Peripheral angiotensin causes salt appetite in rats

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Thunhorst, Robert L., and Douglas A. Fitts. Peripheral angiotensin causes salt appetite in rats. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36): R171–R177, 1994.—The prevailing theory of sodium depletion-induced salt appetite states that angiotensin (ANG) of cerebral origin, not of renal origin, causes the behavior. This assertion depends partly on an experiment that suppressed salt appetite using an intravenous infusion of a dose of angiotensin-converting enzyme (ACE) inhibitor (captopril, 2.5 mg/h) that supposedly blocked both central and peripheral enzyme (17). The present experiments used the same dose of captopril to suppress salt appetite both in 24-h and in 3-h models of sodium depletion-induced behavior. The captopril-infused rats *1*) increased water intake normally after central injections of ANG I given immediately after each salt appetite test, *2*) had no arterial pressor response after intravenously injected ANG I, and *3*) had normal arterial pressor responses after either intravenously injected ANG II or centrally injected ANG I. Thus, although the peripheral ACE was completely blocked in captopril-infused rats, the central ACE was not, because the central thirst and arterial pressor responses to ANG I were indistinguishable from controls. This demonstrates that ANG of renal origin is necessary for the expression of sodium depletion-induced salt appetite.

blood pressure; drinking; thirst; sodium depletion

CONDITIONS AS DIVERSE as water deprivation, lactation, severe physical exertion, and hemorrhage deplete the extracellular fluid spaces of water and sodium. During times of extracellular fluid deficits, physiological and behavioral mechanisms act to restore body water and sodium to normal levels. The hormone angiotensin II (ANG II) is one physiological mechanism that defends the extracellular fluid matrix by its actions on blood vessels, neurohormonal secretions, and the kidneys to maintain blood pressure and promote retention of remaining body water and sodium. Angiotensin II also contributes to the behavioral defense of extracellular fluid by stimulating water thirst and salt appetite.

Angiotensin II derives from renal and cerebral origins. Water drinking during sodium depletion, hypovolemia, or hypotension in the rat appears to be initiated largely by renal ANG II and may involve cerebral ANG II as well (26). Salt appetite, however, has been claimed to result from ANG II of cerebral origin alone because efforts to elicit salt appetite unambiguously with intravenous administration of ANG II (5) or to suppress salt appetite with a blockade of peripheral ANG II receptors (17) have failed.

It is easy to abolish salt appetite by manipulating the brain. A blockade of cerebral ANG II receptors, or of cerebral ANG II synthesis, has been shown repeatedly to reduce salt appetite to sodium depletion (13, 17, 25) or, indeed, to an intravenous infusion of ANG II itself (27). However, the demonstration that cerebral ANG II is necessary for salt appetite to occur during sodium depletion does not rule out a parallel role for peripherally synthesized ANG II. The two may be in series, with peripherally generated ANG II binding to a circumventricular organ, for instance, and provoking efferent neural traffic to angiotensinergic terminal fields inside the blood-brain barrier. Blocking either the receptors or synthesis of ANG II in these neurons would abolish salt appetite even if ANG II continued to be available to the circumventricular organs.

What has not been as easy to demonstrate is whether peripheral ANG II initiates salt appetite during sodium depletion in the rat. Experiments showed that *1*) intravenous ANG II did not synergize with peripheral deoxycorticosterone acetate (DOCA) to provoke salt drinking as centrally administered ANG II did (17), *2*) intravenous administration of a peptide blocker of ANG II receptors did not block the salt appetite to sodium depletion (17), and *3*) intravenous administration of the angiotensin-converting enzyme inhibitor, captopril, inhibited salt appetite, presumably by blocking the central conversion of ANG I to ANG II (13, 17). It was the unproven presumption of the last experiments, that the cerebral synthesis of ANG II was completely blocked by the intravenous infusion of captopril, that led us to conduct this study. If the peripheral captopril did not completely block ANG II synthesis within the blood-brain barrier, then those findings could actually be taken to support the opposite view, that a blockade of peripheral ANG II synthesis alone was sufficient to abolish salt appetite in response to sodium depletion.

