Presystemic influences on thirst, salt appetite, and vasopressin secretion in the hypovolemic rat

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Smith CA, Curtis KS, Smith JC, Stricker EM. Presystemic influences on thirst, salt appetite, and vasopressin secretion in the hypovolemic rat. Am J Physiol Regul Integr Comp Physiol 292: R2089–R2099, 2007. First published January 4, 2007; doi:10.1152/ajpregu.00595.2006.—The present studies investigated the influence of presystemic signals on the control of thirst, salt appetite, and vasopressin (VP) secretion in rats during nonhypotensive hypovolemia. Rats were injected with 30% polyethylene glycol (PEG) solution, deprived of food and water overnight, and then allowed to drink water, 0.15 M NaCl, or 0.30 M NaCl. The PEG treatment, which produced 30–40% plasma volume deficits, elicited rapid intakes in an initial bout of drinking, but rats consumed much more 0.15 M NaCl than water or 0.30 M NaCl. In considering why drinking stopped sooner when water or concentrated saline was ingested, it seemed relevant that little or no change in systemic plasma Na+ concentration was observed during the initial bouts and that the partial repair of hypovolemia was comparable, regardless of which fluid was consumed. In rats that drank 0.15 M NaCl, gastric emptying was fastest and the combined volume of ingested fluid in the stomach and small intestine was largest. These and other observations are consistent with the hypothesis that fluid ingestion by hypovolemic rats is inhibited by distension of the stomach and proximal small intestine and that movement of dilute or concentrated fluid into the small intestine provides another presystemic signal that inhibits thirst or salt appetite, respectively. On the other hand, an early effect of water or saline consumption on VP secretion in PEG-treated rats was not observed, in contrast to recent findings in dehydrated rats. Thus the controls of fluid ingestion and VP secretion are similar but not identical during hypovolemia.

ADAPTIVE BEHAVIORAL AND PHYSIOLOGICAL responses to hypovolemia promote the regulation of blood volume. For example, when subcutaneous injections of polyethylene glycol (PEG) solution in rats were used to disrupt the Starling equilibrium and cause progressive deficits in plasma volume without affecting arterial blood pressure (25, 34), large quantities of water and NaCl solution were consumed in amounts appropriate to form an isotonic fluid mixture sufficient to repair the deficits (27, 30, 32). In addition, there was increased neurohypophysial secretion of vasopressin (VP) (9, 36), which has important pressor actions during hypovolemia (12), as well as activation of the renin-angiotensin-aldosterone system (18, 35).

In previous studies of hypovolemic rats, thirst given access to drinking water immediately after PEG treatment experienced osmotic dilution of systemic body fluids due to renal retention of ingested water. Osmotic dilution inhibited further water intake, despite continued plasma volume deficits (26), whereas consumption of concentrated NaCl solution was stimulated (32). Other studies showed that osmotic dilution of systemic blood also inhibited VP secretion in hypovolemic rats, whereas a salt load increased plasma VP (pVP) (36). Collectively, these studies demonstrate potent systemic influences on thirst, salt appetite, and VP secretion in rats during hypovolemia.

Twenty-five years ago, Thrasher and colleagues (38) reported that a volume-dependent oropharyngeal reflex inhibits intake and VP secretion when dehydrated dogs drink water. Recent studies have suggested that fluid ingestion by dehydrated rats is not constrained by oropharyngeal stimuli but, rather, by a different volume-dependent presystemic inhibitory signal, which is associated with distension of the stomach and small intestine (13). Other recent studies have shown that VP secretion in rats is influenced by a concentration-dependent presystemic signal. Specifically, pVP decreased rapidly when thirsty rats drank water before systemic plasma osmolality (P-osmol) decreased significantly (2, 14, 31), whereas a gastric load of hypertonic NaCl solution stimulated VP secretion in rats before systemic P-osmol increased (5, 16, 29).

We presume that messages from the abdominal viscera, most likely neural, provide the presystemic signals. Afferent fibers of the vagus nerve are known to project from visceral organs to the area postrema and subjacent nucleus of the solitary tract (NTS) in the brain stem (21). If vagal neurons signal incipient hydration before significant osmotic equilibration of ingested water with the systemic circulation, then destruction of those nerves should increase water consumption by thirsty rats until osmotic dilution of systemic body fluids is sufficient to affect cerebral osmoreceptors. The results of several experiments offer strong support of this hypothesis. For example, in response to subcutaneous PEG treatment, rats drank large amounts of water when peripheral sensory fibers (including vagal afferents projecting from the abdominal viscera) had been destroyed by systemic treatment with the neurotoxin capsaicin (6) and also when the area postrema/NTS had been destroyed by surgical lesions (7). In both cases, the thirsty rats behaved as if no early consequence of water intake limited further drinking.

The present studies continue to investigate the presystemic influences on thirst, salt appetite, and VP secretion in rats during nonhypotensive hypovolemia. We imposed a 16-h delay between the subcutaneous injection of PEG solution and the start of the drinking test, by which time the plasma volume deficit was 30–40% and pVP was elevated considerably. As

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expected, the hypovolemic rats consumed fluid avidly, thus allowing us to 1) determine whether presystemic signals inhibit water and NaCl solution intake in this model of thirst and salt appetite, 2) determine whether presystemic signals inhibit VP secretion when hypovolemic rats drink water and stimulate VP secretion when they drink concentrated NaCl solution, and 3) examine the early physiological consequences of water and saline consumption and consider whether observed changes in the gastrointestinal (GI) tract provide the presystemic signals.

