Sodium, Extracellular Fluid Volume, and Cardiac Output Changes in the Genesis of Mineralocorticoid Hypertension in the Intact Dog

KAORU ONOYAMA, M.D., EMMANUEL L. BRAVO, M.D., AND ROBERT C. TARAZI, M.D.

SUMMARY Twenty-four trained, conscious dogs with chronically implanted iliac catheters were used to assess the relative roles of sodium (Na) and volume in the development of mineralocorticoid hypertension. All were maintained on restricted Na diets; supplemental Na was given as slow I.V. infusions of 0.9% NaCl solution. The effect of gradual replenishment of total body sodium on arterial blood pressure (BP), cardiac output (CO), total peripheral resistance (TPR) and extracellular fluid volume (ECFV) was assessed at four separate levels of cumulative Na intake (70, 140, 420 and 840 mEq). The sequence of changes in 12 metyrapone-treated dogs was compared with 12 untreated controls submitted to the same protocol. Changes in fluid volumes and CO were virtually identical in the two groups; however, only dogs previously treated with metyrapone became significantly hypertensive (ΔMAP > 16 and 28 mm Hg, p < 0.001). Within the metyrapone-treated groups, increases in volume and flow led to markedly different TPR responses dependent on level of cumulative sodium intake; at levels ≤ 140 TPR was lowered as output increased, but as levels > 140 TPR and BP rose in the face of increases in output and ECFV that were not greater than those found at 140 mEq cumulative Na intake. These findings suggest that this steroid-induced hypertension in the dog is “resistance-mediated” from its earliest stages, and that restoration of sodium stores rather than volume expansion played the more important role in the initiation of this hypertension. (Hypertension 1: 331-336, 1979)

KEY WORDS • mineralocorticoid hypertension • sodium • extracellular fluid volume • cardiac output • resistance mediated hypertension • autoregulation

It is clearly established that the hypertension induced by electrolyte-active steroids is salt and water dependent. In both the clinical and experimental forms of this disorder, salt loading increases while salt deprivation decreases arterial blood pressure. 1, 2 Since both maneuvers also result in parallel changes of blood and extracellular fluid volumes, it has never been possible to define which of two factors (i.e., sodium or volume loading) play the more crucial role.

The present study was designed to dissociate the two factors and focus on the role of sodium in the genesis of metyrapone-induced hypertension in the conscious dog. To achieve that end, sodium-deprived dogs were used; this allowed a quantitative assessment of hemodynamic and volume alterations associated with gradual replenishment of total body sodium. The sequence of changes in metyrapone-treated dogs was compared with untreated controls submitted to the same protocol; changes in fluid volumes and cardiac output were virtually identical in the two groups. Despite the similarity in response to volume and systemic flow, only dogs previously treated with metyrapone became significantly hypertensive indicating that this steroid hypertension in the dog was “resistance-mediated” from its earliest stages. Furthermore, within the metyrapone-treated group, changes in total peripheral resistance for apparently similar increases in volume and flow were markedly different at separate levels of sodium repletion.

Methods

Experimental Protocol

Studies were performed on 24 trained, conscious dogs with chronic indwelling femoral arterial catheters. After a training period of 2-4 weeks, all dogs received a diet containing 1.4 mEq Na and 60 mEq K per day; half of the dogs received, in addition,
metyrapone orally in doses of 100 mg/kg/day. Two weeks after initiating sodium deprivation, six of the treated dogs received 10 mEq/day of supplemental sodium for 14 days, while the remaining six received 60 mEq/day for the same number of days. The untreated group served as appropriate controls. Supplemental sodium was administered as slow I.V. infusions of 0.9% sodium chloride solution.

A breakdown of the groups investigated is shown in Table 1. In both control and treated groups, sodium stores were gradually replenished allowing a quantitative assessment of the hemodynamic effects of four separate levels of cumulative sodium intakes. Measurements of arterial blood pressure (BP), cardiac output (CO), plasma electrolytes, plasma volume (PV) and extracellular fluid volume (ECFV) were obtained at least twice before the start of sodium replacement, and then at weekly intervals during sodium replacement. All measurements were done 24 hours following the latest saline infusions.

