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Steroid secretion was studied in conscious dogs before and during 4 days of Na depletion. A threefold increase in aldosterone secretion occurred but corticosterone output was unchanged. Plasma renin was markedly elevated. Plasma Na and K concentrations were unchanged until the 4th day of Na depletion at which time plasma Na was decreased and plasma K was elevated. The Na content of the adrenal cortex expressed as fat-free tissue solids was decreased. It seems likely that the adrenocortical Na loss was extracellular; adrenocortical K content was unaltered. It is suggested that the normal rate of corticosterone secretion during Na depletion is maintained by a negative corticosteroid feedback mechanism, since corticosterone output was consistently increased in Na-depleted hypophysectomized dogs. Aldosterone secretion was three times as great in the presence as in the absence of the anterior pituitary during Na depletion. It is concluded that increased activity of the renin-angiotensin system is the primary mechanism leading to hyperaldosteronism during Na depletion and that the adenohypophysis plays an important supportive role.

Methods

The physiological studies were carried out on conscious mongrel dogs. The animals were kept in metabolic-cage balance cages and fed either a diet containing 60 mEq of Na and 18 mEq of K daily or a low-Na diet containing 1-2 mEq of Na and 40 mEq of K daily. Sodium depletion was accomplished by giving the dogs 2 ml of mercychydrin intramuscularly daily for 4 days while they were on the low-Na diet. The urinary bladder was catheterized to end each daily urine collection period and urine was analyzed for Na and K with a flame photometer.

For the blood pressure measurements the dogs were selected and only animals which were extremely quiet were used. A chronic indwelling femoral artery catheter was inserted, and blood pressures were taken daily in the morning with minimal disturbance of the animals. A Statham pressure transducer and a Sanborn recording system were used; heart rate was determined from the tracing.
Hypophysectomy was performed by an oral route (17). Adrenal vein blood was obtained from conscious animals by means of a previously placed lumbaradrenal vein catheter. The concentrations of aldosterone and corticosterone in adrenal vein plasma were measured by the double isotope derivative assay technique of Kliman and Peterson (18).

Tissue electrolytes. Fifteen adult mongrel dogs weighing 15–28 kg were used in the first part of this study; eight of the dogs were fed the synthetic diet containing 60 mEq of Na daily and seven dogs were given the low-Na diet for 1 week and 2 ml of mercurhydrin daily for the first 4 days. At the end of 1 week the animals were killed with a large dose of sodium pentobarbital. The adrenal glands and psoas muscle were removed for analysis of Na, K, water, and fat content by methods previously described (27). Peripheral venous plasma was drawn for measurement of Na and K. FFTS were used as the basis for comparing the water and electrolyte content of tissues from different groups of animals.

On account of the changes observed after 1 week of Na depletion, this study was extended to include nine more adult mongrel dogs weighing 16–24 kg which were given the low-Na diet and 3 ml mercurhydrin intramuscularly for 1 day only. The adrenal glands, adrenal cortex, and plasma were analyzed by the same techniques used in the 7-day Na-depletion series.

Renin and renin substrate. Peripheral venous blood was obtained from a group of normal dogs and a group of dogs depleted of Na for 4 days. The methods of Helmer (14) and Warzynski et al. (25) were used without alteration to prepare plasma for the assay of renin. A third technique was also employed (M. Bumpus, personal communication) in which 20 ml of venous blood were centrifuged at 0 C, the pH of the plasma was adjusted to 6.5 with 1 N HCl, and the plasma was dialyzed against disodium ethylenediaminetetraacetate solution (disodium EDTA, 2.2 g/liter) for 14–18 hr, and then dialyzed against distilled water for 24 hr. The pH of the solution was adjusted to 5.5 with 1 N HCl and the solution incubated for 4 hr at 37 C. Distilled water was added to bring the total volume of the solution to 19 ml, and 1 ml of normal saline was added. The pH was adjusted to 5.0, the material was boiled for 10 min and centrifuged; 0.1 ml of 10 N saline was added to 0.9 ml of the supernatant fluid and the material assayed. In all three procedures, plasma was incubated to produce angiotensin II which was assayed by its pressor activity in the anesthetized, vagotomized rat treated with pentolinium tartrate. The same plasma samples were used for the Bumpus and Helmer methods, but plasmas from different Na-depleted animals were studied by the technique of Warzynski et al. (25). In addition, estimations of renin in plasma prepared by the Helmer technique were made on a group of five normal dogs and on seven dogs given the low-Na diet for 1 day only with one injection of 3 ml of mercurhydrin intramuscularly.