**METHODS**

**Animals.** Adult male Sprague-Dawley rats (Harlan Laboratories) weighing 300–400 g on the day of experiments were housed singly in cages in the Biomedical Research Facility at Florida State University (experiment 1) or in the Department of Neuroscience at the University of Pittsburgh (experiments 2A and 2B). The temperature-controlled (22–23°C) colony rooms were maintained on a fixed light-dark cycle (lights off from 7 PM to 7 AM). All rats had ad libitum access to pelleted laboratory chow (diet no. 5001, Purina), tap water, and 0.15 or 0.30 M NaCl solution for ≥3 days before experiments began. **Experimental protocols.** Experimental protocols were reviewed by and received approval from the Institutional Animal Care and Use Committees of Florida State University and the University of Pittsburgh.

The goal of experiment 1 was to obtain detailed information about the effects of PEG treatment on water, 0.15 M NaCl, and 0.30 M NaCl intake. Ad libitum access to Purina chow powder and water, 0.15 M NaCl, or water and 0.30 M NaCl (n = 8 in each group) for ≥2 wk was allowed to accustom the rats to the special cages in which the behavioral measurements were made (24). A 4 × 4-cm opening on the front of the cages accommodated a jar containing powdered food. The fluid bottles were supported by a rack on the back of the cage. The drinking spouts were recessed ~0.5 cm from the cage; to access each drinking fluid, rats licked through a slot in the cage wall. Licks on each drinking spout activated a contact circuit and were transmitted to a microprocessor and recorded in consecutive 6-s time bins throughout the 1-h session.

At 5 PM on the day before the drinking test, rats were injected with a 30% (wt/wt) solution of PEG (5 ml sc; Compound 20-M, Union Carbide) and then deprived of food and drinking fluids overnight. At 9 AM on the following morning (i.e., 16 h after the injection), the three groups of rats were given access to water, 0.15 M NaCl, or 0.30 M NaCl for 1 h. An analysis program recorded the number and size of each drinking bout during each test session for each rat. For determination of the volumes of fluid consumed, the bottle was weighed before and after each session, and the difference (in g) was taken to be the volume ingested (in ml). Those volumes were divided by the total number of licks in the 1-h session to give the amount of fluid ingested per lick, which was multiplied by the number of licks in each drinking bout to give intakes per bout (150–175 licks usually were required to consume 1 ml, regardless of which fluid was ingested). These computations assumed that the volume consumed per lick was constant throughout the test session.

Our analyses of the initial drinking bouts focused on the pauses between drinks. Because cumulative licks were obtained every 6 s, the same reading in two successive 6-s bins indicated an absence of licking for 0.1 min. We determined the total number of such bins that occurred before the rats stopped drinking for 1.0 min (i.e., 10 successive 6-s bins), which we operationally defined as the end of the drinking bout. These analyses were limited to the intakes during the first 15 min of drinking. During the remainder of the test session, we similarly considered the bouts to be terminated when no licking occurred for 1.0 min. Those drinking bouts were defined as requiring ≥0.2 ml (i.e., 30–35 licks), but there was no set maximum to the bouts, and the volumes consumed varied widely.

**Experiment 2A** determined the effects of PEG-induced hypovolemia on fluid intake by rats during an initial drinking bout and the fate of the ingested fluid. Rats were injected with 30% PEG solution (5 ml sc) at 5 PM on the day before the drinking test and then deprived of food and water overnight. At 9 AM on the following morning, separate groups of rats were given access to water, 0.15 M NaCl, or 0.30 M NaCl in graduated 25-ml burettes (n = 20, 13, and 14, respectively). Each drinking fluid was colored with three drops of green food dye (McCormick, Hunt Valley, MO), which permitted it to be readily visible in the small intestine. Rats were allowed to drink one of the three fluids for 4–10 min, until they paused for 1.0 min consecutively and moved away from the drinking tube. Fluid was removed at this time, and animals were killed immediately (n = 6, 3, and 5, respectively) or after a 15- to 70-min delay (n = 14, 10, and 9, respectively). An additional 18 rats were allowed to drink water, 0.15 M NaCl, or 0.30 M NaCl for variable amounts of time (1.5–5.0 min) before they were interrupted by the experimenter and killed (n = 5, 10, and 3, respectively). Gastric and small intestinal fluid volumes and systemic plasma Na⁺ concentration ([pNa]) were measured in each of these six animals to track the ingested fluid during and at the end of the initial drinking bout. In all cases, rats were killed by decapitation within 10 s after the drinking test ended, and fluid intakes (±0.1 ml) and time spent drinking (±1 s) were recorded.

**Experiment 2B** determined the effect of hypovolemia on the intake of water, 0.15 M NaCl, or 0.30 M NaCl in separate groups of rats during a 60-min test to evaluate the role of presystemic and systemic inhibitory signals on drinking over a longer time period. Gastric and small intestinal fluid volumes and systemic pNa also were measured. The general procedures were the same as those described in experiment 2A. Rats were allowed access to one of the three drinking fluids (n = 7, 6, and 7, respectively), and intakes were recorded every 5 min. Sixteen other rats were given access to 0.15 M NaCl and allowed to drink for 20–65 min before they were killed.

