Effects of subfornical organ lesions on acutely induced thirst and salt appetite

ROBERT L. THUNHORST, TERRY G. BELTZ, AND ALAN KIM JOHNSON

Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242-1407

Thunhorst, Robert L., Terry G. Beltz, and Alan Kim Johnson. Effects of subfornical organ lesions on acutely induced thirst and salt appetite. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R56–R65, 1999.—We examined the role of the subfornical organ (SFO) in stimulating thirst and salt appetite using two procedures that initiate water and sodium ingestion within 1–2 h of extracellular fluid depletion. The first procedure used injections of a diuretic (furosemide, 10 mg/kg sc) and a vasodilator (minoxidil, 1–3 mg/kg ia) to produce hypotension concurrently with hypovolemia. The resulting water and sodium intakes were inhibited by intravenous administration of ANG II receptor antagonist (saralasin, 8 μg·kg⁻¹·min⁻¹) or angiotensin-converting enzyme inhibitor (captopril, 2.5 mg/h). The second procedure used injections of furosemide (10 mg/kg sc) and a low dose of captopril (5 mg/kg sc) to initiate water and sodium ingestion upon formation of ANG II in the brain. Electrolytic lesions of the SFO greatly reduced the water intakes, and nearly abolished the sodium intakes, produced by these relatively acute treatments. These results contrast with earlier findings showing little effect of SFO lesions on sodium ingestion after longer-term extracellular fluid depletion.

Methods

Animals

Male Sprague-Dawley-derived rats (325–350 g) were purchased from Harlan (Indianapolis, IN). They were housed singly 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 where noted. Room lights were on for 12 h/day, and temperature was controlled at 23°C.

Electrolytic Lesion Surgery

Animals were anesthetized with equithesin (0.33 ml/100 g body wt) and were secured in a Kopf 900 stereotaxic instrument. The skull was exposed by midline incision and leveled, and a 3.0-mm trephine hole was made at bregma. Lesions of the SFO were made using three penetrations of an insulated tungsten wire electrode on the midline. The anterior of three mid sagittal stereotaxic coordinates ranged from 0.1 to 0.4 mm posterior to bregma, with the next two being each 0.3 mm posterior to the previous one. The electrode was lowered to points 4.7, 4.5, and 4.3 mm from the top of the exposed mid sagittal sinus, and anodal current was passed through the bare tip at 1 mA for 8 s/penetration. Control lesions were produced by passing anodal current at 1 mA for 8 s/penetration in tissue dorsal to the SFO. Sham lesion surgery consisted of trephination at bregma, insertion of the electrode to points 1 mm above the SFO coordinates, and withdrawal of the electrode without passing current. Experimentation began 2–4 wk later.

Catheter Surgery

Rats received femoral venous and/or arterial catheters for administration of drugs and measurement of arterial blood pressure according to previously published procedures (26). The rats recovered from catheter surgery for at least 3–4 days before experimentation began.
Drugs

Furosemide (Abbott Laboratories, North Chicago, IL) was administered subcutaneously at 10 mg·kg⁻¹·ml⁻¹·h⁻¹. Captopril (SQ-14,225), a gift from the Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ), was dissolved in sterile isotonic saline immediately before the experiment and was administered subcutaneously at 5 mg·kg⁻¹·ml⁻¹·h⁻¹. In one experiment, captopril was dissolved in 5% dextrose in water (DSW) and was administered intravenously at 2.5 mg·h⁻¹. Minoxidil (Sigma, St. Louis, MO) was dissolved in propylene glycol for a stock solution of 10 mg·ml⁻¹. Minoxidil was diluted with isotonic saline and was administered via an arterial bolus at 1 or 3 mg·kg⁻¹·ml⁻¹ over ~60 s. Sarthran ([Sar¹,Thr⁸]ANG II; Sigma) was stored in frozen aliquots and was dissolved in isotonic saline before testing and for intravenous infusion at 8 µg·kg⁻¹·min⁻¹. Nembutal (pentobarbital sodium; Abbott) was administered at 1 mg/ml ip.

General Procedures

The test cages were wooden with aluminum-lined interiors (24 × 29 cm) that extended 31 cm above suspended, stainless steel metabolism cages. The rats were brought into the testing room, placed in the metabolism cages, and attached to a polygraph for ~1 h of adaptation before testing. The arterial catheter was connected to a Cobe transducer by a coiled length of PE-50 tubing for measurement of arterial blood pressure on a polygraph (Dynograph Recorder, model R611; Sensormedics, Anaheim, CA). Mean arterial blood pressure (MAP) was obtained by electronically damping the arterial signal. MAP was averaged over the last 5 min of each 30-min testing period.

