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Effect of Intraventricular Infusions of Artificial Cerebrospinal Fluid on Serum Renin Activity, Aldosterone, Corticosterone, and Prolactin (39869)

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Brain receptors appear capable of initiating alterations of sodium excretion and renin secretion. Direct evidence for a sodium receptor in the brain has come from experiments demonstrating that injection of hypertonic saline into the third ventricle of conscious goats (1) or rats (2) produces large increases in sodium excretion. In contrast, the infusion of hypotonic, hyponatremic artificial cerebrospinal fluid (CSF) into the lateral ventricle of sheep (3) or dogs (4) produced an increase in plasma renin concentration and a decrease in sodium excretion. The infusion of iso-osmotic, hyponatremic artificial CSF produced similar, but lesser, changes (4).

The mechanism for the change in renal sodium excretion is unknown, but alterations in renin and aldosterone secretion may be partly responsible. Aldosterone secretion is increased by four known stimuli: angiotensin, ACTH, increased plasma potassium, and sodium depletion (5). Even though the importance of these factors has been well established, there is suggestive evidence, especially during sodium depletion, that an unknown factor(s), probably cranial in origin, contributes to the regulation of aldosterone secretion (6-8). Rapid sodium repletion of sodium-deficient sheep produced a rapid fall in aldosterone secretion that could not be explained by changes in the known stimuli (9). Abraham *et al.* (10) reported that infusion of hypertonic, hypernatremic (170 mEq/liter) fluid into the lateral ventricle of the sodium-depleted sheep produced a significant fall in blood aldosterone concentration which could not be explained by changes in known regulatory factors. Prolactin has been suggested to be an aldosterone-stimulating (11-14) and sodium-retaining (15-17) hormone. However, plasma prolactin concentration has never been determined during intraventricular infusion of artificial CSF of different osmolalities and sodium concentrations. Thus, changes in plasma prolactin concentration might conceivably mediate some of the chagnes in plasma aldosterone concentration and renal sodium excretion noted with intraventricular infusion. To further evaluate the existence of another aldosterone-stimulating factor(s), possibly prolactin, and to assess changes in serum prolactin concentration during intraventricular infusion, the following studies were performed.

Materials and Methods. Animals. Male Sprague-Dawley rats weighing 160-180 g (Simonsen Laboratories, Calif.) were kept in individual cages in a room with fluorescent illumination cycled with periods of light between 0400 and 1800. The temperature was kept at 23 ± 1°. The rats were fed Purina rat chow containing 0.15 mEq of sodium/g (regular-sodium diet) or ICN Pharmaceuticals low-sodium diet (0.01 mEq of sodium/g) for 7 days prior to the experiment.

Placement of the catheters. Three days before the experiment the rats were anesthesized with ether, and a metal cannula (25-gauge stainless-steel needle) was implanted into the lateral ventricle using the following coordinates: 1.5 mm from the sagittal suture, 0.5-1 mm behind the bregma, and 4.5 mm from the top of the skull. The cannulas were glued to the skull with Eastman 910 adhesive (Eastman Kodak Company) and dental cement.

Experimental procedures. On experimental day, 6 rats were brought to a quiet room at 0900, placed in individual cages, and fasted until 1500, when all the experiments were started. At 1300 a polyethylene tube (PE 20) was connected from the infusion pump (Harvard Instruments) to the ventricular canula. At 1500 the infusions were started at a rate of 1 μl/min. No ill effects or

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evidence of agitation were observed. After 60 min of infusion the catheter was cut, and within 15 sec the rats were quickly removed from the cages and decapitated in the next room. Blood was collected in iced tubes, allowed to clot, and centrifuged. The serum was kept at −20° until assayed. After decapitation 30 μl of a methylene blue solution was injected into the cannula, and the skull was opened to confirm proper placement of the cannula in the lateral ventricle.

