Reduced feeding during water deprivation depends on hydration of the gut

GUUS H. M. SCHOORLEMMER AND MARK D. EVERED
Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada S7N 5E5
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Schoorlemmer, Guus H. M., and Mark D. Evered. Reduced feeding during water deprivation depends on hydration of the gut. Am J Physiol Regul Integr Comp Physiol 283: R1061–R1069, 2002. First published July 8, 2002; 10.1152/ajpregu.00236.2002. — Removal of drinking water at the start of the dark period reduced food intake in freely feeding rats within 45 min. Both first and later meals were smaller during 6 h of water deprivation, but meal frequency did not change. Ingestion of a normal-sized meal (3 g) rapidly increased plasma tonicity when drinking water was withheld, but intravenous infusions of hypertonic NaCl causing similar increases in plasma tonicity did not reduce feeding. Feeding during 6 h of water deprivation was restored by slowly infusing the volume of water normally drunk into the stomach, jejunum, or cecum, but not in the vena cava or hepatic portal vein. The infusions did not alter water or electrolyte excretion or affect food intake in rats allowed to drink. We conclude that the inhibition of feeding seen during water deprivation is mediated by a sensor that is located in the gastrointestinal tract or perhaps in the mesenteric veins draining the gut, but not the hepatic portal vein or the liver. In the absence of drinking water, signals from this sensor provoke the early termination of a meal.

dehydration anorexia; hypertonic; hypotonic; osmoreceptor

Many mammals, including rats (1), dogs (29), cows (32), camels (3), and humans (7), reduce food intake during water deprivation. This inhibition of feeding aids body fluid regulation in two ways. First, there is normally a large volume of osmotically sequestered water in the gut. Inhibition of feeding allows absorption of this water (27, 28, 31). Second, the reduced solute load reduces urinary water loss (31).

There are several ways in which water deprivation might inhibit food intake. First, in the rat at least, drinking is normally closely associated with eating (9, 23) and interfering with the normal pattern of drinking with meals might inhibit food intake. Second, water deprivation causes a dry mouth (37), and this could make eating more difficult (36, 38), especially when the period of water deprivation is long and the food is dry. Third, osmoreceptors or other detectors of body fluid status in the brain, circulation, or gut may be involved. In the rat, various stimuli known to cause thirst and antidiuresis also inhibit feeding, including injection of hypertonic solutions into the gastrointestinal tract or peritoneal cavity, injection of hyperoncotic colloid under the skin, and injection of angiotensin in the brain (13, 19, 25, 30, 39). Research done on cows suggests that hypertonicity of the ruminal content mediates the reduction in food intake during water deprivation in ruminants (32, 35).

We designed a series of experiments to investigate the mechanism by which water deprivation reduces food intake. We started by measuring the effect of short periods of water deprivation on food intake and eating pattern. To investigate the role of plasma tonicity in feeding during water deprivation, we first compared changes in plasma tonicity caused by ingestion of food in the presence and absence of drinking water. We then investigated the effect of changes in plasma tonicity, induced by intravenous infusion of hypertonic and hypotonic solutions, on feeding in the absence of drinking water. To investigate whether the act of drinking is necessary for normal food intake, we deprived rats of drinking water overnight while slowly infusing the water these rats normally drank into the stomach. Finally, we investigated the location of sensors mediating the inhibition of feeding by slowly infusing water in various locations of the body (stomach, jejunum, cecum, hepatic portal vein, and vena cava) during a short period of water deprivation.

METHODS

Animals and Maintenance

We used male Long-Evans rats bought from Charles River Canada, St-Constant, Quebec, Canada, or first-generation offspring of these. Rats weighed 300–600 g during experiments. Rats were housed individually in metabolism cages (diameter 24 cm, 18 cm high, Nalgene 650–0350, Nalge, Rochester, NY) that allowed collection of urine uncontaminated by feces and that were designed to collect all food and water spilled by the rat. The cages were placed in a temperature-controlled chamber (20–22°C), with lights off from 1900 to 0700, except for a dim red bulb giving just enough light for taking measurements. Food (powdered Prolab
RMH3000, Agway, Syracuse, NJ) and tap water were available except where indicated. All experimental procedures were approved by the Animal Care Committee of the University of Saskatchewan and were in compliance with the guidelines of the Canadian Council on Animal Care.