Urine was collected in polypropylene tubes via stainless steel funnels placed beneath the cages. Urine was measured for volume (Uᵥ), and urinary sodium concentration and potassium concentration were determined by ion-specific electrodes (NOVA Biomedical, Waltham, MA) for calculation of urinary sodium excretion (Uₙaᵥ) and potassium excretion (Uᵥₚ). Water balances were calculated by subtracting Uᵥ from the total volume of fluid intake (i.e., water + 0.3 M NaCl). Sodium balances were calculated by subtracting Uₙaᵥ from the ingestion of sodium in the form of 0.3 M NaCl. Some animals were killed with a small animal guillotine for collection of trunk blood for determination of hematocrit (International Micro-Capillary Reader; International Equipment, Needham Heights, MA), plasma protein (National Protometer; National Instrument, Baltimore, MD), plasma sodium and potassium by ion-specific electrodes (NOVA Biomedical), and osmolality by freezing point depression (mOsmette; Precision Systems, Sudbury, MA).

Hypovolemia With Vasodilation

The first series of experiments established a new procedure for producing water and sodium ingestion within a relatively short period of time. Rats used in exp. 1a were randomly selected from a previous study (27) and were used ~1 wk after the previous study was finished. In all other experiments, the rats were experimentally naive.

Experiment 1a: thirst and salt appetite. Rats with arterial catheters were injected with the diuretic furosemide (10 mg/kg body wt sc) to produce diuresis and natriuresis and were injected 30 min later either with minoxidil (1 mg/kg body wt 1a; n = 5) or with isotonic saline (vehicle; n = 5). Access to water and 0.3 M NaCl was provided 30 min later, and intakes were recorded for 3 h. MAP was recorded throughout. Urine samples were not collected.

Experiment 1b: water and electrolyte balance and plasma measures. Rats with arterial catheters were treated with furosemide (n = 4) or with the combination of furosemide and minoxidil (n = 4) as described in Experiment 1a: thirst and salt appetite. Access to water and 0.3 M NaCl was provided 30 min after minoxidil administration, and intakes were recorded for 3 h. Urine samples were collected 1 h after diuretic treatment and again at the end of testing. Animals were killed at the end of testing for collection of trunk blood for measurement of plasma electrolytes and protein and for hematocrit.

Experiment 1c: blockade of the renin-angiotensin system. Rats with both venous and arterial catheters were infused either with the ANG II-receptor antagonist sarthran (8 µg/min, n = 5) or with DSW (9.6 µl/min, n = 5) as the vehicle. One hour later, rats were administered the combination of furosemide and minoxidil (3 mg/kg body wt) as described in Experiment 1a: thirst and salt appetite. Water and 0.3 M NaCl were provided 30 min after minoxidil injections, and intakes were recorded for 2 h. The intravenous infusions continued throughout, and MAP was recorded continuously.

Preliminary work showed that intravenous infusions of sarthran exaggerated the reductions of MAP in rats treated with the combination of furosemide and minoxidil. Therefore, to control for the effects of potentially debilitating reductions in MAP during sarthran infusions, rats were administered a test to assess their behavioral competency (26). Briefly, rats were given access to 5% sweetened condensed milk (Bordon) in glass burettes on three occasions to become familiar with the solution. Three days after the thirst and salt appetite test, the animals were given a second test that was identical to the first except that the rats were offered the 5% sweetened condensed milk at the end, and intakes were recorded for 30 min. Infusions of DSW and sarthran continued throughout. The rats are not obligated to ingest milk in this “dessert” test and presumably would not if the hypotensive treatment made them behaviorally incompetent or induced malaise.

A third group of animals (n = 4) was infused intravenously with a dose of the angiotensin-convert enzyme (ACE) inhibitor captopril (2.5 mg/kg DSW), which prevents the formation of ANG II systemically but does not interfere with ANG II formation inside the blood-brain barrier (8, 24). They were tested for water and salt intakes in response to treatment with the combination of furosemide and minoxidil as described in Experiment 1a: thirst and salt appetite. Their data are presented for comparison of the effects of interference with the renin-angiotensin system by different pharmacological means on water and salt intakes in response to these procedures.

Effects of SFO Lesions on Thirst and Salt Appetite

The second series of experiments examined the effects of SFO lesions on water and sodium ingestion arising acutely during two different, but similar, experimental procedures: after combined treatment with furosemide and minoxidil as described in Experiment 1a: thirst and salt appetite and after combined treatment with furosemide and captopril as described in previous work (6, 12, 27, 28).

Experiment 2a: hypovolemia with systemic inhibition of ACE. Rats were injected initially with furosemide (10 mg/kg body wt sc) to produce diuresis and natriuresis and were injected 10 min later with a low dose of captopril (5 mg/kg body wt sc) to cause blockade of ACE within peripheral tissues (3). One hour later, urine was collected for verification of water and solute loss, and access to water and 0.3 M NaCl was provided from burettes attached to the front of the cages. Intakes were recorded every 30 min for 2 h. Urine was
collected again at the end of testing. MAP was recorded throughout.

Experiment 2b: hypovolemia with vasodilation. Five to ten days later, rats were treated with furosemide (10 mg/kg body wt sc) and minoxidil (1 mg/kg body wt ia) as described in Experimental 1a: thirst and salt appetite. Urine was collected over a period of 30 min. Next, the rats received access to water and 0.3 M NaCl from burettes, and intakes were recorded every 30 min for 3 h. Urine was collected again at the end of testing. MAP was recorded throughout.

