A rapid feedback signal is not always necessary for termination of a drinking bout

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A rapid feedback signal is not always necessary for termination of a drinking bout. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1156–R1163, 1999.—When a pig is deprived of drinking water, a deficit of body water develops that is corrected when the pig drinks to satiation. If food is available during the deprivation, the stimulus to drinking is plasma hyperosmolality. Because of the delay in correction of plasma hyperosmolality as ingested water is slowly absorbed, it has been thought that a rapid inhibitory signal from the digestive tract is necessary to prevent overdrinking. This concept was tested by measuring changes in plasma osmolality before and during drinking after such deprivation and also after infusion of hypertonic saline. As drinking began, there was a rapid fall of plasma osmolality to levels insufficient to drive drinking by the time drinking ended. This fall of plasma hyperosmolality to subthreshold levels while the pig is drinking seems to make a rapid inhibitory control signal from the digestive tract unnecessary to terminate the drinking bout under these conditions.

WHEN AN ANIMAL DRINKS, the stimulus to drinking is often clear, such as plasma hyperosmolality or hypovolemia (3), but the cause of the termination of the drinking bout after an amount approximating body needs has been consumed is not clear (8). Oropharyngeal–esophageal metering of water ingestion is not an immediate or major factor in terminating normal drinking bouts because water far in excess of need is ingested in sham-drinking experiments (4, 6). Ingestion of water is so rapid that it is difficult to conceive of a corresponding rapid absorption of the water with resultant removal of the plasma stimulus by dilution within the time span of the drinking bout. This has led to the concept of a rapidly responding postdigestive inhibitory system initiated in or close to the alimentary canal that would limit the size of each drinking bout to prevent overdrinking. In this case, termination of the drinking bout would not require immediate removal of the plasma stimulus. In this scenario, after the ingested water is absorbed, the stimulus to drinking is corrected and inhibition of drinking persists.

Blood osmolality in the rat can fall rapidly while drinking is occurring in response to osmotic thirst (9, 10), but the significance of this in the control of water intake has not been widely recognized. This rapid fall of blood osmolality during drinking has not been observed in sheep (22), but this is not surprising considering the great differences in digestive tract function in the ruminant.

To investigate this problem further, we deprived pigs of water, but not feed, for a few hours and then allowed them to drink to satiation. As the pigs drank, blood samples were taken in quick succession from implanted catheters to follow the changes in plasma osmolality. The question was, would plasma osmolality fall rapidly enough while the pig was drinking to bring it to a level by the end of the bout that would no longer stimulate drinking? If this were so, a rapid inhibitory signal from the gastrointestinal tract would be unnecessary. In these experiments on pigs, the conditions favored hyperosmolality of the plasma as the primary stimulus to thirst (13). We found in these thirsty pigs that the termination of the initial drinking was correlated with a reduction of plasma osmolality to the predeprivation concentration. A similar set of experiments was performed in which the osmotic thirst was caused by infusion of hypertonic saline.

METHODS AND PROCEDURES

Animals. The subjects of these experiments were 20 young, sexually immature, mixed-breed pigs of both sexes. The pigs were obtained from the Cornell Swine Barn when they were ~15 kg in weight, and they grew during the experiments to ~40 kg. The pigs were housed individually in indoor pens (2.1 × 2.9 m). Room temperature was 22–23°C. Lights were on from 0700 to 2100. The feed was a high-quality pelleted pig starter ration containing 18% protein, 4–4.5% fat, 3–4.5% fiber, and 0.36% sodium (Agway, Ithaca, NY). Feed and water were available operantly at all times at a fixed ratio (FR) of 10, except during the deprivation experiments. During the experiments, a range of FRs were used for drinking, as well as drinking from an open bowl of water. The pigs were handled frequently and soon became quite tame and accustomed to the researchers and the experimental procedures.

