Early osmoregulatory stimulation of neurohypophyseal hormone secretion and thirst after gastric NaCl loads

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INCREASES IN THE EFFECTIVE osmolality of plasma (P_{osmol}) are known to activate neurons in the organum vasculosum of the lamina terminalis (OVLT) in the basal forebrain (29). Lesions of this circumventricular organ markedly attenuate the stimulation of thirst and neurohypophyseal secretion of vasopressin (VP) and oxytocin (OT) by hyperosmolality in rats (17, 26, 36), indicating the importance of the OVLT in mediating these adaptive responses. However, other osmoreceptors may contribute to those effects as well. For example, the existence of peripheral osmoreceptors has been suggested by reports that intragastric intubation of hypertonic saline (ig HS) stimulates thirst and VP secretion in rats before systemic P_{osmol} increases to levels detectable by cerebral osmoreceptors (9, 25). Although relatively small increases in water intake and VP secretion were observed in those studies, it seems plausible that the effects were not fully expressed because euvhydrated animals were used and therefore their brains received a mixed osmoregulatory message, that is, a signal of hydration from cerebral osmoreceptors may have blunted a signal of incipient hyperosmolality from peripheral osmoreceptors. The present experiments tested this hypothesis by determining whether the increase in water intake and plasma VP (pVP) induced by intragastric HS would be more substantial when the gastric load was given either to rats after overnight water deprivation or to rats whose P_{osmol} had been elevated by pretreatment with intravenous infusion of HS (iv HS). Plasma levels of OT (pOT) also were measured because this peptide is known to make important contributions to osmoregulation as a natriuretic hormone in rats (18, 19, 41).

METHODS

Animals. Adult male Sprague-Dawley rats (Zivic Laboratory, Zelienople, PA) weighing 300–350 g were used in this study. They were housed individually in wire-mesh cages in a colony room with ambient temperature of 22–24°C and with lights on from 7:00 AM to 7:00 PM. The rats had ad libitum access to Laboratory Chow pellets (Purina no. 5001) and tap water before experiments began. All procedures for the treatment of animals were in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Procedure. To acclimate the animals to the gastric intubation procedure, intragastric loads of isotonic saline (ig IS) were administered to all rats one time each day for 3 days before testing. A transverse V-shaped metal spring wire was placed between the rats’ front teeth to keep their mouths open, and then a 10-cm length of a polyethylene feeding tube (Pharmaseal, Toa Alta, Puerto Rico) was gently inserted into the esophagus into the stomach. The 4-ml load was administered over ~30 s. Rats showed no visible signs of discomfort by the third load.

Effects of intragastric HS on drinking behavior in water-deprived rats. Three groups were studied to determine the effect of intragastric HS on drinking in water-deprived rats. One group (n = 7) was both water deprived overnight and given a 4-ml gastric load of either 0.15 or 0.5 M NaCl before the drinking test. A second group (n = 10) was not water deprived before receiving either intragastric IS or intragastric HS.
tric HS, whereas a third group (n = 5) was deprived of drinking water overnight but was not given a gastric load before testing. In the first and second groups, the two gastric preloads were administered to each rat in a counterbalanced order with 3–5 days separating the tests. Water was made available immediately after the loads to water-deprived rats, and 5 min after the loads to nondeprived rats, and intakes were recorded every 15 min for 1 h.

Previous reports had indicated that systemic P$_\text{o}_\text{smol}$ is not elevated within 30 min after various intragastric HS treatments in euhydrated rats (6, 9). To test the effect of the present intragastric HS treatment on P$_\text{o}_\text{smol}$ in water-deprived rats, other animals were anesthetized with sodium brevital (50 mg/kg ip, Jones Medical, St. Louis, MO), and a catheter was implanted in the right femoral artery, as described below. The rats were returned to their home cages and, on the following day, they were deprived of water overnight with food available. On the next day, a baseline blood sample (0.5 ml) from each rat was withdrawn via the arterial catheter into tubes coated with EDTA (Vacutainer; Becton-Dickinson, Franklin Lakes, NJ). The tubes were centrifuged immediately (10,000 g for 1 min at −4°C), aliquots of plasma were removed, and P$_\text{o}_\text{smol}$ was measured by freezing-point depression using a microsample osmometer (Micro-Osmette; Precision systems, Natick, MA). The red blood cells were resuspended in an equal volume of 0.15 M NaCl and returned to the animals by injection soon after each sample had been taken. The same procedures were repeated 4 h later, when rats were given a 4-ml gastric load of either 0.15 or 0.5 M NaCl (n = 6 and 7, respectively), and blood samples (0.5 ml) were withdrawn from each rat at 10, 25, and 55 min afterward.

