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# Osmoregulation in water-deprived rats drinking hypertonic saline: effect of area postrema lesions

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**Stricker, Edward M., Carl F. Craver, Kathleen S. Curtis, Kimberly A. Peacock-Kinzig, Alan F. Sved, and James C. Smith.** Osmoregulation in water-deprived rats drinking hypertonic saline: effect of area postrema lesions. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R831–R842, 2001.—Rats drank rapidly when 0.3 M NaCl was the only drinking fluid available after overnight water deprivation, consuming ~200 ml/24 h. Although such large intakes of this hypertonic solution initially elevated plasma osmolality, excretion of comparable volumes of urine more concentrated than 300 meq Na<sup>+</sup>/l ultimately appears to restore plasma osmolality to normal levels. Rats drank ~100 ml of 0.5 M NaCl after overnight water deprivation, but urine Na<sup>+</sup> concentration (U<sub>Na</sub>) did not increase sufficiently to achieve osmoregulation. When an injected salt load exacerbated the initial dehydration caused by water deprivation, rats increased U<sub>Na</sub> to void the injected load and did not significantly alter 24-h intake of 0.3 or 0.5 M NaCl. Rats with lesions of area postrema had much higher saline intakes and lower U<sub>Na</sub> than did intact control rats; nonetheless, they appeared to osmoregulate well while drinking 0.3 M NaCl but not while drinking 0.5 M NaCl. Detailed analyses of drinking behavior by intact rats suggest that individual bouts were terminated by some rapid postabsorptive consequence of the ingested NaCl load that inhibited further NaCl intake, not by a fixed intake volume or number of licks that temporarily satiated thirst.

oxytocin; thirst; vasopressin

THIRST MOTIVATES DEHYDRATED animals to seek and consume water. Complementing water consumption are other adaptive responses to dehydration, such as urinary water conservation and Na<sup>+</sup> excretion (25). These behavioral and physiological responses are flexible in subserving osmoregulation, so that one response may change to compensate for the absence of another. After an osmotic load, for example, rats lacking kidneys consume much more water than intact rats do and drink sufficient amounts to rapidly restore plasma osmolality (P<sub>osmol</sub>) to normal (14). Conversely, intact animals that ingest little water after an osmotic load compensate in part by increasing urine concentration and solute excretion (17).

Inhibition of the ingestion of food and other sources of osmotic particles is another appropriate behavioral response to dehydration (28). For example, water-deprived rats given only concentrated NaCl solution to drink, although thirsty, should avoid further dehydration by not consuming the saline solution. Nonetheless, >50 years ago Adolph (1) reported that rats do consume concentrated NaCl solution when they are deprived of drinking water. More specifically, rats were allowed to drink 0.25, 0.5, or 1 M NaCl after 1 or 2 days of water deprivation, during which they had free access to dry food. They drank large amounts of 0.25 M NaCl in 24 h, little 1 M NaCl, and intermediate volumes of 0.5 M NaCl; urine concentrations were not reported (1). Although ingestion of hypertonic fluid raises P<sub>osmol</sub> and aggravates dehydration, it is possible that subsequent excretion of the ingested osmolytes in concentrated urine may ameliorate the dehydration. If this is so, then the long-term effects of this seemingly inappropriate drinking behavior actually may be adaptive, even though the early effects are counterproductive.

The present experiments confirmed and extended Adolph's pioneering study by characterizing the osmoregulatory responses of water-deprived rats given only concentrated NaCl solution to drink. *Experiment 1A* examined how much the animals consumed under these conditions, how urinary Na<sup>+</sup> excretion was affected by fluid consumption, and how these responses affected P<sub>osmol</sub>. *Experiment 1B* examined the same variables after an injected NaCl load exacerbated the initial dehydration. In *experiment 1C*, urinary Na<sup>+</sup> excretion and drinking were investigated in rats with surgical lesions of area postrema (AP), a circumventricular organ in the caudal brain stem. Rats with AP lesions (APX) have prominent osmoregulatory dysfunctions, such as impaired secretion of the antidiuretic hormone vasopressin (VP) and the natriuretic hormone oxytocin (OT), and impaired inhibition of food and fluid ingestion, including the intake of concentrated NaCl solution (8, 11, 15, 21). Finally, in *experiment 2*, the intake of NaCl solution was closely monitored to determine the factors that influenced the frequency and size

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of the saline drinking bouts. For these purposes, rats were housed in specially designed cages linked to microprocessors that permitted the continuous monitoring of fluid ingestion by recording licks electronically every 6 s (20).

