Circulating angiotensin II mediates sodium appetite in adrenalectomized rats

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Received 30 June 2000; accepted in final form 23 April 2001

Schoorlemmer, G. H. M., A. K. Johnson, and R. L. Thunhorst. Circulating angiotensin II mediates sodium appetite in adrenalectomized rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R723–R729, 2001.—We investigated the role of circulating ANG II in sodium appetite after adrenalectomy. Adrenalectomized rats deprived of their main access to sodium (0.3 M NaCl) for 9 h drank 14.1 ± 1.5 ml of the concentrated saline solution in 2 h of access. Intravenous infusion of captopril (2.5 mg/h) during the last 5 h of sodium restriction reduced sodium intake by 77 ± 12% (n = 5) without affecting the degree of sodium depletion and hypovolemia incurred during deprivation. Functional evidence indicates that this dose of captopril blocked production of ANG II in the peripheral circulation, but not in the brain; that is, injection of ANG I into the lateral brain ventricle stimulated intake of both water and 0.3 M NaCl. Intravenous infusion of ANG II (starting 10–15 min before 0.3 M NaCl became available) in adrenalectomized, captopril-treated rats restored both sodium intake and blood pressure to values seen in rats not treated with captopril. Longer (20 h) infusions of captopril in 22-h sodium-restricted rats also blocked sodium appetite, but reduced or prevented sodium depletion. Intravenous infusion of ANG II after these long captopril infusions stimulated sodium intake, but intake was less than in controls not treated with captopril. These results indicate that most or all of the sodium appetite of adrenalectomized rats is mediated by circulating ANG II.

Mammals have at their disposal several mechanisms to stimulate sodium appetite. Among them is aldosterone (11, 26), which can act directly on the brain to stimulate sodium intake (2, 3). Circulating ANG II is theorized to stimulate sodium intake either by acting directly on the circumventricular organs of the brain (5, 20, 23, 24) or by stimulating the release of aldosterone (2, 14). Signals from vascular sensors monitoring blood pressure and blood volume may also play a role (21, 22).

The role of circulating ANG II in sodium appetite has been controversial. Removal of the kidneys, the source of circulating renin, greatly reduces sodium intake caused by adrenalectomy or subcutaneous formalin (7, 8). However, the reduction in salt intake may be due to the resulting anuria and not to the loss of renin. Intravenous infusions of ANG II are not particularly effective in stimulating salt appetite in sodium-replete animals (4, 8, 14, 27). Furthermore, intravenous infusions of ANG II receptor antagonists sometimes fail to block sodium appetite (13–15). On the other hand, recent evidence suggests that circulating ANG II mediates part of the sodium appetite seen after sodium depletion (5, 19, 20, 24).

Our aim in the present experiments was to investigate the role of circulating ANG II in the sodium appetite seen after adrenalectomy. The lack of aldosterone after adrenalectomy results in large urinary losses of sodium. This renal sodium loss is compensated for by increased intake. Characteristic of adrenalectomy are very large sodium intakes and the absence of one of the known pathways that mediate sodium appetite, namely, aldosterone.

Dalhouse et al. (1) have suggested that circulating ANG II stimulates sodium intake in adrenalectomized rats by a direct action on the brain, but their data provide only weak support for this idea. In contrast, Sakai and Epstein (15) have argued that circulating ANG II does not contribute to sodium appetite in adrenalectomized rats. These authors found that blockade of ANG II production by an intravenous infusion of the angiotensin-converting enzyme inhibitor, captopril (SQ-14,224), prevented sodium appetite in adrenalectomized rats. However, they assumed that this was due to blockade of central conversion of ANG I inside the blood-brain barrier. If this assumption is correct, captopril-treated rats should fail to respond to ANG I injected into the brain. Therefore we repeated their experiment and injected ANG I into the brain at the end of the period of captopril infusion. The central injection of ANG I elicited salt intake in the captopril-treated rats. Next, we restored the circulating ANG II in captopril-treated rats by infusing it intravenously and induced sodium intake. We also measured the effect of the captopril infusion on sodium balance and blood volume. We found that the infusion reduced renal loss of sodium, and this complicated interpretation of our results. To prevent this complication, we
repeated some experiments but reduced the duration of captopril infusion.