METHODS

Animals. Male Long-Evans rats weighing 325–350 g were obtained from the University of Washington Department of Psychology vivarium (n = 21) and from Charles River (Wilmington, MA; n = 10). They were housed singly in hanging, wire cages for at least 1 wk before experimentation. Wayne (Univ. of Washington experiments) or Purina lab chow (Univ. of Iowa experiments), tap water, and 0.3 M NaCl were available ad libitum except during experiments. Room lights were on for 12 h/day, and temperature was maintained at 23°C.

Surgery. Rats were anesthetized with Equi-Thesin (0.35 ml/100 g body weight ip) and fitted with 26-gauge stainless steel cannulas in the left lateral ventricle at stereotaxic coordinates anteroposterior −0.6, lateral +1.4, and dorsoventral −4.3 mm relative to the skull surface and bregma. The cannula was filled by a 33-gauge obturator at all times except during injections. After the animals recovered for at least 3 days, catheters constructed from PE-50 and PE-10 tubing (22) were inserted into the femoral vein under halothane or metofane anesthesia and were tunneled under the skin to exit between the shoulder blades. Some rats (Univ. of Iowa) received a second venous catheter and a femoral arterial catheter as well. Testing began 2–4 days after catheterization surgery.

Drugs. Furosemide (Elkins-Sinn, Cherry Hill, NJ) was injected at 5 mg/0.5 ml sc, undiluted, twice per rat. Captopril (SQ-14,224), a gift from Bristol-Myers Squibb (Princeton, NJ), was dissolved in 5% dextrose in water (D5W) and infused intravenously at a dose of 2.5 mg/h in 0.6 ml/h volume. ANG I (Univ. of Washington: Bachem California, Torrance, CA; Univ. of Iowa: Hypertensin 1, Sigma Chemical, St. Louis) was dissolved in sterile isotonic saline before testing. ANG I was administered intracerebroventricularly at 20 or 40 ng in 2 μl volume and intravenously at 50 and 100 ng/rat in 100 and 200 μl volumes, respectively. ANG II ([Asp^1,Ile^5]ANG II) was a gift from Ciba-Geigy (Summit, NJ).

Behavioral testing during 24-h sodium depletion. On the first day of the experiment, each rat was given two injections of 5.0 mg of furosemide subcutaneously 30 min apart. An infusion into the femoral venous catheter of the angiotensin-converting enzyme (ACE) inhibitor captopril (2.5 mg/h in 0.6 ml/h vol; $n = 7$) or an equal volume of the D5W ($n = 7$) was started immediately after the first injection of furosemide and continued overnight for the next 18 h. The catheters were protected by steel springs. The rats had no food or other source of ambient sodium during this time but did have access to water.

Before the salt appetite test the next morning, the overnight water intake was measured, and the graduated cylinders were replaced with glass burettes containing either water or 0.3 M NaCl. The burettes were calibrated to 0.1 ml and fitted with drinking spouts. The presence of sodium was announced to the rats by sprinkling a few drops of the saline solution on their lips and whiskers. Intakes of both fluids were measured at 30, 60, 90, and 120 min. Infusions continued throughout the test.

Immediately at the end of the 120-min salt appetite test, the NaCl solutions were removed, and injectors containing ANG I were inserted into the intracerebroventricular cannulas. Each injector was connected to a 10-μl Hamilton syringe by a 1-m length of PE-10 tubing filled with sterile isotonic saline except for the immediate injector end, which was filled with a solution of ANG I separated from the saline by a 0.5-μl bubble of air. Ten minutes later a 2-μl bolus injection of either 20 ng ($n = 4$/group) or 40 ng ($n = 3$/group) of ANG I was then made intracerebroventricularly into each rat and the latency to drink was recorded. Intake of water was recorded at 15 and 30 min.

Blood pressure testing during 24-h sodium depletion. A second experiment was conducted to verify that the intravenous infusion of 2.5 mg/h of captopril completely blocked the ACE in the periphery. The experiment was conducted similarly to the salt appetite experiment, except that the rats were fitted with two femoral venous and one arterial catheter before the experiments, and the dependent variables were blood pressure and the overnight fluid balance measures instead of salt appetite. There were five vehicle-treated and four captopril-treated rats.

