Effects of hypotension and fluid depletion on central angiotensin-induced thirst and salt appetite

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Thunhorst, Robert L., and Alan Kim Johnson. Effects of hypotension and fluid depletion on central angiotensin-induced thirst and salt appetite. Am J Physiol Regulatory Integrative Comp Physiol 281: R1726–R1733, 2001.—We examined the effects of hypotension and fluid depletion on water and sodium ingestion in rats in response to intracerebroventricular infusions of ANG II. Hypotension was produced by intravenous infusion of the vasodilator drug minoxidil (25 μg·kg−1·min−1) concurrently with the angiotensin-converting enzyme inhibitor captopril (0.33 mg/min) to prevent endogenous ANG II formation. Hypotension increased water intake in response to intracerebroventricular ANG II (30 ng/h) but not intake of 0.3 M NaCl solution and caused significant urinary retention of water and sodium. Acute fluid depletion was produced by subcutaneous injections of furosemide (10 mg/kg body wt) either alone or with captopril (100 mg/kg body wt sc) before intracerebroventricular ANG II (15 or 30 ng/h) administration. Fluid depletion increased water intake in response to the highest dose of intracerebroventricular ANG II but did not affect saline intake. In the presence of captopril, fluid depletion increased intakes of both water and saline in response to both doses of intracerebroventricular ANG II. Because captopril administration causes hypotension in fluid-depleted animals, the results of the two experiments suggest that hypotension in fluid-replete animals preferentially increases water intake in response to intracerebroventricular ANG II. Because captopril administration causes hypotension in fluid-depleted animals, the results of the two experiments suggest that hypotension in fluid-replete animals preferentially increases water intake in response to intracerebroventricular ANG II and that fluid-depleted animals increase both salt and water intake in response to intracerebroventricular ANG II.

minoxidil; captopril; rats; urine volume; water balance; sodium excretion; sodium balance

THE RENIN-ANGIOTENSIN SYSTEM is a pivotal mechanism used by animals to defend body fluid homeostasis during extracellular dehydration. The effector peptide of the renin-angiotensin system, ANG II, is produced under conditions of reduced arterial blood pressure or extracellular fluid volume (6). ANG II stimulates the consumption of both water and sodium necessary for the repair of extracellular fluid deficits. Exogenous ANG II stimulates water and sodium ingestion on peripheral (10, 12, 14) and central (1, 3–5, 7) administration. Other mechanisms that maintain body fluid balance include mineralocorticoids (20, 35) and baroreceptor afferents (32, 34). In addition to roles in regulating the extracellular fluid space, central ANG II mechanisms appear to have a role in regulation of the intracellular space. Recent evidence suggests that central angiotensin systems mediate thirst owing to increased brain sodium concentration (2) and also natriuresis and pressor responses to central injections of hypertonic saline (22). Therefore, there are multiple considerations when interpreting results from the central administration of ANG II.

The dipsogenic and natriorexigent potency of ANG II depends on the prevailing levels of arterial blood pressure and body fluids. For example, increases in arterial blood pressure during intravenous infusions of ANG II inhibit the concomitant drinking responses (21). The inhibition is probably mediated by arterial baroreceptor afferent nerves (25). Reductions in arterial blood pressure are associated with increased water drinking responses to intravenous infusion of ANG II (9). Arterial blood pressure also modulates the drinking response to intracerebroventricular infusions of ANG II (17, 29, 31). Intravenous ANG II stimulates salt appetite more readily in fluid-depleted rats than in fluid-replete rats (10, 12). The present experiments were conducted to investigate if prevailing levels of arterial blood pressure and body fluids affect salt appetite, as well as thirst, in response to central administration of ANG II. Intracerebroventricular infusions of ANG II were given to rats that were either normotensive or hypertensive and to rats that were either fluid replete or fluid depleted.

METHODS

Animals. Male Sprague-Dawley-derived rats weighing 325–375 g were purchased from Harlan (Indianapolis, IN). They were housed individually in hanging wire cages for at least 1 wk before experimentation. Purina Rat Chow, tap water, and 0.3 M NaCl were available ad libitum except during the experimental periods. Room lights were on for 12 h/day, and temperature was controlled at 23°C.