Histology

Within 1 wk of the conclusion of the experiments, animals that had received electrolytic lesion surgery were deeply anesthetized with an overdose of pentobarbital sodium for exsanguination by transcardial perfusion with isotonic saline followed by 10% formalin saline for fixation. Frozen 40-µm sections through the area of the lesion in each brain were obtained and stained with cresyl violet. The extent of brain damage was determined by light microscopic examination in a single-blind fashion.

Statistics

Data were analyzed by ANOVA appropriate to the experimental designs. Post hoc tests were made with Fischer’s least-significant difference t-tests. Planned comparisons were made with Bonferroni t-tests if the overall F was not significant. All values reported as significant are at the P < 0.05 level.

RESULTS

Hypovolemia With Vasodilation

Experiment 1a: thirst and salt appetite. Some animals (n = 5) received furosemide by itself, whereas others (n = 5) received furosemide in combination with minoxidil. Body weights were not different between the groups (413 ± 11 vs. 416 ± 10 g, respectively; F(1, 8) = 0.05, P > 0.05).

In all experiments, intakes of water and 0.3 M NaCl were analyzed on the basis of milliliters ingested per 30 min (i.e., rate of intake). Water intake was not significantly different between the groups (all F values ≤ 2.70; P > 0.05). However, rats treated with the combination of furosemide and minoxidil drank significantly more 0.3 M NaCl than rats treated only with furosemide [main effect of treatment, F(1, 8) = 5.39; P < 0.05]. Furthermore, this increased ingestion after vasodilator treatment was demonstrated by a significant, fourfold increase in total fluid intake, i.e., water + 0.3 M NaCl, compared with total fluid intake after treatment with furosemide by itself [7.4 ± 2.1 vs. 1.6 ± 0.9 ml; F(1, 8) = 6.36; P < 0.05]. The intakes of water and 0.3 M NaCl are presented in Fig. 1 as cumulative intakes.

Basal MAP was not different between the groups, and MAP did not change in rats treated only with furosemide (Fig. 2). Additional treatment with minoxidil significantly reduced MAP by 60 min after injection, and MAP remained significantly reduced from basal levels and levels of furosemide-treated rats for the duration of testing [F(8, 64) = 5.50, P < 0.05].

Experiment 1b: water and electrolyte balance and plasma measures. Some animals (n = 4) received furosemide by itself, whereas others (n = 4) received furosemide in combination with minoxidil. Body weights were not different between the groups [F(1, 6) = 0.63, P > 0.05; Table 1].

Water intake was significantly greater in rats treated with the combination of furosemide and minoxidil [F(1, 6) = 23.39, P < 0.05] compared with rats treated with furosemide alone, and the fourfold increase in 0.3 M NaCl intake by rats receiving both drugs was significantly greater than control treatment, main effect.

The two groups had equivalent U_v, U_{Na}V, and U_kV in the first hour after administration of furosemide [all F(1, 6) = 0.10, not significant; Table 1]. Although only small amounts of urine were excreted by both groups during the 3 h of fluid access, U_v was significantly reduced in animals treated with minoxidil [2.9 ± 0.4 vs. 1.1 ± 0.2 ml, respectively; F(1, 6) = 21.45; P < 0.05].
Furthermore, although the cumulative $U_V$ for the test was not different between the treatment conditions $[F(1, 6) = 1.44; P > 0.05]$, rats receiving minoxidil finished testing in significantly less negative water balance $[F(1, 6) = 21.60; P < 0.05]$. The group differences in sodium balance did not achieve statistical significance $[F(1, 6) = 4.37, P > 0.05]$. At the end of testing, plasma volume was greater in rats receiving furosemide plus minoxidil, as indicated by significantly reduced hematocrits and plasma proteins $[both F(1, 6) = 28.72, P < 0.05; Table 2]$, probably as a result both of the increased fluid ingestion and the greater reductions in MAP in these animals (see exp. 1a). Plasma sodium, potassium, and osmolality were not different between the groups $[all F(1, 6) ≤ 0.61, P > 0.05]$. Experiment 1c: blockade of the renin-angiotensin system. Groups of animals treated with furosemide and minoxidil received intravenous infusions of the ANG II-receptor antagonist sarthran $[n = 5]$ or DSW vehicle $[n = 5]$. Body weights were not different between the groups $[415 \pm 7$ vs. $424 \pm 8$ g, respectively; $F(1, 8) = 0.98, P > 0.05]$. Sarthran did not affect water intake $[F(1, 8) = 2.44, P > 0.05]$ but significantly reduced $0.3\text{ M }$NaCl intake $[F(1, 8) = 7.27, P < 0.05]$ and the total amount of fluid ingested overall $[F(1, 8) = 7.93, P < 0.05]$ in response to combined treatment with furosemide and minoxidil (Fig. 4). Infusions of captopril completely prevented the ingestion of both water and $0.3\text{ M }$NaCl. The groups had equivalent MAP during the initial hour of intravenous infusion with DSW or sarthran and also during the first 30 min after subcutaneous furosemide (Fig. 5). However, MAP fell progressively after the administration of minoxidil, and the reduction in MAP was significantly greater in rats treated with intravenous sarthran $[interaction, F(6, 42) = 12.26, P < 0.05]$. Infusions of captopril produced similar reductions in MAP.