**Effects of intragastric HS on neurohypophysial hormone secretion in dehydrated rats.** Two different protocols were used to examine the effects of intragastric HS on neurohypophysial hormone secretion in dehydrated rats. In the first protocol, which mirrored the drinking study described above, rats were water-deprived overnight while food remained available. On the following day, they were given a 4-ml gastric load of either 0.15 or 0.5 M NaCl (n = 6 and 7). They were decapitated 25 min later, and trunk blood was collected in ice-cold heparinized tubes (143 USP sodium heparin). For purposes of comparison, blood samples also were taken from other rats given intragastric IS or intragastric HS treatments but not water deprived (n = 6 and 6). All samples were centrifuged, and the plasma was removed. P$_\text{o}_\text{smol}$ was measured immediately in aliquots, as above, while the remainder was frozen for later RIA of VP and OT.

The second protocol examined rats pretreated with intravenous HS. Before the experiment (2 days), rats were anesthetized with sodium brevital, and two catheters were implanted, one (PE-50) in the right femoral artery for blood sampling and one (polyvinyl tubing) in the right femoral vein for infusions via a pump (Harvard Apparatus, South Natick, MA). The free ends of the two catheters were guided subcutaneously along the back to exit between the scapulae. Upon exiting, the catheters were encased in a steel spring to prevent them from being damaged and were connected to a swivel system to allow freedom of movement. The rats were returned to their home cages where experiments occurred. On the morning of the test day, water and food were removed from each cage. Rats were infused (2 ml/h iv) with 0.15 M NaCl solution during a 30-min baseline period, after which the infusate was switched to 1 M NaCl delivered at the same rate for 120 min to stimulate the secretion of VP and OT. Next, the infusion was terminated, and rats were given a 4-ml gastric load of either 0.15 or 0.5 M NaCl (n = 11 and 11). Blood samples (1.5 ml) were taken just before and just after the 120-min intravenous infusion of 1 M NaCl and also 15 and 25 min after the gastric load. All blood samples were withdrawn from indwelling arterial catheters into chilled tubes and centrifuged, and the plasma was removed. P$_\text{o}_\text{smol}$ was measured immediately in aliquots, as above, while the remainder was frozen for later RIA of VP and OT.

**Effects of overnight water deprivation plus gastric loads of NaCl solution on P$_\text{o}_\text{smol}$ in rats**

Table 1. Effect of overnight water deprivation plus gastric loads of NaCl solution on P$_\text{o}_\text{smol}$ in rats

<table>
<thead>
<tr>
<th>Gastric Load Condition</th>
<th>Change in P$<em>\text{o}</em>\text{smol}$ (mosmol/kgH$_\text{2}$O)</th>
<th>10 min</th>
<th>25 min</th>
<th>55 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration + IS</td>
<td>−1 ± 1</td>
<td>+1 ± 1</td>
<td>+1 ± 1</td>
<td></td>
</tr>
<tr>
<td>Dehydration + HS</td>
<td>+2 ± 2</td>
<td>+3 ± 2</td>
<td>+8 ± 2*</td>
<td></td>
</tr>
</tbody>
</table>

Values shown (mosmol/kgH$_\text{2}$O) are mean differences ± SE. Values compared with baseline values. Note that an increase of −6 mosmol/kgH$_\text{2}$O represents the threshold for neurohypophysial hormone secretion and thirst in euvhydrated rats (13, 31). Dehydrated rats were given 4 ml of either 0.15 M NaCl [isotonic saline (IS)] or 0.5 M NaCl [hypotonic saline (HS)] by gastric tube. Baseline blood samples were withdrawn from indwelling arterial catheters just before the intubation and 10, 25, and 55 min later. Access to food or water was not allowed. *P < 0.05 compared with baseline values and values in the deprivation + IS group.
were significantly elevated above both of those control values (all \( P < 0.05 \)). Similarly, when absolute values of \( \text{P}_{\text{osmol}} \) were analyzed rather than changes in \( \text{P}_{\text{osmol}} \), a significant effect was observed at 55 min after intragastric HS (\( P < 0.05 \)) but not at 10 or 25 min.