Cannulation procedures. Animals were anesthetized with Equithesin (0.33 ml/100 g body wt) and secured in a Kopf 900 stereotoxic instrument. The skull was exposed by midline incision and held level. One 26-gauge stainless steel guide cannula was implanted into the left lateral cerebral ventricle...
using standard stereotaxic procedures. Coordinates with respect to bregma were −0.2 mm caudal, −1.4 mm lateral, and −4.5 mm from the surface of the skull. The cannula was secured to the skull with dental acrylic and stainless steel screws and was filled by a 33-gauge obturator at all times except during infusions and injections. Rats were allowed at least 1 wk to recover from the surgery before cannula patency testing. All rats used in the experiments drank >4.0 ml of water within 15 min after intracerebroventricular injection of carbachol (50 ng/2 μl).

Some rats under Equithesin anesthesia received femoral venous and arterial catheters for infusion of drugs and measurement of arterial blood pressure, respectively. Both catheters were made from polyethylene tubing (PE-50) ~25 cm in length that was heat welded to a shorter piece of PE-10. The PE-10 was inserted into the vessel and advanced 4 cm for arterial lines and 3 cm for venous lines. The catheters were tunneled under the skin and secured between the scapula to exit at the base of the neck. When not in use, the catheters were filled with heparinized saline (50 U/ml) and plugged with 33-gauge obturators. The rats were allowed at least 2 days to recover from catheter surgery before experimentation began.

Drugs. Furosemide (Abbott Laboratories, N. Chicago, IL) was administered subcutaneously at 10 mg/kg body wt. Captopril (SQ-14,225; Bristol-Myers-Squibb Pharmaceutical Research Institute, Princeton, NJ) was dissolved in sterile 0.9% NaCl immediately before each experiment. Captopril was infused intravenously at 0.576 ml/h, yielding 0.33 mg·9.6 μl·min⁻¹, or was injected subcutaneously at 100 mg/kg body wt in 1 mg/ml volume. ANG II ((Asp₁Ille₅)ANG II; Sigma, St. Louis, MO), was dissolved in sterile 0.9% NaCl at either 15 or 30 ng/9 μl and stored frozen in small aliquots in polypropylene vials. Fresh samples were thawed before each experiment. The intracerebroventricular infusion rates for ANG II were 0, 15, or 30 ng/h. Minoxidil (Sigma) was dissolved in propylene glycol for a stock solution of 10 mg/ml. It was diluted with sterile 0.9% NaCl or captopril solution immediately before each experiment to achieve an intravenous dose of 25 μg·kg body wt⁻¹·min⁻¹. Carbachol (carbamylcholine chloride; Sigma) was dissolved in sterile 0.9% NaCl at 25 ng/μl and injected intracerebroventricularly in 2-μl volumes.

General procedures. The test cages were wooden (24 × 29 cm) with aluminum-lined interiors that extended 31 cm above suspended, stainless steel metabolism cages.

In one experiment, venous and arterial catheters were connected to pumps and recorders by lengths of coiled PE-50. Arterial blood pressure was recorded on a polygraph (Dyograph Recorder, model R611, Sensormedics, Anaheim, CA) using Cobe transducers. Mean arterial pressure (MAP) was obtained by electronically damping the arterial signal. Venous catheters were connected to 5-ml syringes mounted in a Harvard infusion pump (model 975). In both experiments, 33-gauge injectors were inserted into the intracerebroventricular guide cannulas and were connected by lengths of coiled PE-10 to 100-μl Hamilton microsyringes mounted in Harvard pumps for delivery of ANG II or vehicle.

Urine was collected into polypropylene tubes via stainless steel funnels placed beneath the cages. Urine was measured for urine volume (UV). Urinary sodium and potassium concentrations (UNa and UK) were determined by ion-specific electrodes (NOVA Biomedical, Waltham, MA) and were used for calculation of urinary sodium and potassium excretions (UNaV and UKV). Relative water balances were calculated by subtracting UV from total fluid intake. Relative sodium balances were calculated by subtracting UNaV from sodium ingestion in the form of 0.3 M NaCl. Respiratory and fecal losses of water and sodium were not considered.