**Measurement of Food and Water Intake**

Food intake was measured to the nearest 0.1 g by weighing. Water was provided either in inverted graduated centrifuge tubes or in preweighed drinking bottles with stainless steel spouts. Water intake was measured to the nearest 0.1 ml. Spilled water and food were collected and intakes were corrected for spillage.

**Analysis of Feeding Pattern**

In freely feeding rats, meals are usually separated by intervals of ~1 h during the dark period and ~4 h during the light period (23). To get an impression of the meal pattern during water deprivation, we measured food intake every 15 min by briefly removing the food hopper, weighing it, and returning it to the cage. If a rat ate during a 15-min period, this was counted as one meal. If eating occurred during two consecutive 15-min periods, this was also counted as a single meal. On the rare occasion that a rat ate during three consecutive periods, we assumed these were two meals and one half of the amount eaten during the middle period belonged to the first meal and the other one-half to the second meal. A representative example of the eating pattern in a single rat is shown in Fig. 1. We judged this rat to have eaten five meals during 7.5 h when drinking water was available and six meals during water deprivation.

**Surgery**

Rats were anesthetized with Equithesin (12) (3 ml/kg body wt ip) and were given a postoperative subcutaneous injection of 0.015 mg of the analgesic buprenorphine-hydrochloride (Tengesic, Reckit & Colman Pharmacy, Hull, UK). Instruments were sterilized in a mixture of chlorhexidine and cetrimide (Savlodil, Ayerst Laboratories, Montreal, Canada) and rinsed with boiling water. Sterile drapes were used around incision sites. Cannulas were sterilized by soaking them for 1 h in a mixture of iodine and 70% ethanol. Each rat was equipped with all cannulas in a single session. Rats were allowed at least 10 days to recover. Experiments began only after rats had regained preoperative body weight.

**Vena Cava Cannulas**

Cannulas used for infusions into the vena cava were polyurethane tubing (ID 0.6 mm, OD 0.8 mm, MRE 040, Braintree Scientific, MA) with a 4-cm-long tip of silicone rubber tubing (ID 0.6 mm, OD 1.2 mm, Silastic, Dow Corning, Midland, MI). The silicone end was inserted in the femoral vein and advanced 4 cm to bring the cannula tip in the abdominal vena cava. Cannulas used for blood sampling had a silicone end of 8 cm and were inserted 8 cm to bring the cannula tip in the thoracic vena cava. The free end of the cannula was tunneled under the skin to the area between the shoulder blades and connected to an elbow made of stainless steel tubing. The elbow was cemented with dental acrylic to a circle of polypropylene mesh (~2 cm diameter, 0.25 mm mesh, Small Parts, Miami Lakes, FL). The mesh was implanted under the skin with the open end of the elbow protruding through the skin. Cannulas were filled with heparinized saline (10 U/ml) and closed with a plastic cap.

**Portal Vein Cannulas**

Cannulas were polyurethane tubing (OD 0.8 mm, ID 0.4 mm, MRE 033, Braintree Scientific) with a 3-cm-long silicone rubber tip (OD 0.6 mm, ID 0.3 mm, Dow Corning). We made a small midline incision to open the abdominal cavity and retracted the cecum. The silicone end of the cannula was inserted into the ileocolic vein and advanced 3 cm to bring the cannula tip into the portal vein, ~4 cm from the liver. The cannula was tied in place, anchored to the abdominal muscle wall, and tunneled subcutaneously to the area between the shoulder blades. Muscle and skin incisions were closed with silk suture. The cannula was connected to a metal elbow that protruded through the skin. Cannulas were filled with heparinized saline (10 U/ml) and closed with a plastic cap. Functioning of portal vein cannulas was verified after completion of the experiments by injection of Evans Blue dye through the cannula. The injections invariably discolored the liver.