**Effects of intragastric HS on neurohypophysal hormone secretion in dehydrated rats.** In one study, rats were given intragastric HS either alone or after overnight water deprivation. In both groups, the load had no statistically reliable effects on \( \text{P}_{\text{osmol}} \) measured 25 min later (Fig. 2). Nonetheless, intragastric HS increased pVP and pOT significantly in those animals (all \( P < 0.01 \) compared with values after intragastric IS; Fig. 2). Importantly, larger effects on pVP and pOT were observed when intragastric HS was given after
water deprivation than when it was given alone (pVP, \( P < 0.02; \) pOT, \( P < 0.01 \)). The effects of the gastric loads on pVP and pOT in individual rats are presented in Fig. 3; clearly, the range of P\(_{\text{osmol}}\) values overlapped considerably in rats given intragastric IS or intragastric HS, especially when the loads were given after overnight water deprivation.

In other rats, systemic infusion (2 ml/h iv for 2 h) of 1 M NaCl significantly raised P\(_{\text{osmol}}\), pVP, and pOT above basal values in all rats, as intended (all \( P < 0.01 \)). As shown in Fig. 4, the elevated levels of P\(_{\text{osmol}}\) did not change significantly 15 or 25 min after the rats received intragastric IS or intragastric HS. In contrast, intragastric HS produced further increases in both pVP and pOT, measured both 15 and 25 min later, compared with the levels seen before the loads (all \( P < 0.01 \) except pVP at 15 min) and the levels seen after intragastric IS (all \( P < 0.01 \)). This difference also is seen in Fig. 5, which presents individual values of pVP and pOT in these rats plotted as a function of P\(_{\text{osmol}}\). In water-deprived rats given intragastric IS or intragastric HS, individual values of pVP and pOT in the same blood samples correlated closely with one another (\( r = 0.80, P < 0.001 \)); the linear regression line describing this relationship was pOT = 1.9pVP − 9.3.

**DISCUSSION**

It is well recognized that thirst and VP secretion are stimulated in rats and other animals by increases in the osmolality of blood in the general circulation. An increase in systemic P\(_{\text{osmol}}\) also stimulates neurohypophyseal secretion of OT in rats (34). Cerebral osmoreceptors located in the basal forebrain are known to mediate those adaptive osmoregulatory responses (35). The present results indicate that the same responses occurred in euvhydrated rats after small intragastric HS loads were administered, before an increase in systemic P\(_{\text{osmol}}\) was seen, although the effects obtained were relatively small. These findings are consistent with previous observations (9, 25). The main point of the present report is that a more substantial stimulation of water intake and neurohypophyseal VP and OT secretion occurred when, before the intragastric HS treatment, rats either were water deprived overnight or received intravenous HS to increase P\(_{\text{osmol}}\). Thus coactivation of cerebral osmoreceptors appears to potentiate the effects of peripheral osmoreceptors (or Na\(^+\) receptors) to stimulate thirst and neurohypophyseal secretion in rats.

Kraly et al. (25) reported that rats began to drink water 10–15 min after they received gastric loads of hypertonic NaCl solution, by which time P\(_{\text{osmol}}\) had not yet changed. In fact, it can be calculated that the smallest load given in that study, 2 ml of 0.3 M NaCl, would not have increased P\(_{\text{osmol}}\) in adult rats by 2%, the apparent threshold for thirst (15), even after the entire load had been absorbed and equilibrated with body fluids. Thus it seems unlikely that cerebral osmo-

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**Fig. 3.** Effect of ig IS (○) or ig HS (●) on plasma levels of VP and OT, plotted as a function of the associated P\(_{\text{osmol}}\), in rats that had not (A and C; \( n = 6 \)) or had been (B and D; \( n = 7 \)) deprived of drinking water overnight. Symbols represent values from individual animals, measured 25 min after the gastric loads. Mean values of P\(_{\text{osmol}}\), pVP, and pOT are shown in Fig. 2. Higher values of pVP and pOT were seen when rats were given ig HS instead of ig IS (all \( P < 0.01 \)).
receptors were responsible for stimulating the drinking response. A similar conclusion may be drawn from the results of the present study, in which rats were deprived of water overnight before receiving a 4-ml gastric load of 0.5 M NaCl solution. Those animals drank much more water in 15 and 30 min than water-deprived rats given either intragastric IS or no gastric preload even though significant changes in P_{osmol} were not observed during this time period. Note that, in water-deprived rats given intragastric HS treatment, it can be calculated that the P_{osmol} would have increased by \sim 13 \text{mosmol/kgH}_2\text{O} if the entire load had been absorbed, equilibrated with body fluids, and retained. Because the observed increase in P_{osmol} was only \sim 3 \text{mosmol/kgH}_2\text{O} at 25 min after the load was administered, and only \sim 8 \text{mosmol/kgH}_2\text{O} at 55 min, it seems likely that the load was absorbed very slowly. It can be calculated that the absorption of \sim 1 \text{ml} (i.e., \sim 25\% of the 4-ml load) of 0.5 M NaCl would raise P_{osmol} by \sim 3 \text{mosmol/kgH}_2\text{O} in these animals, which is consistent with previous observations of gastric emptying in water-deprived rats (28).

