Influence of the subfornical organ on meal-associated drinking in rats

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Starbuck, Elizabeth M., and Douglas A. Fitts. Influence of the subfornical organ on meal-associated drinking in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R669–R677, 2001.—A lesion of the subfornical organ (SFO) may disrupt drinking after a meal of dry chow as it does drinking after intragastric administration of hypertonic saline. Food and water intakes of SFO-lesioned (SFOX) and sham-lesioned rats were measured during 90-min tests following various lengths of food deprivation. During the tests, all rats began eating before they began drinking. After 20–24 h of food deprivation, latency to begin drinking after eating had started was longer for SFOX than for sham-lesioned rats. Plasma osmolality was elevated by 2–3% in both lesion groups at 12 min, the latency for sham-lesioned rats to drink, but SFOX rats nevertheless continued eating and delayed drinking. Eating after shorter 4-h food deprivations and ad libitum feeding produced more variable drinking latencies and less consistent effects of SFO lesion. During 24 h of water deprivation, SFO lesion had no effect on the suppression of water intake during eating and in determining drinking patterns and the amount of water ingested during a meal. Angiotensin; osmolality; sodium; water intake

The ingestion of dry chow increases plasma osmolality in the rat by 2–3% within minutes (9), and an increase of this magnitude initiates water intake (4, 9). Plasma osmolality is also increased as rats begin drinking after spontaneous food intake (1). These findings suggest that the water intake that occurs in close temporal relation to eating is due at least in part to an increase in plasma osmolality caused by the ingestion of food. An increase in plasma osmolality following intragastric administration of hypertonic saline also elicits water intake, and this drinking is reduced by a lesion of the subfornical organ (SFO) (26, 31). This water intake is apparently due to an increase in plasma or gut osmolality and not to ANG II (31). A lesion of the SFO could disrupt meal-associated drinking as it does drinking in response to intragastric hypertonic saline, as both increase plasma osmolality. Thus a probable neural substrate for drinking in response to intragastric hyperosmotic loading is the presence of osmoreceptive units at the SFO (21, 22, 24, 28).

Other possible influences on meal-associated drinking include an increase of plasma ANG II (13, 20) and signals carried from the abdomen by gastric vagal afferents (16, 17). Either of these could be interrupted by a lesion of the SFO, because the SFO contains critical dipogenic receptors for circulating ANG II (30) and because it receives afferents from the hindbrain vagal complex (6, 22, 33).

An earlier study found no effect of SFO lesion on drinking during refeeding after 24-h food deprivation (32). In that experiment, water intake was measured only after a 2-h period of eating, and thus any differences in water intake occurring early in the test that could implicate the importance of the SFO would not have been detected. The present study uses a fasting and refeeding schedule similar to that of Weisinger et al. (32), but it includes earlier and more frequent measurements of intakes to allow for detection of subtle disturbances in drinking during eating that may be created by SFO lesion.

In addition to initiating water intake, an elevation of osmotic pressure also decreases dry food intake (2, 3, 7, 27), thus preventing the exacerbation of cell dehydration caused by the ingestion of dry chow when water is not available. The present study also examines whether the SFO is important for the inhibition of food intake during elevated osmotic pressure generated by water deprivation.

METHODS

Animals

Subjects were 76 male Long-Evans rats weighing 300–500 g at the beginning of the experiments. They were obtained from the vivarium at the University of Washington Department of Psychology and housed individually in hanging wire mesh cages with Harlan Teklad laboratory chow and tap water continuously available except during experiments. Room lights were on 12 h per day, and temperature was held at 23°C. Sample sizes after histology are given in the individual experiments. All rats in experiments 1, 3, and 4 were naive to testing. Some of the rats in experiment 2 had previously been used in experiments 1, 3, or 4. All protocols were

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approved by the Animal Care Committee at the University of Washington.