Catheters. Vascular catheters were made of 0.6- to 1-m lengths of silicone rubber tubing 0.20 cm ID × 0.32 cm OD (Silastic; Dow Corning, Midland, MI). Two cuffs of tubing 0.24 cm ID × 0.40 cm OD × 0.5 cm long were attached using silicone medical adhesive (Dow Corning) 2 cm apart starting from 0700 to 2100. The feed was a high-quality pelleted pig starter ration containing 18% protein, 4–4.5% fat, 3–4.5% fiber, and 0.36% sodium (Agway, Ithaca, NY). Feed and water were available operantly at all times at a fixed ratio (FR) of 10, except during the deprivation experiments. During the experiments, a range of FRs were used for drinking, as well as drinking from an open bowl of water. The pigs were handled frequently and soon became quite tame and accustomed to the researchers and the experimental procedures.

Surgery. Anesthesia was induced in a cage using halothane anesthesia with a face mask, and we took care to excite the pig as little as possible. An endotracheal tube was inserted, and the pig was maintained on closed-circuit halothane in oxygen anesthesia. Standard sterile surgical procedures were used. An incision was made through the skin along a line from the manubrium to the angle of the left jaw, the external jugular vein was exposed by blunt dissection, and the catheter was placed into the jugular vein with the tip positioned in
the cranial vena cava close to the entrance of the right atrium. The catheter was secured to the vein and surrounding tissues with ligatures. The free end of the catheter was passed dorsally through the cervical musculature and out through the skin to the back of the neck. When not in use, the catheters were kept filled with heparinized isotonic saline (100 U heparin/ml) containing 0.03 ml/ml gentamicin sulfate solution (100 mg/ml Gentocin; Schering) and were taped to the pig's neck with a collar of adhesive tape. Recovery from anesthesia was complete within an hour or two, and normal eating and drinking resumed. Experiments were begun 5 days later.

Blood analyses. Blood samples were taken into preheparinized sterile tubes and immediately refrigerated. Packed cell volume (PCV) determinations were made in duplicate using a microhematocrit technique with a repeatability of \( \pm 0.1\% \) (microcapillary reader; Damon/IEC Division, Needham Heights, MA). Plasma protein concentration was estimated to be within \( \pm 0.1\% \) with a hand-held refractometer (Veterinary refractometer; American Optical Veterinary Instruments, Buffalo, NY). Plasma osmolality was determined in duplicate on a 2-ml plasma sample using a freezing point osmometer (Osmette A; Precision Systems, Natick, MA). Plasma osmolality was determined in duplicate on a 2-ml plasma sample using a freezing point osmometer (Osmette A; Precision Systems, Natick, MA). Plasma protein concentration was estimated to be within \( \pm 0.1\% \) with a hand-held refractometer (Veterinary refractometer; American Optical Veterinary Instruments, Buffalo, NY). Plasma osmolality was determined in duplicate on a 2-ml plasma sample using a freezing point osmometer (Osmette A; Precision Systems, Natick, MA).

Procedures. Three sets of experiments were performed. In the first set, an initial or control blood sample of 6 ml was taken through the implanted catheter, then the pig was deprived of water, but not food, for 5 h; another blood sample was then taken, food was turned off, and drinking water was made available. Drinking was operant at FR 20, 10, or 1 or from an open bowl. Blood samples were taken at intervals of 1–2 min while the pig was drinking, then at less frequent intervals for about an hour after initiation of drinking. The initial bout of drinking lasted \( \sim 10\) min and was followed by a pause of 5 min or longer. At that point, the water was turned off. Plasma osmolality was measured as well as PCV and plasma protein. Duration of drinking and amount drunk were also recorded.

In the second set of experiments, sterile hypertonic NaCl solutions (20 or 25%) were infused by peristaltic pump to cause a rise of plasma osmolality of \( \sim 10–12\) mosmol/kg H2O, a rise that we had found to be sufficient to stimulate drinking in our previous studies of hypertonic thresholds for drinking. Eating and drinking were operant (FR 10) and were recorded with an event recorder. Food was removed for 1–2 h before the infusion began to avoid having freshly ingested food and water in the alimentary canal. Then the infusion of hypertonic NaCl was made slowly (0.5–0.9 ml/min) until spontaneous drinking began. The rinse blood was reinjected, followed by a few milliliters of heparinized saline.