Although these data suggest that a peripheral signal of thirst resulting from the intragastric HS treatment was potentiated in dehydrated rats, there is an alternative explanation of the findings. The presence of HS in the stomach should increase the concentration of fluid passing to the intestines after rats drank water, thereby reducing the effectiveness of ingested water in providing hydreadial signals that inhibit water intake. To determine whether the observed effects of intragastric HS resulted from an extra excitatory signal or from a diminished inhibitory signal, it was necessary to study this phenomenon under circumstances in which drinking water was not available. That was the protocol used to investigate neurohypophysal hormone secretion, and the results indicate that intragastric HS treatment caused a substantial increase in plasma levels of VP and OT without significantly affecting P_{osmol} in rats pretreated either with overnight water deprivation or with intravenous HS. These findings suggest that an extra excitatory signal was present, which also may have contributed to the increased water intake observed in the drinking experiment.

In a similar experiment, Carlson et al. (6) gave euhydrated rats 2.9 ml of 0.3 M NaCl intragastrically, and pVP increased by \sim 2.5 pg/ml when measured 10 min later. This effect was associated with a significant increase in the P_{osmol} of blood in the hepatic portal vein but not in systemic blood. In the present experiments, euhydrated rats were given 4 ml of 0.5 M NaCl intragastrically, and pVP increased by \sim 4 pg/ml at 25 min after the intubation. In contrast, when rats were water deprived before receiving intragastric HS, pVP increased by \sim 15 pg/ml when measured 25 min later. Values of pOT paralleled those changes, increasing by \sim 10 and by \sim 30 pg/ml in the same blood samples. Similarly, when rats were pretreated with intravenous HS, pVP increased by \sim 12 pg/ml when measured 25 min after intragastric HS, whereas pOT increased by \sim 24 pg/ml. Thus greater increases in neurohypophysal secretion were observed in response to the same intragastric HS treatment when rats were dehydrated rather than euhydrated, despite the absence of further increases in P_{osmol}. The relative magnitude of the neu-

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**Fig. 4.** Effect of 4-ml gastric loads of IS (○) or HS (●) on mean ± SE values of P_{osmol}, pVP, and pOT in rats (n = 11) that had been infused with 1 M NaCl (2 ml/h iv for 2 h). Values of all three variables before the gastric loads were given (0 min) were greater than baseline values before the start of the infusion (BL; all P < 0.01). P_{osmol} did not change significantly in either group during the 25-min test period after either load. In rats given ig IS, pVP did not change significantly, although pOT decreased at 25 min (†P < 0.01) but not at 15 min. In contrast, rats given ig HS had higher levels of pVP and pOT after the load than before the load († all P < 0.01 except for pVP at 15 min) and higher levels of both hormones than rats did after ig IS (∗ all P < 0.01).
rohypophyseal secretions stimulated by intragastric HS resembles the reported effects in rats of HS or hypertonic mannitol solution administered systemically, or of hypovolemia; each of these treatments evokes increases in pOT that are approximately two times greater than the increase in pVP (20, 34).

The present results suggest that intragastric NaCl loads generate peripheral signals that precede significant absorption of NaCl into the general circulation and its detection by cerebral osmoreceptors. Postgastric receptors in the splanchnic area would be ideally located to sample solutions and influence ongoing behavioral and physiological responses before gastric NaCl loads enter the general circulation. In this regard, the hepatic portal vein already has been implicated as a site of osmo- or Na⁺ receptors (27, 39). Vagal afferent nerves responsive to HS infused in the hepatic portal vein are known to project to the nucleus tractus solitarius (NTS) subadjacent to the area postrema (AP) in the brain stem (22, 24). If this neural pathway mediates an early osmoregulatory signal that affects fluid intake and neurohypophyseal hormone secretion, then that signal should be eliminated after destruction of this pathway or its projection sites in the brain stem. Consistent with this expectation, vagotomized rats drank larger volumes of concentrated saline solution than control rats did in response to various stimuli (39), as if they were not receiving an early signal of imminent hyperosmolality. Rats with lesions of the AP/NTS similarly drank larger amounts of concentrated saline solution (10, 33), as did rats with damage to peripheral sensory fibers caused by systemic administration of the neurotoxin capsaicin (11). An analogous effect of AP/NTS lesions to attenuate the increased VP and OT secretion in response to intravenous HS has been reported (20), although such lesions did not blunt the early stimulation of VP secretion by intragastric HS treatment (7, 8, 40).