Direct intra-arterial blood pressure measurements were taken using a Cobe transducer and a polygraph (Sensormedics R611, Anaheim, CA) (22). Basal blood pressure measurements were obtained 4 h after beginning treatment and again the next morning after ~20 h of treatment. After the basal blood pressure reading at 20 h, rats were injected intravenously through the second venous catheter at 5- to 10-min intervals with the following: 50 ng ANG I, 100 ng ANG I, 50 ng ANG II, and D5W vehicle. The agents were placed in the venous line and delivered by a 0.25-ml flush of D5W. The rats also received an injection of 20 ng ANG I icv to test for central blockade of ACE.

Urine was collected overnight in preweighed glass beakers via stainless steel funnels placed beneath the cages. Urine was measured for volume, and urinary sodium and potassium concentrations (U_{Na} and U_K, respectively) were determined by ion-specific electrodes (NOVA Biomedical, Waltham, MA) for calculation of urinary sodium and potassium excretions ($U_{Na}V$ and U_KV, respectively).

Behavioral testing during 3-h sodium depletion. A third experiment tested the generality of the intravenous infusion of 2.5 mg/h of captopril to prevent salt appetite in an experimental model that relies on formation of ANG II within brain tissue located outside the blood-brain barrier. In this model, furosemide and a low dose of captopril (5 mg/kg) are administered simultaneously to produce sodium depletion and inhibition of ACE within the lungs, kidneys, and other peripheral microcirculation (6). However, the high concentrations of ACE within the circumventricular organs (16, 19) are thought to remain functional at this dose of captopril so that conversion of ANG I to ANG II continues there. D5W vehicle was infused intravenously into control rats ($n = 3$) beginning at the time of the furosemide-captopril injections. Captopril was infused into other rats ($n = 4$) at 2.5 mg/h iv in addition to the low dose of captopril they received subcutaneously. Drinking tests began 1 h after the injections of furosemide and captopril and lasted for 2 h. Immediately after the 2-h intake test, 20 ng of ANG I were injected intracerebroventricularly into all rats. The intravenous infusions ran throughout this intracerebroventricular test.

Statistical analysis. Experiments were analyzed with an analysis of variance (ANOVA) appropriate to the individual designs. Planned comparisons were made with Fisher's least-significant difference tests when the global F ratio was significant. A probability of <0.05 was required for significance.

RESULTS

Effects of intravenous captopril on water and saline intakes after 24-h sodium depletion. Water drinking during the overnight period of sodium depletion and fasting averaged 15.1 ± 1.2 (SE) ml in the D5W control group and 5.1 ± 1.1 ml in the captopril group [$t(12) = 6.25$; $P < 0.001$].

Figure 1 shows cumulative saline intake during the salt appetite test. The data were analyzed as raw intakes within each period in an ANOVA, and as expected the infusion of captopril abolished the intake of 0.3 M NaCl solution compared with the robust intake of the D5W group [main effect $F(1,12) = 18.38$, $P < 0.01$]. The interaction of infusion conditions with time was significant [$F(3,36) = 3.32$, $P < 0.05$], indicating that the differences in intakes were greatest in the first 0.5 h of the drinking test.

Figure 2 shows the water intakes generated by intracerebroventricular injections of ANG I immediately after the salt appetite test. The captopril and D5W infusions were continued throughout this test, and the captopril-infused rats drank at least as much water as the D5W controls at both 15- and 30-min time periods and at both the 20- and 40-ng doses. No differences involving infusion groups were significant. The main effect of dose barely missed significance with a two-tailed test [$F(1,10) = 4.27$; $0.05 < P < 0.10$], and the effect of time was significant [$F(1,10) = 20.21$, $P < 0.01$]. The latencies to drink at the 20- and 40-ng doses, respectively, were 5.2 ± 1.0 and 0.8 ± 0.2 min in the D5W-infused control group and 3.8 ± 0.9 and 1.3 ± 0.8 min in the captopril-infused group, which were not