**BEHAVIORAL COMPETENCY TESTING.** Three days after testing, rats received a second test under identical conditions. All of the major main effects were replicated in the second test, including the reduced consumption both of $0.3\text{ M }$NaCl and total fluid overall by sarthran-treated rats compared with vehicle-treated rats. MAP was reduced to levels comparable to those of the first test. When $5\%$ sweetened condensed milk was offered for consumption, the sarthran-treated rats drank three times as much as vehicle-treated rats $[F(1, 8) = 9.78, P < 0.05; Fig. 6]$. Effects of SFO Lesions on Thirst and Salt Appetite

Experiment 2: histology. Based on histological analysis of the locations of the brain lesions, the animals were separated into the following four groups, as described previously (25): 1) animals with essentially complete lesions of the SFO, 2) animals with partial lesions of the SFO, 3) animals with control lesions, and 4) animals with sham lesions. Animals with essentially complete lesions had $≈80–100\%$ destruction of the SFO. Additionally, animals were included in this group if there was less ($≈30–70\%$) damage as long as the rostral extent of the SFO, from which the major efferent projections arise, was clearly destroyed. In the latter case, some of the dorsal median preoptic nucleus was also damaged. Animals with partial lesions of the SFO had $≈20–80\%$ destruction of the nucleus. The degree of damage to the SFO varied considerably in this group, and lesion placement was not consistent and did not include the rostral extent of the SFO. In both groups of animals with SFO lesions, there was also some damage to the thalamic periventricular nuclei and the ventral hippocampal commissure and occasionally damage to the triangular nucleus of the septum and the fornix. Animals with control lesions had damage dorsal and anterior to, but not including, any of the SFO. These lesions encompassed varying portions of the medial and triangular septal nuclei and ventral fornical commissure (see Ref. 25 for photomicrographs of complete SFO lesions and control lesions that are in all respects comparable to typical complete and control lesions in the present study).

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**Table 1.** Body weights and water and sodium excretion and balance measures during furosemide diuresis and vasodilator treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weights, g</th>
<th>Total Fluid Intake, ml</th>
<th>1-h Urine Volume, ml</th>
<th>Total Urine Volume, ml</th>
<th>Final Water Balance, ml</th>
<th>1-h Sodium Excretion, µmol</th>
<th>Final Sodium Balance, µmol</th>
<th>1-h Potassium Excretion, µmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>4</td>
<td>425 ± 15</td>
<td>2.0 ± 0.7</td>
<td>12.8 ± 1.2</td>
<td>15.7 ± 1.0</td>
<td>−13.7 ± 1.7</td>
<td>1.413 ± 153</td>
<td>−1203 ± 230</td>
</tr>
<tr>
<td>Furosemide + minoxidil</td>
<td>4</td>
<td>409 ± 13</td>
<td>8.8 ± 1.6*</td>
<td>12.8 ± 1.1</td>
<td>13.8 ± 1.2</td>
<td>−5.1 ± 0.9</td>
<td>1.386 ± 104</td>
<td>−373 ± 324</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Final sodium balance does not include the small amounts of sodium excreted during the 3-h fluid access. Total fluid intake = water + $0.3\text{ M }$NaCl. *Significant main effect of treatment.
Table 2. Plasma volume and electrolyte measures for rats after furosemide diuresis and vasodilator treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Hematocrit, %</th>
<th>Plasma Protein, g/dl</th>
<th>Plasma Sodium, mmol/l</th>
<th>Plasma Potassium, mmol/l</th>
<th>Plasma Osmolality, mmol/l</th>
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</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>4</td>
<td>52.5 ± 1.0</td>
<td>8.1 ± 0.1</td>
<td>142.6 ± 0.3</td>
<td>6.5 ± 0.2</td>
<td>302.3 ± 1.0</td>
</tr>
<tr>
<td>Furosemide + minoxidil</td>
<td>4</td>
<td>46.2 ± 0.4*</td>
<td>7.3 ± 0.1*</td>
<td>143.0 ± 0.9</td>
<td>6.4 ± 0.2</td>
<td>304.8 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. These measures were obtained at the end of behavioral testing in animals with access to water and 0.3 M NaCl as drinking fluids. *Significant main effect of treatment.

Experiment 2a: Hypovolemia with systemic inhibition of ACE. Rats in the various brain lesion conditions were measured for intakes of water and 0.3 M NaCl and for MAP in a protocol that produced volume depletion concurrently with partial blockade of peripheral sources of ACE. Data were included in the analysis only for those animals from which reliable blood pressure measurements were acquired. The resulting n values were as follows: complete SFO lesion = 12, partial SFO lesion = 12, control lesion = 7, and sham lesion = 9. There were no differences in body weights between the groups of rats in the four different lesion conditions [F(3, 36) = 0.48, P > 0.05; Table 3]. There were significant main effects of lesion condition for both water and 0.3 M NaCl intakes [all F(3, 36) values > 3.26, P < 0.05], and there was a significant lesion condition × time interaction for water intake [F(9, 108) = 2.75, P < 0.05]. All groups with brain lesions drank significantly less water than sham lesion animals in the first 30 min of fluid access, the period when the greatest amounts of water and 0.3 M NaCl were consumed. Rats with complete lesions of the SFO drank significantly less water than all other groups. Rats with either complete or partial SFO lesions drank significantly less 0.3 M NaCl than rats with control or sham lesions. Water and 0.3 M NaCl intakes are presented as cumulative intakes in Fig. 7.