Previous reports indicate that thirst and secretion of pituitary VP and OT are stimulated in an approximately additive fashion in rats when P_osmol is elevated while plasma volume is reduced (32, 34). Those findings have been interpreted to signify that the neural circuits involved in the control of thirst and neurohypophyseal secretion respond to multiple sensory signals with little interaction (also see Ref. 16). The same functional arrangement does not describe the present findings. Although the effects of intragastric HS treatment are relatively small when the animals are well hydrated, they are more substantial when animals are dehydrated or when P_osmol already is elevated. In other words, the osmoregulatory system appears to operate as if there was a gating mechanism that inhibits the peripheral signals when cerebral osmoreceptors detect euhydration and disinhibits them when cerebral osmoreceptors detect dehydration. Whatever the mechanism, the adaptive significance of this functional arrangement is plain. When rats are dehydrated and consume osmolytes in concentrated NaCl solution or in food, it is useful for them not to wait for P_osmol to increase before secreting OT and VP and increasing water consumption, so that they avoid becoming too dehydrated.

When rats are dehydrated and consume water, it is similarly useful for them not to wait for P_osmol to decrease before terminating VP secretion and ongoing water consumption, thereby avoiding overhydration. An early signal of hydration was discussed many years ago (1, 4) to explain why drinking by dehydrated dogs stopped well before the ingested water was absorbed. This anticipatory element in the control of water intake was clarified subsequently in a series of elegant investigations reported by Appelgren et al. (2) and Thrasher et al. (37, 38). Briefly, an early inhibition of thirst and VP secretion was observed after water consumption by dogs fitted with a gastric fistula, which drained the stomach and thereby prevented the possibility of rehydration. The same rapid inhibitory effects occurred when dogs drank HS solution (although, ultimately, when the saline was absorbed and P_osmol was elevated, the dogs became even thirstier and secreted more VP.

Fig. 5. Effect of ig IS (○ and △) or ig HS (● and ▲) on plasma levels of VP (A) and OT (B), plotted as a function of the associated P_osmol, in rats (n = 11) that had been infused with 1 M NaCl (2 mL/h iv for 2 h). Symbols represent values from individual animals at 15 (○ and ●) and 25 (△ and ▲) min after the gastric loads. Mean values of P_osmol, pVP, and pOT are shown in Fig. 4. Higher values of pVP and pOT were seen when rats were given ig HS instead of ig IS (all P < 0.001).
than before, as might be expected). These observations highlight the importance of an early inhibitory signal in the control of water intake and suggest its basis: a neural input to the brain from the oropharynx, associated with rapid swallowing during the act of drinking. This signal, which essentially allowed thirsty dogs to monitor their intake, had a rapid but temporary inhibitory effect on thirst and VP secretion. When ingested water was subsequently absorbed and \( P_{osmol} \) was diluted back to normal levels, a more sustained termination of thirst and VP secretion was produced because of systemic rehydration.

Several other species, including humans (31), also use oropharyngeal signals to inhibit thirst and neurohypophyseal secretion during water consumption. Similarly, in rats pretreated with intravenous HS, water drinking provided a rapid stimulus to inhibit VP and OT secretion before a decrease in \( P_{osmol} \) was seen in systemic blood (21). When thirsty rats drank IS instead of water, however, no change in pVP or pOT was observed (21). Thus the concentration of the fluid that rats consume, not its volume, seems to be the critical variable in providing early inhibitory signals (3, 5). Furthermore, vagotomized rats (23, 25), capsaicin-treated rats (11), and rats with lesions of the AP/NTS (12, 14) all drank much more water than control rats did in response to various thirst stimuli, as if they were not receiving an early satiety signal. The present findings, together with those previous observations, therefore support the hypothesis that in rats peripheral osmo- or \( Na^+ \) receptors detect the osmotic consequences of fluid ingestion, whether increases or decreases in \( P_{osmol} \), and inform the brain of imminent changes in systemic \( P_{osmol} \) before they could be detected by cerebral osmoreceptors.

Remarkably, this early signal of hydration reduces thirst and neurohypophyseal secretion despite the continued stimulation of cerebral osmoreceptors by elevated \( P_{osmol} \). These rapid and potent feedforward effects prepare animals for similar but more gradual signals, much like the familiar autonomic reflex involving the taste and smell of food stimulates insulin secretion before ingested food has been absorbed from the gastrointestinal tract.

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