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The Role of the Adrenal Glands in Sodium Excretion Evoked by Centrally Administered Renin[1] (41467)

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Abstract. Renin or angiotensin, when administered into the central nervous system, increases urinary sodium excretion but the mechanism is undefined. In addition, central angiotensin injections reduce plasma aldosterone concentration. Thus, diminished mineralocorticoid effects on the kidneys may explain the natriuresis. This hypothesis was tested by intracerebroventricular (IVT) renin injections (5 mGU/5 μl) in conscious, hydrated rats pretreated 90 min beforehand with *d*-aldosterone (20 μg/kg ip). Aldosterone did not affect renin-induced sodium output at 3 and 6 hr, although it reduced the Na/K ratio at 3 hr. Other rats were sham-operated or bilaterally adrenalectomized. Four days later, they were pretreated with aldosterone and given an oral water load followed by IVT renin or saline. Basal sodium excretion in adrenalectomized rats was reduced; natriuresis after renin was still evident but of reduced magnitude compared to sham rats. Although IVT renin and angiotensin lower the plasma aldosterone concentration, the natriuretic effect of IVT renin is independent of mineralocorticoids. Likewise, natriuresis can occur in the absence of the adrenal glands, although the magnitude is reduced.

Recent reviews present evidence for a complete renin–angiotensin system (RAS) in brain tissue (1–3). Exogenous administration of renin or angiotensin into the central nervous system markedly affects body hydration. For example, 5-day cerebroventricular (IVT) infusions of angiotensin II in conscious rats increased water intake, urine volume, and sodium excretion; plasma [Na^+] was decreased (4, 5). Also, IVT renin and angiotensin reduced plasma aldosterone concentration and renin activity (4, 6). These results form the basis for the hypothesis that the brain RAS is reciprocally related to the renal RAS and thus functions to defend the body against hypernatremia and hypervolemia (4).

Many investigators have attempted to define the mechanism of the natriuretic response after IVT renin or angiotensin. Brooks and Malvin (7), in anesthetized dogs, clearly showed that natriuresis is not related to systemic blood pressure or whole-kidney hemodynamics. Similar conclusions were reached in anesthetized cats (8) and conscious rats (9). Renal denervation partly reduced natriuresis after IVT angiotensin in anesthetized cats (10), but this procedure did not affect sodium excretion in conscious rats (11). A role for vasopressin has been suggested because homozygous Brattleboro rats exhibit a blunted natriuresis after IVT hypertonic NaCl (12). However, centrally administered vasopressin is not natriuretic in conscious rats (11).

A possible mechanism for natriuresis is withdrawal of mineralocorticoid activity in the kidneys. Biochemical studies support this possibility. IVT renin or angiotensin reduced plasma renin activity and plasma aldosterone concentration (4, 6, 13). The present report examines the importance of aldosterone and the adrenal glands in two ways. First, renin was given IVT to rats pretreated with saline or *d*-aldosterone to determine if excess mineralocorticoid altered sodium excretion. Second, IVT renin was given to adrenalectomized rats (or sham-operated controls) after pretreatment with exogenous aldosterone. These experiments were designed to assess whether the presence of the adrenal glands was an

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absolute requirement for natriuresis evoked by IVT renin or angiotensin.

Materials and Methods. *General procedures.* Adult male Sprague–Dawley rats (300–475 g) were used in all experiments. A cannula (14) was implanted in the left lateral cerebroventricle under pentobarbital anesthesia (40 mg/kg ip); at least 3 days were allowed for recovery. Rats were housed six per cage in a temperature and humidity controlled room with a 12-hr light cycle (lights on 0700). Ventricular cannula placement was confirmed post mortem by examining the distribution of an IVT injection of fast green dye; data were used only if the dye distributed in the lateral, third and fourth ventricles. Drugs used were: *d*-aldosterone (Ciba), renin (U.S. Biochemical Corp.), and dexamethasone phosphate for injection (Elkins-Sinn). Data were analyzed by one- or two-way analysis of variance; the Newman–Keul range statistic was used to evaluate significant ($P < 0.05$) F ratios. Plasma and urine specimens were analyzed for sodium and potassium content by flame photometry.