**Gastric Cannulas**

Cannulas for gastric infusion were made by joining a 3-cm piece of polyethylene tubing (ID 1.2 mm, OD 1.7 mm, PE-190, Clay Adams, Parsippany, NJ) to polyurethane tubing (ID 0.6 mm, OD 1.0 mm, Braintree Scientific). The cannula had a polyethylene flange, ~5 mm diameter, at the tip, and a smaller flange made of silicone glue, 3 mm away. We made a small midline incision to open the abdominal cavity and retracted the stomach. The cannula was implanted in the greater curvature of the stomach and tied in the stomach wall with a purse-string suture between the cannula flanges. The cannula was anchored to the abdominal muscle wall and tunneled subcutaneously to the area between the shoulder blades. Muscle and skin incisions were closed with silk suture. The cannula was connected to a metal elbow that protruded through the skin. The cannulas were filled with water and flushed once a week to prevent plugging.

**Intestinal Cannulas**

Cannulas for infusions in the jejunum or cecum were made from polyurethane tubing (ID 0.8 mm, OD 0.6 mm, Braintree Scientific). The last 3 cm were covered with silicone rubber tubing (ID 0.9 mm, OD 0.5 mm, Dow Corning). Two small flanges, ~3 mm in diameter and 2 mm apart, were made with silicone glue at the end of the silicone tubing. A small purse-string suture was placed in the intestinal wall, a hole was cut in the middle of the suture, and the suture was tightened between the cannula flanges. Jejunal cannulas were placed ~7 cm from the pylorus. Cecal cannulas were implanted in the apex of the cecum. The cannulas were led out of the abdominal cavity through the incision site, anchored to the abdominal muscle wall, tunneled under the skin to the back, and exteriorized between the shoulder blades like the venous cannulas. Muscle and skin incisions were closed with silk suture.
suture. The cannulas were filled with water and closed with a plastic cap. The cannulas were flushed once a week with water to prevent plugging.

Infusion Procedures

Infusions were done in the rat’s home cage unless specified differently. The polyethylene infusion tubing was connected to the elbow on the rat’s back and led through the top of the cage. Sterile distilled water or 0.3 M NaCl solution was infused with a disposable syringe mounted in a calibrated pump. For long infusions, the tubing was protected with a metal spring and was connected through a low-friction water-tight fluid swivel. Tubing, spring, and swivel joint were counterbalanced, allowing rats free movement. To accommodate the rats to the spring and swivel joint they were connected once to the infusion equipment overnight. This training procedure was repeated if it reduced food intake.

Blood Sampling and Analysis

Blood samples (0.35 ml each) were withdrawn from venous cannulas into syringes coated with ammonium heparin. After the sample was collected, we injected 0.35 ml sterile 0.15 M NaCl back through the cannula to reduce changes in extracellular fluid volume due to sampling. Plasma osmolality was measured as freezing point depression (μOsmette model 5004, Precision Systems, Natick, MA). Plasma protein concentration was measured with a flame photometer (model 943, Instrumentation Laboratory, Lexington, MA). Hematocrit was measured in microhematocrit capillary tubes after 5 min centrifugation at 12,700 g. Plasma protein concentration was measured by refractive index.

Changes in blood volume were estimated from \( \frac{bV_2 - bV_1}{bV_1} = (1 - Hct_1)/(1 - Hct_2) \) (18), where \( bV \) is blood volume, \( pV \) is plasma protein concentration, and \( Hct \) is average hematocrit of all blood vessels. This formula assumes the amount of circulating protein does not change but does not make assumptions about red blood cell volume. We multiplied the observed hematocrit values by 0.9 to correct for plasma trapped between cells in the hematocrit tubes and for higher hematocrit in larger blood vessels (8). We assumed that each sample was 1.3% of the total blood volume and corrected for protein lost through sampling by multiplying the result by the fraction of the blood not removed by sampling.

Design of Experiments

Experiment 1. Analysis of feeding during water deprivation. Food intake was measured in 11 rats (body weight 466 ± 8 g) for 7.5 h, starting at the beginning of the dark period (1900), because rats normally do most of their eating during the dark period (22). Every 15 min the food hopper was removed from the cage, weighed, and returned to the cage. Drinking water was not available during the test period in six rats, but the other rats were allowed to drink. Two days later the experiment was repeated, but the treatments were reversed.

In a different group of eight rats (body weight 448 ± 7 g), we tested if the disturbance associated with removing the food hoppers influenced feeding. We measured food intake during the first 6 h of the dark period either every 15 min or only at 0, 2, and 6 h. Rats were tested four times, twice with water available during the test period and twice in the absence of drinking water. Rats were randomly allowed to drink during the first two tests or the last two tests, and the frequency of measurement (every 15 min or only at 0, 2, and 6 h) was counterbalanced. Period between tests was 2 days.