## METHOD

### Animals

Male Sprague-Dawley rats (275–350 g; Zivic-Miller Laboratories, Zelienople, PA) were housed singly in stainless steel wire-mesh cages in the vivarium of the Department of Neuroscience at the University of Pittsburgh (*experiment 1*) or in the vivarium of the Department of Psychology at Florida State University (*experiment 2*). Both colony rooms were maintained at a constant temperature (22–23°C) and with a fixed light-dark cycle (lights on from 7 AM to 7 PM). All rats had  $\geq 1$  wk of ad libitum access to pelleted laboratory chow (5001, Purina) and water before experiments began.

### Experimental Protocols

Rats were deprived of water overnight (~16 h) and then given concentrated NaCl solution to drink for 24 h. Food was withheld during the drinking test. In *experiment 1*, fluid intakes and urine volumes ( $\pm 0.5$  ml) were monitored hourly for 7 h and then at 24 h. *Experiment 2* used the same procedures, except intakes were monitored continuously for 23 h and urine samples were not collected.

*Experiment 1A.* Rats were weighed and then deprived of drinking water beginning at 5–7 PM. Chow was present overnight but was removed at 9–11 AM on the next day, when the test began. Rats drank 0.3 or 0.5 M NaCl solution ( $n = 8$ /group) from calibrated tubes suspended at the front of the cage. Urine was collected from funnels attached beneath the cages. Urine volumes were recorded, and urine Na<sup>+</sup> concentrations ( $U_{Na}$ , in meq/l) were determined using an Na<sup>+</sup>-sensitive electrode (Electrolyte Analyzer II, Beckman Instruments, Fullerton, CA). The cumulative amount of Na<sup>+</sup> (in meq) excreted in urine by each animal was calculated after 7 and 24 h of the test by multiplying the volume of each sample by its  $U_{Na}$  and then summing the results. The  $U_{Na}$  of the entire volume was calculated by dividing the total amount of Na<sup>+</sup> excreted by the total volume of urine.

Four other groups of rats ( $n = 6$ –8/group) were deprived of water overnight and then given 0.3 or 0.5 M NaCl solution to drink for 7 or 24 h before they were killed by decapitation. Trunk blood was collected and centrifuged, plasma was removed, and plasma Na<sup>+</sup> concentration ( $P_{Na}$ ) was measured using the Na<sup>+</sup>-sensitive electrode. (The stomachs contained <1.0 ml of fluid when harvested from rats euthanized 7 h after access to 0.3 or 0.5 M NaCl.) Control values were obtained from rats that were not water deprived or tested ( $n = 6$ ) and from rats that were deprived of water overnight but then given nothing to drink ( $n = 6$ ). In addition to  $P_{Na}$  values,  $P_{osmol}$  was measured by freezing-point depression (micro-Osmette, Precision Systems, Natick, MA) in these latter two groups.

Blood samples were not used for direct measurement of  $P_{osmol}$  in rats that consumed concentrated saline solutions. Instead,  $P_{osmol}$  (in mosmol/kgH<sub>2</sub>O) was estimated from the amounts of water and Na<sup>+</sup> that were consumed in the NaCl solution and excreted in urine by a formula used previously for this purpose (6)

$$P_{osmol} = 1,000 \times \frac{[(0.69)(\text{body weight})(0.306)] + [(2)(Na_{in} - Na_{out})]}{[(0.69)(\text{body weight})] + [H_2O_{in} - H_2O_{out}]}$$

where  $Na_{in}$  is the amount of Na<sup>+</sup> ingested (in meq),  $Na_{out}$  is the amount of Na<sup>+</sup> excreted in urine (in meq),  $H_2O_{in}$  is the volume of fluid consumed (in ml), and  $H_2O_{out}$  is urine volume (in ml). An initial  $P_{osmol}$  was assumed to be 306 mosmol/kgH<sub>2</sub>O, the measured value in control rats after overnight water deprivation, and body water was assumed to be 69% body weight.

*Experiment 1B.* Other water-deprived rats were given an acute NaCl load to determine how it would affect intake of concentrated saline and osmoregulation. Rats were deprived of water overnight, as in *experiment 1A*, but just before the drinking test they were injected intraperitoneally with hypertonic saline (HS; 2 ml of 2 M NaCl). Food was removed, and then rats were allowed to drink 0.3 or 0.5 M NaCl ( $n = 7$  and 6, respectively). Intakes, urine volumes, and  $U_{Na}$  were measured hourly for 7 h and then again at 24 h. Blood samples were not taken from HS-treated rats, but  $P_{osmol}$  was estimated using the formula described previously.