Protocol for aldosterone experiment. Rats with ventricular cannulae were weighed and placed in individual metabolism cages. At 0900 half of the rats received *d*-aldosterone (20 μg/ml/kg ip) or an equal volume of vehicle (10% ethanol in 0.85% saline). Half the rats receiving each pretreatment were injected IVT 90 min later with renin (5 mGU/5 μl) or saline (5 μl), followed by 20 ml/kg po water. Urine was collected at 3 and 6 hr; the bladder was emptied at 6 hr by gentle suprapubic massage. Food and water were not available during the experiment.

Protocol for adrenalectomized rats. At the time of ventricular cannulation, the adrenal glands were approached through a midline abdominal incision, isolated from perinephric fat and left in place (sham-surgery) or removed (adrenalectomized). The muscle and skin layers were closed with silk sutures and topical Neosporin powder was applied. On the day of surgery, and 2 days later, adrenalectomized rats were injected sc with 4 mg/kg dexamethasone phosphate and 20 μg/kg *d*-aldosterone. Sham-operated rats received vehicle injections. After surgery, all rats were kept in individual metabolism cages. Adrenalectomized rats received 0.45% saline to drink; sham rats were given water. Daily intake of fluid and food, and output of urine volume, sodium, and potassium were recorded. Four days after surgery, all rats were given *d*-aldosterone, 20 μg/kg ip, 90 min before IVT renin or saline injections and po water (20 ml/kg). Urine was collected at 3 and 6 hr. Drinking tubes and food were removed from the cages at the time of IVT injection. Some intact, sham-operated and adrenalectomized rats were killed 90 min after *d*-aldosterone administration. Blood was collected for the assessment of $[Na^+]$, $[K^+]$, and hematocrit to assess basal hydration before IVT injections.

Results. *Effects of aldosterone on the renin response in intact rats.* The effects of aldosterone on the renal response to IVT renin in intact, hydrated rats are illustrated in Fig. 1. Two-way analysis of variance of urine volume revealed no significant F ratios at 3 hr ($P > 0.05$); renin treatment increased urine volume at 6 hr ($F = 30$, $P < 0.01$), whereas aldosterone pretreatment was associated with a decrease ($F = 11$, $P < 0.01$). There was no interaction between pretreatments and IVT injections ($F = 1$, $P > 0.05$). Absolute sodium and potassium excretion was increased by renin at 3 and 6 hr (all F's > 15, $P < 0.01$). Evaluation of the urinary Na/K ratio at 3 hr revealed significant ($P < 0.05$) renin and interaction terms. Comparison of individual means showed that the vehicle–saline and aldosterone–saline groups differed from all other means ($P < 0.05$). Only a renin effect was significant for the Na/K ratio at 6 hr ($F = 18$; $P < 0.01$).

Effects of adrenalectomy on the renin response in aldosterone-pretreated rats. The urinary response to IVT renin in hydrated sham and adrenalectomized rats pretreated with aldosterone is summarized in Fig. 2. Urine volume at 3 hr was decreased by renin ($F = 7.4$, $P < 0.05$) and there was a reduction associated with surgery ($F = 4.5$, $P < 0.05$). Adrenalectomized rats had a reduced urine volume ($F = 7$, $P < 0.05$) at 6 hr; there was a tendency for interaction ($P = 12\%$), most likely related to the low output in the adrenalectomized rats given IVT