Lesion Surgery

Rats received either an electrolytic lesion of the SFO or a sham-lesion surgery (31). Rats were anesthetized with Equithesin (0.35 ml/100 g ip) and secured in a Kopf stereotaxic instrument. Lesions of the SFO were made by lowering an electrode on the midline through the midsagittal sinus. Four penetrations were made with the use of a 31-gauge tungsten wire electrode insulated with Teflon except for the cross-sectional area at the tip. One milliamp of current was passed for 7 s on each penetration. Sham lesions were made by advancing the electrode through the midsagittal sinus and brain parenchyma to approximately the level of the corpus callosum without passing current. An intramuscular injection of 0.2 ml Gentamicin and topical Betadine were administered after surgery to control infection. Experimentation began at least 2 wk after surgery.

Histology

After experimentation, rats were deeply anesthetized with pentobarbital sodium and perfused through the heart with saline for exsanguination followed by 10% formalin for fixation. Brains were removed and stored in 10% formalin until cutting on a freezing microtome in 50-μm sagittal sections. Sections were mounted on glass slides, stained with thionine, placed under a coverslip, and examined with the use of a microprojector or light microscope. The person evaluating the lesions had no knowledge of the behavioral results during the analysis.

Rats were considered to have good lesions of the SFO if at least the entire rostroventral pole containing the efferent fibers from the SFO was destroyed (19). Figure 1 shows representative photomicrographs of complete SFO lesions, lesions of the rostroventral pole, and lesions that missed the SFO. Twenty rats were eliminated from the analyses because of misplaced lesions.

Water and Food Intakes

Water intakes during all 90-min tests and throughout the baseline and rehydration phases of experiment 4 were measured to the nearest 0.1 ml. Water intakes during all other periods were measured to the nearest 1.0 ml. Latency to drink was determined as the time from the presentation of water until the rat licked sufficiently at the spout to cause a bubble to rise. Food intakes were accurate to 0.1 g and corrected for spillage. Powdered chow was made by grinding the rats’ regular Teklad diet in a food blender.

Experiment 1

Food and water intakes after mild and extended food deprivation. The effect of SFO lesion on drinking during eating was examined in six SFO-lesioned (SFOX) and six sham-lesioned rats under two circumstances 2 wk apart: 1) after 20-h overnight food deprivation with the test conducted during the light part of the cycle, and 2) after a mild 4-h fast with the test beginning at the onset of the dark cycle with the use of a red light. The 4-h fast and the presentation of food at the onset of the dark cycle were intended to synchronize the beginning of the rats’ next meal. All testing was done in the rats’ home cages. The rats were weighed and deprived of food with water available. In the 20-h food-deprivation test, the rats were weighed again at 19 h, and overnight water intake was recorded. In both tests, rats were presented with fresh water 1 h before testing. Pelleted chow was presented, and latency to begin eating and latency to begin drinking after eating had started were recorded. Water intakes were recorded every 5 min for the first hour and at 75 and 90 min, and food intake was recorded at 90 min.

Fig. 1. Photomicrographs of midsagittal sections of complete lesions of the subfornical organ (SFO) (left), a lesion of the rostroventral pole of the SFO (top right), and a misplaced lesion (bottom right). Bar = 0.5 mm.
Experiment 2

Blood and plasma values. We tested whether a lesion of the SFO affects the changes in hematocrit, plasma osmolality, plasma protein, or sodium concentrations that may occur during eating according to the procedure of experiment 1. Rats were anesthetized rapidly with halothane, and blood was sampled from the heart with a 23-gauge, lightly heparinized needle. Plasma osmolality was measured by freezing-point depression, and protein concentration was determined by refractometry. Plasma sodium was analyzed by flame photometry. Hematocrit was measured by the microhematocrit method. All determinations were made in duplicate.

Rats were either naive or had been previously used as noted below. At least 10 days elapsed between any previous treatment and blood sampling. In group A, basal blood values were determined in naive rats (SFOX n = 6; sham n = 6) while they were not eating or drinking but had ad libitum access to food and water. In group B, rats that had previously been used in experiment 1 (SFOX n = 6; sham n = 6) were sampled just as they began drinking during refeeding after 20-h food deprivation. Because this resulted in SFOX and sham-lesioned rats being sampled at very different times, we repeated the method used for group B with a third group, group C. All rats in group C (SFOX n = 5; sham n = 7) were sampled at 12 min, which was the median latency to drink for sham-lesioned rats in group B. Rats in group C had previously been used in the experiment that is labeled experiment 4. All measurements were made during the light part of the cycle.