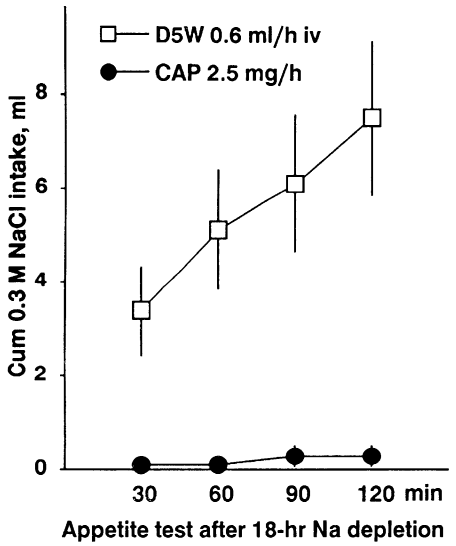

Fig. 1. Cumulative intake of 0.3 M NaCl solution beginning 18 h after furosemide-induced sodium depletion in groups of naive rats receiving continuous intravenous infusions of 5% dextrose in water (D5W) vehicle or captopril (CAP) during entire period of depletion and testing. Values are means ± SE. Captopril infusion completely suppressed sodium depletion-induced salt appetite. This replicates Sakai et al. (17), who found partial suppression with 2.5 mg/h iv captopril in rats that had previously been depleted twice before testing.

different statistically. The effect of dose on the latency to drink was significant [$F(1,10) = 15.61, P < 0.01$].

Water intake during the salt appetite test was also greater in the D5W-infused control group than in the captopril-infused group, probably as a result of osmotic changes induced by the ingestion of hypertonic saline.

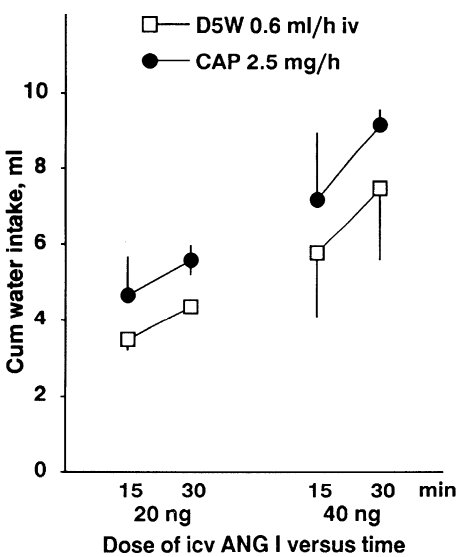

Fig. 2. Cumulative water intake at 15 and 30 min after an intracerebroventricular injection of 20 or 40 ng of angiotensin I (ANG I) immediately after salt appetite test in groups of rats receiving intravenous infusions of D5W vehicle or 2.5-mg/h captopril continuously from time of furosemide injection until end of this drinking test. Values are means ± SE. Peripheral captopril totally failed to block synthesis of angiotensin II (ANG II) from ANG I in brain at any dose or time even though dose completely suppressed salt appetite during previous 2 h. Thus salt appetite depended on synthesis of peripheral rather than cerebral ANG II.

Cumulative water intakes over the 120-min experiment were 1.9 ± 0.8 and 0.3 ± 0.2 ml in the D5W and captopril groups, respectively.

Effects of intravenous captopril on urinary excretion measures and water intake during 24-h sodium depletion. There were no significant effects of intravenous captopril treatment on any of the urinary excretion measures obtained acutely (*hours 0–2*) after furosemide-induced diuresis (Table 1). A significant interaction of infusion condition with time [$F(1,7) = 12.28, P < 0.01$] revealed that the captopril group did not increase urine volume (UV) during the overnight period of sodium depletion and fasting (*hours 2–20*) compared with the D5W control group, probably because the captopril group drank less water overnight than the control group [$F(1,7) = 47.14, P < 0.001$]. While the total, cumulative UV of the captopril group before testing was reduced compared with controls [$F(1,7) = 27.21, P < 0.01$], the overall water balance before testing, calculated as water intake plus infusion volume minus UV, was not different between the infusion conditions [$F(1,7) = 1.11, P > 0.05$].