Experiment 2. Effect of the presence of drinking water on changes in plasma tonicity and blood volume induced by eating. Because it is difficult to take blood samples in spontaneously feeding rats without interrupting feeding, especially in the dark, we took blood samples from rats that had been food deprived and allowed the rats to eat a normal-sized meal. We used seven rats (468 ± 21 g) with venous cannulas. Rats were deprived of food for 19 h. The length of the deprivation period was based on a preliminary test that showed that rats reliably ate 3 g of food after this period. After 19 h of food deprivation, rats were transferred to restraining cages (31) and offered 3 g food in the presence (\( n = 4 \)) or absence (\( n = 6 \)) of drinking water. Drinking water was removed as soon as the rat had ingested all the food (within 20 min). Blood samples (0.35 ml each) were taken from the venous cannulas just before food was given (−1100) and 10, 30, 60, and 240 min after food was returned. Period between experiments was at least 10 days.

Experiment 3. Effect of rapid intravenous infusion of water or 0.3 M NaCl on food intake. To test the sensitivity of feeding to changes in plasma tonicity, we used eight rats (body weight 511 ± 13 g) that had a cannula in the thoracic vena cava. To measure sensitivity of feeding to a fall in plasma tonicity, these rats were deprived of drinking water for 18 h (from 1700 to 1100). Then food was removed, cannulas were connected, and sterile water (0, 5, 10, or 15 ml) was infused into the vena cava at a rate of 1.1 ml/min. Food (but not drinking water) was returned 6 min after the end of the infusion. In a preliminary test, these rats ate 16.1 ± 0.9 g of food during the 18-h period of water deprivation, compared with 25.8 ± 1.6 g when having access to water. All rats received all infusions in counterbalanced order, with 2 or 3 days between infusions.

To measure sensitivity of feeding to an increase in plasma tonicity, these rats were deprived of food for 10 h (from 0100 to 1100). Then water was removed, and 0.3 M NaCl solution (0, 5, 10, or 15 ml) was infused through the venous cannula at a rate of 1.1 ml/min. Food (but not drinking water) was returned 6 min after the end of the infusion. In a preliminary test, these rats ate 10.3 ± 0.7 g of food during the 10-h deprivation period. All rats received all infusions in counterbalanced order, with 2 or 3 days between infusions.

The same rats were used to measure the effect of the infusions on plasma tonicity. We infused 10 ml water or 0.3 M NaCl solution at a rate of 1.1 ml/min and took blood samples (0.35 ml each) just before the start of the infusion and 6 min after the end of the infusion (at the start of the eating period). Rats were food and water replete at the start of the experiment, but were not allowed to eat or drink during the experiment.

Experiment 4. Effect of slow infusions of water in the stomach of rats not allowed to drink. To determine whether the act of drinking is necessary for normal food intake, we used eight rats (body weight 504 ± 22 g) with gastric cannulas. They were water deprived from 1730 to 0900 the next morning. During this period, water was infused intragastrically (3 ml/h). This amount was slightly larger than those rats normally drank during this period. In control experiments, cannulas were connected, but no water was infused. In other control experiments, rats were allowed to drink while water was infused. Treatments were in counterbalanced order, 2 or 3 days apart.

Experiment 5. Effect of infusion of water in various body locations on food intake of rats not allowed to drink. To locate the sensors that mediate the effect of hydration on feeding
we slowly infused water in various locations in the body. We used eight rats (body weight 552 ± 13 g) that had cannulas in the stomach and abdominal vena cava (experiment 5A), eight rats (368 ± 12 g) with cannulas in the stomach and jejunum (experiment 5B), eight rats (404 ± 3 g) with cannulas in the stomach and cecum (experiment 5C), and nine rats (413 ± 16 g) with cannulas in the portal vein and abdominal vena cava (experiment 5D). In each experiment, food intake was recorded when 1) drinking water was available and no infusion was made, 2) drinking water was withheld and no infusion was made, and 3 and 4) drinking water was withheld but water was slowly infused through one of the cannulas. Experiments started at the onset of the dark period (1900) and lasted 6 h. Infusions were 10 ml in 6 h, about two-thirds of the normal water intake during this period. When no infusions were made, cannulas were not connected to infusion tubing. Treatments were given in counterbalanced order, at least 2 days apart. We collected urine voided during the 6-h test period and determined the volume (experiment 5A-D) and sodium and potassium content (experiment 5B). To test for nonspecific effects of the intravenous and intraportal infusions, we measured the effect of these infusions on feeding in rats allowed to drink freely.