METHODS

Animals and Cages

We used male Long-Evans rats from Harlan (Indianapolis, IN) that weighed 350–500 g. Rats were housed individually, in a temperature (23°C)- and humidity-controlled room. Lights were on between 0600 and 1800. Tap water and 0.3 M NaCl were provided in graduated glass burettes or plastic tubes equipped with metal spouts. Rats had free access to fluids and to Purina 5012 rat chow except where stated. Each gram of this chow contains about 110 μmol Na⁺ and 260 μmol K⁺.

A few days before a rat was used in an experiment, it was transferred to a metabolic cage. These test cages were wooden with aluminum-lined interiors (24 × 29 cm) that extended 31 cm above the suspended, stainless steel metabolism cages. Urine was collected in preweighed glass beakers via stainless steel funnels placed beneath the cages. Water, 0.3 M NaCl, and food intakes were recorded throughout. The regular chow was ground for use in these cages.

Surgery

We used an Equithesin-like compound (Ref. 9; 2.5 ml/kg body wt for adrenalectomy or surgery on adrenalectomized animals, otherwise 3 ml/kg body wt) for surgical anesthesia. Instruments were disinfected by soaking them in chlorhexidine (Nolvasan, Ft. Dodge Laboratories, Ft. Dodge, IA). Canulas were soaked for 30 min in 70% ethanol. Rats were allowed to recover from surgery for at least 10 days.

Adrenalectomy

The adrenal glands were exposed by a retroperitoneal approach. A small lateral incision was made, and the adrenal glands were visualized by spreading the muscles. The adrenal glands were isolated and surgically excised along with a small amount of surrounding fat. The muscle layer and subcutaneous fascia were closed separately with suture, and the skin was closed with wound clips. Adrenalectomized rats received dexamethasone, 20 μg/day sc, dissolved in 0.1 ml 98% saline-2% ethanol. We did not attempt to confirm the completeness of adrenalectomy histologically; rather, we assumed all adrenalectomies were effective because intake of 0.3 M NaCl gradually increased to at least 18 ml/day in all rats.

Brain Cannulas and Injections into the Brain

To implant brain cannulas, we mounted the rat in a stereotaxic instrument with the skull level between lambda and bregma. A stainless steel guide tube (12 mm long, 0.7-mm OD) was implanted so that the tip was just above a lateral ventricle (1.5 mm lateral from midline, 1.2 mm posterior of bregma, and 4.0 mm ventral of dura). Guide tubes were anchored with dental acrylic to stainless steel obturators. To make intracranial injections the obturator was removed and an injector, made of 30-gauge hypodermic tubing, was inserted so that the injector tip protruded 0.5 mm out of the guide tube. Drugs were injected slowly (1 μl/min) with a hand-held 10-μl Hamilton syringe.

Venous Cannulas, Venous Infusions, and Blood Sampling

Venous cannulas were made from silicone rubber tubing (0.6-mm ID, 1.2-mm OD, Dow Corning, Midland, MI) connected to polyurethane tubing (0.6-mm ID, 1.0-mm OD, MRE 040, Braintree Scientific, Braintree, MA). The silicone rubber end was inserted in the femoral vein and advanced 8 cm, which places the cannula tip in the thoracic vena cava. The polyurethane end was led under the skin to the area between the shoulder blades and connected to an elbow of hypodermic tubing that protruded through the skin. The cannula was filled with 0.15 M NaCl containing heparin (50 U/ml) and penicillin G (1.3 mg/ml), and the elbow was closed with a plastic cap.

To make infusions, the metal elbow was connected with polyethylene tubing to a low-friction fluid swivel joint. The tubing was led through a metal spring to prevent the rat from damaging it. Spring and swivel hung on a movable, counterbalanced arm. This gave the rat complete freedom of movement. Infusion lines and fluid swivels were filled with 70% ethanol when they were not used. Infusions were made with disposable syringes equipped with a sterile Millipore filter (0.22-μm mesh). The syringes were mounted in a syringe pump (model 22, Harvard Apparatus, Holliston, MA). The infusion assembly was disconnected from the elbow on the rat’s back when no infusions were made.

Blood samples (0.3–0.4 ml each) were collected from the venous cannulas. Because the volume of blood withdrawn in any single experiment was small, we did not attempt to return saline or red blood cells. Blood to be used for hemoglobin determination was kept separate; the rest of the sample was mixed with heparin and centrifuged for 5 min at 16,000 g. Plasma was stored at −20°C until analysis.