Rats with SFO lesions excreted slightly, but significantly, less U₉ during the first hour after injection with furosemide compared with the other groups [F(3, 36) = 7.15, P < 0.05] and significantly less cumulative U₉ for the test [F(3, 36) = 5.34, P < 0.05; Table 3]. However, when cumulative U₉ was subtracted from the significantly reduced fluid intake [F(3, 36) = 12.53, P < 0.05] of rats with SFO lesions, there were no differences in overall water balance between the groups [F(3, 36) = 2.12, P > 0.05]. Rats with SFO lesions also had significantly reduced U₉ within the first hour after injection with furosemide compared with the other groups [F(3, 36) = 5.94, P < 0.05] as well as significantly reduced total U₉ within the test [F(3, 36) = 4.90, P < 0.05]. However, the final sodium balances for the test were not different between the groups [F(3, 36) = 1.07, P < 0.05]. There were no effects of lesion condition on U₉ within the test [all F(3, 36) ≤ 2.73, P < 0.05].

The effects of furosemide/captopril treatment on MAP varied between the lesion conditions and were not as large as those obtained in previous work (27). Only rats with complete lesions of the SFO and those with control lesions had significant reductions in MAP in response to furosemide/captopril treatment [lesion condition × time interaction, F(18, 216) = 1.84, P < 0.05; Fig. 8]. The MAP of rats with partial lesions of the SFO or sham lesions remained essentially unchanged during testing. Rats with complete or partial lesions of the SFO had reduced basal MAP compared with rats with control lesions, but no groups had MAP different from sham lesion animals at this time. The MAP of rats with control lesions never differed from MAP of rats with sham lesions. MAP of rats with complete or partial

Fig. 4. Experiment 1c: Intake of water (A) and 0.3 M NaCl (B) in 2 h of fluid access in rats infused iv with 5% dextrose in water as vehicle (D5W; n = 5) or with ANG II receptor antagonist (sarthran, 8 µg·kg⁻¹·min⁻¹; n = 5) and subsequently treated with furosemide in combination with minoxidil. Values of rats infused with angiotensin-converting enzyme (ACE) inhibitor (captopril, 2.5 mg/h; n = 4) and subsequently treated with furosemide and minoxidil are presented for comparison. Values are means ± SE. *Significantly reduced compared with control treatment.

Fig. 5. MAP in rats during iv infusions with D5W (n = 5) or with ANG II receptor antagonist (sarthran, 8 µg·kg⁻¹·min⁻¹; n = 5) and subsequently treated with furosemide in combination with minoxidil. Furosemide and minoxidil were injected at −60 and −30 min, respectively. Fluid access was provided at 0 min. Values of rats infused with ACE inhibitor (captopril, 2.5 mg/h; n = 3) and subsequently treated with furosemide and minoxidil are presented for comparison. Values are means ± SE. *Significantly reduced compared with control treatment.
lesions of the SFO differed at 90 min postaccess to fluids.

Experiment 2b: hypovolemia with vasodilation. Rats in the various brain lesion conditions were tested for water and 0.3 M NaCl intakes and for MAP in a protocol that employed volume depletion and vasodilation. Excluding animals from which adequate blood pressure measurements could not be obtained, the final n values were as follows: complete SFO lesion = 10, partial SFO lesion = 9, control lesion = 5, and sham lesion = 9. There were no differences in body weights between the groups of rats in the four different lesion conditions [F(3, 29) = 0.20; P > 0.05, Table 4].

Rats with either complete or partial lesions of the SFO drank significantly less of both water and 0.3 M NaCl solution than rats with either control or sham lesions [main effects of lesion condition, both F(3, 29) = 4.06; P < 0.05]. Water and 0.3 M NaCl intakes are presented as cumulative intakes in Fig. 9. The group differences were evident in the first 30-90 min of fluid access [main effects of time, both F(5, 145) = 2.39; P < 0.05].

There were no differences in U_, U_, or UK during the first hour after diuretic treatment or during the 3 h of fluid access, in which there was virtually no excretion, so that there also were no differences in total U_, U_, or UK for the test [all F(3, 29) ≤ 2.12, P > 0.05]. However, because rats with either complete or partial lesions of the SFO had significantly reduced 0.3 M NaCl intake and significantly reduced fluid ingestion overall [F(3, 29) = 11.42, P < 0.05], these groups had significantly reduced (i.e., more negative) water and sodium balances by the end of testing compared with control and sham lesion groups [both F(3, 29) ≥ 5.85, P < 0.05]. MAP was not significantly different between rats with complete SFO lesions, control lesions, or sham lesions when analyzed either as raw pressures or as change in MAP from basal levels (Fig. 10). Rats with partial lesions of the SFO had slightly elevated basal MAP and had greater reductions in MAP in response to intravenous minoxidil compared with the other groups.