Experiment 3

Rates of nocturnal food and water intake. The difference in the effect of SFO lesion on drinking between the 20-h and 4-h conditions in experiment 1 might have been due to a difference in the rats’ rate of eating after the different lengths of food deprivation. Additionally, the two parts of experiment 1 were conducted during different phases of the light cycle. Experiment 3 was conducted entirely during the dark phase and examined the effect of SFO lesion on water intake and rate of eating.

Rats (SFOX n = 10; sham n = 9) were adapted to a partially reversed light cycle for 2 wk before experimentation began, with lights on from midnight to noon. After light cycle adaptation, rats were placed in metabolic cages and provided with water and powdered Teklad diet. Daily food and water intakes were measured through three dark cycles to assure that eating and drinking were stable. Observations were made with the use of a red light.

Two adaptation trials of the testing procedure were conducted. At dark cycle onset, water and powdered food were presented, and intakes were recorded every 15 min during 90-min tests. Food and water intakes were also measured for a 90-min period at the beginning of the dark cycle in the absence of experimenters to test for experimenter interference.

Beginning 3 days later, three 90-min tests were conducted that were procedurally identical to the adaptation trials, except that water and food intakes were measured every 10 min. Rats were deprived of food for 0, 4, and 24 h, during which water was available. The tests following the 0- and 4-h food deprivations were conducted on consecutive days, and the 24-h deprivation test followed 2 days later. At least 4 days later, rats were deprived of food for 24 h in their home cages with water available. Intakes of water and pelleted food were then measured as in experiment 1.

Experiment 4

Water deprivation and rehydration. Osmoreceptors at the SFO may be important for detecting a need for water during a meal and thereby function to decrease food intake during water deprivation. We tested the effects of SFO lesion on food intake during water deprivation and on food and water intakes during subsequent rehydration.

Rats (SFOX n = 5; sham n = 7) were maintained on a partially reversed light cycle for 19 days before experimentation began, with lights on from midnight to noon. Rats were weighed and placed into metabolic cages with water and powdered diet for measurement of 24-h intakes. Baseline water and food intakes were measured every 30 min for 3 h, hourly through 6 h, and at 23 h beginning at lights out with the use of a red light. Urine was collected at 2, 4, 6, and 23 h. After 24 h of baseline recording, rats were weighed, water was removed, and food intake and urine volume were measured during water deprivation as during the baseline period. After 24-h water deprivation, rats were weighed and water was returned for a test of food and water intakes during rehydration. Latency to begin drinking and latency to begin eating after drinking had occurred were recorded. Food and water intakes were measured at 30, 60, 90, and 120 min.

Urine was collected in graduated cylinders calibrated to the nearest 0.25 ml. Samples were sealed and frozen for later determination of sodium and potassium concentrations by flame photometry. Water balance was calculated as the total water intake minus urine volume. Sodium balance was calculated as the total sodium intake from food minus urinary sodium excretion. The Teklad diet contained 87 μmol of sodium per gram of food by our analysis.

Statistical Analysis

Data were analyzed with the use of ANOVA appropriate to the individual designs, except for latency data that were compared using the Mann-Whitney’s U test. A probability of <0.05 was required for significance. Planned comparisons were made with the use of Fisher’s protected least-significant difference test following a significant F ratio or the Bonferroni test if the F was not significant. Data are expressed as means ± SE, except for latency data that are presented as medians. Cumulative intake data are presented for clarity, but the uncumulated data were analyzed.