Arterial Cannulas and Blood Pressure Measurement

Cannulas were made from polyethylene tubing (PE-10, 0.6-mm OD, 0.3-mm ID; Beckton Dickinson, Sparks, MD), heat welded to larger polyethylene tubing (1.0-mm OD, 0.6-mm ID, PE-50, Beckton Dickinson). The PE-10 end was inserted in the femoral artery and advanced 4 cm, which places the cannula tip in the abdominal aorta, below the renal arteries. The PE-50 end was led subcutaneously to a metal elbow between the shoulder blades. The cannula was filled with 0.15 M NaCl containing heparin (50 U/ml) and penicillin G (1.3 mg/ml), and the elbow was closed with a plastic cap. The operated legs always looked healthy; the cannula and surgery did not noticeably alter leg function.

To record blood pressure we connected the arterial cannulas with PE-50 tubing to Cobe blood pressure transducers. The signal from the transducers was amplified, sampled at 200 Hz or higher, and converted to a 12-bit digital signal (1401 plus, CED, Cambridge, UK). Data were stored on a computer and analyzed with Spike2 software (CED, Cambridge, UK). Tubing and transducers were filled with 70% ethanol when they were not used.

Drugs

Captopril (SQ-14,225, Squibb & Sons, Princeton, NJ) was dissolved to make a concentration of 25 mg/ml in 5% d-glucose or in 75 mM Na₂CO₃. This last solution is isotonic, with a pH of ~7. The control solution was either 5% d-glucose or 150 mM NaCl. These solutions were used the same day. ANG I, ANG II, phenylephrine, and antidiuretic hormone (ADH; all from Sigma Chemical, St. Louis, MO) were diluted with saline from concentrated stock solutions and were used the same day.
**Analysis of Blood and Urine Samples**

Urine volume was measured gravimetrically (using 1 g = 1 ml). Urinary and plasma [Na\(^+\)] and [K\(^-\)] were measured with a flame photometer (IL 343, Instrumentation Laboratory, Watertown, MA). To measure blood hemoglobin concentration we mixed blood immediately after its collection with Drabkin’s reagent (diagnostics kit 525, Sigma Chemical, St. Louis, MO) and measured absorbance with a spectrophotometer.

**Data Analysis**

Data are presented as means ± SE. With blood pressure and heart rate, the test period was divided in 1-min intervals, and the average of each interval was calculated. The 1-min averages were smoothed over a 5-min period, which removed much of the scatter but had no noticeable effect otherwise. The smoothed signal was plotted every minute.

Intakes of water and saline were analyzed as rate of intake, i.e., milliliters per 15 minutes, with two-way ANOVA using time and treatment as within-subjects variables when the number of subjects was equal for treatments, and time as a within-subjects variable when the numbers were not equal between treatments. Blood pressure, heart rate, plasma [Na\(^+\)], and blood hemoglobin were analyzed with a two-way ANOVA, with time but not treatment as a within-subjects factor, because the number of subjects differed with some treatments. If the effect of treatment, or the interaction of treatment and time, differed significantly from the null hypothesis, we located the differences by using at each time point Tukey’s multiple comparison test. All other data were analyzed by paired t-tests.

**Design of Experiments**

At the time of experiments, rats had been adrenalectomized between 3 wk and 6 mo. In all experiments rats served as their own controls. Treatment order was randomized. The period between treatments varied between 2 and 5 days. Rats were used for experiments intermittently for periods up to 6 mo.

**Effects of long-duration captopril infusions.** The basic design of this experiment closely resembles the original experiment done by Sakai and Epstein (15). Adrenalectomized rats (n = 11) had access to 0.3 M NaCl for 2 h a day (1000–1200). After rats had been on this schedule for 2 wk, either captopril (2.5 mg/h iv in 0.1 ml/h) or vehicle was infused, starting 2 h into the depletion period. The next morning at 1000, 0.3 M NaCl was offered, and food was removed. Intakes of water and 0.3 M NaCl were measured 15, 30, 60, 90, and 120 min later. Blood pressure was recorded in three of these rats during the drinking period. Urine produced during the 22-h depletion period was collected.

Upon completion of the drinking experiment, rats received an intracerebroventricular (icv) injection of 10 ng ANG I, dissolved in 1 μl 150 mM NaCl. Intakes of water and 0.3 M NaCl were measured 30 min later. The infusion of captopril stopped at the end of this period.