### Table 1. Breakdown of the Groups Investigated

<table>
<thead>
<tr>
<th>Group</th>
<th>Daily Na replacement (mEq)</th>
<th>Cumulative Na intake (mEq)</th>
<th>Serum Na (mEq/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I A</td>
<td>10 (X 7 days)</td>
<td>70</td>
<td>144 ± 1</td>
</tr>
<tr>
<td>I B</td>
<td>10 (X 14 days)</td>
<td>140</td>
<td>144 ± 1</td>
</tr>
<tr>
<td>II C</td>
<td>60 (X 10 days)</td>
<td>420</td>
<td>146 ± 1</td>
</tr>
<tr>
<td>II D</td>
<td>60 (X 10 days)</td>
<td>840</td>
<td>145 ± 1</td>
</tr>
</tbody>
</table>

Each group consisted of 12 dogs (6 treated with metyrapone and 6 untreated). Sodium replacement was intravenously administered as 0.9% NaCl solution. Sixty mEq is the normal daily Na intake of a dog. Values for serum Na are expressed as Mean ± SE. None of the differences achieved statistical significance.

### Hemodynamic Measurements

All investigations were performed in a quiet laboratory in the morning with the dogs fasting and resting comfortably on a padded laboratory bench. Blood pressure was measured with Statham P23Db transducers; CO was determined in triplicate using indocyanine green dye (5 mg) as previously described. Dye-dilution curves were obtained by the usual method and blood was rein infused immediately after curve inscription to avoid blood loss. Cardiac output was calculated by the Stewart Hamilton method and expressed in ml/kg; total peripheral resistance (TPR) was obtained by dividing mean arterial pressure (MAP) by CO × 1000, and expressed in arbitrary units. Variations in the measurement of BP and CO are 2% and 8%, respectively.

### Analytical Methods

The ECFV was measured as the distribution volume of bromine-82 after 30 minutes of equilibration and PV with Evans blue dye after 10 minutes of equilibration as previously described. Variations in the measurement of ECFV and PV are 3% and 6%, respectively. Plasma renin activity (PRA) was measured by the radioimmunoassay technique for angiotensin I described by Haber et al. The PRA values for conscious dogs receiving a daily sodium intake of 60 mEq average 1.1 ± 0.4 (SE).

### Statistical Analysis

The Student's t test was used to assess statistical significance of differences between groups; however, for analysis of changes within each group, paired t test was applied and results expressed as mean ± SE.

### Results

Baseline measurements in treated and untreated dogs before initiation of studies are summarized in Table 2. Hemodynamically, the two groups did not differ in either BP, CO or calculated TPR; only resting heart rate was significantly higher in untreated dogs. Similarly, the groups did not differ in either weight, plasma or ECF volumes, or in resting PRA.
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Relationship of BP Responses to Changes in Fluid Volumes

With increasing cumulative sodium intake, similar directional changes in plasma and ECF volumes occurred in both groups (fig. 1). However, the rise in ECFV with higher levels of Na was limited, in part, by some degree of natriuresis indicated by return of intravascular volume in both groups to control values after an initial rise. Concomitant changes in body weight occurred during this phase. The return of PV toward control values was completely dissociated from the BP response indicating that the change was, in large part, brought about by volume-mediated mechanisms, acting directly on the kidney.

Relationship of BP Responses and Changes in Cardiac Output to Cumulative Na Intake

Arterial pressure in treated dogs did not begin to rise significantly (ΔMAP = 16 ± 2 mm Hg, p < 0.01) until more than 140 mEq cumulative sodium intake was attained. At a cumulative sodium intake of 840 mEq (60 mEq × 14 days), MAP increased further (ΔMAP = 28 ± 3 mm Hg, p < 0.001). Blood pressure remained unchanged in all untreated dogs at all levels of cumulative sodium intake (fig. 2).