RESULTS

Experiment 1

Food and water intakes after mild and extended food deprivation. At the beginning of the 20-h fast, average body weights of SFOX and sham-lesioned rats were 477 ± 28 and 417 ± 46 g, respectively, and did not differ between groups. Weight loss as a consequence of overnight food deprivation was ~7% for both lesion groups. Water intakes during the overnight period were not significantly different (SFOX 9 ± 2 ml; sham 15 ± 3 ml). After the 20-h fast, all rats began eating before they began drinking, and the median latency to begin eating was similar between lesion groups (SFOX 1.3 min; sham 1.5 min). Total food intake measured at the end of the 90-min test was also similar (SFOX 5.4 ± 0.8 g; sham 4.3 ± 0.6 g). Median latency to begin drinking water after eating had started was over twice as long in SFOX rats (29.0 min) than in sham-lesioned rats (8.7 min), U(6,6) = 3, P < 0.05.
or overnight fasting and refeeding

Table 1. Plasma measures in sham-lesioned or SFOX rats after ad libitum feeding or overnight fasting and refeeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Food and water</th>
<th>Body Wt, g</th>
<th>Body Wt Loss, PM, g</th>
<th>Water Intake, PM, ml</th>
<th>Food Intake Test, g</th>
<th>Latency to Eat, min</th>
<th>Latency to Drink After Eat, min</th>
<th>Plasma, mosm/L kgH2O</th>
<th>PNa, mmol/l</th>
<th>Hct, %</th>
<th>PP, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>ad libitum</td>
<td>7</td>
<td>426 ± 10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>302 ± 1</td>
<td>130.8 ± 0.5</td>
<td>45.5 ± 0.4</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>SFOX</td>
<td></td>
<td>6</td>
<td>458 ± 11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>302 ± 2</td>
<td>131.0 ± 0.9</td>
<td>45.8 ± 0.5</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Refed until</td>
<td>first drink</td>
<td></td>
<td>6</td>
<td>436 ± 43</td>
<td>27 ± 3</td>
<td>11.2 ± 2.2</td>
<td>2.6 ± 0.5</td>
<td>308 ± 1†</td>
<td>134.6 ± 1.1†</td>
<td>49.5 ± 0.7†</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td>6</td>
<td>518 ± 22</td>
<td>32 ± 6</td>
<td>9.0 ± 2.7</td>
<td>5.7 ± 0.4*</td>
<td>310 ± 2†</td>
<td>134.7 ± 1.0†</td>
<td>48.1 ± 1.7†</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>SFOX</td>
<td></td>
<td></td>
<td>6</td>
<td>398 ± 14</td>
<td>—</td>
<td>22.3 ± 3.7</td>
<td>2.8 ± 0.2</td>
<td>n.d</td>
<td>n.a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refed 12 min</td>
<td>without water</td>
<td></td>
<td>7</td>
<td>390 ± 10</td>
<td>25 ± 1</td>
<td>18.8 ± 3.8</td>
<td>2.4 ± 0.2</td>
<td>n.d</td>
<td>n.a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE except for median latencies. n.d., Not determined; n.a., not allowed; SFOX, subfornical organ lesioned; PNa, plasma sodium; Hct, hematocrit; PP, plasma protein; Sham, sham lesioned. *P < 0.01 vs. Sham. †P < 0.001 vs. ad libitum.
vated in groups B and C compared with group A, \(F(2,28) = 14.69, P < 0.001\), but plasma protein concentrations did not differ. In group B, which was sampled for blood at the onset of drinking, the SFOX group ate more than twice as much as the sham-lesioned group before drinking, \(t(10) = 4.86, P < 0.001\), and had a longer median latency to begin drinking, \(U(6,6) = 0, P < 0.01\). These drinking data repeat the findings of experiment 1. Because of the larger food intake of the SFOX group and its delayed drinking, these rats might be expected to have higher plasma osmolalities than sham-lesioned rats that ate less food by drinking onset. The mean plasma osmolality of this SFOX group was the highest observed in this experiment, but the difference from similarly treated sham-lesioned rats was not significant. In group C, both lesion groups ate an amount in the 12 min allowed that was similar to the amount eaten in 12 min by the sham-lesioned rats of group B. The lesion groups had similarly elevated plasma osmolalities, demonstrating that a lesion of the SFO does not affect the rise in plasma osmolality during refeeding.