**DISCUSSION**

The main findings of the present experiments are 1) The administration of a vasodilator (minoxidil) to hypovolemic rats readily stimulated the ingestion of water and sodium, and this ingestion was inhibited by antagonism of the renin-angiotensin system, and 2) lesions of the SFO greatly reduced the water and sodium ingestion caused by treating hypovolemic rats either with a vasodilator or with low doses of ACE inhibitor.

The first series of experiments characterized the basic features of an acute-onset model of thirst and salt appetite that uses injections of furosemide and minoxidil to produce a condition of hypovolemia with concurrent hypotension. Rats made hypovolemic by injections of furosemide and treated subsequently with minoxidil had lower MAP than rats treated only with furosemide. The robustness of the water and sodium intakes produced by this treatment was demonstrated in three separate experiments. These results provide support for the idea that modest reductions in arterial pressure facilitate salt appetite (27) as they do thirst (4, 26). Minoxidil-treated rats also finished testing with two- to threefold improvements in water and sodium balance, and with significantly expanded plasma volume, compared with rats treated only with diuretic. The improved balance and plasma measures of the hypoten-

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**Table 3.** Body weights and water and sodium excretion and balance measures during furosemide diuresis and angiotensin-converting enzyme blockade in rats with various electrolytic brain lesions

<table>
<thead>
<tr>
<th>Lesion Condition</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Total Fluid Intake, ml</th>
<th>1-h Urine Volume, ml</th>
<th>Total Urine Volume, ml</th>
<th>Final Water Balance, ml</th>
<th>1-h Sodium Excretion, µmol</th>
<th>Total Sodium Excretion, µmol</th>
<th>Final Sodium Balance, µmol</th>
<th>1-h Potassium Excretion, µmol</th>
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</thead>
<tbody>
<tr>
<td>Sham</td>
<td>9</td>
<td>421 ± 8</td>
<td>7.1 ± 0.8</td>
<td>14.8 ± 0.4</td>
<td>18.1 ± 0.7</td>
<td>−11.0 ± 0.6</td>
<td>1,748 ± 51</td>
<td>2,195 ± 102</td>
<td>−1,498 ± 229</td>
<td>682 ± 47</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>420 ± 10</td>
<td>6.0 ± 1.1</td>
<td>14.8 ± 0.7</td>
<td>17.7 ± 1.2</td>
<td>−11.7 ± 1.5</td>
<td>1,744 ± 119</td>
<td>2,129 ± 182</td>
<td>−1,597 ± 231</td>
<td>651 ± 70</td>
</tr>
<tr>
<td>Partial</td>
<td>12</td>
<td>420 ± 8</td>
<td>3.3 ± 0.6</td>
<td>14.2 ± 0.3</td>
<td>16.8 ± 0.6</td>
<td>−13.5 ± 0.7</td>
<td>1,690 ± 57</td>
<td>2,029 ± 81</td>
<td>−1,849 ± 109</td>
<td>620 ± 27</td>
</tr>
<tr>
<td>SFO X</td>
<td>12</td>
<td>410 ± 7</td>
<td>1.5 ± 0.6</td>
<td>12.3 ± 0.4*</td>
<td>14.8 ± 0.3*</td>
<td>−13.3 ± 0.7</td>
<td>1,399 ± 68*</td>
<td>1,710 ± 67*</td>
<td>−1,568 ± 93</td>
<td>525 ± 41</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. SFO X, complete subfornical organ lesion. Total fluid intake = water + 0.3 M NaCl. *Significantly different from all other groups.
sive animals cannot be explained by the minimal differences in urinary excretion of water and sodium compared with rats treated only with furosemide. Rather, the improved balance and plasma measures are almost certainly due to the increased ingestion of water and sodium by the hypotensive animals. Plasma sodium, potassium, and osmolality were equivalent between groups by the end of testing, but we do not have data for these measures in the absence of fluid intake.

It is likely that the water and sodium intakes produced by combined treatment with furosemide and minoxidil, i.e., the model presented here, are angiotensin dependent. Volume depletion and reductions of arterial pressure are both stimuli for renin secretion (2), so it is likely that rats treated with the combination of diuretic and vasodilator had increased plasma renin activity and increased levels of circulating ANG II. Intravenous infusions of ANG II receptor antagonist sarthran reduced overall fluid intake, and particularly sodium intake, of rats treated jointly with furosemide and minoxidil. Because sarthran is a peptide analog of ANG II and is unlikely to cross the blood-brain barrier (18), these results support the idea that ANG II receptors located on the blood side of the blood-brain barrier are responsible for supporting at least part of the water and sodium intakes in the present work. The behavior was completely prevented by intravenous administration of ACE inhibitor. It is not uncommon to find more complete attenuation of ANG II-induced responses by peripherally administered ACE inhibitor than by peripherally administered ANG II receptor antagonists (e.g., see Refs. 9, 11, 16, 17), possibly due to partial agonist properties associated with the antagonists and the difficulty in choosing a criterion for effective doses (see Ref. 5 for discussion). The intravenous sarthran did not reduce salt intake by making animals behaviorally incompetent, because sarthran-treated rats actually drank significantly more, not less, milk than controls in the dessert test.