Assays. Serum corticosterone and aldosterone concentrations were measured by radioimmunoassay (RIA) (18, 19). Serum renin activity (SRA) was measured by RIA of angiotensin I generated after incubation of the serum in the presence of diisopropylfluorophosphate and EDTA at pH 6.35 for 1 hr (20). Serum prolactin concentrations were measured by radioimmunoassay (21) using a kit kindly provided by the Rat Pituitary Program of the National Institute of Arthritis, Metabolic and Digestive Diseases, Bethesda, Maryland.

Experimental groups. The dietary sodium consumed by each experimental group (A–F) and osmolality and sodium concentration of the artificial CSF infused intraventricularly are given in Table I. The iso-osmotic, isonatremic artificial CSF contained 305 mOsm/liter and 150 mEq of Na^+/liter; the hyperosmotic CSF contained 405 mOsm; the hyponatremic CSF contained 25 mEq of Na^+/l; the hypernatremic CSF contained 210 mEq of Na^+/l. In each case the artificial CSF infused contained 3.0 mEq of potassium, 2.5 mEq of calcium, 1.6 mEq of magnesium, 25 mEq bicarbonate, 135 mEq of chloride, and 0.5 mEq of phosphate per liter. When necessary the osmolality was increased by the addition of glucose. The solutions were freshly prepared from stock solutions and gassed for 2 hr with 95% oxygen, 5% carbon dioxide. The pH was determined before each experiment and adjusted to pH 7.4 when needed. The osmolality of each artificial CSF was checked with a Hewlett-Packard 302B vapor pressure osmometer.

On the day of the experiment, six rats from two groups were infused at a time. All six rats were eating food of the same sodium content. Serum aldosterone concentrations were determined in one assay for the regular-sodium-diet rats and another assay for the low-sodium-diet rats. Serum renin activity and prolactin and corticosterone concentrations were each determined in one assay. Statistical analysis was performed using Student's *t* test.

Results. The results are summarized in Table I.

Regular sodium diet. Serum renin activity was higher ($P < 0.05$) in rats infused with the hyponatremic artificial CSF. Serum aldosterone, corticosterone, and prolactin concentrations were not statistically different in the two groups and were within normal values previously reproted (21).

Low sodium diet. Serum renin activity and serum aldosterone and prolactin concentrations were not statistically different in the different infusion groups. Serum corticosterone concentration was significantly el-

TABLE I. SERUM RENIN ACTIVITY, ALDOSTERONE, CORTICOSTERONE, AND PROLACTIN (MEAN ± SEM) AFTER INTRAVENTRICULAR INFUSIONS.

Group	No.	CSF infused	SRA (ng/ml/hr)	Aldosterone (ng/dl)	Corticosterone (ng/dl)	Prolactin (ng/dl)
			Regular-sodium diet			
A	13	Iso-osmotic, isonatremic	3.1 ± 0.3	12.7 ± 2.2	31.7 ± 2.5	20.6 ± 6.2
B	12	Iso-osmotic, hyponatremic	4.9 ± 0.6*	14.9 ± 3.0	37.7 ± 3.4	18.5 ± 2.9
			Low-sodium diet			
C	12	Iso-osmotic, isonatremic	8.1 ± 0.8	269.0 ± 47.4	23.1 ± 4.9	14.3 ± 4.3
D	8	Iso-osmotic, hyponatremic	8.2 ± 0.7	309.0 ± 80.0	44.5 ± 3.6**	24.7 ± 9.8
E	11	Hyperosmotic, hypernatremic	8.1 ± 0.8	294.6 ± 71.8	41.5 ± 5.3**	17.7 ± 5.1
F	13	Hyperosmotic, isonatremic	8.8 ± 1.0	217.6 ± 52.0	30.7 ± 3.8	16.4 ± 4.7

* $P < 0.05$ (A vs B).
** $P < 0.01$ (D,E vs C).

evated ($P < 0.01$) during infusion of hyponatremic, iso-osmolar artificial CSF (group D) and hyperosmolar hypernatremic artificial CSF (group E). Serum renin activity and serum aldosterone concentration increased, as expected, during low-sodium diet ($P < 0.001$). Serum prolactin concentration did not increase with low-sodium diet, as previously reported (21), but at variance with another report (17).