Renin substrate was measured in plasma from six normal dogs and from the same five Na-depleted dogs used above for plasma renin measurements by the methods of Helmer and Bumpus. The method for measuring renin substrate has been described previously (15, 16).

RESULTS

Steroid response to 4 days of Na depletion. A standard regimen for Na depletion was used and the average values for the response in seven dogs are presented in Fig. 1. Aldosterone secretion was significantly elevated after 1 day of Na depletion during which Na loss was 101 mEq. Aldosterone secretion remained high but failed to increase further during continued Na depletion for 4 days. Cumulative negative Na balance was 193 mEq at the end of 4 days. Corticosterone secretion was unaffected by Na depletion. Plasma Na and K concentrations were not significantly changed until the 4th day, at which time plasma Na was low and plasma K was high.

Peripheral plasma renin and renin substrate. Measurements of renin were made to provide evidence of renin activity in plasma under the conditions used to produce Na depletion. After 4 days of Na depletion, the plasma level of renin was increased fourfold by the Bumpus technique and eightfold by the method of Helmer (Table 1). In another series of normal and Na-depleted dogs, a sixfold elevation in plasma renin was observed by the technique of Warzynski (Table 1). With all three techniques, the experimental values were statistically significantly higher than the normal values.

In another group of dogs, after 1 day of Na depletion plasma was prepared for assay by the technique of Helmer. An average value of 15.7 ng of angiotensin was formed per milliliter of plasma. This indicates a fourfold elevation in plasma renin when compared with the value

![Fig. 1. Alterations in steroid secretion and plasma electrolytes in response to 4 days of Na depletion.](http://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/)
TABLE 1. Plasma renin and net sodium loss after 4 days of sodium depletion*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Negative Sodium Balance for Sodium-Depleted Dogs, mEq</td>
<td>Normal dogs</td>
<td>Sodium depleted</td>
<td>Normal dogs</td>
</tr>
<tr>
<td>Mean</td>
<td>112</td>
<td>3.9</td>
<td>20.1</td>
</tr>
<tr>
<td>SEM</td>
<td>±1.2</td>
<td>±1.8</td>
<td>±2.8</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Values for both normal and sodium-depleted dogs, in all three methods, are expressed as ng angiotensin-like activity formed per ml plasma. * Sodium depletion was effected with a low-Na diet plus 2 ml of mercuhydrin im daily.

TABLE 2. Plasma electrolytes, adrenal weights, and water and electrolyte content of adrenal cortex of normal and sodium-depleted dogs

<table>
<thead>
<tr>
<th>Total Negative Sodium Balance, mEq Na</th>
<th>Plasma Electrolytes, mEq/liter</th>
<th>Adrenal Gland</th>
<th>Adrenal Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>K</td>
<td>Na</td>
</tr>
<tr>
<td>Normal dogs</td>
<td>142</td>
<td>3.8</td>
<td>0.0805</td>
</tr>
<tr>
<td>SEM</td>
<td>±1.1</td>
<td>±0.2</td>
<td>±0.004</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Dogs depleted of sodium for 1 day</td>
<td>Mean</td>
<td>92</td>
<td>3.8</td>
</tr>
<tr>
<td>SEM</td>
<td>±2.1</td>
<td>±0.2</td>
<td>±0.03</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Dogs depleted of sodium for 1 week</td>
<td>Mean</td>
<td>179</td>
<td>4.7</td>
</tr>
<tr>
<td>SEM</td>
<td>±1.4</td>
<td>±0.2</td>
<td>±0.004</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pi</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

P represents statistical comparison with the series of normal dogs. Pi represents statistical comparison with dogs depleted of Na for 1 day.

of 3.9 for normal dogs (Table 1), and this difference is significant (P < 0.01).

No significant difference was found between the levels of renin substrate in plasma from normal and Na-depleted dogs. The mean values for renin substrate in peripheral plasma from normal and Na-depleted dogs were 82 and 70 ng, respectively, of angiotensin formed from 0.1 ml of plasma in 1 hr.

Water and electrolyte changes in tissues. Analyses of adrenal cortex and psoas muscle for electrolyte and water content revealed significant changes. The Na content of adrenal cortex expressed in milliequivalents per 100 g of FFTS was significantly decreased after 1 week of Na depletion (Table 2). This change was associated with a significant reduction in plasma Na concentration and an increase in the plasma K level. Since Na depletion for 1 week was severe enough to produce a reduction in the plasma Na level, measurements were made of tissue electrolytes after only 1 day of Na depletion. In this situation, adrenocortical Na content was reduced but plasma Na concentration was normal (Table 2). The decrease in adrenocortical Na was no greater after 1 week of Na depletion than after 1 day of Na loss, although net Na loss was twice as great after 1 week as after 1 day. The K content, the water content, and FFTS of adrenal cortex were unaltered during Na depletion. Adrenal weight in grams per kilogram of initial body weight was increased in both the 1-day and the 1-week Na-depletion series.