**Tissue analyses.** Trunk blood was collected in ice-cold heparinized tubes (143 USP heparin sodium; Becton Dickinson, Franklin Lakes, NJ) and kept on ice until stomachs and intestines were removed. The abdomen was opened, and hemostats were placed at the junction of the stomach with the pylorus, at the junction of the stomach with the esophagus, and at the most distal site of visible green dye in the small intestine, in that order. This portion of the surgical procedure took <2 min. Each stomach was removed from the abdominal cavity, the intestinal contents were removed, and the intestinal contents were placed in separate beakers. The beakers were covered with Parafilm until they were placed in an oven and dried to constant weight at 60°C for 2–3 days. All blood samples were centrifuged (10,000 g for 10 min at 4°C), plasma was harvested, pNa was measured (±1 meq/l) using an Na⁺-sensitive electrode (Schwann ELISE model 4410, Beckman Coulter, Brea, CA), and plasma protein concentration was measured (±0.1 g/dl) using a refractometer.

Finally, 18 other rats that were not used in behavioral studies were killed for control values of blood and GI tissue. Thirteen rats were treated with PEG as described above but were not given access to drinking fluid, and the other five rats served as nondeprived, nontreated controls. In 10 of these control animals (n = 5 and 5, respectively) and 29 PEG-treated rats from experiment 2A, the remaining small samples were stored at −80°C until they were assayed for pVP.

These tissue analyses collectively sum to a total of 475 individual measurements (i.e., 3–6 measurements in each of 118 rats), of which 10 were lost as a result of procedural errors (2.1%; 4 measurements of intestinal fill, 5 of pNa, and 1 of pVP).

**Radioimmunoassay.** The procedures for measurement of pVP have been described previously (23). Briefly, duplicate 250-μl plasma samples were extracted using solid-phase columns (1-ml, 50-mg Sep-Pak C₁₈ cartridges, Waters, Milford, MA), and VP was measured...
by radioimmunoassay in aliquots of these extracts. The assay sensitivity was 2.5 pg/ml, and the intra-assay variations were <10%.

Calculations. Gastric emptying was computed as the difference between the measured fluid intake and the estimated amount of ingested fluid that remained in the stomach and was expressed as a percentage of the intake. For estimation of the amount of ingested fluid that remained in the stomach, it was necessary to distinguish that fluid from water already in the gastric chyme. To address this issue, we evaluated the gastric chyme of eight PEG-treated rats not allowed access to drinking fluid and found that a consistent amount of water was associated with dry matter. Specifically, we prepared a scatterplot of the stomach liquids of individual animals (in ml, on the y-axis) expressed as a function of stomach solids (in g, on the x-axis) and used the associated trend line \( y = 2.0475x + 0.1331 \) to correct for the amount of fluid associated with gastric solids in each rat, which was subtracted from the water content of the chyme of rats allowed to drink for computation of the volume of ingested water that had not yet emptied from the stomach. Of 113 PEG-treated rats, only 27 had food residues in gastric chyme at the start of the drinking test (see above). These animals were injected with 30% polyethylene glycol (PEG, 5 ml sc) and then deprived of food and fluid for 16 h before the test. There were few pauses in drinking during the first 3–5 min, regardless of which fluid was consumed; then the rats began to drink increasingly more slowly until they stopped. Equations of linear regression lines and correlation coefficients when rats drank slowly (data not shown) were as follows: \( y = 1.93x - 8.57 \) \( (r = 0.99, P < 0.001) \), and \( y = 0.79x - 2.75 \) \( (r = 0.98, P < 0.001) \) for 0.15 M NaCl from 5–15 min, and \( y = 2.56x - 7.26 \) \( (r = 0.99, P < 0.001) \) for 0.30 M NaCl from 3 to 6 min. Slope of the trend line for 0.15 M NaCl was much more shallow than the slopes of the trend lines for water and 0.30 M NaCl (both \( P < 0.001) \), and slopes of the latter trend lines also were significantly different from one another \( (P < 0.01) \).

RESULTS

Experiment 1. PEG-treated rats invariably ingested fluid soon after it became available, with few pauses in drinking initially, regardless of which fluid was consumed. However, within a few minutes the rats began to drink increasingly more slowly, and after 5–20 min of drinking they stopped. The progressive slowing is displayed in Fig. 1 as an increase in the number of 6-s pauses per minute as a function of time. Different functions were observed for water, 0.15 M NaCl, and 0.30 M NaCl. More specifically, when rats ingested water, there were 0–2 pauses/min in the first 5 min of the initial bout, 3–5 pauses/min in the next 2 min, 7–9 pauses/min in the next 2 min, and 9–10 pauses/min thereafter. Rats stopped licking for 1.0 min consecutively by ~8 min of the bout. A similar drinking pattern was observed when PEG-treated rats ingested 0.30 M NaCl, instead of water, except pauses began to increase ~2 min sooner and licking stopped after ~6 min of the test session. In contrast, rats that ingested 0.15 M NaCl slowed drinking more gradually and did not stop licking until ~15 min of the test session. The total intakes during the first drinking bout were 9.9 ± 0.4 ml of water, 6.7 ± 0.9 ml of 0.30 M NaCl, and 14.9 ± 1.5 ml of 0.15 M NaCl (all \( P < 0.01 \) compared with one another).