U_{Na} and $U_{Na}V$ were significantly reduced overnight compared with the acute diuresis period after furosemide treatment [main effects of time, both $F(1,7)$ values $> 286.10, P < 0.001$] (Table 1). However, these measures were not different between the infusion conditions, and the cumulative loss of urinary sodium before testing was equivalent between the groups [all $F(1, 7)$ values $< 4.16, P > 0.05$]. The captopril group increased U_K in the overnight period [interaction, $F(1,7) = 12.03$, $P < 0.05$]. This was a function of the reduced water intake and urine output rather than increased U_KV in the captopril-infused rats because the groups increased U_KV equally overnight [main effect of time, $F(1,7) = 47.42$, $P < 0.001$]. The cumulative loss of urinary potassium before testing was also equivalent between the groups [$F(1,7) = 0.81, P > 0.05$].

Effects of intravenous captopril on blood pressure during 24-h sodium depletion. Captopril significantly reduced blood pressure after the furosemide diuresis compared with D5W controls at both 4 h (92 ± 3 vs. 108 ± 4 mmHg) and 20 h (73 ± 6 vs. 112 ± 7 mmHg) [both $F(1,8)$ values $\geq 8.97, P < 0.05$].

The blood pressure readings for each of the injection conditions for the captopril and D5W groups are shown in Table 2. The pressor responses to intravenous vs. intracerebroventricular injections were analyzed separately. The 50- and 100-ng doses of ANG I produced dose-related increases in mean arterial pressure in the D5W group but not in the captopril-infused group [interaction, $F(3,21) = 21.88, P < 0.001$]. A 50-ng dose of ANG II produced equal pressor responses in the two groups, indicating that the vascular responsiveness to ANG II was normal in captopril-treated animals. Vehicle injections produced small increases in blood pressure in both groups that probably were due to remaining traces of ANG II in the lines. A 20-ng bolus intracerebroventricular injection of ANG I caused equal pressor responses in the groups [$F(1, 7) = 0.48, P > 0.05$].

Table 1. *Water balance and electrolyte excretions in D5W- and CAP-infused rats during 2-h diuresis and 18-h overnight periods preceding blood pressure measurements of experiment 2*

	D5W (n = 5)			D5W+CAP (n = 4)		
	0–2 h	2–20 h	0–20 h	0–2 h	2–20 h	0–20 h
Body wt, g	372 ± 12			382 + 13		
UV, ml	18.9 ± 1.3	37.4 ± 3.8	56.3 ± 2.8	18.2 ± 1.5	16.6 ± 1.9†	34.8 ± 3.0†
D5W iv, ml	1.2 ± 0.0	10.8 ± 0.0	12.0 ± 0.0	1.2 ± 0.0	10.8 ± 0.0	12.0 ± 0.0
Water intake, ml		31.8 ± 3.8	31.8 ± 3.8		13.3 ± 4.3‡	13.3 ± 4.3‡
Water balance, ml	−17.7 ± 1.2	5.2 ± 2.7	−12.5 ± 1.8	−17.0 ± 1.5	7.4 ± 0.7	−9.6 ± 2.1
U_{Na}, μmol/ml	120 ± 3	12 ± 2		110 ± 5	13 ± 2	
$U_{Na}V$, μmol	2,260 ± 132	437 ± 45	2,697 ± 120	1,992 ± 170	225 ± 66	2,217 ± 219
U_K, μmol/ml	33 ± 2	46 ± 5		38 ± 3	85 ± 4*	
U_KV, μmol	609 ± 38	1,702 ± 182	2,311 ± 170	690 ± 85	1,404 ± 142	2,094 ± 166

Results are expressed as means ± SE; n = no. of animals. 5% Dextrose in water (D5W) served as intravenous (iv) vehicle for 2.5 mg/h captopril (CAP). UV, urinary volume; U_{NA} and U_K, urinary sodium and potassium concentrations, respectively; $U_{Na}V$ and U_KV, urinary sodium and potassium excretions, respectively. CAP vs. D5W controls: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

Effects of intravenous captopril on water and saline intakes during 3-h sodium depletion. Vehicle-infused control rats drank significantly greater amounts of water and saline solution than captopril-infused rats in the 2 h of fluid access after furosemide-captopril treatment (Table 3). In response to intracerebroventricular injection of 20 ng of ANG I, the reverse was true; rats treated with intravenous captopril drank a considerably greater amount of water than the rats treated with a control infusion of D5W, probably because the latter rats had just ingested 18 ml of fluid in the preceding test. Both groups of animals had previously drunk in response to intracerebroventricular ANG II, so the cannulas were patent.