*Experiment 1C.* After recovery of preoperative body weight and behavioral assessment of the effectiveness of the lesions, rats with APX were in the same weight range as the neurologically intact rats in *experiments 1A* and *1B*. Rats with APX similarly were deprived of water overnight and then given only 0.3 or 0.5 M NaCl to drink. APX had been produced by vacuum aspiration, as described previously (11). Briefly, each rat was anesthetized with Equithesin [3.0 ml/kg body wt ip of a solution containing pentobarbital sodium (0.98 g/dl), chloral hydrate (4.25 g/dl), and MgSO<sub>4</sub> (2.12 g/dl)], and its head was placed in a stereotaxic instrument with the nose pointed down. A small, dorsal midline incision was made, the foramen magnum was enlarged, and the meninges were incised. AP was visualized through an operating microscope and aspirated with a blunted 25-gauge needle. Muscle and skin then were sutured, and a broad-spectrum antibiotic (penicillin, 30,000 U im) was administered.

Some rats required sweetened liquid foods for several weeks after APX to encourage eating and thereby prevent extreme loss of body weight. When rats recovered their preoperative body weights, up to 6 wk later, the effectiveness of the lesions was assessed by the failure of LiCl to suppress water intake elicited by water deprivation (10) and by spontaneous intakes of 0.5 M NaCl in the range of 21–57 ml daily (mean 37 ml) (8).

The rats with chronic APX ( $n = 15$ ) were deprived of water overnight as in *experiment 1A*. Food was removed from their cages, and the animals were given access to 0.3 M NaCl to drink. Intakes, urine volumes, and  $U_{Na}$  were measured hourly for 7 h and once again at 24 h. One week later, a subset of rats with APX ( $n = 7$ ) again was deprived of water overnight, and food was removed from their cages, but this time they were given 0.5 M NaCl to drink. Fluid intakes, urine volumes, and  $U_{Na}$  were measured hourly for 7 h. The  $P_{osmol}$  of the rats with APX in these two studies was estimated as described in *experiment 1A*. After completion of testing, rats with APX were anesthetized with an overdose of Equithesin and perfused intracardially with 0.15 M NaCl followed by 10% formalin solution. Brain stems were removed and cut into 33- $\mu$ m sections along the rostral-caudal extent corresponding to AP. Sections were mounted and stained for Nissl substance with neutral red or cresyl violet.

*Experiment 2.* To obtain insight into how dehydrated rats consumed concentrated NaCl solution under these testing

conditions, individual drinking bouts were examined in detail. Five rats were maintained on ad libitum food (powdered Purina chow) and water for 1 wk to accustom them to the special cages in which studies were conducted (20). Then they were deprived of drinking water overnight, as in *experiment 1A*. On the following morning, food was removed from the cages, and rats were allowed access to one of four drinking fluids for 23 h: 0.3 M NaCl, 0.5 M NaCl, 0.7 M NaCl, or water, studied in that order at weekly intervals. (The last hour of each 24-h block was used to clean the cages, refill the food bins and bottles, and weigh the animals.) Subsequently, the test fluid was removed, and food and water were returned and remained available ad libitum until the next test.

Strip charts were plotted for each animal, depicting an overall pattern of eating and drinking during the 23-h period. Bout and episode criteria for food and fluid ingestion were established as described previously (21). Briefly, drinking bouts were defined operationally as a period of fluid consumption that contained at least eight licks ( $\sim 0.05$  ml). Feeding bouts were defined as a period in which the rat's head interrupted the photobeam above the food jar for  $\geq 8$  s (i.e., a minimum of  $\sim 45$  mg of food was consumed). Neither drinking nor feeding bouts had upper limits, and both varied widely. A given intake bout ended when the animal switched from consuming one substance to another or when there was a pause of  $\geq 5$  min between ingestive bouts. An ingestive episode contained one or more of these feeding or drinking bouts and ended when no such activity occurred for 5 min.

Analysis of data collected on the 4 test days focused on the size and frequency of the drinking bouts that occurred during the 12-h dark period. Additional analyses were made of the eating and drinking bouts during the first ingestive episode that occurred when food and water were returned to the cage after each test day. Mean food and water intakes on the days preceding each test also were noted.

### Calculations

The formula mentioned previously was used to calculate the amount of  $\text{Na}^+$  that must be excreted in urine ( $\text{Na}_{\text{out}}$ ) to void the ingested NaCl load and restore  $P_{\text{osmol}}$  to normal values, given observed fluid intakes and urine volumes. Theoretical lines relating urine volume and  $U_{\text{Na}}$  were computed for each group in *experiment 1*.

Body weight-matched control rats that were not water deprived had basal  $P_{\text{osmol}}$  of  $299 \pm 1$  mosmol/kg and a mean body weight of  $348 \pm 7$  g. If it is assumed that body water contributes 69% of total body weight (14), control rats had 240 ml of body water ( $348 \text{ g} \times 0.69 \times 1 \text{ ml/g}$ ) containing 71.8 mosmol (i.e.,  $240 \text{ ml} \times 0.299 \text{ mosmol/ml}$ ). Rats deprived of water overnight had measured  $P_{\text{osmol}}$  of  $306 \pm 1$  mosmol/kg. To simplify the calculations, we assumed that only water was lost during the deprivation period, in which case total body water was reduced by 5.4 ml ( $71.8 \text{ mosmol}/0.306 \text{ mosmol/ml} = 234.6 \text{ ml}$ ). Thus rats must conserve that amount of water while excreting concentrated urine to restore body fluid osmolality to normal. [Rats actually lose osmolytes and water during the 24-h deprivation period (19). However, if rats were assumed to attain a  $P_{\text{osmol}}$  of 0.306 mosmol/ml by losing reasonable amounts of osmolytes and water, then the following estimates of  $U_{\text{Na}}$  would be only  $\sim 1\%$  less than those indicated.]