The second series of experiments established the SFO as a critical brain site for the elaboration of water and sodium intake produced by the two acute procedures presented here. Both procedures appear to stimulate behavior through activation of the renin-angiotensin system. Water and salt intakes after treatment with furosemide and minoxidil (expt. 1) likely result from increased renin secretion and formation of circulating ANG II. Water and sodium ingestion caused by combined treatment with furosemide and captopril (expt. 2) is hypothesized to result from the formation of ANG II within the circumventricular organs of the brain (6, 27, 28) under the special conditions when formation of ANG II is partially prevented in peripheral tissues and blood by the administration of a low dose of captopril (3). In fact, low doses of captopril are by themselves dipsogenic (3, 13, 25) and appear to produce water drinking by activation of neurons within circumventricular organs (13). Regardless of the precise location where the ANG II is formed, this model clearly depends on the formation of ANG II and the activation of ANG II receptors for generating behavior (27, 28).

The observation that SFO lesions eliminate the water and sodium ingestion during both procedures used here is consistent with the idea that the SFO has ANG II receptors that are critical for hypovolemic thirst and, possibly, salt appetite. Partial destruction of the SFO was nearly as effective as complete destruction of the nucleus in eliminating the behavioral responses to water and sodium loss.

The present findings contrast with earlier studies investigating the role of the SFO in salt appetite. Masson and Fitts (12) found that knife cuts of the rostral afferent/efferent pathway of the SFO significantly reduced water intake, but had no effect on salt intake, in response to nearly identical treatment with furosemide (10 mg/kg ip) plus captopril (6 mg/kg ip) as that used here (see expt. 2a). In the present work, we find that electrolytic lesions that destroy, either completely or partly, the body of the SFO nearly abolish salt intake and water intake in this model. Lesions of the body of the SFO may remove additional pathways, integrative

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**Fig. 7.** Experiment 2a: Intakes of water (A) and 0.3 M NaCl (B) in 2 h of fluid access after treatment with furosemide and ACE inhibitor in rats with sham lesions (n = 9), control lesions (n = 7), and either partial (n = 12) or complete (n = 12) lesions of the subfornical organ (SFO). SFO X, complete SFO lesion. Values are means ± SE. *SFO X group significantly reduced compared with all other groups, main effect. **Both SFO groups significantly reduced compared with sham lesion and control lesion groups, main effect.

**Fig. 8.** MAP in rats with sham lesions (n = 9), control lesions (n = 7), and either partial (n = 12) or complete (n = 12) lesions of the SFO after treatment with furosemide and ACE inhibitor. Furosemide and ACE inhibitor were injected at −60 and −50 min, respectively. Fluid access was provided at 0 min. Control lesion and SFO X rats had significantly reduced MAP compared with basal (i.e., −60 min) levels. Values are means ± SE.
mechanisms, or other factors necessary for the elaboration of salt appetite that are not removed by transection of rostral pathways that spare the SFO itself. Other differences in experimental design between the studies could contribute to the different findings. Possible important differences between the studies include more extensive pretest exposure to the saline solution and larger group n values in the present study, which provide greater statistical power.

Thunhorst et al. (25) tested the effects of SFO lesions on thirst and salt appetite of rats after 24 h of water deprivation. The drinking water provided for rehydration was adulterated with a low concentration of captopril. Oral ingestion of captopril causes exaggerated water drinking and saline intakes in dehydrated animals (15, 19, 25). Lesions of the SFO largely prevented the exaggerated water drinking under these conditions but did not significantly reduce the concomitant overconsumption of 0.3 M NaCl. However, of the experimental groups employed in that study, rats with complete lesions of the SFO had the smallest increases in salt appetite after ingesting captopril. It is possible that the negative findings resulted from insufficient statistical power due to the relatively small sample sizes. Group sizes in the present work are approximately double those of the previous study.

The SFO is more critical to water and sodium intake generated by the acute procedures used here than by more chronic treatments used in earlier studies. For example, when the measure of salt appetite is the amount of saline consumed over periods of 24 h or longer, lesions of the SFO, or transections of its rostral fibers, have no observable effect on salt appetite (12, 20, 25). When the measure of salt appetite is the amount of saline consumed in 2- to 3-h periods in tests of 24 h or shorter duration, ablation of the SFO markedly reduces salt appetite (21, 23, 29). Notably, in the two procedures presented here, lasting only 3-4 h each, the salt appetite responses of rats with complete or partial lesions of the SFO were nearly abolished. Together, these results support the idea that additional mechanisms, perhaps associated with other brain structures (e.g., amygdala), become engaged over the course of more prolonged salt appetite tests that compensate for the destruction of the SFO and its associated mechanisms (23).