DISCUSSION

The present experiments used intravenous infusions of the ACE inhibitor, captopril (2.5 mg/h), to suppress salt appetite both in 24-h and in 3-h models of sodium depletion-induced behavior. In addition, the captopril-infused rats *1*) drank water normally after central injections of ANG I given immediately after each salt appetite test, *2*) had no arterial pressor response after intravenously injected ANG I, and *3*) had normal arterial pressor responses after either intravenously injected ANG II or centrally injected ANG I. These findings indicate that there was complete, functional blockade of peripheral ACE in captopril-infused rats, yet central ACE was not blocked because the central thirst and arterial pressor responses to ANG I were indistinguishable from controls. These findings provide strong evidence that ANG of peripheral origin is necessary for depletion-induced salt appetite.

The intravenous captopril significantly reduced water intakes in the overnight period between the furosemide injection and the onset of testing in both experiments using the 24-h depletion protocol. This significant reduction in water intake in the captopril groups suggests that the ACE in the subfornical organ (SFO) was completely blocked during the postdiuresis period because a dose of captopril that does not completely block the SFO greatly increases water intake (11, 18, 21). The finding that water intake was actually reduced instead of enhanced suggests that the overnight intake of water after furosemide is mediated in part by circulating ANG II acting at the SFO. This is partially confirmed by the finding that rats with lesions of the SFO have reduced water intake compared with sham-lesioned rats during the 2 h immediately after a furosemide injection (20). However, those rats did not have reduced intakes during the remaining 20 h of the 22-h depletion. This discrepancy probably resulted from the fact that the rats in the study of Thunhorst et al. (20) had low sodium diet to eat overnight, whereas the rats in the present study were food deprived and therefore had no prandial drinking overnight.

The intravenous captopril virtually abolished the intake of saline when it was offered the morning after

Table 2. *Effect of ACE inhibition on pressor responses to ANG I and ANG II*

		Change in MAP from Baseline, mmHg				
	n	ANG I (50 ng iv)	ANG I (100 ng iv)	ANG II (50 ng iv)	Vehicle	ANG I (20 ng icv)
D5W	5	17 ± 2	31 ± 2	37 ± 2	5 ± 2	20 ± 1
D5W + CAP	4	3 + 3*	0 + 1*	35 ± 5	6 ± 2	21 ± 1

Results are expressed as means ± SE; n = no. of animals. D5W served as intravenous vehicle for 2.5 mg/h captopril. ACE, angiotensin-converting enzyme; ANG I, angiotensin I; ANG II, angiotensin II; MAP, mean arterial pressure. *$P < 0.05$ vs. D5W controls but not vs. baseline measurements.

Table 3. *Effect of intravenous captopril on drinking and salt appetite after subcutaneous injections of furosemide and captopril and on subsequent water drinking response to an intracerebroventricular injection of ANG I*

	D5W Vehicle	Captopril
Furosemide + captopril		
Water intake, ml	12.3 ± 1.1	4.8 ± 1.1*
Saline intake, ml	5.4 ± 1.8	0.9 ± 0.7*
ANG I (20 ng icv)		
Water intake, ml	0.9 ± 0.5	4.2 + 1.5

Results are expressed as means ± SE. D5W served as intravenous vehicle for 2.5 mg/h captopril. Captopril vs. D5W controls: *$P < 0.01$.

depletion. This replicates the conditions and findings of Sakai et al. (17) that an intravenous infusion of 2.5 mg/h captopril blocks depletion-induced salt appetite. In addition, however, the captopril-infused rats showed no deficit either in intake or in latency to drink after an intracerebroventricular ANG I injection. Thus the ACE within the blood-brain barrier was not blocked by 2.5 mg/h captopril infusions.