Data Analysis

Data are presented as means ± SE. For statistical analysis we used the SigmaStat 2.03 package (SPSS, Chicago, IL). Differences in cumulative intakes were tested with a t-test or one-way ANOVA at each time point. ANOVAs were followed by Dunnett’s multiple comparison test when there was evidence of significant effects. All other differences were tested with a t-test, one-way ANOVA, or two-way ANOVA, as appropriate, followed by Tukey’s multiple comparison test. We used designs for repeated measures wherever all rats subjected to an experiment received all treatments. Differences between means were considered significant if the probability that they occurred by chance was < 0.05.

RESULTS

Experiment 1. Analysis of Feeding During Water Deprivation

To determine the effects of short periods of water deprivation on feeding, we removed drinking water at the beginning of the dark period because rats take most of their daily intake of water and food during the dark period. Food intake fell rapidly during water deprivation (Fig. 2). The earliest significant difference was observed after 45 min of water deprivation, at which time the rats had eaten 1.9 ± 0.3 g, compared with 2.8 ± 0.3 g when allowed to drink (P < 0.05).

Measurement of food intake every 15 min allowed us to get an impression of the meal pattern (see METHODS for our operational definition of a meal). Figure 2 shows that rats ate as many meals when water deprived, but meals were smaller. Even the first meal was smaller when water was withheld (2.1 ± 0.3 vs. 2.9 ± 0.3 g, P < 0.05).

Similar findings were obtained in a second group of eight rats (Fig. 3A). These animals also ate less when water deprived, although the reduction in intake did not reach statistical significance until 105 min (2.9 ± 0.3 vs. 4.2 ± 0.3 g), perhaps because the n value was smaller. Once again we observed that rats ate smaller meals during water deprivation (2.1 ± 0.3 vs. 1.4 ± 0.1 g, P < 0.01) and that the number of meals did not change significantly (5.6 ± 0.5 meals when water was available; 6.5 ± 0.7 meals when water deprived). Also, as observed previously, the first meal during water deprivation tended to be smaller (1.4 ± 0.2 g compared with 2.0 ± 0.4 g when water was available). Similar inhibitory effects of water deprivation on food intake were observed in these same animals when measurements were taken only at 2 and 6 h, showing that the frequent disturbance of the rats in the previous experiments had little effect on the findings (Fig. 3B).
Experiment 2. Effect of the Presence of Drinking Water on Changes in Plasma Tonicity and Blood Volume Induced by Eating

Rats deprived of food but not water for 19 h rapidly ate the 3 g of food provided whether water was available or not. Eating in the absence of water rapidly increased plasma sodium concentration (Fig. 4). Also, plasma osmolality increased and blood volume fell, but these changes were slower to reach statistical significance (Fig. 4). Plasma osmolality, plasma sodium concentration, and blood volume did not change if rats were allowed to drink during the 20-min eating period (mean water intake: 4.6 ± 0.9 ml at 10 min, 7.4 ± 0.8 ml by 20 min).

Experiment 3. Effect of Rapid Intravenous Infusion of Water and 0.3 M NaCl on Food Intake

In this experiment we infused intravenously 5, 10, or 15 ml water at a rate of 1.1 ml/min in rats that had been deprived of water (but not food) for 18 h. The feeding period started 6 min after the end of the infusion. Intravenous infusion of water caused a dose-dependent stimulation of feeding (Fig. 5). Intravenous infusion of 10 ml water reduced plasma osmolality by 4.4 ± 0.9% (P < 0.01), from 295 ± 1 just before the infusion started to 282 ± 2 mosmol/kgH2O at the start of the feeding period. Plasma sodium concentration fell by 4.3 ± 0.9% (P < 0.01), from 140 ± 1 to 134 ± 1 mM. Plasma collected after intravenous infusion of 10 ml water was clear, indicating very little or no hemolysis.