In three rats we tested whether salt appetite after treatment with captopril could be restored by intravenous infusion of ANG II. The rate of infusion of ANG II was adjusted throughout the experiment to keep blood pressure close to the level seen in rats depleted of sodium but not treated with captopril. However, if the infusion reduced heart rate below 220 beats/min, the rate of infusion was reduced. The drinking period started 13 ± 2 min (range 8–18 min) after the first entry of ANG II in the blood. In control experiments we infused the same volume of 150 mM NaCl. In other control experiments we measured intakes of 0.3 M NaCl while we infused phenylephrine to restore blood pressure.

To determine the effects of infusion of captopril on blood solutes we repeated the experiment with some modifications. Rats (n = 7) had free access to water, food, and 0.3 M NaCl for 2 days. A venous blood sample was then collected at 1000, and 0.3 M NaCl was removed. Two hours later an intravenous infusion was started with either captopril (2.5 mg/h in 0.1 ml/h) or vehicle. A second blood sample was collected at 1000 the next morning, and 0.3 M NaCl was returned. A third sample was collected at 1200 (the end of access to 0.3 M NaCl). No venous blood could be withdrawn from two rats treated with vehicle.

**Effects of shorter-duration captopril infusions.** Preliminary experiments showed that infusion of captopril only during the last 5 h of the 22-h restriction period had little effect on electrolyte excretion. However, rats did poorly with these infusions; apparently the sodium deficits that accrued during the restriction period produced nonspecific behavioral debilitation unless ANG II was present. Therefore we reduced the period of sodium restriction from 22 to 9 h.

For these 9-h depletion experiments we used five rats that had access to 0.3 M NaCl at all times except during experiments. During experiments, 0.3 M NaCl was removed at midnight, infusion of captopril (2.5 mg/h in 0.1 ml/h) or vehicle started 4 h later, and 0.3 M NaCl was returned at 0900 the next morning. In these experiments, we measured the effect of captopril infusion on sodium balance (n = 5), intake of water and 0.3 M NaCl (n = 5), and blood hemoglobin (captopril n = 4, vehicle n = 3), but not plasma sodium concentration. As before, we measured sodium intake during intravenous infusion of ANG II in captopril-treated rats (n = 5). However, we used ADH, rather than phenylephrine, as a control treatment to raise blood pressure, because some rats subjected to adrenalectomy and captopril treatment in this experiment responded to phenylephrine infusion with severe bradycardia and hypotension. Data from one experiment in which infusion of ADH caused hypotension were discarded.

**RESULTS**

**Effects of Long-Duration Captopril Infusions**

Adrenalectomized rats drank 0.3 M NaCl avidly after a period of 22 h of sodium restriction (Fig. 1). Intake of 0.3 M NaCl was blocked by intravenous infusion of captopril (2.5 mg/h) during the last 20 h of the restriction period. Water intake during the 2-h drinking test was low. Arterial pressure was low after captopril treatment, probably because of the lack of circulating ANG II in these hypovolemic rats (Fig. 1).

Rats drank little during the last half hour of the drinking period. Injection of 10 ng ANG I into the lateral brain ventricle caused the animals to start drinking again, whether or not they had received captopril (Fig. 2). Injection of ANG I stimulated intake of both water and 0.3 M NaCl.

To test if restoration of the circulating ANG II restored salt appetite, ANG II was infused intravenously in adrenalectomized, sodium-depleted rats treated with captopril. We adjusted the rate of infusion to elevate blood pressure to the level seen in adrenalectomized, sodium-depleted rats not treated with captopril (Figs. 1 and 3). The average rate of infusion was...
19 ± 3 ng/min, but the rate of infusion was higher at the start of the drinking period (≈25 ng/min) and slowly tapered off. Total amount of ANG II infused was 2.6 ± 0.5 μg/2.3 h in a volume of 0.9 ± 0.2 ml. Intra-venous infusion of ANG II significantly stimulated intake of 0.3 M NaCl (Fig. 3), but intake was less than in rats with an intact renin-angiotensin system (Fig. 1). In contrast to the increased intake induced with ANG II, infusion of phenylephrine (4.0 ± 1.9 mg/min, or 540 ± 260 μg in 2 h in a volume of 1.4 ± 0.7 ml) did not stimulate sodium appetite, although it restored blood pressure. Rats drank no water during intravenous infusions of ANG II, phenylephrine, or vehicle.