The third set of experiments addressed a corollary problem in the deprivation experiments. We had assumed that when a pig drank water after a period of water deprivation, it replaced the deficit in the initial bout of drinking, but this has not been studied in the pig. To do this, we compared the amount of water drunk spontaneously during a 5-h control period when both water and food were available with the amount drunk at the initial bout after a similar 5-h period but with water deprivation. The question was whether the initial bout would approximate the amount of water that was drunk during the same 5-h period during control measurements with water and feed ad libitum. The time, or pause, after that initial rehydration bout of drinking until the next drinking bout was also measured. In these experiments, the pigs were operantly drinking and eating at FR 10 and all eating and feeding was recorded on an event recorder.

Plasma osmolality changes. The results of these experiments were summarized as changes in plasma osmolality plotted against time. Such summary plots were derived from the repeated experiments on each of several pigs. Each individual experiment consisted of data from blood samples taken at intervals of \( 1–3\) min while the pig was drinking (generally for \( \sim 10\) min) and then at longer intervals for up to \( 60\) min. The aim was to combine these results from repeated experiments into a summary figure. However, the blood samples were not taken exactly the same time point after the drinking bout began. To estimate blood values that could be used to summarize these results of repeated experiments, the following procedure was used.

First, the blood osmolality data from each experiment were plotted against time, and a curve was drawn through the points showing the fall that occurred during and after that drinking bout. This best-fit curve was drawn using an irregular (French) curve. The same type of curve was developed for each of the repeated experiments done on each pig. Second, from the curve of each experiment, the values at 5-min intervals were read off and those values at each time point were averaged for all the repeated experiments on a particular pig. Finally, these 5-min mean values at each time point for each pig were averaged to give mean values for all pigs. These overall mean values were plotted to give figures that summarized all the data on the set of pigs used in a particular experimental procedure (Figs. 1–5).

Blood volume changes. Although absolute blood volumes were not directly measured in these experiments, changes in blood volume could be estimated between the beginning and end of each stage of the experiments using changes in PCV and plasma protein values. This estimate is in the form of a ratio of blood volume at the beginning of the period (\( V_1 \)) to that at the end of the period (\( V_2 \)). The result is then expressed as a percentage change of blood volume. The formula used is:

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\frac{V_2}{V_1} = \frac{(1 - PCV_1)(PlPr_1)}{(1 - PCV_2)(PlPr_2)}
\]

where PCV is packed cell volume expressed as a fraction, PlPr is plasma protein concentration, and subscripts 1 and 2 indicate initial and final values for the period selected for estimation of blood volume change. The stages of the experiments subjected to these calculations were 1) during the 5-h water deprivation or the period of infusion of hypertonic NaCl; 2) when actually drinking; and 3) for 30–35 min after drinking stopped.

Statistical analyses. The JMP statistical program of the SAS Institute was used for comparison of means. The data from the various experiments were similar in character, and where samples were sufficiently large, a quantile analysis using JMP indicated a normal distribution.
fore assumed that the data were generally normally distributed. The procedure was to apply ANOVA and, if significance was indicated, pairs of means of interest were compared by t-test. A probability level of 0.05 or less was considered significantly different.

RESULTS

Effect of drinking on plasma hyperosmolality after water deprivation. In all these experiments, the 5-h water deprivation with food available resulted in an elevated plasma osmolality and in thirst. After drinking began, there was a rapid fall of plasma osmolality. Plasma osmolality decreased during the drinking bout at least to the predeprivation level (arrows in Figs. 1–4). The ease of drinking in these experiments was varied by presenting the water operantly at FRs of 20, 10, or 1 or from an open bowl. The duration of drinking varied correspondingly. Figures 1-3 show the results of drinking after the 5-h deprivation when pigs were drinking operantly with FRs of 20, 10, and 1, respectively. Figure 3 conformed to the pattern of the other figures, and, although the limited results at FR 1 gave an ANOVA with $P < 0.10$, the paired t-test indicated significance among the points at 0, 5, and 10 min. Figure 4 shows the results when pigs were drinking freely from an open bowl of water. In all cases, the fall of plasma osmolality during drinking was dramatic, and drinking terminated when plasma osmolality decreased to or below the control, i.e., predeprivation, level. The mean ratio of osmolality at the end of the drink to the preinfusion value was $1.00 \pm 0.002$ for all four drinking conditions.