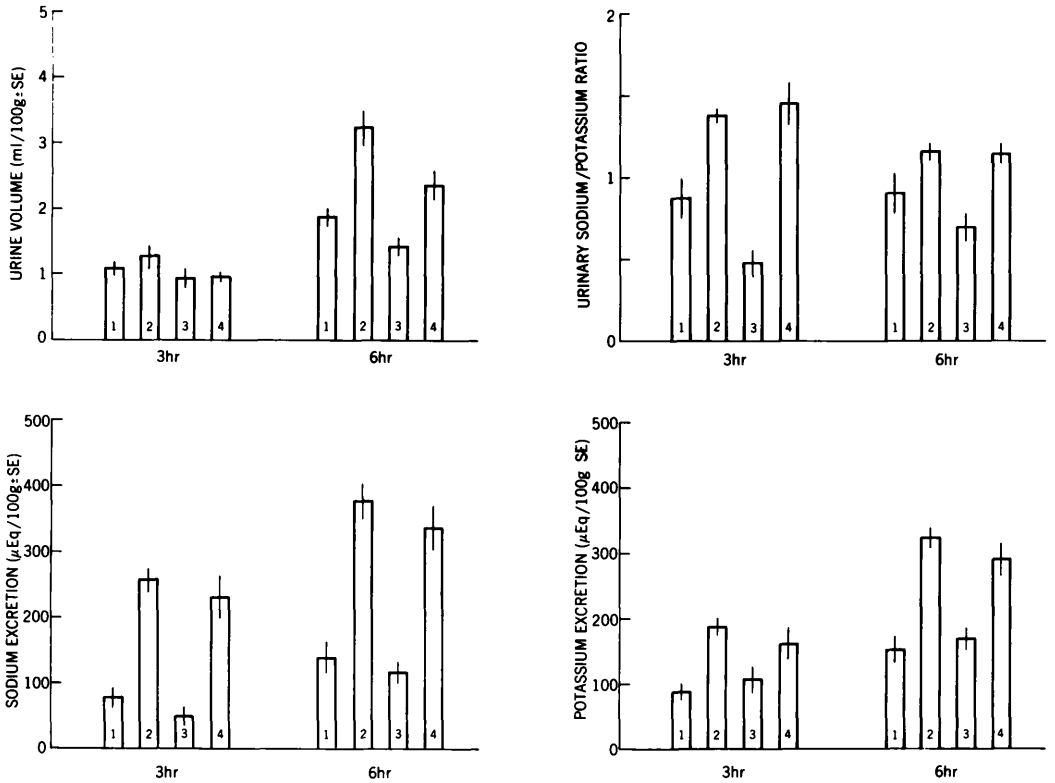

FIG. 1. Effects of aldosterone pretreatment on the urinary response to IVT renin. Groups of rats (n = 10–14) were given ip aldosterone (A) or vehicle (V) followed by IVT renin (R) or saline (S) and po water 90 min later. Groups: 1 = V–S; 2 = V–R; 3 = A–S, 4 = A–R. Significant ($P < 0.05$) F ratios from two-way analysis of variance were: volume—A and R effects at 6 hr; Na^+ and K^+—R effect at 3 and 6 hr; Na/K ratio—R and interaction at 3 hr (groups 1, 3 differ from all means), R effect at 6 hr.

renin. Renin increased sodium excretion whereas a decrease was associated with adrenalectomy at 3 and 6 hr (all F's > 12, $P <$ 0.01). There was also a significant interaction at 3 and 6 hr (F's > 8, $P < 0.01$); both renin groups differed from all means. Renin-induced kaliuresis was significant at 3 and 6 hr (F's > 20, $P < 0.01$). There was a tendency for a reduction in potassium output associated with surgery at 3 hr (P = 10%); this reduction was significant at 6 hr ($F = 6.5$, $P < 0.05$).

Table I summarizes the effects of adrenalectomy on the hydration status of rats up to the time when renin was injected IVT. Adrenalectomized rats lost body weight 4 days after surgery (358 ± 9 to 318 ± 9 g ± SE, $P < 0.05$, paired t); sham-operated rats weighed the same (359 ± 6 to 364 ± 6 g ± SE, $P > 0.05$, paired t). Adrenalectomized rats maintained normal plasma [Na^+] and [K^+] but hematocrit increased, thereby suggesting the presence of hypovolemia. Adrenalectomized rats increased urinary sodium excretion by an amount similar to the content of their drinking fluid (0.45% NaCl). Potassium loss in the urine of adrenalectomized rats was similar to sham-operated animals, but adrenalectomized rats consumed only about 60% as much food.