Analyses of psoas muscle also showed a reduction in Na content from 11.5 to 0.9 mEq/100 g FFTS for the 1-week Na-depleted dogs (P < 0.05). As with adrenal cortex, the K concentration, water content, and FFTS were unchanged in psoas muscle after 1 week of Na depletion. Steroid response of hypophysectomized dogs to Na depletion. This experiment was carried out to investigate the role of the corticosteroid feedback mechanism in the control of the rate of corticosterone secretion during Na deple-
The results of 4 days of Na depletion in a hypophysectomized dog are presented in Fig. 2. In this dog, the increase in aldosterone production was accompanied by a 10-fold increase in corticosterone output. The average response to 4 days of Na depletion for all six hypophysectomized animals is presented in Fig. 3; corticosterone secretion was significantly increased throughout the 4-day period. There was a decrease in plasma Na and an increase in plasma K, as in the dogs with an intact pituitary, and the steroid changes occurred before the plasma electrolyte changes.

The importance of the anterior pituitary for the steroid response to Na depletion is also demonstrated by comparing the values observed for aldosterone secretion in intact dogs with the data obtained in hypophysectomized dogs (Fig. 4). The basal control values before Na depletion in the hypophysectomized dogs were about one-third of normal. During the 4 days of Na depletion, a definite increase in aldosterone secretion occurred in the hypophysectomized dogs, but the rate of aldosterone secretion was only approximately one-third that observed in the presence of the anterior pituitary. This difference appears to be related to anterior pituitary hormones, since the plasma electrolyte changes were essentially the same in normal and hypophysectomized Na-depleted dogs.

Alterations in arterial pressure and heart rate during sodium depletion. Arterial pressure and heart rate were measured in three dogs before, during, and following 4 days of Na depletion (Table 3). There was no change in mean arterial pressure, but a definite decrease in pulse pressure occurred in all three animals.

DISCUSSION

Evidence for the role of the renin-angiotensin system in the control of aldosterone secretion during Na depletion has been obtained by study of the effects of nephrectomy (8). In hypophysectomized Na-depleted dogs, hypersecretion of aldosterone was a common occurrence, and bilateral nephrectomy reduced aldosterone secretion to a very low level. This finding implies that the primary mechanism leading to increased aldosterone secretion during Na depletion in the dog is dependent on the kidney. Also, bilateral nephrectomy of hypophysectomized dogs with hyperaldosteronism secondary to thoracic caval constriction markedly reduced aldosterone secretion (8). Although the first studies (1, 2) of the effect of nephrectomy in anesthetized, hypophysectomized sheep failed to demonstrate a fall in aldosterone secretion, recently Denton (12) reported that nephrectomy reduced aldosterone production in conscious Na-
depleted sheep to a very low level within 4–5 hr, and he suggested that the renin-angiotensin system is the primary mechanism leading to hypersecretion of aldosterone during Na depletion. Additional evidence for the role of the renin-angiotensin system in the hypersecretion of aldosterone during Na depletion was provided by Brown and associates (3, 4) and by Winer (26). These workers reported that the peripheral plasma level of renin was elevated in man during either a low-Na intake or Na depletion.

The present data support the earlier evidence that the renin-angiotensin system is the primary mechanism leading to increased aldosterone production during Na depletion. From the present observations after only 1 day of Na depletion, a high plasma level of renin and a marked increase in aldosterone secretion were present. After 4 days of Na depletion an eightfold rather than a fourfold increase in plasma renin was evident during measurements of renin in plasma prepared for assay by the Helmer technique (14). Since the completion of the present experiments, a marked increase in both plasma renin and aldosterone output has been observed within 2 hr after the intramuscular administration of mercuhydrin (5); these changes were associated with a striking loss of Na and water. The close temporal changes in plasma renin and aldosterone secretion strongly suggest a causal relationship.

In the present study, three different techniques were used for assay of plasma renin. It is of interest that dogs subjected to the same regimen for Na depletion showed a marked elevation in plasma renin by all three techniques. In all three procedures the final product formed by incubation was assayed for its pressor activity. It has been demonstrated previously (16) that angiotensin II is formed during preparation of plasma by the Helmer technique. For these procedures to be a valid measure of renin, it is necessary to demonstrate that renin substrate is not a limiting factor for the formation of angiotensin II in normal dog plasma. The present data show that the level of renin substrate was not significantly different between plasma from normal and from Na-depleted dogs.