As noted in RESULTS, rats drinking 0.3 M NaCl ingested  $\sim 20$  ml more than the volume they excreted in urine. Thus to restore body fluid osmolality, it was necessary for rats to conserve 4.37 mosmol for the extra 14.6 ml to be in osmotic balance ( $4.37 \text{ mosmol}/14.6 \text{ ml} = 0.299 \text{ mosmol/ml}$ ). With

those values, it is possible to estimate the  $U_{\text{Na}}$  required for osmoregulation over a range of intake volumes. For example, if a rat drinks 30 ml of 0.3 M NaCl (which contains 18.0 mosmol) and retains 20 ml of fluid containing 4.37 mosmol, then it must excrete 13.63 mosmol in 10 ml of urine (1,363 mosmol/l, or 682 meq  $\text{Na}^+$ /l) to osmoregulate. If a rat drinks 100 ml of 0.3 M NaCl (60 mosmol) and retains 20 ml containing 4.37 mosmol, it must excrete 55.63 mosmol in 80 ml of urine (695 mosmol/l, or 348 meq  $\text{Na}^+$ /l). If a rat drinks 200 ml of 0.3 M NaCl (120 mosmol) and retains 20 ml containing 4.37 mosmol, it must excrete 115.63 mosmol in 180 ml of urine (642 mosmol/l, or 321 meq  $\text{Na}^+$ /l).

Similarly, rats drinking 0.5 M NaCl ingested  $\sim 2$  ml more than the volume they excreted in urine, so for osmoregulation their urine had to contain 1.1 mosmol plus 1 mosmol for every 1 ml they consumed [ $(71.8 - 1.1) \text{ mosmol}/(234.6 + 2.0) \text{ ml} = 0.299 \text{ mosmol/ml}$ ]. Thus, for example, a rat that drinks 12 ml of 0.5 M NaCl (12.0 mosmol) must excrete 13.1 mosmol in 10 ml of urine (1,310 mosmol/l, or 655  $\text{Na}^+$  meq/l). A rat that drinks 102 ml of 0.5 M NaCl (102.0 mosmol) must excrete 103.1 mosmol in 100 ml of urine (1,031 mosmol/l, or 515.5  $\text{Na}^+$  meq/l).

Rats with APX drinking 0.5 M NaCl ingested  $\sim 2$  ml less than the volume they excreted in urine, so for osmoregulation their urine had to contain 2.3 mosmol plus 1 mosmol for every 1 ml they consumed [ $(71.8 - 2.3) \text{ mosmol}/(234.6 - 2.0) \text{ ml} = 0.299 \text{ mosmol/ml}$ ]. Thus, for example, a rat with APX that drinks 8 ml of 0.5 M NaCl (8.0 mosmol) must excrete 10.3 mosmol in 10 ml of urine (1,030 mosmol/l, or 515  $\text{Na}^+$  meq/l). A rat that drinks 98 ml of 0.5 M NaCl (98.0 mosmol) must excrete 100.3 mosmol in 100 ml of urine (1,003 mosmol/l, or 501.5  $\text{Na}^+$  meq/l).

Finally, for rats drinking 0.3 or 0.5 M NaCl after injection of 2 ml of 2 M NaCl, the theoretical  $U_{\text{Na}}$  may be calculated by adding 2 ml (the injected volume) to the initial body fluid volume and 8 mosmol (the injected NaCl load) to the total initial number of milliosmoles contained in that volume. As might be expected, the calculated  $U_{\text{Na}}$  paralleled the values computed when rats were not treated with HS but were higher at each urine volume.

In each case, the urinary retention of a hypotonic solution containing a fixed amount of milliosmoles and volume from the ingested fluid will produce theoretical  $U_{\text{Na}}$  values that exceed the concentration of the NaCl solution that is consumed. Furthermore, those  $U_{\text{Na}}$  values will progressively diminish toward the concentration of the NaCl solution that is consumed as intake and urine volumes increase.

