Water ingestion provides an early signal inhibiting osmotically stimulated vasopressin secretion in rats

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Received 20 January 2000; accepted in final form 24 March 2000

Dehydration is well known to stimulate thirst, with the induced water intake allowing rehydration of body fluids. Dehydration also stimulates secretion of the antidiuretic hormone vasopressin (VP), which permits water conservation in urine and thereby reduces further dehydration. Osmoreceptors sensitive to changes in the effective osmolality of circulating blood have been located in the basal forebrain and are presumed responsible for these two familiar effects (34). However, it takes time for ingested water to be absorbed and to alter plasma osmolality (P_{osmol}). Thus, unless some early stimulus signals inhibition of thirst and VP secretion, dehydrated animals should continue to drink and to conserve urinary water in amounts exceeding need. It is therefore noteworthy that overhydration does not occur when access to water is restored to dehydrated animals (2).

A rapid inhibitory effect of fluid ingestion on thirst and VP secretion has been documented in studies using water-deprived dogs as experimental subjects (35). Specifically, satiety for thirst and inhibition of VP secretion occurred within minutes after drinking began, before substantial amounts of the ingested water had been absorbed. Similar results were obtained when dogs drank isotonic saline instead of water. These rapid inhibitory effects occurred (albeit only for 10–20 min) even when a gastric fistula drained stomach contents and thereby prevented rehydration. In contrast, no effect on VP secretion was observed when a water load was delivered directly to the stomach, bypassing the oropharynx. Remarkably, a rapid but temporary inhibition of thirst and VP secretion also occurred when dehydrated dogs drank hypertonic NaCl solution instead of water; as expected, enhanced thirst and VP secretion occurred once the ingested fluid was absorbed and P_{osmol} became elevated (4). Collectively, these findings suggest that the swallowing component of fluid consumption provides an early signal that inhibits VP secretion and thirst in dehydrated dogs.

A similar conclusion regarding the control of VP secretion has been drawn from studies of human (19, 30) or nonhuman primates (5, 38). In rats, however, the picture appears to be different. The usual reduction in the rate of water intake by thirsty rats early in a drinking episode does not occur when vagal afferents (14), or their projection sites in the caudal brain stem (15), have been destroyed. Furthermore, small but reliable decreases in plasma VP (pVP) levels have been observed without substantial changes in P_{osmol} when a water load was given to rats intragastrically rather than by normal ingestion (6). These results suggest the existence of visceral receptors, perhaps in the small intestines, hepatic portal vein, and/or liver, which signal hydration before significant absorption of ingested water into the general circulation could have occurred and allowed detection by cerebral osmoreceptors.

The present experiments investigated the influence of fluid consumption on VP secretion in rats. This effect was studied in rats after substantial VP secretion had been stimulated by intravenous infusion of hypertonic NaCl solution. To control for the act of drinking and to avoid a direct effect of the ingested fluid on P_{osmol}, other rats were given isotonic saline to drink instead of...
water. The effect of these procedures on pituitary secretion of oxytocin (OT) was also investigated because, in rats, OT secretion is regulated much like VP secretion when P_osmol is elevated (33). In addition, OT acts physiologically as a natriuretic hormone and thereby contributes importantly to osmoregulation after a NaCl load (20, 37).

METHODS

Animals. Adult male Sprague-Dawley rats (Zivic Laboratory, Zelienople, PA), weighing 330–380 g, were used in this study. They were housed individually in wire mesh cages in a colony room with ambient temperature maintained at 22–24°C and with lights on from 0700 to 1900. The rats had ad libitum access to pelleted Laboratory Chow (Purina, #5001) and tap water before experiments began.

Procedure. The day before experiments, all rats were anesthetized with Sodium Brevital (50 mg/kg ip, Jones Medical Industries, St. Louis, MO). One catheter (PE-50) was implanted in the right femoral artery for blood sampling, and a second catheter (polyvinyl tubing) was implanted in the right femoral vein for infusions. The free ends of the two catheters were guided subcutaneously along the back to exit between the scapulae. Upon exiting, the catheters were encased in a steel spring to prevent them from being damaged and were connected to a swivel system to allow freedom of movement. The rats were returned to their home cages where experiments occurred.

On the following morning, water and food were removed from each cage, and the free end of the venous catheter was connected to an infusion pump (Harvard Apparatus, South Natick, MA). During a 30-min baseline period, 0.15 M NaCl solution was infused (2 ml/h) and a basal blood sample (1.5 ml) was collected via the arterial catheter. Then the infusate solution was infused (2 ml/h) and a basal blood sample (1.5 ml) was collected via the arterial catheter. After 220 min, the solution was switched to 1 M NaCl, delivered at the same rate for 240 min. Additional blood samples were taken 5 and 15 min after the end of the drinking period and remained that low in mean P_osmol was increased to 321–324 mosmol/kgH2O from baseline values of 303–307 mosmol/kgH2O (P < 0.01). Plasma levels of VP and OT were increased by the infusion of hypertonic saline (Fig. 1, B and C) in accordance with the elevated P_osmol.