The nature of the signal to the kidney for release of renin remains a complicated unresolved problem. The present observations failed to show a drop in mean arterial pressure. Pulse pressure was consistently decreased, but there is convincing evidence (11, 22) that decreased pulse pressure is not the signal for release of renin. Recently, Vander and Miller (24) have suggested that the sensor in the kidney is the macula densa which responds to a decreased Na load or some related parameter. They reported that chlorothiazide and osmotic diuretics blocked the acute release of renin which occurs in response to aortic constriction. The finding (5) that the acute response to mercuhydrin results in increased renin and hypersecretion of aldosterone during a marked natriuresis seems incompatible with the hypothesis of Vander and Miller. It is possible, however, that chlorothiazide, which Vander and Miller reported blocked renin release, acts at a different renal tubular site from mercuhydrin.

It is clear that a low plasma Na or a high plasma K concentration stimulates aldosterone secretion by a direct action on the adrenal cortex (1, 10). However, neither hyponatremia nor hyperkalemia is a primary mechanism during Na depletion because plasma electrolytes are usually normal. In the present study, plasma Na and K were not altered consistently until the 4th day of Na depletion. At this time, it seems likely that alterations in both plasma Na and K contributed to the increase in aldosterone secretion.

Another possible mechanism which might be operative
during Na depletion and increased aldosterone secretion is a change in intracellular electrolytes. Since it is not possible to measure accurately intracellular Na or K with available methods, it was decided to measure the Na and K content of tissues and to relate tissue Na and K to the FFTS. One significant finding during Na depletion was the decrease in Na content of both the adrenal cortex and psoas muscle. Measurements of the Na content of the adrenal cortex showed that decreased Na in terms of FFTS occurred after 1 day and after 1 week of Na depletion. Since the K content of the adrenal cortex expressed in terms of FFTS was unaltered, the data are more consistent with an extracellular than an intracellular loss of Na. It is of interest that the Na and K content of the adrenal cortex in terms of FFTS was unaltered in dogs with hyperaldosteronism secondary to thoracic caval constriction and in dogs with experimental heart failure (27); in both experimental situations, the renin-angiotensin system provides the primary mechanism for control of aldosterone secretion (6-8, 11, 15, 16).

There have been recent conflicting reports on the role of the anterior pituitary in the control of aldosterone secretion during Na depletion. Blair-West and associates (2) pointed out the potent aldosterone-stimulating action of ACTH in sheep and showed that aldosterone secretion declined after hypophysectomy in sheep with mild Na deficiency. On the other hand, Slater and associates (23) were unable to detect a fall in aldosterone secretion 18 hr after apparent hypophysectomy of six dogs deprived of NaCl. Since the secretion rates for cortisol were 70, 74, and 233 mcg/min in three of these six animals after the hypophysectomy procedure, the data suggest that hypophysectomy was incomplete and release of ACTH from the remaining anterior pituitary could account for failure of aldosterone secretion to decrease. In one of the remaining three dogs, aldosterone secretion clearly decreased from 23.7 to 8.5 mcg/min. It should also be mentioned that measurements 18 hr after hypophysectomy might be associated with decreased pressure and flow of blood through the kidney; in such instances, increased renin release would be expected.

The role of the anterior pituitary in the regulation of adrenal steroid production during Na depletion is evident from the present data. Corticosterone secretion increased in hypophysectomized dogs but was unchanged in normal animals during Na depletion. This finding is consistent with the view that a negative corticosteroid feedback mechanism is responsible for the low rate of corticosterone secretion during chronic Na depletion in intact normal dogs.

The importance of the anterior pituitary in mediating the increase in aldosterone secretion during Na depletion is also demonstrated by comparative data in normal intact dogs and in hypophysectomized animals. The rate of aldosterone secretion was three times as great in intact Na-depleted as in hypophysectomized Na-depleted animals. This finding is in agreement with an earlier observation (9) that hypophysectomy resulted in a marked fall in aldosterone secretion in dogs with hyperaldosteronism secondary to thoracic caval constriction; repeated daily observations were made in these conscious dogs over a period of several days after hypophysectomy. It is suggested that the anterior pituitary plays a supportive role in aldosterone production during Na depletion, as in caval constriction, while the renin-angiotensin system provides the primary mechanism. A plausible relationship of the anterior pituitary and the renin-angiotensin system to the adrenal cortex in the control of aldosterone secretion is depicted in Fig. 5.
REFERENCES