**Experiment 3**

Rates of nocturnal food and water intake. At the beginning of the experiment, average body weight was similar between SFOX (412 ± 7 g) and sham-lesioned (436 ± 15 g) groups. Average food and water intakes over the three nights of adaptation and the two 90-min adaptation trials were similar between groups. The 90-min food and water intakes in the absence of experimenters were compared with the 90-min intakes following the 0-h food-deprivation test. There was no effect of experimenter interference on food or water intakes in either lesion group.

SFO lesion did not affect the median latency for rats to begin eating on the return of food after 0-, 4-, or 24-h food deprivation. Median latencies to begin eating were <2 min for both lesion groups in all tests, and all rats always began eating before they began drinking. Median latency to begin drinking was significantly increased by SFO lesion after the 4-h fast [51.7 vs. 14.6 min, \(U(9,10) = 10, P < 0.01\)] and 24-h fast [19.0 vs. 8.9 min, \(U(9,10) = 23, P < 0.05\)]. Median latency to begin drinking was longer in the SFOX group than in the sham-lesioned group even after the 0-h fast (40.3 vs. 10.5 min), however, this difference was not significant due to large within-group variability in both lesion conditions.

Food and water intakes were analyzed by ANOVA with lesion as the between-subjects factor and substance (g of food or water) as the within-subjects factor (see Fig. 3). After both the 0- and 4-h deprivations, SFOX rats drank less water than sham-lesioned rats by the end of the 90-min tests, lesion \(\times\) substance interactions (both \(P < 0.05\)). The 90-min water intake was not significantly affected by SFO lesion after the 24-h fast.

The 90-min food intake was not significantly reduced by SFO lesion after either the 0- or 4-h fasts (Fig. 3). However, even if food intake had been lower in the SFOX group, this still could not account for the SFOX rats’ low water intake in either test because, whereas sham-lesioned rats ingested as much water as food by 90 min, SFOX rats ingested significantly less water than food \((P < 0.05)\). Furthermore, at 20 min, which was the nearest time point measured to the median latency for SFOX rats to begin drinking after the 24-h deprivation (19 min, dotted line), SFOX rats had consumed significantly more food and less water than sham-lesioned rats.
rats had ingested as much water as food, but SFOX rats had ingested significantly less water than food.

During the test with pelleted food after 24-h food deprivation, all rats began eating before they began drinking, and median latency to eat and mean 90-min food intakes were similar between lesion groups (SFOX 2.7 min, 6.6 ± 0.7 g; sham 2.3 min, 7.3 ± 0.7 g). Median latency for SFOX rats to begin drinking after they had started eating (23.4 min) was significantly longer than for sham-lesioned rats (11.4 min), 11.4 ± 0.9 s. During hours 6-23 of water deprivation, rats ate only half their baseline intake (Table 2). Food intakes of SFOX and sham-lesioned rats did not differ significantly at any time.

Food and water intakes during the rehydration period did not differ significantly at any time between lesion groups. Latency to drink (SFOX 1.1 min; sham 0.5 min) and latency to begin eating after drinking (SFOX 40.3 min; sham 21.9 min) also did not differ significantly.

**DISCUSSION**

A lesion of the SFO repeatedly created a delay in drinking as rats ate dry chow after 20- to 24-h food deprivation. At the latency for sham-lesioned rats to begin drinking during refeeding, SFOX and sham-lesioned rats developed similar increases in plasma osmolality. Despite this increase, SFO lesion more than doubled the median latency to begin drinking. A lesion of the SFO had less consistent effects on drinking during normal nocturnal feeding and had no effect on consumption of food or water during dehydration-induced anorexia or rehydration from water deprivation.