The special cages also allowed detailed analysis of the intake patterns later in the 60-min test. PEG-treated rats ingesting water drank 4.0 ± 1.0 ml in 2.4 ± 0.5 additional bouts, which averaged 1.7 ± 0.3 ml in volume. Similarly, rats ingesting 0.30 M NaCl drank 4.8 ± 0.8 ml in 2.1 ± 0.4 additional bouts of 2.3 ± 0.3 ml [all \( P = \) not significant (NS)], whereas rats ingesting 0.15 M NaCl consumed 7.6 ± 2.6 ml (both \( P = \) NS) in 1.5 ± 0.5 additional bouts (both \( P = \) NS) of 5.1 ± 0.7 ml (both \( P < 0.001 \) vs. rats drinking the other fluids). In other words, bout frequency was comparable in the three groups, but bout size was unusually large when rats drank 0.15 M NaCl. The total amounts of water, 0.30 M NaCl, and 0.15 M NaCl consumed in 60 min, including the initial bouts, were 13.9 ± 1.2, 11.5 ± 1.2, and 22.5 ± 1.9 ml, respectively \( (P < 0.01 \) for 0.15 M NaCl vs. water and 0.30 M NaCl).
Experiment 2A. After a 16-h delay, PEG-treated rats drank readily when given access to water, 0.15 M NaCl, or 0.30 M NaCl in one-bottle tests. They consumed each fluid at a similar rate during the first bout, regardless of the duration of the bouts (Fig. 2), and all the drinking rates fell on a single linear trend line \( r = 0.91, P < 0.001 \). The mean drinking rate was 1.1 ml/min after 5 min and slightly slower thereafter (because of an increasing number and duration of pauses while drinking).

As might be expected, gastric fluid volume increased as a result of fluid ingestion. Nonetheless, ingested fluid began to empty quickly into the small intestine in amounts (in ml) that depended in part on the concentration of the fluid consumed. Figure 3A displays gastric emptying of individual rats as a function of time after one of the three test fluids was consumed in an initial bout. The slopes of the regression lines for the data from rats drinking 0.15 or 0.30 M NaCl differed significantly \( P < 0.001 \), whereas the data from rats drinking water and 0.30 M NaCl were similar. However, when gastric emptying of the ingested NaCl solutions was plotted in milliequivalents of Na\(^+\) as a function of time, it is evident that rats emptied Na\(^+\) at the same rate whether 0.15 or 0.30 M NaCl was ingested (Fig. 3B).

Intestinal fluid volume (i.e., fluid in the lumen of the small intestine between the pylorus and the most distal portion of the intestine in which green dye was visible) increased exponentially as a function of the volume consumed in the initial bout, regardless of which fluid was ingested (Fig. 4; \( r = 0.80, P < 0.001 \)). The intestinal volumes were inversely correlated with gastric fluid volumes when both were expressed as a percent-

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**Figure 2.** Intake of water (diamonds), 0.15 M NaCl (squares), or 0.30 M NaCl (triangles) by PEG-treated rats plotted as a function of time spent drinking during the initial bout. Each symbol represents intake of a single animal; solid symbols represent the 14 rats that drank until they paused; open symbols represent the 18 rats whose drinking was interrupted to determine the fate of ingested fluid. Intakes were highly correlated with time spent drinking, regardless of which fluid was consumed or for how long rats drank \( y = 0.8228x + 1.6475, r = 0.91, P < 0.001 \).

**Figure 3.** Gastric emptying of ingested water, 0.15 M NaCl, or 0.30 M NaCl by PEG-treated rats plotted as a function of time spent drinking. Symbols represent animals shown in Fig. 2. Linear regression lines and correlation coefficients are as follows: \( y = 0.7386x - 0.1047 \) \( r = 0.90, P < 0.001 \) for 0.15 M NaCl and \( y = 0.3166x + 0.3855 \) \( r = 0.88, P < 0.001 \) for 0.30 M NaCl in A. In B, data from rats drinking 0.15 or 0.30 M NaCl were not significantly different from one another, so they were combined for analysis: \( y = 0.1075x + 0.0273 \) \( r = 0.90, P < 0.001 \). Rats emptied 0.15 M NaCl in milliliters very rapidly, water less rapidly, and 0.30 M NaCl very slowly, whereas 0.15 and 0.30 M NaCl emptied similarly in milliequivalents of Na\(^+\).
age of intake for rats drinking water or 0.15 M NaCl (not shown; $r = 0.80, P < 0.001$). The distance traveled by the three ingested fluids into the small intestine bore the same logarithmic relation to the volume contained in the intestine (Fig. 5; $r = 0.81, P < 0.001$), to time spent drinking (not shown; $r = 0.87, P < 0.001$), and to the amount consumed (not shown; $r = 0.82, P < 0.001$). Thus mean intestinal distension (i.e., volume per length) increased similarly in all three groups as a function of intestinal fill (Fig. 6; $r = 0.92, P < 0.001$), time (not shown; $r = 0.85, P < 0.001$), and intake (not shown; $r = 0.90, P < 0.001$).

Dye-colored fluid was not actually distributed uniformly throughout the small intestine when rats drank water or 0.30 M NaCl. Instead, intervals containing little or no fluid punctuated the dye-colored segment. In contrast, the dyed fluid was present more or less continuously within the intestine when rats consumed 0.15 M NaCl.