Experiment 1: effects of hypotension on water and saline intakes in response to intracerebroventricular ANG II. Water intake. 0.3 M NaCl intake, and MAP were measured in the same animals (n = 6). Rats received intracerebroventricular ANG II under conditions when MAP was either at or below resting levels in tests separated by 2–3 days. Test order was counterbalanced. Rats were weighed, connected to intravenous, arterial, and intracerebroventricular lines, and then placed in the test cages at least 30 min before the experiment began. Sterile 0.9% NaCl (9.6 μl/min) was infused intravenously for 30–45 min to allow the rats to acclimate. The intravenous test infuses followed immediately and consisted of a vehicle solution containing captopril (0.33 mg·9.6 μl·min⁻¹), which did not affect MAP, or a mixture of minoxidil (25 μg·kg·min⁻¹) plus captopril, which reduced MAP. This dose of captopril has been shown to prevent the endogenous formation of ANG II in the periphery and in the brain (29). At this time (t₀), funnels were placed beneath the cages for collection of urine, and glass burettes filled with water and 0.3 M NaCl were secured at the fronts of the cages. Central (intracerebroventricular) infusions of ANG II (30 ng/h) began 60 min later (t₀) and ran concurrently with the intravenous infusions for another 3 h.

Experiment 2: effects of fluid depletion on water and saline intakes in response to intracerebroventricular ANG II. Separate groups of rats were tested with intracerebroventricular ANG II under fluid-replete or fluid-depleted conditions. Rats were weighed, then placed in the test cages and given two subcutaneous injections ~10 min apart. Some rats were acutely depleted of body fluids by injections of furosemide (10 mg/kg body wt), which caused diuresis and natriuresis. A vehicle injection followed. Other rats were similarly depleted of body fluids by injections of furosemide and also received injections of a high dose of captopril (100 mg/kg body wt) to completely prevent the endogenous formation of ANG II in the periphery and partially prevent endogenous formation in the brain (8). As the formation of ANG II is essential for maintaining MAP in hypovolemic animals (e.g., Ref. 30), these animals were also likely to be mildly hypotensive. Additional rats received two subcutaneous injections of isotonic saline vehicle to control for the effects of repeated subcutaneous injections. The intracerebroventricular injectors were inserted after the second subcutaneous injection, and burettes of water and 0.3 M NaCl were provided at this time. Intakes were recorded every 30 min for 4 h. The intracerebroventricular infusions of ANG II at 0, 15, or 30 ng/h began 60 min after the first subcutaneous injection and continued for 3 h. Collection funnels were placed under the cages before the first subcutaneous injection, and urine was collected at 60, 150, and 240 min after the first subcutaneous injection. Thus there were three subcutaneous treatment conditions consisting of two injections: furosemide and vehicle, furosemide and captopril, and vehicle injected twice. There were three intracerebroventricular infusion conditions, namely, ANG II at 0, 15, or 30 ng/h. We did not infuse 0 ng/h ANG II into rats injected subcutaneously twice with vehicle, so there were eight separate groups of rats in all (n = 5–6/group).

Statistical analysis. Data were analyzed by ANOVA appropriate to the experimental designs. Planned comparisons were made with Fisher’s least significant difference tests when the global F ratio was significant and with the Bonferroni correction when it was not. All values reported as significant are at the P < 0.05 level.
RESULTS

Experiment 1: effects of hypotension on water and saline intakes in response to intracerebroventricular ANG II. There were no differences in body weight of the rats during testing with intravenous vehicle vs. minoxidil (body wt 440 ± 29 vs. 438 ± 29 g, respectively). Infusions of the captopril solution that served as the vehicle did not affect MAP (Fig. 1). Infusions containing minoxidil progressively reduced MAP below control levels (interaction, $F(8,40) = 11.87, P < 0.05$). Blood pressure was reduced ~30 mmHg by the start of intracerebroventricular ANG II ($t_{96}$).

Negligible amounts of water and saline were ingested before the intracerebroventricular ANG II infusions began. Hypotension nearly doubled average water intakes in the first 90 min of intracerebroventricular ANG II. However, water intakes of normotensive rats caught up to those of hypotensive rats in the next 90 min so that there was no treatment difference overall ($F(1,5) = 0.92, P > 0.05$; Fig. 2). Planned comparisons on water intakes in the first 90 min showed that reductions in MAP were associated with increased water intakes (Bonferroni, all $t$ values $\geq 3.08, P < 0.05$). Hypotension did not affect saline intakes ($F(1,5) = 0.52, P > 0.05$).