**Table 2.** Water and sodium balance data for hours 0–6 and 6–23 during baseline and water-deprivation days in sham-lesioned and SFOX rats

<table>
<thead>
<tr>
<th></th>
<th>Hours 0–6</th>
<th>Hours 6–23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>SFOX</td>
</tr>
<tr>
<td>Baseline day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water intake</td>
<td>6.6 ± 0.7</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>Urine volume</td>
<td>4.2 ± 0.5</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>Water balance</td>
<td>2.4 ± 0.8</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>Food intake</td>
<td>6.9 ± 0.8</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Na intake</td>
<td>594 ± 66</td>
<td>670 ± 37</td>
</tr>
<tr>
<td>UNaV</td>
<td>390 ± 71</td>
<td>515 ± 43</td>
</tr>
<tr>
<td>Na balance</td>
<td>203 ± 71</td>
<td>155 ± 68</td>
</tr>
<tr>
<td>Deprivation day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water balance</td>
<td>−4.2 ± 0.2</td>
<td>−4.0 ± 0.2</td>
</tr>
<tr>
<td>Food intake</td>
<td>5.8 ± 0.4</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>Na intake</td>
<td>500 ± 34</td>
<td>563 ± 24</td>
</tr>
<tr>
<td>UNaV</td>
<td>615 ± 50</td>
<td>615 ± 66</td>
</tr>
<tr>
<td>Na balance</td>
<td>−115 ± 76</td>
<td>−53 ± 66</td>
</tr>
</tbody>
</table>

Data are means ± SE. Units: water in ml, food in g, Na in mmol.

*P < 0.05 vs. Sham. UNaV, urine sodium excretion.

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**Fig. 4.** Cumulative 360-min food intake of SFOX and sham-lesioned rats during eating with water (baseline) and without water (dehydration) available. SFOX lesion did not affect food or water intakes during either test. Food intake during dehydration was reduced from baseline in both lesion groups.
The total amount of water consumed in 90 min was similar between SFOX and sham-lesioned groups in some of the present experiments, but not in others. Weisinger et al. (32) found no effect of SFO lesion on 2-h water intake after 24-h food deprivation, and we found no effect of SFO lesion on 24-h ad libitum water intake. However, closer examination of intakes in experiment 3 revealed finer effects of the lesion. For example, two effects of SFO lesion often occurred at the median latency for SFOX rats to drink: 1) water intake was significantly lower in rats with SFO lesion, whereas food intake was not significantly reduced; and 2) sham-lesioned rats consumed similar amounts of water and food, whereas SFOX rats consumed less water than food. Thus food and water intakes were coupled in sham-lesioned rats, and these substances were consumed in closely equivalent amounts over the course of testing. This coupling was compromised in SFOX rats (Fig. 3).

There are several possible explanations for how the SFO may be involved in the water intake that occurs in response to the ingestion of food: 1) the SFO contains receptors that are sensitive to changes in osmotic pressure of the plasma (21, 22, 24, 28); 2) the SFO contains dipsogenic receptors for ANG II (30); and 3) the SFO receives afferents from the hindbrain vagal complex (6, 22, 33). Our measurements in this study focused on osmotic changes, however, these mechanisms are not mutually exclusive, and any or all could be operating.

**SFO osmoreceptors**

Experiment 2 demonstrated that plasma osmolality was similarly elevated in both lesion groups by 12 min after the initiation of eating. This was the approximate median latency for sham-lesioned rats to begin drinking. When SFOX rats were allowed to continue eating until they began to drink at 34 min, they ate significantly more food and had plasma osmolalities that were slightly, but not significantly, higher than those of sham-lesioned rats sampled at 12 min. This implies that 34 min was sufficient time for SFOX rats to excrete some of the excess solute as concentrated urine, perhaps thereby compensating for their delayed latency to drink. The elevated plasma sodium concentration of group B compared with group A demonstrates the contribution of sodium to the increase in osmolality. Importantly, sodium is largely excluded from the intracellular compartment, thus its increases would activate osmoreceptors.

We previously observed that an intragastric load of hypertonic saline (2 ml of 900 or 1,200 milliosmolar NaCl) increased plasma osmolality and sodium concentration by 15 min after administration (31). Sham-lesioned rats in that study drank water within 15 min of loading, but SFOX rats drank later and less. In other studies, both intravenous infusion (8, 24) and intraperitoneal injection (5) of hypertonic saline increased c-fos expression in the SFO. These results, taken together with the results of the present study, strongly imply that the delay in drinking onset in SFOX rats following intragastric hypertonic saline or food intake is due to the removal of osmoreceptive units at the SFO.