Thirst caused by infusion of hypertonic NaCl. As hypertonic NaCl was slowly infused intravenously, plasma osmolality rose until drinking began. There was a rapid fall of plasma osmolality during the drinking bouts similar to that seen in the post-water deprivation experiments (Fig. 5). The elevated level of osmolality at the end of the infusion, at which point drinking began, was significantly different from the preinfusion level. The fall of osmolality during drinking was also significant. Note, however, that the osmolality attained at the end of the drinking bout did not quite reach the preinfusion level and was still significantly different from the preinfusion level ($P < 0.01$).

Amounts drunk immediately after 5-h water deprivation period compared with expected water deficit. Figure 6 shows the mean amounts of water drunk in the

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**Fig. 1.** Drinking operantly at fixed ratio (FR) 20 after water deprivation: effects on plasma osmolality. Data from 4 pigs, 11 trials, means ± SE. ANOVA for 0-, 5-, and 10-min points, $P < 0.02$. Zero time point significantly different from 5-min point, and 5-min point significantly different from 10-min point ($P < 0.01$). Arrow indicates plasma osmolality at start of 5-h deprivation period that ended at time 0. Ratio of osmolality at termination of drinking to predeprivation level was 0.997. Duration of drinking is indicated by hatched bar.

**Fig. 2.** Effect of drinking on plasma osmolality with operant drinking set at FR 10. Data from 12 pigs, 34 trials, means ± SE. ANOVA for 0-, 5-, and 10-min points, $P < 0.001$. Zero time point is significantly different from 5-min point, and 5-min point is significantly different from 10-min point ($P < 0.01$). Arrow indicates predeprivation osmolality. Ratio of osmolality at termination of drinking to predeprivation value was 1.00.

**Fig. 3.** Drinking operantly with FR 1 after water deprivation: effects on plasma osmolality. Data from 3 pigs, 8 trials, means ± SE. ANOVA for 0-, 5-, and 10-min points, $P < 0.10$, but zero time point is significantly different from 5-min point, and 5-min point is significantly different from 10-min point ($P < 0.01$). Arrow indicates predeprivation osmolality. Ratio of osmolality at termination of drinking to predeprivation level was 0.996.
initial drinking bout at the end of the 5-h deprivation period (Fig. 6A) and the feed consumed during the 5-h deprivation period (Fig. 6B). Also shown are the amounts of water drunk and food eaten in the control 5-h periods, when both water and feed were freely available. The repeated values for each pig were averaged, and these were used to calculate all pig means as plotted in Fig. 6. The differences between water drunk during control 5-h periods and the initial bouts at the end of the 5-h deprivation periods were statistically significant, as were the differences between food eaten during the 5-h control periods and during the 5-h water deprivation periods. However, the ratio of water intake to food intake tends to be constant in young pigs (5). Here the ratio of water intake to feed intake in the control period was 1.5, and the ratio of water intake in the initial bout after deprivation to food intake during the deprivation period was 1.6. When the amounts...
drunk were adjusted to be proportional to the amount of food eaten during the 5-h periods, there was no significant difference between control and initial post-deprivation water intakes ($P < 0.9$).

Also shown in Fig. 6C is the mean pause in drinking between the end of the initial bout after the 5-h water deprivation periods and the next drinking bout. This reveals a pattern of a large drinking bout when water was presented at the end of the water deprivation period followed by a long pause, averaging 49 min, before the next drinking occurred.

Estimated blood volume changes. These calculations, summarized in Fig. 7, were made for the experiments in which drinking occurred after a 5-h water deprivation period, either while pigs were drinking operantly with an FR of 10 (Fig. 7A) or from an open bowl (Fig. 7B), and for the times at which the drinking was caused by infusion of hypertonic NaCl solutions (Fig. 7C).

Blood volume changes were estimated for each stage: during the water deprivation or infusion of hypertonic NaCl, during drinking, and for ~30 min afterwards. These blood volume changes were in the form of changes from the volume at the end of the previous stage of the experiment.