The sum of the fluid in the stomach and small intestine increased in proportion to fluid intake during the initial drinking bout (Fig. 7). The solid line in Fig. 7 represents the volume of fluid in the stomach and small intestine if no net absorption had occurred. When rats drank water or 0.15 M NaCl, all points fell to the right of this line, except when very small volumes were consumed; since no green dye was found in the cecum, these results indicate that some fluid usually was absorbed from the small intestine into the systemic circulation. More specifically, the volume of fluid absorbed increased as a function of intake, ranging from 5% to 25% of the fluid ingested during the initial drinking bout. In contrast, GI fill was significantly greater when rats consumed 0.30 M NaCl instead of water or 0.15 M NaCl ($P < 0.01$). Individual values usually fell to the left of the “no-absorption” line, presumably indicating osmotic movement of water into the small intestine from the systemic circulation.

Gastric and GI distension also were measured when PEG-treated rats were allowed to drink only an initial bout and killed after a delay. Rats that consumed water or 0.15 M NaCl emptied up to 62% or 90%, respectively, of the ingested fluid from their stomachs by the end of the first drinking bout and 80–100% by 30 min (not shown). Nonetheless, 80–85% of the ingested fluid remained in the stomach and small intestine by the end of the first drinking bout. GI fill then decreased steadily to 15–40% by 30 min and to 5–20% by 60 min as the water or 0.15 M NaCl was absorbed (not shown). Rats that consumed 0.30 M NaCl, in contrast, emptied only 10–40% of the ingested fluid from their stomachs by the end of the first drinking bout ($P < 0.05$ vs. water or 0.15 M NaCl) and only ~60% by 30 min (not shown). The amount of fluid in the stomach and small intestine actually was greater than the volume of 0.30 M NaCl ingested during the first 30 min of the test, although it decreased rapidly to 10–20% by 60 min (not shown).

The plasma protein concentration was significantly elevated after 30% PEG treatment (Table 1), as expected (25), and it was reduced comparably whether rats drank water, 0.15 M NaCl, or 0.30 M NaCl; that is, plasma protein was negatively
correlated with fluid intake (not shown; \(r = -0.61, P < 0.001\)). Gastric emptying in each group was smallest when plasma protein concentration was most elevated (Fig. 8), and emptying increased comparably as the plasma volume deficit diminished (\(r = -0.86, -0.78, \) and \(-0.77\), respectively, in relation to plasma protein concentration, all \(P < 0.001\)). Because hypovolemia had such a substantial effect on gastric emptying, in a post hoc analysis we focused on animals with overlapping plasma protein concentrations within 7.9 – 9.8 g/dl.

Significant differences in gastric emptying were observed between the three groups (\(P < 0.05\), water vs. 0.15 M NaCl; \(P < 0.01\), water vs. 0.30 M NaCl; \(P < 0.001\), 0.15 M NaCl vs. 0.30 M NaCl).

The pNa of PEG-treated rats measured during and immediately after the first bout of water drinking were slightly but significantly lower than pNa of PEG-treated control rats (Table 1), and values decreased subsequently even when rats were denied further access to water (140.0 \(\pm\) 0.5 meq/l, \(P < 0.001\) vs. control). When 0.15 or 0.30 M NaCl was consumed, pNa after the first drinking bouts was not significantly different from pNa of PEG-treated control rats (Table 1). However, values from rats that drank concentrated saline increased subsequently even when they were denied further access to drinking fluid (146.6 \(\pm\) 0.3 meq/l, \(P < 0.001\) vs. control).

Finally, pVP increased exponentially as a function of increasing plasma volume deficits in PEG-treated rats deprived of drinking fluid (Fig. 9), as expected (36). When PEG-treated rats were killed during or after an initial drinking bout of water, 0.15 M NaCl, or 0.30 M NaCl, pVP decreased exponentially in rats deprived further access to water (140.0 \(\pm\) 0.5 meq/l, \(P < 0.001\) vs. control). When 0.15 or 0.30 M NaCl was consumed, pVP after the first drinking bouts was not significantly different from pVP of PEG-treated control rats (Table 1). However, values from rats that drank concentrated saline increased subsequently even when rats were denied further access to drinking fluid (146.6 \(\pm\) 0.3 meq/l, \(P < 0.001\) vs. control).

Experiment 2B. The separate groups of PEG-treated rats drank similar amounts of the three fluids during the first 15 min of the 1-h test before statistically significant differences were observed (Fig. 10). Rats drank 0.15 M NaCl steadily throughout the remainder of the test, whereas rats consumed very little water or 0.30 M NaCl after the initial bout. Therefore, intake of 0.15 M NaCl was much greater than intake of water or 0.30 M NaCl by the end of the test (24.7 \(\pm\) 1.2, 11.2 \(\pm\) 0.9, and 9.7 \(\pm\) 0.6 ml, respectively; both \(P < 0.001\)).