The effects of intravenous captopril infusion on blood pressure in the second experiment strengthen this conclusion. First, the reduced basal blood pressure of the captopril group suggests that the production of circulating ANG II was inhibited sufficiently to compromise the maintenance of normal pressure under these hypovolemic conditions. Second, the intravenous captopril prevented the pressor response to bolus intravenous injections of ANG I but not to central injections of ANG I. These findings further indicate that peripheral, but not central, stores of ACE were inhibited by 2.5 mg/h captopril.

It has been argued that systemically administered captopril can leak across a damaged blood-brain barrier to inhibit ACE inside the brain for up to 2 wk after brain cannulation surgery and thereby inhibit the development of depletion- and captopril-induced salt appetite (2, 13). Therefore, our finding that intracerebroventricular ANG I produced completely normal pressor and drinking responses after nearly 20 h of intravenous captopril infusion is especially striking given that the rats were cannulated only 5–6 days before testing. Our results agree with those of Rowland and Fregly (15), who found no impairment in captopril-induced salt intake in rats cannulated only 7 days before testing. Collectively, the data offer no support for the idea that captopril can cross a leaky blood-brain barrier to inhibit ACE inside the brain.

The water and electrolyte balance data from the 2-h diuresis and the 18-h overnight periods show that the D5W and captopril groups maintained equal hydromineral status throughout this pretest period. Water intakes were much lower in the captopril group, but urine volumes were reduced accordingly to yield a similar negative balance in that group compared with the controls. Urinary potassium concentrations were elevated in the captopril group, but this was simply a function of the reduced urine volumes, and total excretions of both sodium and potassium did not differ from controls. Finally, the blood pressures of the captopril group were lower than those of the control group in the second experiment because of the loss of circulating ANG II. This alone could not account for the difference in drinking because the mean arterial pressures in the captopril group were not low enough to debilitate the animals to the point that they would not drink (22).

The third experiment generalized the present findings to a 3-h model of depletion-induced behavior that uses simultaneous injections of furosemide and low doses of captopril to cause increased delivery of ANG I to the brain (18). The results indicate that the additional dose of intravenous captopril blocked ACE that is necessary for generating the intakes of water and saline in this model and that this ACE is accessible from the peripheral circulation. Because rats infused with captopril drank in response to intracerebroventricular injections of ANG I, these results indicate that intravenous captopril did not block ACE inside the blood-brain barrier. Therefore, the water and salt intakes by rats receiving combined furosemide-captopril treatment but not intravenous captopril rely on the generation of ANG II by ACE that is located on the blood side of the blood-brain barrier.

Sakai et al. (17) used a sustained intravenous infusion of captopril at 2.5 mg/h to block salt appetite induced by furosemide diuresis. They assumed that the dose was sufficient to block both peripheral and central conversion of ANG I to ANG II based on citations (1, 13) rather than observation and concluded from the experiment that the source of the ANG II generating the salt appetite was within the blood-brain barrier. The references cited did not use the same experimental protocols as Sakai et al. (17) and in one case did not use the same dosage. Our data show that the results of their experiment actually support the opposite conclusion, that the origin of ANG II participating in salt appetite is in fact the peripheral circulation.

Sakai et al. (17) presented two major experiments to support their conclusion that peripheral ANG II is not the cause of salt appetite in the rat. The first showed that combined, peripherally administered DOCA and ANG II do not evoke sodium intake in the replete rat. DOCA was administered subcutaneously for 5 days, and then ANG II was infused intravenously for 6 h. The peripheral ANG II infusions generated water drinking but no saline intake. Based on prior findings showing robust saline intake when the DOCA pretreatments were combined with a pulse intracerebroventricular injection of ANG II, the investigators concluded that an activation of central rather than peripheral ANG II was essential for the saline-drinking response.

It has recently become clear that water intake aroused by ANG II is easily inhibited by hypertension (3, 4, 9, 10, 14), and the same may be true for salt intake. The rise in mean arterial pressure of the intravenously infused ANG II group in the first experiment of Sakai et al. (17) must have been much greater than that of the intracerebroventricularly infused group (3), especially in rats already made hypervolemic by daily DOCA injections. This difference in pressor responses could account for the failure to provoke salt appetite with peripheral infusions.

