained thereafter. By the twelfth day of study mean blood pressure for the group averaged 122 mm Hg. Similar doses of aldosterone administered subcutaneously had no effect on blood pressure. Similarly, i.c.v. infusion of artificial CSF did not alter blood pressure over the period that it was infused.

The associated hemodynamic and neurohumoral responses are shown in Table 1. In dogs fed normal dietary sodium the predominant factor associated with elevations in blood pressure induced by i.c.v. aldosterone infusion was a rise in total peripheral resistance. Cardiac output and total blood volume were unchanged from the outset. Heart rate tended to decrease as blood pressure rose. No significant changes were apparent in the plasma levels of catecholamines, vasopressin, aldosterone, renin activity, and potassium.

Dogs on low dietary sodium also exhibited significant increases in blood pressure with i.c.v. aldosterone infusion. However, the rise in blood pressure was not evident until about the ninth day of infusion. In addition, the maximum blood pressure attained was significantly lower than that obtained in dogs receiving normal dietary sodium (122 ± 2 vs 108 ± 2 mm Hg; p < 0.01).

**Discussion**

The present study has demonstrated that 1) a dose of aldosterone that was too small to produce changes in arterial pressure when administered subcutaneously produced hypertension when infused into the lateral cerebral ventricle of the dog, 2) the hypertension was due primarily to increases in systemic vascular resistance, and 3) sodium deprivation attenuated but did not completely inhibit the development of hypertension.

These studies confirm and extend the findings of Gomez-Sanchez in the rat. In that study, a dose of aldosterone (0.005 μg/hr) equivalent to doses used in the present study produced hypertension when infused intracerebroventricularly but not when given systemically. In the rats studied the polydipsia and polyuria characteristic of the hypertension induced by systemic administration of mineralocorticoids were not present, indicating that the elevation in arterial pressure was not dependent on an increase in circulating blood volume. In addition, neither urine volumes nor body weights differed significantly between rats that became hypertensive with i.c.v. aldosterone infusion and those that remained normotensive with s.c. aldosterone infusion.

The observation that the elevated arterial pressure was due to increased total peripheral resistance suggests that it may have resulted from altered vascular sensitivity. Central administration of aldosterone may alter vascular smooth muscle sensitivity by either humoral or neurogenic pathways. Bohr has proposed the hypothesis that mineralocorticoids produce a change in plasma membrane calcium binding of a pressure-regulating center in the hypothalamus. The resultant changes in sodium fluxes alter efferent activity of the center, initiating the elevation of arterial pressure. Extensive experimental evidence has accrued in support of a putative hormone that can be released from the hypothalamus during changes in salt balance. This hormone is postulated to inhibit Na⁺, K⁺—adenosine triphosphatase activity of plasma membrane of vascular smooth muscle. This inhibition leads to elevations of intracellular sodium, to increased cytosolic free calcium, and ultimately, to increased vascular resistance.

Most experimental evidence from animal studies suggests that mineralocorticoids indirectly increase membrane permeability to sodium and elevate intracellular sodium concentration. By partially depolarizing the muscle cell membrane, the abnormalities of cation turnover lead to vasoconstriction and elevated vascular resistance. Such changes also increase metabolic activity and provide an early signal for vascular smooth muscle hypertrophy that, when combined with
elevated arterial pressure, could lead to thickening of the media and so raise the wall-to-lumen ratio. This structural adaptation, implying enhanced reactivity, could be crucial for both potentiating and maintaining the hypertensive process.16–18

Abnormal neurogenic influences from altered tone of the putative pressor center could cause changes in vascular smooth muscle sensitivity. These neurogenic influences could stem from alterations in norepinephrine release and uptake19 or changes in membrane potential of vascular smooth muscle.20 The vascular smooth muscle of the spontaneously hypertensive rat has been shown to have a less negative resting membrane potential, a reduced K+ equilibrium potential, and an enhanced electrogenic component to the resting membrane potential. These aberrations are associated with increased adrenergic influence on the vasculature. Some studies have also shown that intrathecally administered 6-hydroxydopamine prevents the increased vascular response to norepinephrine and arginine vasopressin in deoxycorticosterone acetate–saline hypertensive rats,21 but not when given by the intravenous22 or intraspinal route.23 In the present studies, plasma catecholamine concentrations did not change during the development and maintenance of hypertension. However, this finding does not preclude abnormal neurogenic influences, since changes in baroreceptor reflex function may occur without changes in circulating plasma catecholamine concentration.

The hypertension induced by i.c.v. aldosterone infusion differs in certain aspects from that obtained by systemic administration of mineralocorticoids. In the former, neither blood volume nor cardiac output is altered during the development and maintenance of hypertension. In addition, hypertension develops (albeit modified) despite severe salt restriction. In the latter, the development of hypertension is paralleled by salt and water retention with expansion of intravascular volume and is reversed on a low salt diet.5,24 However, both share a common hemodynamic basis for the elevation of arterial pressure (i.e., by increases in total peripheral resistance). Whether hypertension induced by i.c.v. aldosterone infusion has relevance to so-called mineralocorticoid hypertension remains to be clarified.

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