![Fig. 7. Sum of fluid in stomach and small intestine (GI fill) of individual rats](https://www.ajpregu.org).
Other PEG-treated rats given continuous access to water for 60 min did not ingest much additional fluid after the initial drinking bout (not shown), similar to the results depicted in Fig. 10. These animals had considerably less fluid in their stomachs and intestines than rats that were killed at the end of the initial bout of water ingestion (1–3 and 5–7 ml, respectively, \(P < 0.001\)) and had significant hyponatremia (137.9 ± 0.6 meq/l, \(P < 0.001\) vs. control values from PEG-treated rats). In contrast, although rats given 0.30 M NaCl to drink for 60 min also did not ingest much additional fluid after the initial bout, amounts of fluid in their stomachs and intestines (7–9 ml) were similar to those of rats drinking 0.30 M NaCl that were killed at the end of the initial bout. They also had significant hypernatremia (149.7 ± 0.4 meq/l, \(P < 0.001\) vs. control values from PEG-treated rats). Finally, half of the PEG-treated rats given continuous access to 0.15 M NaCl for 20–60 min had about as much fluid in their stomachs and intestines (13–16 ml) as did the rats that drank the largest volumes of 0.15 M NaCl in the initial bout, whereas the other half had 5–6 ml less fluid (Fig. 11). Furthermore, the relationship between intestinal fill and either intestinal distance (Fig. 5) or mean intestinal distension (Fig. 6) was similar to that after the initial drinking bout. Plasma protein concentrations (range 6.2–8.0 g/dl) in these animals were reduced in proportion to the intake of 0.15 M NaCl (range 10–34 ml; not shown; \(r = -0.74\), \(P < 0.01\)), but observed values (6.9 ± 0.2 g/dl, \(P < 0.01\) vs. control values from nondeprived nontreated rats of 6.2 ± 0.1 g/dl) indicate that plasma volume deficits up to 22% persisted in most animals.

**DISCUSSION**

Recent studies of dehydrated rats (29, 31) have supported earlier findings that neurohypophysial VP secretion can be altered by a presystemic concentration-dependent signal resulting from gastric fluid loads (2, 5). Other recent observations have suggested that thirst in dehydrated rats is inhibited by a presystemic volume-dependent signal associated with distension of the stomach and small intestine by the ingested fluid (13). The present experiments suggest that early volume- and concentration-dependent signals inhibit thirst and salt appetite in the model of severe and sustained hypovolemia that is produced by subcutaneous injection of PEG solution in rats. In contrast, these experiments indicate that a presystemic signal does not influence VP secretion when hypovolemic rats consume water or 0.30 M NaCl.

**Early signals terminate the initial bout of water or saline drinking by hypovolemic rats.** At 16 h after subcutaneous PEG treatment, hypovolemic rats consumed 0.15 M NaCl avidly during an initial drinking bout. Rats ingested more or less continuously for 4–5 min, but then pauses in drinking began to appear increasingly in the drinking bout until intake ended, after 10–20 min (Fig. 1). By then ~15 ml had been consumed. Nonetheless, plasma volume deficits of 25–30% still were
evident in other PEG-treated rats that drank comparable amounts in experiment 2A (Table 1), so the cessation of intake clearly can be attributed to inhibition, rather than to the loss of the excitatory stimulus. But what signal inhibited further intake? When rats drank 0.15 M NaCl, ~25% of the ingested fluid was still in the stomach at the end of the drinking bout, while ~60% was present in the small intestine (the remainder likely was absorbed, since there was no evidence of green dye in the cecum). Indeed, the intestine was swollen with fluid and had distended in proportion to the fluid it contained (Fig. 6). Recent studies have suggested that thirst in dehydrated rats is inhibited by a volume-dependent signal associated with con-joint distension of the stomach and small intestine (13; also see Ref. 8). The present observations are consistent with that idea and suggest that cumulative volumes of 12–15 ml are sufficient to inhibit hypovolemic thirst in these testing conditions.

We depended on a detailed analysis of drinking records to anticipate when the initial drinking bout ended. This analysis revealed that pauses in fluid ingestion became longer and more frequent until intake stopped for >1.0 min, so we used a consecutive pause time of 1.0 min to operationally define the end of the bout. At that time, the cumulative pause time in the bout amounted to ~2 min, which is the criterion we have used in other recent studies of fluid ingestion by rats with thirst or salt appetite (Bykowski MR, Smith JC, Stricker EM, unpublished observations; 13, 28).

PEG-treated rats also consumed water for 4–5 min before pauses in drinking began to increase, but intake ended after 8–9 min (Fig. 1). By then, a total of ~10 ml had been consumed. Nonetheless, plasma volume deficits of ~30% were observed in other PEG-treated rats that drank comparable amounts of water in experiment 2A, which were slightly larger than those seen when rats stopped drinking 0.15 M NaCl (Table 1). However, GI fill was much smaller when rats stopped drinking water than when they drank saline (only 4–7 ml vs. 12–16 ml), suggesting an additional inhibitory signal. One signal that is known to inhibit thirst during hypovolemia is a decrease in systemic P_{osmol} (26). Statistically significant decreases in systemic pNa were observed at the end of the initial water-drinking bouts, but this effect probably was too small (only ~1%) to cause a marked suppression of thirst. Instead, we propose that a presystemic signal related to the concentration of ingested fluid contributed to the inhibition of the initial bout. This signal presumably was derived from the water in the small intestine when the bout ended.

Generally similar observations also were made when PEG-treated rats drank 0.30 M NaCl. Thus these animals consumed concentrated saline for 3–4 min, but then they began to pause increasingly until intake stopped 2–3 min later (Fig. 1). Only 6–8 ml were consumed in total (associated with GI fill of 7–9 ml), and substantial plasma volume deficits remained in PEG-

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**Fig. 10.** Cumulative intake of water (○), 0.15 M NaCl (■), or 0.30 M NaCl (▲) by PEG-treated rats plotted as a function of time spent drinking (n = 7, 6, and 7, respectively). Values are means ± SE. Rats drank at a similar rate for the first 5 min of the drinking test, but by 25 min and for the remainder of the test, 0.15 M NaCl intake was significantly greater than water or 0.30 M NaCl intake: *P < 0.05; **P < 0.01; #P < 0.001. Values do not reflect episodic nature of drinking bouts by individual animals.