Evidence for an involvement of the SFO in response to rapid osmotic loading was provided by Han and Rowland (8). They identified c-fos expression in the SFO, organum vasculosum laminae terminalis (OVLT), hypothalamic paraventricular nucleus, and supraoptic nuclei in response to an intravenous infusion of 12 mmol/kg NaCl over either 1 h or 10 min. The rapid infusion caused greater c-fos expression in the SFO and OVLT than the slow infusion. They also found that a smaller load, 6 mmol/kg NaCl, induced c-fos expression in the paraventricular and supraoptic nuclei but not in SFO or OVLT. Thus the SFO and possibly OVLT may be more affected by large, rapid increases in plasma osmolality than by small, slow increases. These results and those of the present study imply that the SFO and OVLT may be especially important for a quick response to an osmotic challenge created by the rapid ingestion of food or hypertonic saline. Furthermore, osmoreceptors at the OVLT or other nuclei may be partly responsible for osmotic mediated drinking in rats with SFO lesion.

Different lengths of fasting may produce different rates of eating and, consequently, different osmotic environments in the gut and extracellular fluids. Kraly et al. (17) observed that longer fasts produced greater food and water intakes and more reliable meal-related drinking than shorter fasts. In experiment 1, plasma osmolality presumably increased more rapidly during rapid eating after the 20-h fast than during slower eating after the 4-h fast. Slow eating after the 4-h fast eventually produced a drinking response, but this may be dependent on osmoreceptors other than those at the SFO. We conducted experiment 3 with powdered chow in part to determine the rates of eating and drinking after different lengths of food deprivation. Eating rate was faster in both sham-lesioned and SFOX rats after the 24-h fast than after the 4- or 0-h fasts. However, the SFOX rats in that particular experiment were always slower to drink after eating regardless of the length of deprivation or ad libitum feeding. Thus it is not clear that the rate of eating was the only controlling variable. Perhaps in changing from pelleted chow to powdered chow to observe feeding with greater resolution, we changed the rats’ pattern of eating and thus altered the participation of the SFO in that process.

Ordinarily, when rodents are deprived of water, they reduce their intake of dry food (2, 3, 7, 27) and increase excretion of concentrated sodium. This excretion during intracellular dehydration serves to increase the amount of water available to the intracellular compartment, thus better allowing the rats to continue eating during the dehydration. Weisinger et al. (32) found that SFOX rats ate significantly more food than sham-lesioned rats during 24 h of water deprivation, which suggests that this regulation of food intake and sodium excretion might be defective in SFOX rats. In experiment 4 of the present study, rats in both lesion groups reduced their food intake to approximately half of their baseline intake throughout 23 h of food access during...
water deprivation, excreted similar amounts of sodium, and ended the dehydration period in similar negative sodium balance. With the return of water after 24 h of water deprivation, rats in both lesion conditions began drinking and recommenced eating with similar latencies. Thus we did not replicate the enhanced eating in SFOX rats observed by Weisinger et al. (32). However, we did observe elevated food intake in SFOX rats during the first 20 min of refeeding from 24-h food deprivation even though the rats displayed reduced water intake through the same period (experiment 3; Fig. 3, bottom). SFOX rats also frequently ate more food than sham-lesioned rats before taking their first drink during refeeding from food deprivation. Therefore, our data are in partial agreement with Weisinger et al. (32) in that SFOX rats may sometimes inappropriately overeat in the face of osmotic dehydration.

**SFO and ANG II**

Water may account for nearly half of gut contents for up to 6 h after a meal of dry chow regardless of whether rats have water to drink during the meal (18). Other studies have also found that a closely equivalent ratio of fluid and food is present in the stomachs of rats after a meal of dry chow without water available (25), and this 1:1 ratio may continue for up to 2 h after the end of a dry meal (23). These results suggest that if water is not drunk during a meal, it must be obtained at the expense of body fluid, either extracellular, intracellular, or both.