Similarly, we infused intravenously 5, 10, or 15 ml 0.3 M NaCl solution at a rate of 1.1 ml/min in rats that had been deprived of food for 10 h. The feeding period started 6 min after the end of the infusion. These infusions appeared to reduce feeding dose dependently, although only the 15-ml infusion had a significant effect on food intake (Fig. 5). Infusion of 10 ml 0.3 M NaCl increased plasma osmolality by 4.4 ± 0.7% (from 295 ± 1 to 308 ± 2 mosmol/kgH2O at the onset of the feeding period, P < 0.01) and plasma sodium concentration by 5.5 ± 1.0% (from 139 ± 0.3 to 147 ± 0.7 mM, P < 0.01). These changes are much larger than those seen in rats eating 3 g food (Fig. 4).

Experiment 4. Effect of Slow Infusions of Water in the Stomach of Rats Not Allowed to Drink

To determine whether the act of drinking is necessary for normal food intake, we removed drinking water for 17 h, and during this period we slowly infused into the stomach the amount of water these rats normally drank. Water deprivation reduced overnight food intake, as in previous experiments. Food intake was restored, however, when water was infused intragastrically (Fig. 6). The total volume infused over the 15.5 h experiment, 47 ml, or ~3 ml/h, was slightly more than the amount these rats drank when water was freely available (40 ± 4 ml). The gastric infusions did not alter food intake in rats allowed to drink.
Experiment 5. Effect of Infusion of Water in Various Body Locations on Feeding of Rats Not Allowed to Drink

We tried to localize the site of the sensors responding to water infusion by depriving rats of drinking water during the first 6 h of the dark period while slowly infusing water into either the stomach, jejunum, cecum, portal vein, or vena cava. The volume infused, 10 ml/6 h, was less than the average amounts drunk by these rats voluntarily when water was available (14.3 ± 0.6 ml).

Infusion of water in the stomach, jejunum, or cecum restored feeding in rats not permitted to drink, but infusions into the hepatic portal vein or abdominal vena cava did not (Fig. 7, A-D). The effect of the infusions on urinary losses was small and not statistically significant (Table 1).

Neither infusions in the vena cava nor portal intravenous infusions altered food intake in rats allowed to drink. Rats with cannulas in the vena cava ate 12.9 ± 0.6 g/6 h without infusion and 12.9 ± 0.6 g/6 h when water was infused in the vena cava. Rats with portal cannulas ate 10.1 ± 0.5 g/6 h without infusion and 9.7 ± 0.3 g/6 h when water was infused into the portal vein.

DISCUSSION

We investigated the inhibition of feeding caused by water deprivation. Our main findings are as follows. 1) Water deprivation reduced meal size, including the first meal. 2) Intravenous infusions of 0.3 M NaCl that increased plasma tonicity and sodium concentration to levels greater than those seen after eating caused little or no reduction in food intake. 3) Feeding in rats not allowed to drink was restored when amounts of water similar to that normally drunk were infused slowly into the stomach, jejunum, or cecum but not when the water was infused into the vena cava or portal vein.

Effect of Water Deprivation on Eating

As observed previously, water-deprivation reduced food intake in rats (1, 28, 31, 38). Rats ate smaller meals during water deprivation, but meal frequency did not change, at least during the short periods of water deprivation that we used. Even the first meal during water deprivation was smaller. A reduction of meal size, including the first meal, has been observed also in cows (32). This suggests that feeding is very sensitive to the availability of water.

Role of Plasma Osmolality in Inhibition of Feeding

Our results show that intake of a meal in the absence of drinking water caused a rapid increase in plasma osmolality and sodium concentration (see also Ref. 6).

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**Figure 6.** Effect of intragastric infusion of water (47 ml/15.5 h) on food intake. During the infusion period rats were either allowed to drink freely or were deprived of drinking water. Data are means ± SE. *P < 0.001, Tukey’s test, n = 8.