Discussion. Recent investigations have documented the presence of all components of a renin–angiotensin system (RAS) in brain tissue (1–3), but the endogenous role of brain-synthesized angiotensin remains unknown. It has been proposed that the brain RAS is reciprocally related to the renal RAS (4). The basis for this proposal was that IVT angiotensin increased urinary sodium loss and, in sodium-deficient rats, the peptide stimulated water intake and

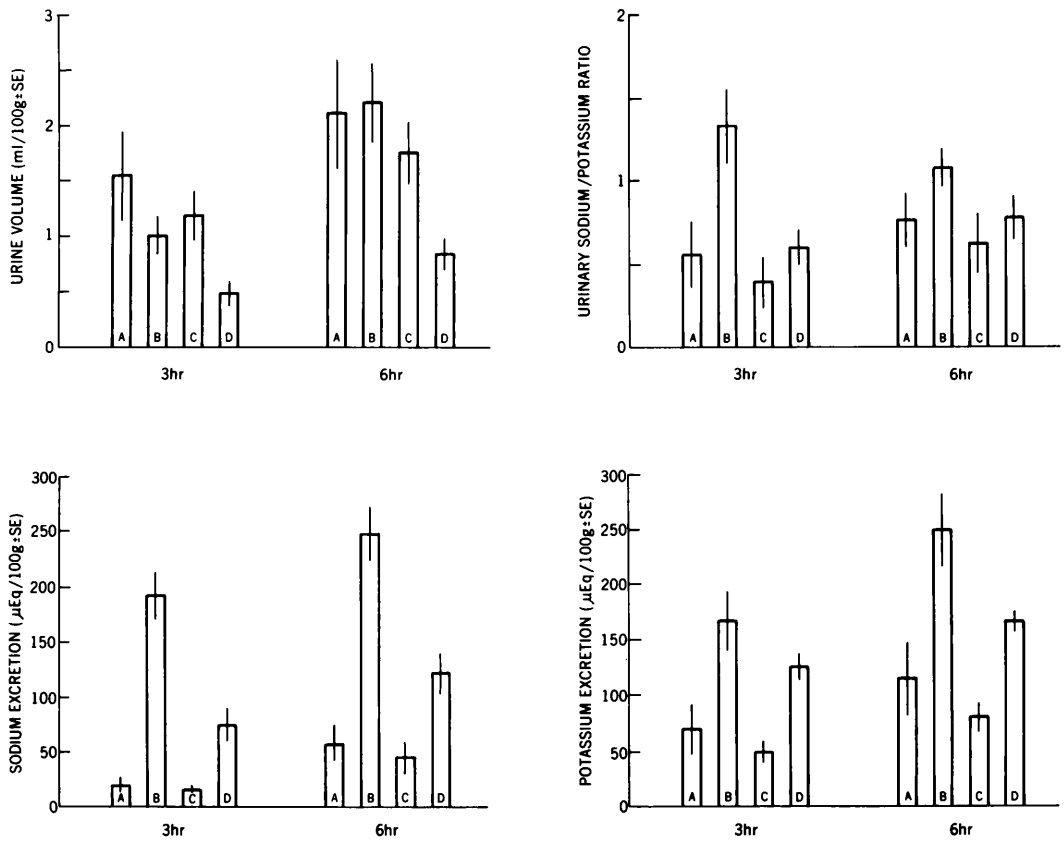

FIG. 2. Urinary response to IVT renin in sham and adrenalectomized (Ax) rats. Groups of sham and Ax rats (n = 6–8) were pretreated with aldosterone followed by IVT renin (R) or saline (S) and po water 90 min later. Groups: A = Sham–S; B = Sham–R; C = Ax–S; D = Ax–R. Significant ($P < 0.05$) F ratios from two-way analysis of variance were: volume—Ax and R effects at 3 hr, Ax at 6 hr; Na^+—Ax, R, and interaction at 3 and 6 hr (groups B, D differ from all means); K^+—R effect at 3 and 6 hr, Ax effect at 6 hr; Na/K ratio—Ax and R effects at 3 hr.