### Statistics

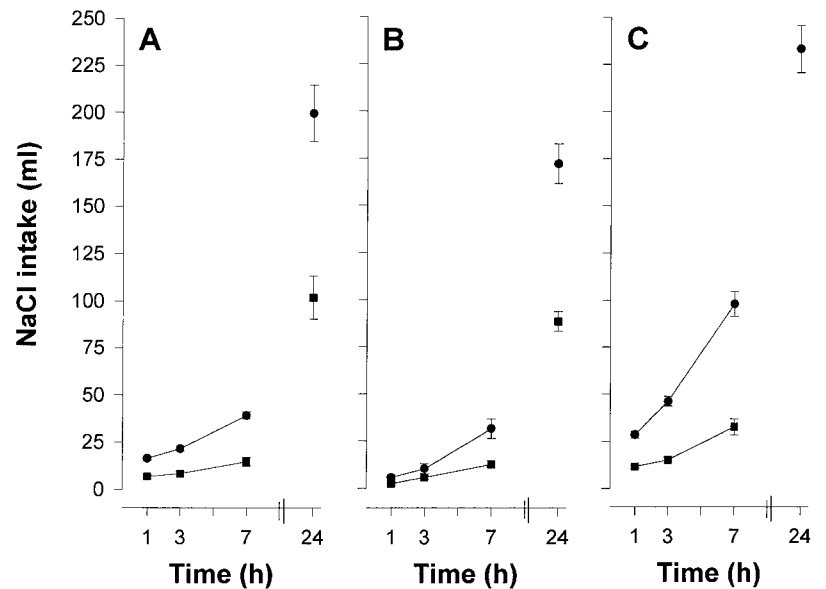
Values are group means  $\pm$  SE or individual data points. Statistical comparisons were made using the appropriate ANOVA methods, *t*-tests, or  $\chi^2$  analysis of percent incidence or, when variability was greater, by the corresponding non-parametric methods (Kruskal-Wallis 1-way ANOVA on ranks or Mann-Whitney *U* test). When a statistically significant difference ( $P < 0.05$ ) existed, pairwise comparisons were made using the Student-Newman-Keuls or Tukey's honestly significant difference method. Pairwise differences were considered to be statistically significant when  $P < 0.05$ .

## RESULTS

### Experiment 1A

Figure 1A depicts the cumulative 24-h intakes of 0.3 and 0.5 M NaCl by rats after overnight water deprivation. Rats consumed substantial quantities of these

Fig. 1. Cumulative volumes of 0.3 M (●) or 0.5 M NaCl solution (■) consumed by rats in 24 h after overnight water deprivation ( $n = 8-15/\text{group}$ ). **A:** rats drank substantial amounts of saline, especially during the final 17 h. **B:** other water-deprived rats were injected with hypertonic saline (HS; 2 ml of 2 M NaCl ip) just before the test. Drinking was reduced in the first 3 h but not subsequently. **C:** intakes of rats with area postrema lesions (APX). These rats consumed large amounts of both fluids in the first 7 h of the test. Values are means  $\pm$  SE.



two fluids:  $200 \pm 15$  ml of 0.3 M NaCl and  $102 \pm 11$  ml of 0.5 M NaCl ( $P < 0.001$  compared with one another). Most of the drinking occurred during the last 17 h of the test, which included the 12-h dark period. Rats also excreted urine in substantial volumes that paralleled fluid intakes (Fig. 2):  $180 \pm 13$  ml in 24 h when drinking 0.3 M NaCl and  $101 \pm 11$  ml when drinking 0.5 M NaCl. After the first 2 h, the  $U_{\text{Na}}$  was greater by  $\sim 100$  meq/l when rats drank 0.5 M NaCl instead of 0.3 M NaCl (Fig. 3A). The  $U_{\text{Na}}$  of the individual samples from the eight rats that drank 0.3 M NaCl always was  $>300$  meq/l (i.e., the concentration of the drinking fluid) after the 1st h. In contrast, the  $U_{\text{Na}}$  of the eight rats that drank 0.5 M NaCl was  $>500$  meq/l in fewer than one-third of the individual samples collected dur-

ing the first 7 h and in only one of the eight samples collected thereafter.

Calculated  $U_{\text{Na}}$  of urine excreted in 7 and 24 h was plotted as a function of cumulative urine volumes at those times. For rats drinking 0.3 M NaCl,  $U_{\text{Na}}$  ranged