**Figure 7.** Effect of infusion of water into the stomach, jejunum, cecum, hepatic portal vein, or abdominal vena cava (10 ml/6 h) on food intake by rats not allowed to drink. Volume of water infused was less than the amount normally drunk. A: rats with cannulas in the stomach and the vena cava, n = 8; B: rats with cannulas in the stomach and the jejunum, n = 8; C: rats with cannulas in the cecum, n = 8; and D: rats with cannulas in the vena cava and the hepatic portal vein, n = 9. Data are means ± SE, *P < 0.05, **P < 0.01, Tukey’s test.
Allowing rats to drink water during the feeding period reduced or abolished this change, which would be consistent with a role for sensors monitoring plasma tonicity controlling feeding. To investigate this possibility, we measured the effect of intravenous infusions of 0.3 M NaCl on feeding induced by 10 h food deprivation. Although these infusions increased plasma osmolality and sodium concentration by amounts much greater than the changes caused by eating, they had little or no effect on food intake. We interpret these findings cautiously, however, because the factors controlling feeding after food deprivation may differ from those active in rats eating ad libitum. Nevertheless, these results suggest that the reduction of the size of the first meal eaten during water deprivation was probably not caused by hypertonicity of the plasma.

Although increasing plasma tonicity by intravenous infusion of hypertonic saline did not inhibit feeding, food intake was stimulated by lowering plasma tonicity (infusing water intravenously) in rats that had been water deprived for 18 h. This would be consistent with the hypothesis that sensors monitoring plasma tonicity control feeding. Again, the results must be treated cautiously because these effects of rapid water infusion after a fairly long period of water deprivation may be quite different from what happens during ad libitum feeding. Also, the volume of water needed to stimulate feeding (10 ml) was much larger than the volume of a normal drink (<4 ml; Ref. 23). Furthermore, these infusions do not permit localization of the sensors involved. We addressed these issues by studying the effects of slow, continuous infusion of water into various sites in rats not allowed to drink.

**Splanchnic Location of the Sensor Inhibiting Feeding During Water Deprivation**

In contrast to the effects noted above, slow, continuous infusion of water intravenously into rats not allowed to drink did not restore normal food intake. Food intake was restored, however, when water was infused at the same rate into the stomach. It is unlikely that the failure of the intravenous infusion to restore food intake was caused by some unspecified inhibition of feeding, because similar intravenous water infusions did not affect food intake in rats allowed to drink. Neither did the intravenous water infusions alter urinary water loss.

The restoration of feeding by intragastric infusion of water demonstrates that dehydration anorexia in rats is not caused by the behavioral linkage between eating and drinking. Rather, the effectiveness of intragastric infusions in restoring feeding suggests the sensor that mediates dehydration anorexia in rats is located in the gastrointestinal tract or perhaps in the circulation that drains this area. It should be noted that the amount of water infused that restored normal food intake was smaller than the amount normally drunk by rats during this period. Although the pattern of delivery would differ from normal drinking, it cannot be argued that the amounts given were unphysiological.

Food intake was also restored by slow, continuous infusion of water into the jejunum or the cecum. It surprised us that infusions into the cecum were as effective as infusions into the stomach or small intestine, because it is highly unlikely that the water drunk with a meal normally changes the hydration of the cecum. On the other hand, it has been shown that sensors responding to hypertonicity are present throughout the small intestine, as well as in the vasculature draining this area, because application of 0.3 M NaCl to the outside surface of the small intestine or mesenteric vasculature causes a rapid increase in the activity of the hypothalamo-hypophysial system (2). Our finding that infusion of water into the cecum restored feeding is compatible with the idea that sensors responding to hydration state are distributed through much of the gastrointestinal tract.

We considered that the infusion of water in proximal or distal gut sites could be effective because the sensor was in the liver. This seems unlikely, though, because infusion of water in the portal vein failed to restore food intake in rats not allowed to drink. Once again, this cannot be attributed to some unspecified inhibition of feeding, because similar portal water infusions did not affect food intake in rats allowed to drink. It should also be noted that these water infusions would not be