The 5-h water deprivation periods resulted in a small fall of blood volume (Fig. 7, A and B). During drinking, there was a partial restoration of volume during the short drinking bout from an open bowl (Fig. 7B) or complete restoration of blood volume during the longer drinking bout at FR 10 (Fig. 7A). By the end of the 30- to 35-min postdrinking period, there was an apparent overcompensation of volume when pigs were drinking from an open bowl. During the infusions of hypertonic NaCl (Fig. 7C), there was the expected sharp increase in blood volume, but then there was little further change from that expanded blood volume, that is, the expanded blood volume persisted throughout the drinking and after-drinking periods.

**DISCUSSION**

The results of these experiments indicate that a rapid feedback inhibitory signal is not always necessary to terminate a drinking bout, at least not in the young pig. In particular, when the stimulus to drinking is predominantly plasma hyperosmolality, plasma osmolality can decline precipitously while the animal is drinking to levels that are no longer sufficient to stimulate drinking, so terminating the drinking bout.

This phenomenon is illustrated in Figs. 1–4, in which the ease of drinking was varied by decreasing the FR of the operant system over a range from 20 to 1 or by providing water in an open bowl. Drinking duration under these conditions varied from a mean 9.7 min at FR 20 to 3.4 min from an open bowl, yet in all cases plasma osmolality fell to a level at the end of the drink that would be expected to be insufficient to stimulate drinking. That level was close to the predeprivation level (arrows in Figs. 1–4), and the ratio of the plasma osmolality when drinking stopped to the predeprivation level was 1.00 under all four of the drinking conditions. The predeprivation level of osmolality varied but was apparently subthreshold for thirst because the pigs were not voluntarily drinking at that time.

When the pig drank, it drank an amount equal to the water deficit (Fig. 6). This was sufficient to reduce
plasma osmolality to a level below the threshold for thirst, but plasma osmolality continued to decrease in the postdrinking period. Presumably, at the end of the drinking bout, the bulk of the ingested water was still in the gastrointestinal tract, and as absorption of water continued, plasma osmolality continued to decline. It appears that when the drinking was slowed by higher FRs (Figs. 1 and 2), plasma osmolality tended to level off as the absorbed water was distributed throughout body fluid compartments. When water was ingested more rapidly (Figs. 3 and 4), plasma osmolality fell more rapidly and the postdrinking fall was also accentuated with a later rise of osmolality, possibly due to slower distribution of the absorbed water from the extracellular fluid (ECF) into the cellular compartment.

The rapidity of the fall of plasma osmolality while pigs were ingesting water has been described in the rat by Hatton and Bennett (10), and earlier similar rapid changes in brain fluids have been described by Novin (17), but their significance in the control of drinking appears not to have been generally recognized.

In the first series of experiments, the hypertonic stimulus for thirst was caused by depriving the pig of water, but not food, for 5 h. We had earlier found that these deprivation conditions result in a rise in plasma osmolality and little or no change in blood volume, despite the inevitable loss of body water during the deprivation period (13). To confirm the dramatic effect of water drinking on plasma osmolality, a second series of experiments was performed in which hypertonic NaCl solutions were slowly infused intravenously to cause water drinking. In this case, the hypertonic osmotic stimulus was caused without any deprivation period and so with no appreciable loss of body water before drinking began. Here, instead of blood volume falling, it rose (Fig. 7C) as the hypertonic ECF osmotically drew water from cells throughout the body, and this expanded blood volume persisted while the pig drank and for at least 30 min afterwards. Although a fall in blood volume can be a stimulus to drinking, a moderate rise has little inhibitory effect in the dog (19). However, in these infusion experiments on pigs, as osmolality fell, drinking stopped before the preinfusion osmolality level was reached (Fig. 5). The persistent rise of blood volume by 7% may have had a slight inhibitory effect, resulting in a small rise of the osmotic threshold for drinking. This concept of increased blood volume depressing thirst sensitivity to hyperosmolality has been supported by work on humans (21, 24). Nevertheless, the primary objective of these hypertonic NaCl infusion experiments was to determine if a fall in plasma osmolality would be as rapid in these infusion experiments while the pig drank as in the first set of deprivation experiments. This it was, indicating that under these circumstances in which the hypertonicity is caused directly by the salt infusion, the same direct effect of drinking on plasma osmolality influenced the termination of drinking.