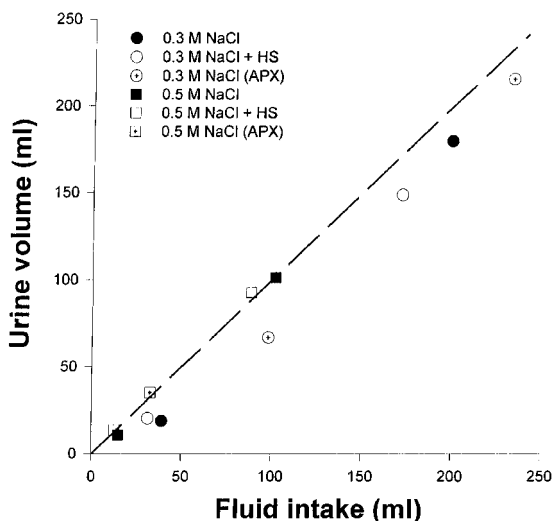


Fig. 2. Mean urine volumes plotted as a function of mean intakes of 0.3 or 0.5 M NaCl by water-deprived rats after 7 or 24 h of the test. Some rats were pretreated with HS, others had APX. Intakes are shown in Fig. 1. Symbols represent different groups at the two times; 7-h values are at *bottom left*. Diagonal line represents equality between fluid intake and urine volume.

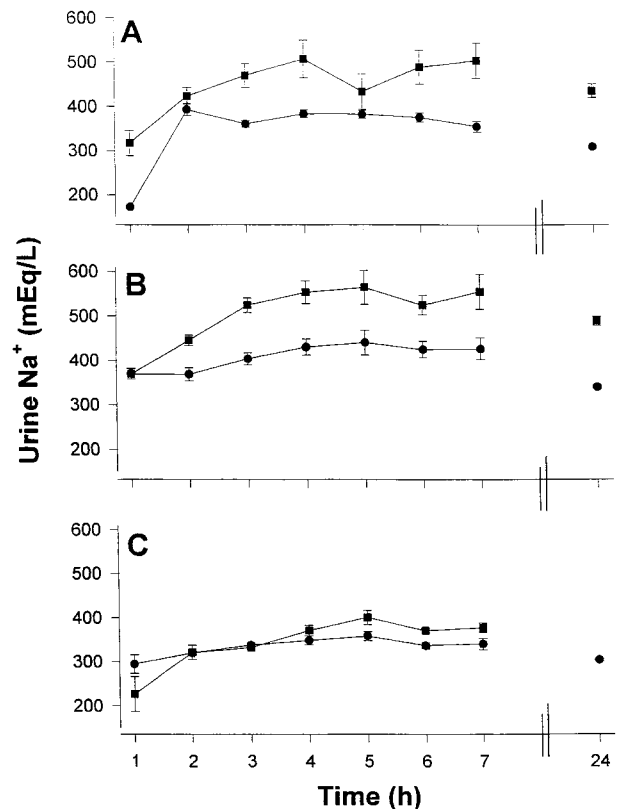


Fig. 3. **A:** urine  $\text{Na}^+$  concentrations ( $U_{\text{Na}}$ ) of water-deprived rats drinking 0.3 M (●) or 0.5 M NaCl solution (■) in samples collected hourly for 7 h and then after 24 h ( $n = 6-8/\text{group}$ ). Also shown are similar values for rats pretreated with HS (**B**) and rats with APX (**C**). Intakes are shown in Fig. 1, and urine volumes are shown in Fig. 2. Values are means  $\pm$  SE.

from ~330 to ~400 meq/l at 7 h, with greater urine volumes associated with lower  $U_{Na}$  (Fig. 4A). These values were much lower than the theoretical  $U_{Na}$  required for osmoregulation, but by 24 h they approximated those values (Fig. 4A). When rats drank 0.5 M NaCl, the  $U_{Na}$  at 7 h similarly was much lower than the theoretical  $U_{Na}$  required for osmoregulation, but by 24 h it asymptoted ~100 meq/l below the values required for osmoregulation (Fig. 5A).

As expected, overnight water deprivation caused measured  $P_{Na}$  to increase above control values from nondeprived rats ( $P < 0.05$ ). As shown in Table 1, the estimated  $P_{osmol}$  of rats that drank 0.3 M NaCl was significantly elevated after 7 h ( $P < 0.01$ ), but not 24 h, compared with values from rats that were not water deprived. In contrast, the  $P_{osmol}$  of rats that drank 0.5 M NaCl was higher than control values after 7 and 24 h (both  $P < 0.01$ ). Parallel effects in  $P_{Na}$  also were observed.

### Experiment 1B

After overnight water deprivation, rats injected intraperitoneally with HS suppressed their intakes of 0.3 M NaCl during the first 3 h of the test (Fig. 1B;