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**Fig. 11.** Sum of measured fluid in stomach and small intestine (GI fill) of individual rats plotted as a function of 0.15 M NaCl intake. Symbols represent individual animals. Solid line represents GI fill if no net absorption had occurred. GI fill was highly correlated with fluid intake when rats were killed during or immediately after the initial drinking bout (γ = 0.753x + 0.2036, r = 0.97, P < 0.001), as shown in Fig. 7. Rats killed later in the 60-min test could be segregated into 2 subgroups according to volume in the gastrointestinal tract, perhaps reflecting how recently they had consumed fluid before death.
treated rats with comparable intakes in experiment 2A (Table 1). Systemic pNa was not significantly increased in those animals, so evidently it did not provide a signal that inhibited intake. Instead, we propose that inhibition of salt appetite resulted from a combination of presystemic signals related to the concentration and volume of the ingested fluid, as has been suggested recently by studies of other models of salt appetite in rats (unpublished observations; 28).

It has long been known that mechanoreceptors monitoring stomach fullness send neural signals to the brain stem, which play an important role in the early inhibition of water intake (39). These signals are similar whether gastric distension results from a load of water, isotonic saline, or hypertonic saline (20). However, gastric emptying clearly was concentration dependent in the present study, and gastric distension was not uniform within or among the groups. On the other hand, regardless of which fluid was consumed, more fluid was present in the small intestine when less was in the stomach, and vice versa. Figures 4 and 5 display results from individual PEG-treated rats and provide a picture of fluid movement as the animals drank, with the passage of time generally conveyed by the progression of symbols from the lower left- to the upper right-hand portions of Figs. 4 and 5. These data followed a single pattern showing that as fluid was consumed, increasingly large amounts emptied from the stomach and moved down the small intestine. After 3–5 min of drinking, intestinal transit slowed and intestinal distension began to increase, and the latter development may have provided a volume-dependent inhibitory stimulus that supplemented gastric distension.

In considering the mechanism by which GI fill may have inhibited thirst in rats during hypovolemia, it seems relevant that mechanoreceptors in the small intestine are known to influence the vagal afferent nerves signaling gastric distension (10). Furthermore, sensory signals from the stomach and small intestine project to overlapping sites within the NTS (40), which may provide another anatomic basis for integrating information about distension of these visceral organs. More direct evidence in support of an early signal inhibiting water ingestion in PEG-treated rats is the finding that the size of an initial drinking bout of water is increased when such signals are eliminated experimentally (6, 7). This observation resembles findings of increased meal sizes when liquid food is consumed by neurotrophin-4-deficient mice, which are missing 90% of intraganglionic mechanoreceptors from the duodenum (11).

If GI fill generally inhibited fluid intake, then one might expect a single trend line relating GI distension to intake. However, the regression line relating GI fill to fluid intake was not uniform within or among the groups. On the other hand, regardless of which fluid was consumed, more fluid was present in the small intestine when less was in the stomach, and vice versa. Figures 4 and 5 display results from individual PEG-treated rats and provide a picture of fluid movement as the animals drank, with the passage of time generally conveyed by the progression of symbols from the lower left- to the upper right-hand portions of Figs. 4 and 5. These data followed a single pattern showing that as fluid was consumed, increasingly large amounts emptied from the stomach and moved down the small intestine. After 3–5 min of drinking, intestinal transit slowed and intestinal distension began to increase, and the latter development may have provided a volume-dependent inhibitory stimulus that supplemented gastric distension.

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If GI fill generally inhibited fluid intake, then one might expect a single trend line relating GI distension to intake. However, the regression line relating GI fill to fluid intake was not uniform within or among the groups. On the other hand, regardless of which fluid was consumed, more fluid was present in the small intestine when less was in the stomach, and vice versa. Figures 4 and 5 display results from individual PEG-treated rats and provide a picture of fluid movement as the animals drank, with the passage of time generally conveyed by the progression of symbols from the lower left- to the upper right-hand portions of Figs. 4 and 5. These data followed a single pattern showing that as fluid was consumed, increasingly large amounts emptied from the stomach and moved down the small intestine. After 3–5 min of drinking, intestinal transit slowed and intestinal distension began to increase, and the latter development may have provided a volume-dependent inhibitory stimulus that supplemented gastric distension.

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If GI fill generally inhibited fluid intake, then one might expect a single trend line relating GI distension to intake. However, the regression line relating GI fill to fluid intake was not uniform within or among the groups. On the other hand, regardless of which fluid was consumed, more fluid was present in the small intestine when less was in the stomach, and vice versa. Figures 4 and 5 display results from individual PEG-treated rats and provide a picture of fluid movement as the animals drank, with the passage of time generally conveyed by the progression of symbols from the lower left- to the upper right-hand portions of Figs. 4 and 5. These data followed a single pattern showing that as fluid was consumed, increasingly large amounts emptied from the stomach and moved down the small intestine. After 3–5 min of drinking, intestinal transit slowed and intestinal distension began to increase, and the latter development may have provided a volume-dependent inhibitory stimulus that supplemented gastric distension.