Effects of hypotension on water and sodium excretions and balances during intracerebroventricular ANG II. Hypotension reduced UV at each collection period compared with normotensive conditions [all $F_{s}(1,5) \geq 8.93, P < 0.05$; Fig. 3]. The reduced UVs and initially increased water intakes of hypotensive rats greatly increased their cumulative water balances compared with normotensive controls. Hypotension reduced $U_{Na}$ during intracerebroventricular ANG II and decreased $U_{NaV}$ at all collection times, resulting in significantly increased sodium balances compared with normotensive conditions [all $F_{s}(1,5) \geq 7.64, P < 0.05$]. Hypotension did not affect $U_{K}$; however, it reduced $U_{KV}$ during intracerebroventricular ANG II [cumulative $U_{KV}$ for minoxidil vs. vehicle infusions $= 130 \pm 37$ vs. $809 \pm 53$ μmol, all $F_{s}(1,5) \geq 29.64, P < 0.05$].

Experiment 2: effects of fluid depletion on water and saline intakes in response to intracerebroventricular ANG II. There were no differences in body weight across the treatment conditions. Average body weights for rats treated subcutaneously with vehicle, furosemide, and furosemide plus captopril were $434 \pm 9, 436 \pm 6$, and $424 \pm 9$ g, respectively, without regard to intracerebroventricular condition. There was no ingestion of water or saline in the 60 min before intracerebroventricular infusions. Rats infused intracerebroventricularly with isotonic saline drank an average of 0.7 ml of water across treatments (Fig. 4). Infusions of ANG II at 15 ng/h caused significantly more water drinking in rats treated with the combination of furosemide and captopril (furosemide/captopril) compared with the other groups [main effect, $F(7,34) = 9.90, P < 0.05$]. Infusions of ANG II at 30 ng/h stimulated significantly greater water intakes in both furosemide-treated and furosemide/captopril-treated rats compared with vehicle-treated rats.

Rats infused intracerebroventricularly with isotonic saline drank an average of 0.1 ml of 0.3 M NaCl across treatments (Fig. 4). Rats treated with furosemide/captopril drank significantly more saline than the other groups at both 15 and 30 ng/h doses of intracerebroventricular ANG II [main effect, $F(7,34) = 4.37, P < 0.05$]. Furosemide-treated and vehicle-treated rats did not differ in saline intake.
Effects of fluid depletion on water and sodium excretions and balances during intracerebroventricular ANG II.

There were significant main effects for UV and UNaV but not for UNa. Cumulative UV and UNaV were significantly greater across the experimental session in rats receiving subcutaneous furosemide or furosemide/captopril compared with rats receiving subcutaneous vehicle [all Fs(7,34) ≥ 19.05, P < 0.05; Fig. 5]. These measures were significantly more negative in furosemide-treated rats than in furosemide/captopril-treated rats at most collection times. The negative water balances of furosemide-treated and furosemide/captopril-treated rats improved, depending on the dose of intracerebroventricular ANG II, whereas the sodium balances became more negative or remained unchanged. U_K was reduced and U_KV was increased for rats receiving furosemide, either alone or in combination with captopril, in the first hour after injection, and in the first 90 min of central infusion [main effects, all Fs(7,34) ≥ 4.36, P < 0.05]. Cumulative U_KV for rats receiving vehicle, furosemide, or furosemide plus captopril were 363 ± 37, 794 ± 98, and 800 ± 65 μmol, respectively.

DISCUSSION

The first experiment showed that hypotension was associated with immediate, increased water drinking responses to intracerebroventricular infusions of ANG II. There were significant main effects for UV and UNaV but not for UNa. Cumulative UV and UNaV were significantly greater across the experimental session in rats receiving subcutaneous furosemide or furosemide and captopril together compared with rats receiving subcutaneous vehicle [all Fs(7,34) ≥ 14.55, P < 0.05; Fig. 5]. In the 60 min before intracerebroventricular infusions, UV and UNaV were significantly greater in rats receiving furosemide compared with those receiving furosemide plus captopril. The reduced UV and UNaV of rats receiving furosemide/captopril compared with rats receiving only furosemide probably resulted from the hypotension that occurs during furosemide/captopril treatment. However, in the first 90 min of intracerebroventricular ANG II, furosemide/captopril-treated rats excreted more water and sodium than similarly infused furosemide-treated rats, either as a delayed response to furosemide treatment or because of their significantly greater water and sodium intakes during this time. The water and sodium balances of rats receiving furosemide and furosemide/captopril were significantly reduced in the hour before intracerebroventricular infusion compared with rats receiving vehicle (main effects, both Fs(7,34) ≥ 19.05, P < 0.05; Fig. 5). These measures were significantly more negative in furosemide-treated rats than in furosemide/captopril-treated rats at most collection times. The negative water balances of furosemide-treated and furosemide/captopril-treated rats improved, depending on the dose of intracerebroventricular ANG II, whereas the sodium balances became more negative or remained unchanged. U_K was reduced and U_KV was increased for rats receiving furosemide, either alone or in combination with captopril, in the first hour after injection, and in the first 90 min of central infusion [main effects, all Fs(7,34) ≥ 4.36, P < 0.05]. Cumulative U_KV for rats receiving vehicle, furosemide, or furosemide plus captopril were 363 ± 37, 794 ± 98, and 800 ± 65 μmol, respectively.