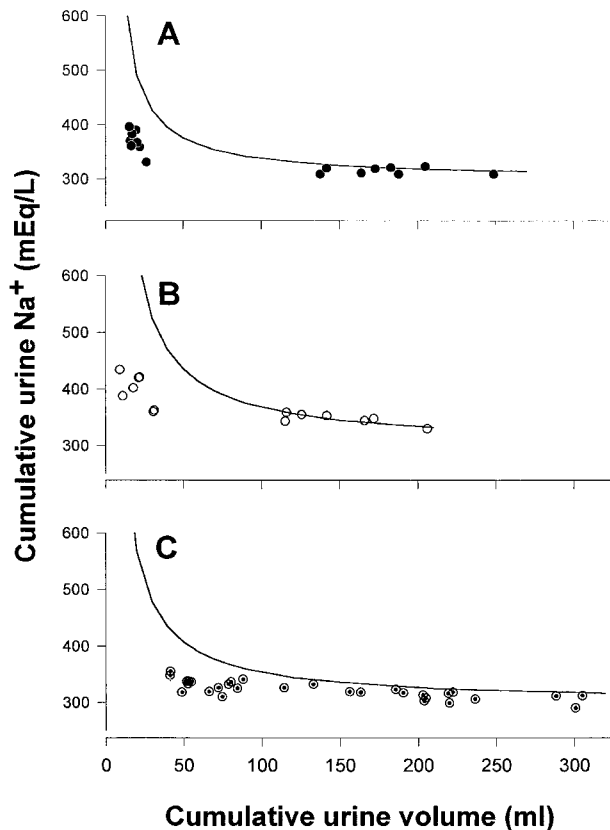


Fig. 4. A: calculated  $U_{Na}$  in 7 and 24 h by water-deprived rats drinking 0.3 M NaCl plotted as a function of cumulative urine volumes at those times. Also shown are similar values for rats pretreated with HS (B) and rats with APX (C). Fluid intakes were shown in Fig. 1, urine volumes in Fig. 2, and hourly  $U_{Na}$  in Fig. 3. Symbols represent individual animals; 7-h values are clustered to left of 24-h values. Lines represent theoretical  $U_{Na}$  required for osmoregulation.

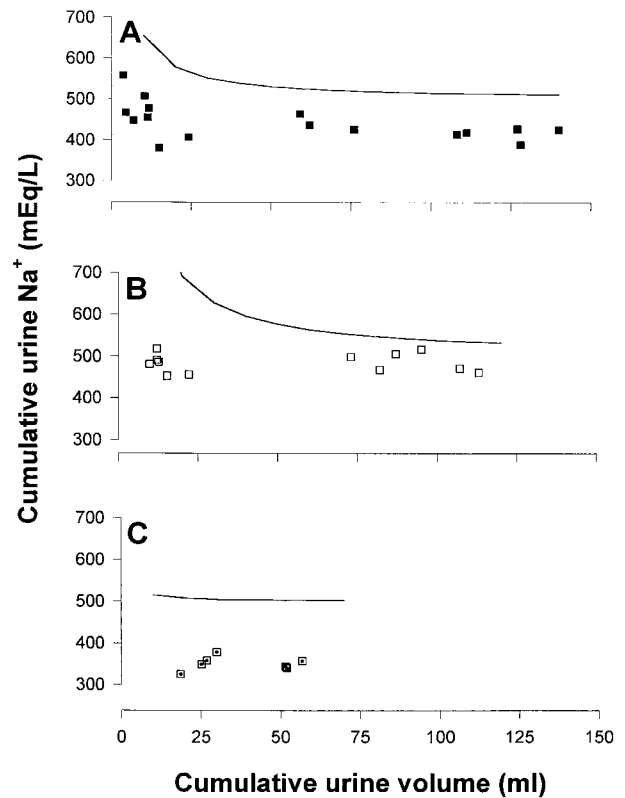


Fig. 5. A: calculated  $U_{Na}$  in 7 and 24 h by water-deprived rats drinking 0.5 M NaCl plotted as a function of cumulative urine volumes at those times. Also shown are similar values for rats pretreated with HS (B) and rats with APX (C). Fluid intakes are shown in Fig. 1, urine volumes in Fig. 2, and hourly  $U_{Na}$  in Fig. 3. Symbols represent individual animals; 7-h values are clustered to left of 24-h values. Lines represent theoretical  $U_{Na}$  required for osmoregulation.

$P < 0.01$  compared with volumes consumed by rats not given HS). However, these two groups drank comparably large volumes in 24 h. Similarly, rats injected with HS consumed less 0.5 M NaCl in 3 h ( $P < 0.05$ ), but not in 24 h, than did rats not given HS (Fig. 1B).