There are two main hypotheses concerning the role of the SFO in the stimulation of salt appetite. The first is that ANG II receptors within the SFO are critical for eliciting salt appetite in response to circulating ANG II in sodium-deplete rats (29). This proposal is an extension of the known role of ANG II receptors in the SFO to mediate other central nervous system responses to circulating ANG II, including thirst, vasopressin secretion, and the central component of its pressor response (10, 14). The second proposal is that the SFO is critical for eliciting hypovolemic thirst in sodium-deplete rats (21), and the ingestion of water in response to hypovolemic thirst then secondarily releases the expression of

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### Table 4. Body weights and water and sodium excretion and balance measures during furosemide diuresis and vasodilator treatment in rats with electrolytic lesions of the subfornical organ

<table>
<thead>
<tr>
<th>Lesion Condition</th>
<th>Body Weight (g)</th>
<th>Total Fluid Intake (ml)</th>
<th>1-h Urine Volume (ml)</th>
<th>Total Urine Volume (ml)</th>
<th>Final Water Balance (ml)</th>
<th>1-h Sodium Excretion (µmol)</th>
<th>Total Sodium Excretion (µmol)</th>
<th>Final Sodium Balance (µmol)</th>
<th>1-h Potassium Excretion (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>9</td>
<td>432 ± 7</td>
<td>10.6 ± 1.5</td>
<td>14.1 ± 0.5</td>
<td>15.8 ± 0.4</td>
<td>−5.2 ± 1.3</td>
<td>1.726 ± 0.74</td>
<td>1.926 ± 0.73</td>
<td>−500 ± 315</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>427 ± 13</td>
<td>11.9 ± 1.7</td>
<td>14.4 ± 0.7</td>
<td>15.8 ± 0.6</td>
<td>−3.9 ± 1.6</td>
<td>1.773 ± 0.74</td>
<td>1.942 ± 0.88</td>
<td>−340 ± 377</td>
</tr>
<tr>
<td>Partial</td>
<td>9</td>
<td>428 ± 7</td>
<td>3.7 ± 1.2*</td>
<td>13.9 ± 0.6</td>
<td>14.8 ± 0.7</td>
<td>−11.1 ± 1.6*</td>
<td>1.634 ± 0.6</td>
<td>1.718 ± 0.81</td>
<td>−1,401 ± 214*</td>
</tr>
<tr>
<td>SFO X</td>
<td>10</td>
<td>424 ± 6</td>
<td>3.6 ± 0.9*</td>
<td>14.6 ± 0.7</td>
<td>15.6 ± 0.6</td>
<td>−11.9 ± 1.0*</td>
<td>1.742 ± 1.03</td>
<td>1.845 ± 0.98</td>
<td>−1,593 ± 152*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Total fluid intake = water + 0.3 M. *Significantly different from the control lesion and sham-lesion groups.
salt appetite subsequent to osmotic dilution (22). The second proposal, then, restricts the role of ANG II receptors in the SFO to stimulating thirst, but not salt appetite directly, under hypovolemic conditions.

The present experiments do not address which of these two proposed mechanisms explains how SFO lesions impair salt appetite. However, neither mechanism may adequately explain the impaired salt appetite of rats in the present studies. First, the stimulation of salt appetite by short-term procedures such as those presented here does not appear to depend on osmotic dilution, or, at least, the ingestion of water. In the studies by Masson and Fitts (12), animals with knife-cut transections of the major afferent/efferent pathways of the SFO drank normal amounts of saline to furosemide/captopril treatment even though water intake was completely prevented by the surgical transections. If osmotic dilution through the ingestion of water was required to “release” salt appetite under these short-term conditions, then the negligible water intake by rats in that study should have precluded salt intake. Second, attempts to stimulate salt appetite by direct administration of ANG II into the SFO have so far produced mixed results, even though water drinking is always readily elicited (1, 7). Thus, while receptors in the SFO support thirst, current evidence is equivocal on their capacity to support salt appetite. Therefore, the reason why lesions of the SFO eliminate salt appetite as well as thirst in acute procedures such as those presented in the present work remains to be determined.

The results of the present experiments, together with those of previous work (23, 29) examining the effects of lesions of the SFO on water and sodium ingestion in sodium-depleted animals, suggest two things: 1) that lesions of the SFO have more pronounced effects on acute, or rapidly developing, sodium ingestion/salt appetite than on chronic or more slowly developing salt appetite and 2) that lesions of the SFO may eliminate additional mechanisms that knife-cut transections of SFO afferent/efferent connections do not remove that control water and sodium ingestion of sodium-deficient rats. Additionally, the fact that the acute models employed here produced varying degrees of hypotension in sodium-deplete animals may indicate that the SFO is involved in producing salt appetite in pathological states.

The research was supported by National Institutes of Health Grants MH-50260, HL-54292, and HL-57472, National Aeronautics and Space Administration Grant NAG5-6171, and Office of Naval Research Grant NO0014-97-1-0145 (to A. K. Johnson). Address for reprint requests and other correspondence: R. L. Thunhorst, Dept. of Psychology, Univ. of Iowa, 11 Seashore Hall E., Iowa City, IA 52242-1407 (E-mail: thunhors@blue.weeg.uiowa.edu).

Received 14 October 1998; accepted in final form 16 March 1999.

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