Sodium deficits were lower in captopril-treated rats. Intravenous infusion of captopril did not alter food intake but reduced excretion of Na⁺, K⁺, and water (Table 1). As a result, captopril-treated rats did not have the degree of hypovolemia (increase in blood hemoglobin concentration) and hyponatremia seen in control rats (Fig. 4). Therefore, the reduced sodium intakes of rats treated with captopril may partially reflect their reduced level of sodium depletion.

Effects of Shorter-Duration Captopril Infusions

Table 2 summarizes intake of water and food, and urinary excretion of water and salt during 9 h of sodium restriction. Infusion of captopril during the last 5 h of the restriction period had no significant effect on water and salt balance. Blood hemoglobin concentration increased by 6% during sodium restriction, whether captopril (n = 4) or vehicle (n = 3) was infused.

After 9 h of sodium restriction, rats ingested a substantial volume of 0.3 M NaCl (Fig. 5). Salt intake was almost abolished when captopril (2.5 mg/h iv) was infused during the last 5 h of the restriction period. Little water was ingested during this time under either infusion condition.

As before, we infused ANG II intravenously to restore blood pressure in captopril-treated rats to normal levels. ANG II infusion restored sodium appetite to normal levels and reduced sodium intake by 10.220.33.1 on October 14, 2017 http://ajpregu.physiology.org/ Downloaded from

Table 1. Effect of captopril infusion on intake and excretion of water and solutes during 22 h of sodium depletion

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Captopril</th>
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<tbody>
<tr>
<td>Food intake, g</td>
<td>20.5 ± 1.2</td>
<td>21.1 ± 0.5</td>
</tr>
<tr>
<td>Water intake, ml</td>
<td>30.6 ± 1.6</td>
<td>32.3 ± 1.9</td>
</tr>
<tr>
<td>Urine volume, ml</td>
<td>37.8 ± 3.9</td>
<td>23.0 ± 1.9*</td>
</tr>
<tr>
<td>Na⁺ excretion, mmol</td>
<td>07.9 ± 0.8</td>
<td>05.0 ± 0.6*</td>
</tr>
<tr>
<td>K⁺ excretion, mmol</td>
<td>04.3 ± 0.3</td>
<td>02.9 ± 0.3*</td>
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Values are means ± SE; n = 11. Adrenalectomized rats were depleted of sodium for 22 h. Captopril (2.5 mg/h) or vehicle was infused during the last 20 h of the deprivation period. Difference between treatments with captopril and vehicle: *P < 0.05.

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**Fig. 1.** A and B: cumulative intakes of water and saline by adrenalectomized rats after 22 h of sodium depletion. During the last 20 h of the depletion period, and continuing during the drinking period, either captopril or vehicle was infused intravenously. C and D: blood pressure and heart rate before and during the period of access to saline. Difference between captopril and vehicle: *P < 0.05; n = 11 (A and B) and 3 (C and D).

**Fig. 2.** Water and saline intake before and after injection of 10 ng ANG I into the lateral brain ventricle of adrenalectomized rats. Rats were pretreated with captopril (A; 2.5 mg/h iv for 20 h) or vehicle (B), and this infusion continued throughout the drinking period. Difference between before and after ANG I: *P < 0.05, n = 11.

**Fig. 3.** Effect of intravenous infusion of ANG II on sodium intake (A), blood pressure (B), and heart rate (C) in adrenalectomized rats that had been depleted of sodium for 22 h. During the last 20 h of the depletion period, and continuing throughout the drinking period, captopril (2.5 mg/h iv) was infused. Infusion of ANG II started 10–15 min before saline was returned. Difference from vehicle: *P < 0.05. Difference between ANG II and phenylephrine: +P < 0.05; n = 3.
The average amount infused was 24 ± 5 ng/min, but the rate of infusion at the beginning of the drinking period was higher (~30 ng/min), and the infusion slowly tapered off. Total amount infused was 3.3 ± 0.7 mg in 2.3 h in a volume of 1.1 ± 0.2 ml. These infusions completely restored salt appetite. Infusion of ADH (6 ± 1 ng/min, total amount infused was 0.7 ± 0.2 mg in 1.8 ± 0.6 ml in 2.3 h) significantly inhibited sodium intake, although it increased blood pressure comparably. Water intake during infusion of ANG II, vehicle, or ADH was negligible (1.2 ml or less). The volume of urine produced during these infusions was small (saline 0.7 ± 0.3 ml, ANG II 2.8 ± 0.9 ml, ADH 1.0 ± 0.5 ml/2 h; n = 4, 4 and 3, respectively).