An alternative hypothesis is that the extra volume in the small intestine associated with osmotic influx of water, seen when PEG-treated rats drank 0.30 M NaCl, did not contribute to the inhibitory signal but is simply correlated with that signal. Instead, the inhibitory signal may have been generated only by fill in the duodenum, where mechanoreceptors are especially dense (3, 22), and the osmotic movement of water into the small intestine may have occurred more distally. Another view is that an inhibitory signal may have resulted not from intestinal distension but, rather, from pyloric metering of fluid delivery into the duodenum (19), which would functionally resemble the oropharyngeal signals generated when thirsty dogs swallow liquids (38). Further studies are needed to evaluate these possibilities.

Systemic signals inhibit drinking of water and 0.30 M NaCl by hypovolemic rats after the initial bout. In experiment 2B, little water was ingested by PEG-treated rats after the initial bout. These rats had considerably less fluid in their stomachs and intestines at the end of the 60-min test than did rats killed at the end of the initial water-drinking bout, so presystemic signals probably contributed little to the very slow rate of drinking. Instead, the slowing of water ingestion after the initial bout presumably resulted largely from a systemic signal, no doubt derived from osmotic dilution of body fluids (26).

When PEG-treated rats consumed 0.30 M NaCl, GI fill likely was sustained after the initial bout, because intestinal absorption was counterbalanced by the osmotic influx of water into the small intestine (1). However, the volume of intestinal fluid may not have contributed much to the continued inhibition of 0.30 M NaCl intake, because most of it was present in the distal small intestine. Instead, the progressive increase in systemic pNa or P-osmol probably was responsible for the inhibition of 0.30 M NaCl ingestion (37).

In contrast, the intake of isotonic saline during the 1-h test clearly was not affected by concentration-dependent signals, so we focused our attention on volume-dependent signals that may have limited intake. One such signal undoubtedly was systemic and reflected a reduction in the excitatory stimulus of thirst; that is, the plasma protein concentrations were decreased but remained elevated in most PEG-treated rats, despite sizable intakes of 0.15 M NaCl during the 1-h test. In addition, a presystemic signal likely inhibited intake in the saline-drinking bouts. In this regard, GI fill was 12–15 ml after the initial drinking bout, and rats lost ~12% of GI fill every 10 min when they were subsequently deprived of fluid. In other words, they lost ~5 ml of GI fill in 30–35 min. Thus the observed intakes of one or two additional drinking bouts of 5.1 ± 0.7 ml during the test by PEG-treated rats with continuous access to 0.15 M NaCl is consistent with the idea that this intake replaced previously ingested fluid that had been absorbed from the small intestine. The rats with a relatively small GI fill after 20–60 min of drinking 0.15 M NaCl had 5–6 ml less GI fill than the rats with a larger GI fill (Fig. 11), as if that volume constituted a threshold for loss of inhibition and renewed drinking.

An early signal does not influence VP secretion when hypovolemic rats consume water or 0.30 M NaCl. In the present studies, pVP increased exponentially as a function of plasma protein concentration in PEG-treated rats, as reported previously (9, 36). However, in contrast to the concentration-dependent presystemic signals for the inhibition (2) or stimulation of VP secretion in dehydrated rats (29) provided by
gastric loads of water or concentrated saline, respectively, the present results provided little evidence for presystemic inhibition or stimulation of VP secretion in hypovolemic rats. That is, the initial drinking bout reduced pVP in association with the partial restoration of plasma volume, regardless of which fluid was consumed. Ingestion of water did not cause an exaggerated reduction of pVP, as might have resulted from a presystemic concentration-dependent signal, nor did ingestion of 0.30 M NaCl increase pVP. Instead, intake of water or 0.30 M NaCl decreased pVP, as did ingestion of 0.15 M NaCl.

In considering these differences from previous findings, it seems unlikely that pVP in hypovolemic rats was too high to be inhibited by a presystemic concentration-dependent signal, because water ingestion rapidly eliminated VP secretion in rats with comparably elevated pVP after intravenous infusion of hypertonic saline (14). Alternatively, the visceral osmoreceptors that mediate early inhibition of VP secretion may not have been able to detect sufficient quantities of ingested water because of some consequence of the severe hypovolemia. If this is the case, the problem is likely to be postgastric, since gastric emptying of water in hypovolemic rats was comparable to that reported in dehydrated rats (40–60% of the ingested volume) (12, 30). In contrast, the apparent inability of 0.30 M NaCl ingestion to rapidly stimulate additional VP secretion may reflect the very slow gastric emptying of hypertonic saline in hypovolemic rats.

**Perspective**

The present results add to accumulating evidence that a volume-dependent signal derived from GI distension provides a potent early inhibition of thirst and salt appetite in rats. It seems plausible that mechanoreceptors located on the outer walls of the stomach and the proximal small intestine sense the distension during fluid intake and provide a signal that inhibits further intake. This presystemic signal would allow animals to avoid large changes in systemic P\textsubscript{osmol} due to consuming and emptying substantial quantities of ingested water because of some consequence of the severe hypovolemia. If this is the case, the problem is likely to be postgastric, since gastric emptying of water in hypovolemic rats was comparable to that reported in dehydrated rats (40–60% of the ingested volume) (12, 30). In contrast, the apparent inability of 0.30 M NaCl ingestion to rapidly stimulate additional VP secretion may reflect the very slow gastric emptying of hypertonic saline in hypovolemic rats.

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