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II. The enhanced drinking was accompanied by substantial retention of water and sodium. The rate of water drinking during hypotension slowed as the experiment progressed, so there were no significant differences in cumulative water intakes between conditions by the end of the experiment. Saline drinking was not affected by hypotension. The second experiment showed that fluid depletion did not substantially affect water and 0.3 M NaCl intakes in response to intracerebroventricular ANG II. However, fluid depletion combined with blockade of endogenous formation of ANG II increased ingestion of both 0.3 M NaCl and water in response to intracerebroventricular ANG II. The latter treatment reduces arterial blood pressure as well as blood volume. Together, these experiments suggest that hypotension concomitant with fluid depletion is associated with increased water and sodium ingestion in response to intracerebroventricular ANG II.

Previous work in this laboratory assessed the effects of hypotension on water drinking responses to intracerebroventricular ANG II (29). In that work, reductions in MAP during intravenous infusions of minoxidil were associated with a doubling of the 90-min drinking responses to two doses of ANG II (4 and 16 ng/h) compared with drinking obtained under normotensive conditions. The additional drinking was not due to endogenous formation of ANG II, either peripherally or in the brain, because the dose of captopril (i.e., 0.33 mg/min) employed concurrently with minoxidil was sufficient to prevent such formation. Furthermore, the hypotensive treatment by itself did not stimulate drinking, i.e., in the absence of intracerebroventricular ANG II. It was suggested that hypotension provides a neural signal from arterial baroreceptors that, while not effectively dipsogenic itself, modulates the dipsogenic activity of intracerebroventricular ANG II (29).

The goal of the first experiment presented here was to determine the effects of hypotension on the ability of intracerebroventricular ANG II to stimulate salt intake in addition to water intake. The experiment employed identical infusions of minoxidil mixed with captopril used previously (29). A higher dose of ANG II was infused intracerebroventricularly for twice the duration as in prior work because the salt appetite response to intracerebroventricular ANG II, as well as to other stimuli (e.g., subcutaneous polyethylene glycol; Refs. 26 and 27), is less robust than the water drinking response and occurs with a longer latency. The results of the first 90 min of testing replicate the earlier work in that hypotension was associated with a near doubling of the water drinking response to intracerebroventricular ANG II. However, the drinking responses of normotensive rats caught up to those of hypotensive rats in the next 90 min of testing. It is important to note that hypotension did not increase saline ingestion in response to intracerebroventricular ANG II. The considerable urinary retention of water and sodium during minoxidil infusion resulted in positive water and sodium balances that may have provided signals that slowed additional ingestion.

The goal of the second experiment was to determine the effects of fluid depletion on water and sodium intakes in response to intracerebroventricular ANG II. Injections of furosemide were used to produce acute extracellular fluid depletion by causing water and sodium excretion. Fluid depletion (i.e., furosemide treatment) increased water drinking only in response to the highest dose of intracerebroventricular ANG II (30 ng/h) and did not significantly affect saline intake in response to either dose of ANG II. However, fluid depletion in the presence of captopril increased water and saline intakes in response to intracerebroventricular ANG II compared with fluid-replete (i.e., subcutaneous vehicle) and fluid-depleted (i.e., subcutaneous furosemide) conditions. In fluid-depleted animals, administration of captopril at the dose used here results in modest hypotension (~10–25 mmHg; Refs.