TABLE 1. EFFECTS OF ADRENALECTOMY ON CONSUMATORY BEHAVIOR AND URINE OUTPUT[a]

	Intact(I)	Sham(S)	Adrenalectomy(A)	AOV[b]
Food intake (g)	20 ± 2.1	15.6 ± 1.8	10.0 ± 1.8	All differ
Fluid intake (ml)	33.6 ± 3.2	28.6 ± 1.2	37.2 ± 2.5	A ≠ I,S
Urine volume (ml)	14.3 ± 1.2	13.3 ± 0.7	29.3 ± 2.1	A ≠ I,S
Na^+ excretion (μeq)	2912 ± 263	1924 ± 325	4331 ± 676	All differ
K^+ excretion (μeq)	3756 ± 460	2987 ± 476	2919 ± 312	NS
Plasma $[Na^+]^c$ (meq/l)	143 ± 0.9	145 ± 0.3	143 ± 0.6	NS
Plasma $[K^+]^c$ (meq/l)	6.8 ± 0.4	6.7 ± 0.3	6.6 ± 0.3	NS
Hematocrit[c] (%)	40.6 ± 0.8	40.4 ± 0.9	45 ± 0.9	A ≠ I,S

[a] Values shown are average 24 hr values ± SE (n = 5 or more) for the 4-day interval from surgery to experimentation. Data are shown for unoperated sham and adrenalectomized rats. The latter group received dexamethasone and aldosterone (see text) and were given 0.45% NaCl to drink.

[b] One-way analysis of variance and Newman–Keul test; $P < 0.05$.

[c] Values from rats killed 90 min after ip aldosterone on the experimental day.

vasopressin release while reducing plasma renin activity (4). Brooks and Malvin (6) showed that IVT angiotensin antagonists, by themselves, produced effects opposite to those of IVT angiotensin. In particular, Brooks and Malvin (6) found that IVT angiotensin reduced, whereas saralasin increased, plasma aldosterone concentrations in sodium deprived dogs. Therefore, reduction of plasma aldosterone levels by IVT angiotensin could be a reasonable explanation for increased urinary sodium loss. Evaluation of this explanation was the goal of this research.

Renin, injected IVT when the kidneys were likely to be under the maximal influence of exogenous aldosterone (15), caused a natriuretic effect equal in magnitude to control rats. A marked reduction of the Na/K ratio at 3 hr by aldosterone provided *in vivo* evidence that the kidneys were under mineralocorticoid influence when natriuresis occurred. These results clearly show that natriuresis is unrelated to the known reduction in plasma aldosterone concentration evoked by IVT renin (see also Ref (7)).

Adrenalectomized rats given an oral water load undergo an incomplete water diuresis; urine osmolality and plasma vasopressin levels are higher than corresponding sham-operated animals (16). Impaired urine output was likewise observed in our adrenalectomized rats and under the influence of aldosterone, very little sodium excretion occurred. Nevertheless, within the two adrenalectomized groups, IVT renin clearly evoked natriuresis. Our results suggest that the adrenal glands are not an absolute requirement for renin-evoked natriuresis. A contribution of the adrenal glands cannot be entirely ruled out in intact rats because of the reduced effect of renin and the known difference in renal handling of water in adrenalectomized rats (16).

Changes in systemic and renal hemodynamics, renal nerve activity, and central vasopressin effects have been excluded as absolute requirements for natriuresis after IVT renin or angiotensin. Our results further exclude withdrawal of mineralocorticoid activity in the kidneys as an efferent mechanism. Natriuresis, albeit reduced in magnitude, occurs in the complete absence of the adrenal glands.

While the mechanism of sodium excretion must await further developments, currently available information raises the possibility that IVT angiotensin releases a natriuretic hormone. Natriuretic hormone can be recovered from plasma and urine under conditions of extracellular volume expansion and it may play a role in hypertensive mechanisms (17). Because chronic IVT angiotensin reduces plasma [Na^+] (5) and may act as a defense against hypernatremia (4), it is reasonable to suggest, as a working hypothesis, that angiotensin releases a natriuretic substance.

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