Discussion. In agreement with previous investigations in both sheep (2) and dogs (3), we have shown that in the rat on regular-sodium diet, hyponatremic CSF induces an increase in serum renin activity. This increase was not accompanied by an increase in serum aldosterone concentration in either rats or sheep (2), as might be expected from the known stimulation of aldosterone secretion by angiotensin (5). In sodium-depleted rats a hyponatremic infusion did not change serum renin activity. This may reflect a baseline of maximum renin stimulation by sodium depletion, masking any effect of the hyponatremic infusion.

Hypernatremic intraventricular infusion in sodium-depleted rats did not produce any significant changes of serum renin activity or aldosterone concentration, the latter in disagreement with previously reported results with sheep (10). The reason for this discrepancy is not clear, but it may be that the more-prolonged infusion done by Abraham *et al.* (10) might have decreased serum aldosterone concentrations simply by allowing slow diffusion of sodium into the systemic circulation since the decreased aldosterone concentration did not occur until after 3 hr of infusion. Tuck *et al.* (22) have demonstrated that infusion of relatively small amounts of saline can lead to suppression of plasma aldosterone concentration.

During hypernatremic CSF infusion, serum corticosterone increased in both sheep (10) and rats (group E). Since ACTH is a potent stimulus of aldosterone secretion, even during saline infusion (23), it is surprising that serum aldosterone concentration did not change. Similarly, in group B (hyponatremic CSF infusion, regular-sodium diet) serum aldosterone concentration did not change in response to an increase in serum renin activity. The failure of serum aldosterone concentration to increase in response to these two known stimuli suggests that another central nervous system factor(s) which may inhibit aldosterone secretion may be operative. Similar conclusions in favor of an inhibitor of aldosterone secretion have recently been reached by Denton *et al.* (24). Alternatively, the metabolic clearance rate of aldosterone may have increased so that no change in aldosterone concentration resulted. Paradoxically, serum corticosterone concentration also increased during infusion of iso-osmotic, hyponatremic CSF (group D, low-sodium diet). The reason for the increased corticosterone in both group D and group E is unknown, but it raises the possibility that infusion of hyponatremic or hypernatremic artificial CSF into sodium-depleted rats produces nonspecific stress.

Serum prolactin concentration did not change with any type of CSF infusion and did not increase with low-sodium diet. Other investigators have reported that prolactin does not stimulate aldosterone secretion (25), does not respond to osmotic stimuli (26, 27), as previously reported (28), and does not cause renal sodium retention (25). A generous body of evidence thus suggests that prolactin does not play a significant role in sodium and water homeostasis in the rat or in man. In addition, it appears highly unlikely that the observed changes in renal sodium excretion after intraventricular infusion are mediated via aldosterone or prolactin. The mechanism for these changes remains uncertain.

Summary. Renal sodium excretion has been noted to increase with intraventricular infusion of hypernatremic fluid and to decrease with infusion of hyponatremic fluid. To further explore the mechanism of these changes, serum renin activity and serum aldosterone, corticosterone, and prolactin concentration were measured after 1-hr infusion of artificial cerebrospinal fluid of varying sodium concentration and osmolality into the lateral ventricles of unanesthetized rats which had been consuming regular-sodium or low-sodium diets. Serum aldosterone and prolactin concentrations did not change significantly. Serum renin activity increased with infusion of iso-osmotic,

hyponatremic fluid during regular-sodium diet. Serum corticosterone concentration increased during low-sodium diet with infusion of iso-osmotic, hyponatremic and hyperosmotic, hypernatremic fluid. The failure of serum aldosterone concentration to increase concomitant with increases in serum renin activity and corticosterone concentration suggests that an additional central nervous system factor(s) may influence aldosterone secretion. The mechanism for the changes in renal sodium excretion during intraventricular infusion remains uncertain, but it does not appear to be mediated through aldosterone or prolactin.

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