### Table 1. Effects of water deprivation and water infusions on urine volume and electrolyte excretion

<table>
<thead>
<tr>
<th>Experiment 5A: rats receiving infusions in the stomach (g) and abdominal vena cava (v)</th>
<th>Water Replete</th>
<th>Water Deprived</th>
<th>Water Deprived + Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume</td>
<td>6.0 ± 0.9</td>
<td>6.5 ± 0.3</td>
<td>6.3 ± 0.9(g)</td>
</tr>
<tr>
<td>Urine volume</td>
<td>7.0 ± 1.0</td>
<td>5.9 ± 0.6</td>
<td>6.5 ± 0.6(g)</td>
</tr>
<tr>
<td>Na⁺ excretion</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1(g)</td>
</tr>
<tr>
<td>K⁺ excretion</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>2.0 ± 0.1(g)</td>
</tr>
<tr>
<td>Experiment 5C: rats receiving infusions in the stomach (g) and in the cecum (c)</td>
<td>Water Replete</td>
<td>Water Deprived</td>
<td>Water Deprived + Infusion</td>
</tr>
<tr>
<td>Urine volume</td>
<td>7.6 ± 0.6</td>
<td>6.6 ± 0.5</td>
<td>6.9 ± 0.5(g)</td>
</tr>
<tr>
<td>Urine volume</td>
<td>4.3 ± 0.5</td>
<td>4.7 ± 0.9</td>
<td>4.4 ± 1.2(v)</td>
</tr>
</tbody>
</table>

Data are means ± SE, urine volume in ml/6 h, solute excretion in mmol/6 h. Infusions were 10 ml/6 h. Experiment 5, A–C: n = 8; experiment 5D: n = 5. Within experiments, differences between treatments were not significant (1-way repeated-measures ANOVA).
expected to produce a large change in the tonicity of portal blood. Assuming a blood flow through the portal vein of 10 ml/min (4), the infusions used by us (28 μl/min) would have reduced portal blood tonicity by <0.3%. We expect that infusions of water at the same rate into the gut would have similar small effects on the tonicity of portal blood entering the liver.

In conclusion, our findings show that sensors responsive to hypertonicity of the gastrointestinal tract can inhibit feeding. We would expect the sensors specifically responsible for the reduction in food intake during water deprivation to be a subpopulation of these located in the proximal gut, because this is the region most likely to be affected by the ingestion of normal size drinks. These sensors are probably in the stomach, in the first part of the small intestine, or in the vasculature that drains these areas, before the liver. In the absence of drinking water, signals from these sensors provoke an early termination of the meal.

Our findings supporting a role for a gut sensor in dehydration anorexia are consistent with observations made by other research groups. Houpt et al. (16) found that infusion of small amounts of hypertonic NaCl in the duodenum rapidly reduced food intake in pigs not allowed to drink, but similar infusions into the portal vein did not. The feeding inhibition was blunted after vagotomy (16). The mechanism appears sensitive to tonicity rather than sodium concentration (17) and may play a role in the normal termination of a meal. Houpt et al. also showed that large changes in duodenal tonicity do indeed occur after eating (15). Recent experiments indicate that hypertonicity of the rumen is important in reducing feeding during water deprivation in ruminants (35). First, in cows, as we observed in rats, already the first meal eaten during water deprivation was smaller (32), although changes in systemic osmolality tend to develop only slowly. Second, tonicity of the ruminal fluid at the end of a meal was higher when drinking water was not available (35), even though the meal ingested in the absence of drinking water was smaller. Third, feeding was stimulated in cows not allowed to drink by infusing water into the rumen (35). Additional information indicates that brain osmoreceptors are not important in dehydration anorexia. For example, forebrain lesions that block drinking to hypertonic solutions fail to block the inhibition of feeding caused by administration of hypertonic NaCl (10). In addition, lesions of the subfornical organ do not affect feeding during water deprivation (34). In fact, forebrain function is not necessary for a reduction in feeding after intraperitoneal or intravenous administration of hypertonic NaCl, because it occurs in decerebrate rats (11).

**Perspectives**

These findings are consistent with the view that postdigestive, preabsorptive, negative-feedback information from the gut has a primary and direct role in the control of feeding (33). They suggest that hydration of the gastrointestinal tract is one of the important variables monitored by the gut mucosal receptors and that the information is carried by afferent fibers to the caudal brain stem rather than through changes in the tonicity of the circulation monitored by forebrain or other nonsplanchnic sites. Information from splanchnic sensors monitoring gut hydration may also play a role in the control of drinking (24, 26), release of vasopressin (5) and oxytocin (19), salt excretion (14), and gastric emptying (21). Whether effects on gastric emptying are responsible for dehydration anorexia remains to be explored.

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**REFERENCES**