**DISCUSSION**

The main finding of this study is that, first, sodium appetite in adrenalectomized rats can be blocked almost completely by an intravenous infusion of captopril that does not block activity of the angiotensin-converting enzyme (ACE) in the brain. Second, salt intake can be restored completely or almost completely by introduction of circulating ANG II. Our data suggest that essentially all the sodium appetite in adrenalectomized rats is mediated by circulating ANG II. In addition, our data suggest that some of the evidence that has been used previously to argue against a role of circulating ANG II in sodium appetite may have to be reevaluated.

**Effect of Intravenous Captopril on Brain ACE**

It has been shown that hypovolemic rats with intact adrenals drink water after injection of ANG I into the brain, whether they are treated with captopril (2.5 mg/h iv for 18 h) or vehicle (20). The present study shows that icv injection of ANG I induces both thirst and sodium intake, and both mechanisms seem intact after intravenous infusion of captopril. The saline drinking is not simply a continuation of sodium ingestion from the 2-h drinking test because there is a significant increase in salt intake in the 30 min after icv ANG I compared with the last 30 min of the access period. In fact, the amount of saline consumed by captopril-treated rats in 30 min after ANG I is nearly twice as much as during the entire 2 h of sodium access.
before the ANG I injection. Because ANG I has to be converted to ANG II to stimulate drinking (6), it follows that intravenous infusion of captopril at the present dose did not block ACE activity in the brain (i.e., inside the blood-brain barrier).

The fact that intravenous infusions of ANG II stimulated the salt appetite of captopril-infused rats also supports the idea that the captopril infusions did not completely block ACE inside the brain (5). Central infusions of captopril potently reduce salt appetite of sodium-depleted animals even when the peripheral synthesis of ANG II is intact (25, 27). That is, the actions of central ANG II appear to be critical for the expression of salt appetite in sodium-depleted rats. As argued in previous work (5), intravenous infusion of ANG II should not have been capable of stimulating salt appetite if the brain synthesis of ANG II was blocked by the peripheral infusions of captopril.

In conclusion, it seems that the intravenous infusions of captopril used here block neither conversion of ANG I injected into the brain nor the conversion of ANG I produced endogenously inside the brain by the pathways subserving sodium appetite. The lack of salt appetite after captopril treatment in our experiments was likely due to captopril inhibiting formation of circulating ANG II and not by a direct effect of captopril on production of ANG II inside the blood-brain barrier. Our data do not support Sakai and Epstein’s (15) conclusion that to inhibit sodium appetite in adrenalectomized rats by intravenous infusion of captopril it is necessary to interfere with brain ACE. Blockade of the peripheral renin-angiotensin system is sufficient to interfere with sodium appetite.

**Effect of Intravenous Captopril on Sodium Intake**

Infusion of captopril for 20 h significantly reduced the sodium deficit of adrenalectomized rats. This is probably due to retention of sodium by the kidneys in response to lowered arterial pressure. Therefore, the low sodium intake after 20 h of captopril infusion compared with infusion of vehicle may reflect the lack of direct stimulatory effects of ANG II on sodium ingestion, or the reduced need for sodium, or both. The same cannot be said for adrenalectomized rats deprived of sodium for 9 h and receiving captopril infusions of shorter duration (i.e., 5 h). Under these circumstances, rats treated with captopril lost as much sodium and urine volume as rats receiving vehicle, yet their sodium ingestion was much less. Their reduced sodium intakes cannot be ascribed to reduced sodium deficits compared with vehicle-treated rats.

Although treatment with captopril sharply reduced sodium intake (by 77% in both experiments), rats invariably drank some saline. We do not know if this remaining intake is mediated by circulating ANG II. For example, it may be that the captopril infusions used by us do not completely block the production of ANG II in the circumventricular organs of the brain. Alternatively, signals from low- or high-pressure baroreceptors in the circulation might directly stimulate brain pathways subserving sodium appetite (21, 22).