The present experiments showed that the drinking latency for SFOX rats after extended food deprivation was two to three times as long as for sham-lesioned rats. Furthermore, at the time of drinking onset in each group, SFOX rats had eaten significantly more food than sham-lesioned rats. Thus SFOX rats required twice as much time or twice as much food as sham-lesioned rats before drinking occurred, indicating that a prolonged deviation of body fluid distribution may be necessary for the stimulation of thirst in the absence of the SFO.

Increased plasma ANG II levels and plasma renin activity have been found in rats after eating dry laboratory chow or dry salted crackers (15, 20, 25). This synthesis of ANG II may have been due to activation of the renal sympathetic nerves (15) or to an osmotically driven influx of water into the gut at the expense of the systemic circulation as discussed above. Sympathetic stimulation of renin secretion may occur during a meal, and if ANG II is thereby formed peripherally, the SFO would be a logical site for it to act in stimulating water intake. Our blood and plasma measures of experiment 2 provide some evidence of extracellular fluid depletion at the time of drinking onset as estimated by hemocrit, although plasma protein concentrations were unchanged from baseline values. The discrepancy between these two indicators of plasma volume suggests that these variables need not change simultaneously. This inconsistency makes it difficult to draw conclusions from these data about changes in plasma volume during eating.

**SFO and Gut Osmoreceptors**

Subdiaphragmatic vagotomy reduces drinking in response to hypertonic saline administration and to the ingestion of an osmotically dense meal (11, 17, 29). Kraly and colleagues (12, 14) have demonstrated that drinking after intragastric hypertonic saline or after eating occurs before any elevation of plasma osmotic pressure. Kraly and colleagues suggested that an osmoreceptive control system in the gastrointestinal or hepatic-portal regions detects the potential for increases in systemic plasma osmolality and then attenuates or precludes these changes by stimulating drinking (14).

The SFO receives both direct and indirect afferents from the hindbrain vagal complex (6, 22, 33), and these may stimulate drinking in response to the ingestion of food. Destruction of the SFO may compromise the effectiveness with which the rat can respond to signals from local gut osmoreceptors. Given sufficient time, SFOX rats eventually drink normally during refeeding, as evidenced by the 90-min water intakes after 20–24 h of fasting in experiments 1 and 3. Perhaps the gradual elevation of plasma osmolality activates other osmoreceptive units within the blood-brain barrier to generate this drinking.

The blood data of experiment 2 fail to support the hypothesis that drinking is initiated in advance of an increase in plasma osmolality (12, 14). However, the brief delay between drinking initiation and our blood sampling may have been sufficient for further elevation of plasma osmolality to occur. Whether or not plasma osmolality was elevated, these data do not refute the hypothesis that gut osmoreceptors may also stimulate drinking, perhaps via the SFO, because a gut-brain mechanism could operate parallel or in synergy with a plasma osmotic mechanism to stimulate water intake.

**Perspectives**

The SFO is somehow involved in making fine adjustments to water intake as plasma osmolality increases. For example, although osmolality increased equivalently in SFOX and sham-lesioned rats by the time sham-lesioned rats began drinking during a meal, drinking onset was delayed by the lesion. This suggests that the SFO is necessary either for the prompt detection of deviations in plasma osmolality or for the early onset of osmotically mediated drinking, thus limiting the length of time a rat must tolerate a departure from normal plasma osmotic pressure. Likewise, SFO lesion tended to decrease water intake only through the early part of testing during intravenous hypertonic saline infusion (10), further indicating that a sustained increase in osmolality may be required to initiate drinking in the absence of the SFO. If stimulation of osmoreceptors occurs earlier at areas outside of the blood-brain barrier rather than inside, the SFO may inform
structures such as the paraventricular and supraoptic nuclei of increases in plasma osmolality before direct stimulation of hypothalamic osmoreceptors occurs. Such a signal from the SFO could thereby induce thirst and perhaps vasopressin secretion earlier in intact rats than in rats with SFO lesion. The present study demonstrates a regulatory mechanism for meal-associated water intake, with the SFO as an important initiator of this drinking.

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