Changes in CO were virtually identical at all levels of sodium repletion in both groups. The difference in BP response can, therefore, only be attributed to the response of TPR during sodium repletion. Although untreated dogs responded appropriately to increased output by fall in resistance, metyrapone-treated dogs had either a rise in TPR or were unable to vasodilate in the face of increasing flow.

These relationships in metyrapone-treated dogs are best summarized in figure 3. The uninterrupted line describes a full range of relationships between output and resistance when MAP is 87 mm Hg. The interrupted line describes a similar range of relationships when the MAP is 120 mm Hg. Both isolines were derived from actual experimental data. Starting from similar levels of output and TPR, metyrapone-treated dogs that attained cumulative sodium intakes of 70 and 140 mEq did not develop hypertension despite an increase in CO because of reciprocal alterations in TPR. In dogs attaining cumulative sodium intakes of 420 and 840 mEq, cardiac output rose to similar values as in the lower intake, but hypertension developed because of concomitant increases in TPR.

Discussion

Restoration of sodium stores failed to alter arterial blood pressure in untreated dogs while, in contrast, it
led to significant hypertension in metyrapone-treated dogs. Changes in cardiac output and fluid volumes were virtually identical at every stage in the normotensive and hypertensive dogs. These findings suggest that changes in flow, which occurred in all dogs following salt and water administration, were not responsible for the subsequent rise in arterial pressure. The increased arterial pressure in metyrapone-treated dogs was clearly due to a rise in TPR when these dogs attained a critical amount of cumulative sodium intake.

Two conclusions emerge: first, that this steroid "salt and water dependent" hypertension was clearly "resistance-mediated" from its early stages; and second, and possibly more important, that the association of sodium repletion with mineralocorticoid excess rather than the volume expansion associated with intake of salt, played the more important role in its initiation. The latter conclusion is based upon the demonstration that despite the similarity in response of volume and systemic flow to sodium repletion, only dogs previously treated with metyrapone became significantly hypertensive. These conclusions were based on actual determinations of both volume (PV, ECFV) and flow (CO) and not on extrapolations from one variable to another since such extrapolations are not warranted.

These studies have allowed a quantitative evaluation of the relationship between sodium intake and TPR changes in the presence of excess electrolyte-active steroids. Increases in CO by as much as 20% were not associated with increased TPR or BP when cumulative sodium intake was limited to 140 mEq. In contrast, equivalent increases in CO and in ECF volumes were associated with a rise in TPR and BP only at much higher cumulative sodium intakes. Of additional considerable importance was the demonstration that the rise in BP was not time-dependent, but related entirely to the amount of sodium given. Thus, infusion of 10 mEq Na for 14 days (total cumulative sodium = 140 mEq) failed to modify BP although both volume and CO were elevated, while the administration of 60 mEq Na for 7 days (total cumulative sodium = 420 mEq) produced significant increases in BP at levels of ECFV and CO not significantly different from those produced by 140 mEq cumulative sodium intake.

Current speculations about the mechanism(s) underlying the increase of peripheral vascular resistance in mineralocorticoid hypertension include the following: 1) total body vascular autoregulation in response to increased cardiac output and tissue perfusion, 2) enhanced activity of the peripheral sympathetic nervous system, 3) direct or indirect myogenic effect of electrolyte-active steroids and 4) altered ion distribution in the vascular smooth muscle cell, both active and passive.

The volume expansion associated with sodium retention has been proposed as the pivotal factor in the pathogenesis of this type of hypertension via the resulting increases in blood volume and cardiac output. The results of the present study indicate that neither changes in fluid volumes nor in cardiac output were the essential mechanisms responsible for the development of hypertension. The demonstration of similar effects of salt and water repletion on fluid volumes and cardiac output, regardless of blood pressure response under these conditions, is consistent with this conclusion. Results from other studies provide additional evidence in support of the concept that a phase of high CO is not always essential for the development of hypertension induced by electrolyte-active steroids. The pig treated with deoxycor-
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tosterone acetate (DOCA) develops hypertension within 3 to 5 days without any demonstrable increases in CO. In both the DOCA-saline and metyrapone-treated hypertensive dogs, the rise in arterial pressure was not uniformly associated with increases in CO nor did prevention of rises in CO by beta-blockade inhibit the development of hypertension.