Fig. 5. Cumulative UV (A), relative water balance (B), UNaV (C), and relative sodium balance (D) in response to icv infusions of ANG II (0, 15, or 30 ng/h) after subcutaneous injections of isotonic saline vehicle, furosemide, or Furo/Cap. Central infusions begin at 60 min. *Significantly different from vehicle, $P < 0.05$. **Significantly different from Furo/Cap, $P < 0.05$. Values are means ± SE.
11 and 30). The present excretion data provide functional evidence consistent with reductions in MAP in rats receiving furosemide/captopril treatment. The diminished diuresis and natriuresis of rats receiving furosemide/captopril compared with furosemide by itself are comparable to excretions observed in prior work using identical injections of these drugs and in which MAP was reduced in rats receiving furosemide/captopril (30). The reduced urinary excretion is likely attributable to reduced MAP, which causes renal retention of water and sodium (16). These results suggest that hypotensive, fluid-depleted animals have increased water and saline drinking responses to ANG II administered into the brain ventricles.

Reductions in arterial blood pressure can disrupt normal Starling forces at the capillary, resulting in blood volume expansion. Past work using minoxidil infusions such as those employed here (experiment 1) to reduce MAP resulted in significantly decreased hematocrit and plasma protein, indicating expansion of blood volume by about 5–6% (31). Minoxidil infusions in the present work clearly resulted in retention of water and sodium and positive water and sodium balances during testing. Therefore, rats infused with minoxidil in the present experiments must have had significantly expanded volume compared with their normotensive control conditions. Hypotension in these fluid-replete rats did not increase the salt appetite response to intracerebroventricular ANG II as it does the thirst response (29), at least not in the acute experiment described here. It is possible that the volume expansion that must have ensued during hypotension (note the increased water and sodium balances) inhibited further ingestion of water and saline. The results of experiment 2 speak to this possibility. In the second experiment, rats were depleted of fluid, i.e., placed in negative water and sodium balance, before infusion of intracerebroventricular ANG II. Rats receiving captopril likely were mildly hypotensive in addition to being fluid depleted. Thus we had two groups of hypotensive rats [rats infused with minoxidil (experiment 1) and rats treated with furosemide/captopril (experiment 2)]. One group was likely volume expanded and the other was volume contracted. Rats receiving furosemide/captopril drank as much saline in 1 h of intracerebroventricular ANG II (30 ng/h) as rats infused with minoxidil drank during the entire test. It is reasonable to conclude that the unloading of volume in furosemide/captopril-treated rats, in which blood pressure was also reduced, favored the increased ingestion of fluids. Although rats treated with either furosemide or furosemide/captopril were in negative water and sodium balances, only furosemide/captopril-treated rats, which were presumably hypotensive, had increased water and saline drinking responses to ANG II. Because only the hypotensive, volume-contracted rats drank additional saline in response to intracerebroventricular ANG II, the second experiment suggests that volume expansion prevented increased saline intakes in minoxidil-treated rats. These results support a role for volume receptors, i.e., cardiopulmonary baroreceptors, in the control of salt appetite during intracerebroventricular infusion of ANG II.

Aldosterone is another factor that must be considered in the enhanced sodium intake in the second experiment. Systemic mineralocorticoids potentiate the salt appetite response to centrally administered ANG II (15). Aldosterone levels increase approximately threefold in response to furosemide/captopril treatment (33), so it is possible that increased circulating aldosterone contributed to the increased salt appetite under the present experimental conditions. Aldosterone may directly synergize with ANG II to potentiate salt appetite (15) or may suppress secretion of oxytocin, an agent hypothesized to be inhibitory to salt appetite (28). It is noteworthy that aldosterone levels increase comparably in rats receiving furosemide alone and in combination with captopril (33), so increased aldosterone levels alone do not account for the increased salt appetite response to intracerebroventricular infusions of ANG II.

Reductions in arterial blood pressure were associated with both experimental situations in which ingestion was increased, so a contribution by arterial baroreceptors must be considered. Increases in arterial blood pressure inhibit water drinking responses to ANG II (21, 24), while reductions in blood pressure increase drinking responses to ANG II (9, 29). It is possible that the small reductions in blood pressure that must have occurred in rats receiving furosemide/captopril treatment in the present experiments may have reduced inhibitory signals arising from baroreceptors, thus permitting greater responsiveness to intracerebroventricular ANG II. However, interruption of arterial blood pressure information to the brain after sinoaortic baroreceptor deaerentation (SAD) does not change water drinking responses to intracerebroventricular ANG II, even when MAP is experimentally increased or decreased (31). Perhaps salt appetite is affected by arterial blood pressure levels more than water intake is. Removal of arterial baroreceptor input to the brain diminishes by half the salt appetite response to overnight fluid depletion (32). We have not tested whether SAD affects salt appetite responses to intracerebroventricular ANG II.