How could this rapid restoration of normal plasma osmolality be explained? Absorption of water can be fast; in rats, ~20–30% of an ingested amount is absorbed in the few minutes during which drinking is occurring (10). But even 30% restoration of a body water deficit would seem inadequate to cause the fall in plasma osmolality observed. The fall of plasma osmolality would involve either addition of water to ECF or removal of osmotically active ions and molecules. The effectiveness of the absorption of a relatively small fraction of the ingested water in bringing plasma osmolality down would be enhanced if most of that absorbed water were restricted to the ECF compartment.

Studies of the regulation of cell volume when cells are exposed to either hyperosmotic or hypoosmotic media have weakened the traditional view of cells as perfect osmometers (16). When exposed to hypotonic solutions, they do not swell by osmotic inward movement of water as much as would be expected. In the intact animal, this tendency to regulate cell volume in the face of osmotic changes in the ECF may persist for many minutes or even hours (15). This would restrict water absorbed from the intestines to the extracellular compartment, tending to rapidly return any elevated osmolarity toward isotonic levels that would no longer stimulate thirst.

However, in addition, there would be a movement of electrolytes from plasma across the intestinal epithelium down their concentration gradients into the water that is accumulating in the intestine as drinking proceeds. The early demonstration of the dynamic relations of sodium and other ions between blood and gut fluid make such shifts of electrolytes from blood to gut conceivable (23), with the duodenum and proximal jejunum being the most likely sites for such ionic movement. The net secretion of sodium and other osmoles from blood to gut has been demonstrated in the guinea pig small intestine (18). Furthermore, in a study of duodenal osmolality in pigs after drinking water, we found that there was a rapid recovery of duodenal osmolality (12). As ingested water entered the duodenum, osmolality fell from ~300 to 50 mosmol/kg in 3 min but then rose at a rate of 15 mosmol/min. This indicates a rapid movement of osmoles into the gut water. Both the addition to the ECF of absorbed water from the gut plus diffusion of small ions and molecules from the plasma into the gut water would lower plasma (and ECF) osmolality, and these processes could be part of the explanation for the rapid fall of osmolality during drinking. Hypothetically, a decrease in renal free water clearance could also contribute to a fall of plasma osmolality.

In these experiments, it was at first assumed that after a period of water deprivation, the initial bout of drinking to satiation would approximate the amount of the deficit, as it has been shown in dogs drinking after similar moderate water deprivation (1). To test this assumption, the amounts of water drunk in 5-h control periods with food and water available were compared.
with the amounts of water drunk after 5-h water deprivation periods with food available (Fig. 6). There was a trend for less water to be drunk after the deprivation periods than in the control periods. However, less food was also eaten during the deprivation period. Food and water intake are usually positively correlated (5, 7, 14), and the depression of food intake during the deprivation period with lower water consumption afterward would be expected. The water deficit during the 5-h deprivation period adjusted proportionally to feed intake was little different from control amounts, and the differences were statistically insignificant. That this reaction was satiation of the thirst is further supported by the fact that the initial bout was followed by a long pause averaging 49 min (Fig. 6C). The next drinking bout presumably represented a resumption of the normal cycle of eating and drinking and not a further replacement of the deficit. Regardless of whether the initial drinking bout matched the body water deficit, the amounts drunk were adequate to cause the rapid fall of plasma osmolality that presumably played a part in the termination of the drinking bouts.

Some animals replace a moderate water deficit developed during a period of water deprivation by a series of short drinking bouts, tapering off to smaller and smaller bouts over a period of time, and the deficit may not be fully replaced for hours (2). Rats and humans fall into this category. The pigs in our study, however, replaced most or all of the deficit in the first bout, and they fall into the other category of animals that promptly replace a water deficit in the first drinking bout. Examples of this group are the dog and donkey (1, 2, 19).

In summary, the results of these experiments indicate that when drinking is stimulated by blood hyperosmolality, the drinking can be terminated by direct correction of the elevated osmolality, removing the stimulus to drinking. Further investigation will be needed to establish the mechanism of this rapid dilution of ECF under these circumstances.

**References**