Since neither increases in fluid volumes nor in CO can sufficiently account for the rise in TPR, alternative explanations must be sought. The sympathetic nervous system, although important, does not appear to be essential in the development of this type of hypertension in the dog. We have previously shown that, in this model, plasma norepinephrine (NE) concentrations were decreased rather then increased. In addition, the prior administration of either centrally or peripherally acting adrenergic blocking drugs could not prevent the development of hypertension, although the values of plasma NE concentrations were reduced further.

Enhanced sensitivity of vascular smooth muscle to vasoconstrictor substances during administration of electrolyte-active steroids has been demonstrated in a number of studies. Schmid et al. showed that constriction of resistance and capacitance vessels in response to NE is potentiated by treatment with 9α-fluoro-hydrocortisone. Berecek and Bohr found a distinct lowering of threshold doses for both angiotensin II and NE during the early stages of the development of hypertension in the DOCA hypertensive pig. Hinke demonstrated hyperresponsiveness to exogenous vasopressin in DOCA hypertension of rats, while Mohring and co-workers recently reported some observations suggesting a vasopressor role of antidiuretic hormone in the pathogenesis of malignant, but not benign, DOCA hypertension of rats. It also appeared unlikely that the increased TPR was related to the renal pressor system since plasma renin activity was suppressed in these dogs before and more so during studies.

This increased vascular reactivity could be theoretically related to changes in potassium since decreases in plasma potassium (< 3.0 mEq/liter) were found to increase vascular resistance. However, in our studies plasma potassium in hypertensive dogs averaged 3.9 mEq/liter. In addition, other studies have shown that the local vascular changes encountered in the DOCA-saline hypertensive rat were independent of potassium balance. Providing supplemental potassium in these rats countered weight loss and symptomatology without influencing the progress of vascular changes and hypertension. Similar results were obtained by Conway and Hatton in the DOCA-saline hypertensive dog.

The foregoing considerations led us to examine the evidence that, in this experimental model, primary changes in vascular smooth muscle could account entirely for increased TPR and the eventual rise in arterial blood pressure. A possible sequence of events is depicted in figure 4. Considerable evidence has accumulated indicating that induction of hypertension in animals with DOCA leads to altered membrane properties of vascular smooth muscle. This evidence has accrued largely from the work of Jones and Hart, Friedman and Friedman and Berecek and Bohr. This altered membrane property has been identified as an increase in membrane permeability that, in turn, leads to abnormal cation turnover. Abnormalities of cation turnover could, by several mechanisms, lead to vasoconstriction, increased peripheral vascular resistance, and finally to increased arterial blood pressure. Increased cation turnover would be expected to increase metabolic activity and may provide an early signal for vascular and smooth muscle hypertrophy; combined with rising arterial blood pressure the results of these stimuli could lead to thickening of the media and increase the wall:lumen ratio. This structural change, with its enhancement of vascular reactivity, could be crucial for both potentiating and maintaining the hypertensive process. Of considerable interest is the observation made by Jones and Hart that neither salt nor DOCA alone led to the vascular changes but that their simultaneous administration produced the altered ion transport. In addition, they have found that such changes preceded the onset of hypertension.

Our findings in the intact, conscious animal confirm and extend the growing evidence accumulated from in vitro studies indicating that alterations in vascular smooth muscle sensitivity may have a primary role in the initiation of hypertension. Furthermore, they emphasize that sodium, apart from its effect on fluid volumes, could interact with electrolyte-active steroids on vascular smooth muscle to alter ion transport resulting in increased vascular resistance and hence, arterial blood pressure.

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