**Restoration of Circulating ANG II Restores Sodium Intake**

It is difficult to reliably stimulate salt appetite in sodium-replete animals with short-term intravenous infusions of ANG II (4, 8, 14, 27). Because sodium-replete animals do not need sodium, the difficulty may be that counterregulatory mechanisms become engaged that inhibit sodium ingestion under those conditions (5, 20). For example, the pressor response to intravenous ANG II may be inhibitory to sodium appetite much as it is to water drinking (12). Likewise, counterregulatory mechanisms may explain why infusion of ANG II in rats treated with captopril for 20 h failed to bring sodium intake to the level seen in controls not treated with captopril. These long captopril infusions reduced solute excretion and prevented the hypovolemia and hyponatremia seen after sodium depletion. Infusion of ANG II during the subsequent period of access to saline did not restore saline intake, but intake was substantial (9.5 ml), presumably enough to make the animal hypervolemic and hypernatremic. Signals caused by hypernatremia (18) and hypovolemia would be attractive candidates to explain the lack of potency of ANG II after 20-h captopril infusions.

On the other hand, sodium ingestion is readily stimulated by intravenous ANG II in sodium-depleted animals (5, 24). In the present work, sodium ingestion was greatly increased by intravenous ANG II in sodium-depleted, adrenalectomized rats. The effect was significant within 30 min of the start of the ANG II infusion and within 15 min of saline access. Similar intravenous infusions of ADH and phenylephrine failed to elicit sodium intake; in fact, sodium intake was reduced during ADH infusions, so the increased consumption of sodium is specific to infusions of ANG II.

The plasma ANG II concentration of adrenalectomized rats, depleted of sodium for 22 h, is ~1,000 ng/ml (15). The infusions used by us would probably bring plasma ANG II concentrations close to this value. In our 22-h depletion experiment, the average rate of infusion at the beginning of the drinking period was ~25 ng/min, which would be expected to raise plasma ANG II concentration in a 400-g rat by ~500 ng/ml (10). The initial infusion rate in the 9-h depletion experiment was slightly faster (30 ng/min), which would increase plasma ANG II concentration by 600 ng/ml.

In theory, there are several ways in which circulating angiotensin can stimulate sodium intake. First, ANG II stimulates release of aldosterone, which may directly act on the brain to stimulate sodium appetite (2, 3). As the rats in our study did not have adrenal glands, it follows that changes in plasma aldosterone concentration do not account for sodium appetite in our study.

Second, the restoration of blood pressure caused by intravenous infusion of ANG II might reduce malaise,
and this might unmask an existing sodium appetite. For several reasons it seems unlikely this mechanism accounts for our results. During the depletion period, water and food intake were similar, whether or not captopril was infused. Because reductions in water and food intake are commonly regarded as signs of malaise, it seems unlikely that the reduction in sodium intake after infusion of captopril was due to a nonspecific disruption of ingestive behavior. Also, the blood pressure observed here in captopril-blocked, adrenalectomized rats (~70 mmHg) is higher than what is argued to be frankly debilitating in acute experiments (~35–50 mmHg; Refs. 16 and 17). With respect to the stimulation of sodium intake by intravenous infusion of ANG II, we found that intravenous infusion of ADH or phenylephrine failed to stimulate sodium intake, although the infusions increased blood pressure. It follows that relief of incapacitating hypotension is not the cause of the sodium appetite seen after intravenous infusion of ANG II.

Third, circulating ANG II might act directly on angiotensin receptors in the circumventricular organs to stimulate brain pathways involved in sodium appetite. This mechanism accounts for at least a part of the sodium appetite caused by treatment with the diuretic furosemide (5, 19, 20). Our results are completely compatible with this theory and suggest that this is a powerful mechanism. Circulating ANG II accounts for much, possibly all, of the sodium appetite in adrenalectomized rats.

Perspectives

The ingestion of sodium as a result of high levels of circulating ANG II is a common phenomenon observed widely among mammals (it occurs in cows, sheep, rabbits, mice, and rats). Our results indicate that it is a quantitatively important mechanism, and circulating ANG II may account for all of the sodium appetite in adrenalectomized rats. Because ANG II is a peptide molecule that cannot penetrate the blood-brain barrier, our results also suggest that the sensory circumventricular organs of the brain must have key roles in the neural basis of adrenalectomy-induced sodium appetite.

This research was funded by National Heart, Lung, and Blood Institute Grants HL-54292 to R. L. Thunhorst and HL-14388 and HL-57472 to A. K. Johnson and by Office of Naval Research Grant N00014–97–1–045 to A. K. Johnson. G. H. M. Schoorlemmer was partly supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo.

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