RESULTS

Systemic infusion of 1 M NaCl (2 ml/h iv) significantly raised P_osmol in all three groups of rats (Fig. 1A), as intended. After 220 min of infusion (i.e., just before the drinking period), mean P_osmol was increased to 321–324 mosmol/kgH2O from baseline values of 303–307 mosmol/kgH2O (P < 0.01). Plasma levels of VP and OT were increased by the infusion of hypertonic saline (Fig. 1, B and C) in accordance with the elevated P_osmol.

These elevated values of P_osmol, pVP, and pOT remained stable for 20 min in rats that were not allowed to drink (Fig. 1, A, B, and C). In contrast, rats given water to drink consumed 5.5 ± 0.2 ml in 5 min, and their pVP and pOT were significantly lower than those of rats not allowed to drink both at 10 and 20 min after the onset of drinking (Fig. 1, B and C; all P < 0.05). These striking effects occurred without significant changes in P_osmol (Fig. 1A). On the other hand, although rats consumed 0.15 M NaCl in amounts comparable to the water intake (5.6 ± 0.3 ml), their pVP and pOT were significantly higher than those of rats drinking water (Fig. 1, B and C; all P < 0.05 at 10 and 20 min after the onset of drinking) and were similar to those of rats not allowed to drink. As expected, P_osmol was not affected by consumption of isotonic saline (Fig. 1A). The values of P_osmol, pVP, and pOT in all three groups were not significantly different between the two times of measurement after the drinking period (Fig. 1A).

Individual values of pVP and pOT presented as a function of associated P_osmol are shown in Fig. 2. Values from rats drinking water were clearly lower than those from the other two groups, although the range of observed P_osmol was comparable in the three groups. In contrast, saline ingestion did not lower pVP or pOT.

DISCUSSION

The principal focus of this investigation was the effect of water intake on pVP in rats given an intravenous infusion of hypertonic NaCl solution. A substantial effect was found: pVP fell by >50% within 5 min after the drinking period and remained that low in samples taken 10 min later. Given that the half-life of VP in the circulation of rats is only a few minutes (16), these observations suggest that water ingestion had a large and rapid inhibitory effect on VP secretion.

Infusion of 1 M NaCl for ~4 h increased P_osmol by ~5%, which exceeds the threshold documented to stimulate VP secretion in rats (29). Because pVP values are proportional to P_osmol in rats (17, 33), a sudden decrease in pVP could reflect a rapid drop in P_osmol caused by the ingestion of water. However, direct measurements indicated that P_osmol was not altered significantly by water consumption in the present experiments due to the limited time allotted for drinking and gastric water absorption. Moreover, only one-third of the ingested water load would be expected to be absorbed in 15 min (28), and the estimated osmotic dilution resulting from that small effect (calculated to be about −2 mosmol/kgH2O) would be buffered by the
increase in $P_{\text{o smol}}$ from the continued infusion of 1 M NaCl. Thus the changes in pVP observed after rats drank water appear to reflect an inhibitory signal that was not generated by noticeable changes in the osmolality of blood in the general circulation.

The results of studies in dehydrated dogs suggest that an oropharyngeal signal associated with swallowing mediates the rapid inhibition of VP secretion that accompanies fluid ingestion (35). This inhibitory effect was observed even when a decrease in the elevated $P_{\text{o smol}}$ was not possible, such as when dogs drank water with an open fistula (35) or when they drank hyper-

Fig. 1. Effect of drinking on mean ± SE values of plasma osmolality ($P_{\text{o smol}}$; A), plasma vasopressin (pVP; B), and plasma oxytocin (pOT; C) in rats infused with 1 M NaCl (2 ml/h iv for 240 min). Baseline values (BL) before start of the infusion and of the drinking test (0 min) are given. A drinking test began after 220 min of infusion, at which time each of the 3 variables was already significantly increased (all $P < 0.01$). Then rats were given either water ($n = 6$), 0.15 M NaCl solution ($n = 4$), or nothing to drink ($n = 8$) for 5 min (horizontal bar), and additional blood samples were taken 5 and 15 min later. Differences in $P_{\text{o smol}}$ among the 3 groups were not statistically significant. pVP and pOT each decreased abruptly in rats drinking water (all $P < 0.05$), but no significant changes were observed in the other 2 groups.

Fig. 2. Effect of drinking on pVP (A) and pOT (B) in rats infused with 1 M NaCl (2 ml/h iv for 240 min). Symbols represent individual animals at 10 (triangles) and 20 min (circles) after the onset of the 5-min drinking test, plotted as a function of the associated $P_{\text{o smol}}$. Mean values of $P_{\text{o smol}}$ were shown in Fig. 1, and mean values of pVP and pOT were shown in Fig. 1, B and C.
tomic NaCl solution instead of water (4). If the act of drinking similarly decreased VP secretion in rats, as suggested by electrophysiological studies (3), then a decrease in pVP would be expected in rats ingesting 0.15 M NaCl, similar to that seen in rats consuming water but unlike values obtained from rats not allowed to drink. However, no changes in pVP were observed when rats drank saline in amounts comparable to those of rats drinking water. These results do not support the hypothesis that an oropharyngeal stimulus associated with water consumption inhibits VP secretion in rats, as it apparently does in dogs.