The second experiment is similar to other work assessing the effects of central infusions of ANG II on thirst and salt appetite responses after extracellular fluid depletion. In work by Fitts et al. (13), rats were depleted of body fluids by subcutaneous injections of furosemide and subsequently infused intracerebroventricularly with ANG II (438 ng/h) or vehicle. The dose of ANG II employed by Fitts et al. (13) was considerably higher than those used here. The intracerebroventricular infusions of ANG II caused additional water and saline drinking compared with infusions of vehicle in the depleted rats. This earlier work did not compare infusions of ANG II in fluid-depleted vs. fluid-replete rats. Thus the present work differs from Fitts et al. (13) and shows that, in rats infused centrally with ANG II, fluid depletion has little effect on water and saline intakes in response to centrally administered ANG II.
compared with intakes observed in fluid-replete rats infused centrally with ANG II. Thus centrally administered ANG II increases water and sodium consumption of fluid-depleted animals, but fluid depletion does little to change the potency of intracerebroventricular infusions of ANG II to stimulate water and sodium intake.

In other work, Fitts et al. (11) used procedures similar to those reported here to test the effectiveness of ANG II to generate water and saline drinking when infused directly into forebrain structures under hypotensive conditions. ANG II (80 ng/h) was infused into the subfornical organ (SFO), organum vasculosum laminae terminalis (OVLT), or the third ventricle. Small reductions of arterial pressure (~20 mmHg) after a low dose of minoxidil (0.75 mg/kg sc) were associated with increased water intake when ANG II was infused into the third ventricle. Greater reductions in arterial pressure (~40 mmHg) after a higher dose of minoxidil (2.25 mg/kg sc) were associated with decreased intakes of both water and saline when ANG II was infused at any of the sites. Fitts et al. (11) attributed the suppression of behavior to detrimental effects of reduced arterial pressure or to direct effects of the injected minoxidil.

Our work here suggests the alternative possibility that the greater reduction in MAP after the higher dose of minoxidil caused significant volume expansion that suppressed the behavior. Fitts et al. (11) also reduced arterial blood pressure by depleting animals of fluid with furosemide in the presence of a high dose of captopril. They did not find increased water or saline drinking responses to ANG II infused into the SFO or OVLT under hypotensive conditions compared with intakes observed in animals that received only captopril injections (i.e., presumably normotensive animals). The route of infusions did not include the brain ventricles, so it is difficult to make direct comparison with the present work that administered ANG II directly into the lateral ventricle. However, ANG II infused into ventricles reaches tissues in addition to the SFO or OVLT (18, 19, 23) that may be responsible for generating greater intakes under hypotensive/hypovolemic conditions.

In summary, the results do not support a role for either blood pressure- or blood volume-related signals acting alone to augment salt appetite stimulated by intracerebroventricular infusions of ANG II. Neither unloading of arterial baroreceptors alone (experiment 1) nor of venous baroreceptors alone (experiment 2) substantially affected the ability of centrally administered ANG II to stimulate salt appetite. Rather, the experiments suggest that unloading of both sets of baroreceptors is required to augment the ability of ANG II to stimulate salt appetite. Unloading only of arterial baroreceptors is sufficient to increase water drinking responses to intracerebroventricular ANG II. These conclusions are tempered by the fact that we did not produce conditions in which arterial blood pressure was reduced without affecting changes in blood volume (if it is granted that blood volume was probably increased during minoxidil infusions).

**Perspectives**

Multiple factors are involved in the control of thirst and salt appetite behaviors, including stimulatory and inhibitory mechanisms (28). In determining the role of a particular factor, the role of other factors operating at the same time must be evaluated. Central infusions of ANG II into fluid-replete animals in normal water and sodium balance and with normal levels of arterial blood pressure may underestimate the importance of ANG II to stimulate thirst and sodium appetite. Intracerebroventricular infusions of ANG II elicit greater thirst and salt appetite responses under hypotensive/hypovolemic conditions that better mimic the natural circumstances in which ANG II is likely to contribute to these behaviors.

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