The second major experiment of the study of Sakai et al. (17) showed the effects of peripheral vs. central interference with ANG II action on depletion-induced salt appetite. Their captopril protocol was replicated in our experiment. They also showed that intracerebroventricular infusions of the ANG II receptor-blocking peptide Sarile ([Sar,^{1} Ile^{8}] angiotensin II) reduced depletion-induced salt appetite in a dose-dependent manner but that peripheral infusions did not. These infusions began 2 h before the salt appetite test instead of continuing all night as in the captopril experiment.

A major problem with the interpretation of the peripheral infusions of Sarile during sodium depletion in this experiment has to do with the expected renin and ANG II concentrations in the plasma. The two doses of Sarile given in the experiment, 498 or 600 μg/h, were not very different, so a lack of a dose relationship on salt intake is not surprising. The investigators conclude that the infusions were ineffective because the full 2-h intake of the Sarile and control groups were not different. However, it is apparent from their graph that the means of the two Sarile-infused groups are each about 3 SEs below the mean of the control group at 15 min. This could indicate an early suppression of salt appetite that gradually decayed.

An infusion of Sarile in sodium-depleted rats would likely reduce the blood pressure and eliminate the negative feedback on renin secretion normally provided by ANG II. For instance, ligation of the vena cava above the renal veins produced renin secretion and ANG II formation, and a simultaneous blockade with the ANG II receptor blocker saralasin intravenously increased the plasma ANG II concentration from below 1,000 to above 4,000 fmol/ml (12). Consequently, it must be expected that renin secretion and plasma ANG II levels in the Sakai et al. study (17) were much higher in the Sarile-infused group than in the control group or in the intracerebroventricularly infused groups. Thus the salt appetite aroused by the combination of Sarile and sodium depletion may have been impossible to block with the given doses of Sarile. Also, agonist effects of the Sarile on salt intake cannot be ruled out from the data presented.

Can the findings of this study be explained by assuming that there are at least two dipsogenic pools of ANG II in the brain, one supporting water drinking and accessible to the ventricles and another supporting salt appetite and inaccessible to the ventricles? If so, an intracerebroventricular injection of ANG I might cause water drinking even though the ACE in the more remote salt appetite system was completely blocked. This happens not to be the case because salt appetite is easily blocked with intracerebroventricular receptor blockers or with both intracerebroventricular and peripheral captopril (6, 13, 17). This demonstrates that the ANG II system in the brain that supports salt appetite, both the receptors and the sites of synthesis, are readily accessible from the cerebral spinal fluid and are not blocked if the thirst system is not blocked.

The peripheral ANG II probably exerts its effects on salt appetite by acting at forebrain circumventricular organs. This conclusion is supported by evidence that ANG II does not cross the blood-brain barrier and that ANG II infused into the forebrain but not the hindbrain ventricles elicits salt appetite (6). The preponderance of evidence suggests that the organum vasculosum laminae terminalis (OVLT) is the circumventricular organ site for a peripheral action of ANG II in the arousal of salt appetite because infusions of ANG II into the OVLT but not the SFO elicit salt appetite (7) and because lesions of the OVLT suppress salt appetite aroused either by a low dose of oral captopril or by sodium depletion (8). Lesions of the SFO reduce salt appetite aroused by sodium depletion (20, 23), but the mechanism related to this effect is unknown. The fact that salt appetite is affected at all by manipulations of these circumventricular organs suggests that hormonal mediators such as circulating ANG II must be considered. Similar conclusions were reached by Weisinger et al. (24), who found that intravenous infusions of captopril reduced by one-half the salt appetite of sodium-deficient sheep. Subsequent intravenous infusions of ANG II restored the salt appetite to baseline levels or above, indicating that ANG II receptors on the blood side of the blood-brain barrier were responsible for reestablishing the salt appetite.

In summary, the present experiments have shown that it is possible to block the arousal of depletion-induced salt appetite in rats with an intravenous infusion of captopril that blocks all peripheral ACE, including that in the circumventricular organs, without blocking the ACE inside the blood-brain barrier. This demonstrates that ANG II of peripheral origin is necessary for the expression of salt appetite in response to sodium depletion.

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