Administration of hypertonic NaCl solution to rats is known to stimulate pituitary secretion of OT as well as VP (8, 33). Also like pVP, pOT values increase in proportion to increases in $P_{\text{osmol}}$, although the slope of the regression line is twice as steep as the line relating pVP to $P_{\text{osmol}}$ (21, 33). In rats, this effect is adaptive, because OT has potent natriuretic effects (20, 37). Thus it was of considerable interest that a rapid inhibitory effect of water drinking on OT secretion was also seen in these rats. This inhibition of OT secretion was not seen when rats drank isotonic saline. We conclude that the inhibitory signal likely was related to the low osmolality of drinking water rather than to the act of drinking.

Accumulating evidence indicates that there is an early response to decreases in the osmolality (or Na$^+$ concentration) of fluid absorbed from the gastrointestinal tract. For example, intragastric water loads were observed to decrease VP secretion in water-deprived rats without substantial changes in systemic $P_{\text{osmol}}$, an effect blunted by hepatic vagotomy (6). Presumably, this effect resulted from a neural message sent first to the caudal brain stem, perhaps including the area postrema (22, 26), and then relayed to magnocellular neurosecretory neurons in the hypothalamus. It remains to be determined whether hepatic vagotomy or area postrema lesions also blunt the inhibitory effect of water drinking on VP and OT secretion in rats given intravenous infusions of hypertonic NaCl solution.

A rapid inhibitory effect of water ingestion on VP secretion clearly is adaptive to dehydrated animals because it prevents overhydration resulting from the undesirable urinary conservation of water in excess of need. If the same early signal inhibits thirst as well as VP secretion, then this early signal additionally has the useful effect of limiting a behavior once it is no longer necessary. In fact, the early effects of water ingestion to inhibit VP secretion were paralleled by a rapid inhibition of thirst in dogs (35). The same effect likely occurs in rats. For example, water intake by water-deprived rats was reported to be suppressed much more when water was infused into the hepatic portal vein instead of the jugular vein (23). Conversely, dehydrated rats overdrank water when peripheral sensory fibers, including hepatic afferents, were destroyed by systemic treatment with the neurotoxin capsaicin (14). A similar overconsumption of water was observed when water-deprived rats with area postrema lesions were allowed to drink (15; also Refs. 18, 27, 32), and the rats behaved as if they were not detecting an early inhibitory consequence of water intake. Thus these observations allow the hypothesis that ongoing drinking by thirsty rats, like VP secretion, is moderated by postgastric detection of ingested water by intestinal or hepatic portal portal receptors before cerebral osmoreceptors could detect changes in the osmolality of circulating plasma.

The present results confirm and extend many previous reports suggesting that an early detection of ingested water inhibits neurohypophyseal hormone secretion and thirst (e.g., 2, 5, 9, 10, 35). Complementing these findings are more numerous observations on the role of peripheral receptors in stimulating VP secretion and other osmoregulatory responses in rats. For example, small gastric NaCl loads evoke VP secretion (7, 11) and thirst (24) without producing measurable increases in $P_{\text{osmol}}$. Increases in the electrophysiological activity of hepatic afferent nerves and in c-Fos expression in the area postrema are associated with the delivery of NaCl to the stomach and hepatic portal vein (1, 12, 25). Conversely, area postrema lesions blunt the increases in VP and OT secretion stimulated by systemic NaCl loads (13, 21, 22). Moreover, infusions of NaCl into the hepatic portal vein inhibit NaCl intake (36), whereas systemic treatment with capsaicin blunts the normal postingestive inhibition of NaCl intake by rats with salt appetite (14).

Together with the present results, these findings suggest that peripheral Na$^+$ receptors (or osmoreceptors) provide early periprandial sensory information that affects many aspects of osmoregulation in rats, including stimulation of VP and OT secretion, thirst, and inhibition of salt appetite. The apparent location of these receptors in the intestines and/or hepatic portal vein certainly is well situated to provide an “anticipatory” or feed-forward signal to influence osmoregulation before water or osmolytes have been absorbed into the general circulation and detected by cerebral osmoreceptors. The major role of the peripheral receptors may be modulatory rather than stimulatory; that is, they may act to rapidly influence an established secretion or intake or to amplify an incipient response rather than to initiate these responses independently. Such effects may be seen in the substantial reduction in pVP and pOT that occurred in response to the ingestion of water in the present experiments and the substantial increase in water intake that occurred in previous experiments when systemic capsaicin treatment or area postrema lesions disrupted some peripheral satiety signal in dehydrated animals (14, 15). In short, multiple contributions to osmoregulation may be served by peripheral Na$^+$ or osmoreceptors that function as a feed-forward system, complementing the well-known negative feedback system mediated by osmoreceptors in the basal forebrain.

We acknowledge the technical assistance of Ruwani Bandaranayake, Carrie A. Smith, and Eric Logue. This research was supported in part by a National Institute of Mental Health Grant MH-25140.
A preliminary version of this report was presented at the national meeting of the Federation of American Societies for Experimental Biology in San Diego, CA in April 2000.

REFERENCES