Table 1.  $P_{Na}$  and estimated  $P_{osmol}$  in water-deprived rats given 0.3 or 0.5 M NaCl to drink for 7 or 24 h

Drinking Fluid	7 h		24 h	
	$P_{Na}$ , meq/l	Estimated $P_{osmol}$ , mosmol/kg	$P_{Na}$ , meq/l	Estimated $P_{osmol}$ , mosmol/kg
0.3 M NaCl	$146 \pm 1$	$315 \pm 2^*$	$143 \pm 1$	$301 \pm 2$
+HS		$332 \pm 2^\dagger$		$304 \pm 1$
APX		$315 \pm 2^*$		$306 \pm 3$
0.5 M NaCl	$147 \pm 1$	$318 \pm 4^*$	$158 \pm 4^\dagger$	$366 \pm 9^\dagger$
+HS		$326 \pm 2^\dagger$		$326 \pm 3^\dagger$
APX		$345 \pm 7^\dagger$		

Values are means  $\pm$  SE. Some rats were injected intraperitoneally with 2 ml of 2 M NaCl [hypertonic saline (HS)], and some rats had area postrema lesions (APX). Rats were killed by decapitation immediately after the drinking test, and trunk blood samples were taken for analysis of plasma  $Na^+$  concentration ( $P_{Na}$ ,  $n = 4-6$ /group). Plasma osmolality ( $P_{osmol}$ ) was computed from water and  $Na^+$  balances during the drinking tests ( $n = 7-15$ /group). Measured values for water-deprived rats given nothing to drink:  $143 \pm 1$  meq/l and  $306 \pm 1$  mosmol/kg; values for nondeprived control rats:  $141 \pm 1$  meq/l and  $299 \pm 1$  mosmol/kg. \* $P < 0.01$ ;  $^\dagger P < 0.001$  vs. nondeprived control rats.

HS-treated rats also excreted urine in volumes that paralleled fluid intakes (Fig. 2). Hourly  $U_{Na}$  at 3–7 h of the test (Fig. 3B) was higher than  $U_{Na}$  from rats not given HS (Fig. 3A), whether rats drank 0.3 M NaCl ( $\chi^2 = 11.91$ ,  $P < 0.001$ ) or 0.5 M NaCl ( $\chi^2 = 5.99$ ,  $P < 0.02$ ). Similar results were observed at 24 h ( $\chi^2 = 15.00$ ,  $P < 0.001$  when rats drank 0.3 M NaCl;  $\chi^2 = 5.53$ ,  $P < 0.02$  when rats drank 0.5 M NaCl). After the first 2 h, the  $U_{Na}$  was  $\sim 120$  meq/l higher when rats drank 0.5 M NaCl instead of 0.3 M NaCl. After 7 h, the  $U_{Na}$  of HS-treated rats drinking 0.3 or 0.5 M NaCl was much lower than the theoretical  $U_{Na}$  required for osmoregulation (Figs. 4B and 5B). In contrast, after 24 h, the  $U_{Na}$  of HS-treated rats drinking 0.3 M NaCl approximated the values required for osmoregulation, whereas it was  $\sim 60$  meq/l below those values when rats drank 0.5 M NaCl (Figs. 4B and 5B).

As shown in Table 1, water-deprived HS-treated rats that drank 0.3 or 0.5 M NaCl had an estimated  $P_{osmol}$  that was significantly elevated at 7 h (both  $P < 0.001$ ). The estimated  $P_{osmol}$  of rats that drank 0.3 M NaCl had returned to levels at 24 h that were comparable to those of rats not given HS treatment. In contrast, the estimated  $P_{osmol}$  of rats that drank 0.5 M NaCl remained elevated then ( $P < 0.05$ ) but much less markedly than in rats not given HS treatment ( $P < 0.001$ ).

#### Experiment 1C

As shown in Fig. 1C, rats with APX drank  $233 \pm 13$  ml of 0.3 M NaCl in 24 h after overnight water deprivation ( $P < 0.05$  compared with the intakes of neurologically intact rats shown in Fig. 1A). Rats with APX ingested  $33 \pm 4$  ml of 0.5 M NaCl during the 7-h test period, which was twice the amount consumed by intact rats ( $P < 0.001$ ).

The cumulative urine volumes excreted by rats with APX were significantly greater than those of intact rats (all  $P < 0.001$ ) in association with their larger intakes (Fig. 2). During the first 7 h of the test, the hourly urine samples of rats with APX had much lower  $Na^+$  concentrations than the  $U_{Na}$  from intact rats, whether 0.3 or 0.5 M NaCl was consumed (both  $P < 0.001$ ). In marked contrast to the other groups, the  $U_{Na}$  from rats with APX was comparable regardless of which fluid they drank (Fig. 3C). The  $U_{Na}$  when rats with APX drank 0.3 M NaCl for 7 h was 50–75 meq/l lower than the theoretical  $U_{Na}$  required for osmoregulation, but after 24 h it was only  $\sim 15$  meq/l below those values (Fig. 4C). When rats with APX drank 0.5 M NaCl for 7 h,  $U_{Na}$  was  $\sim 150$  meq/l below the values required for osmoregulation (Fig. 5C).

As shown in Table 1, the estimated  $P_{osmol}$  of water-deprived rats with APX when drinking 0.3 M NaCl was comparable to that of control rats at 7 and 24 h. However, the estimated  $P_{osmol}$  of rats with APX when drinking 0.5 M NaCl was much more elevated at 7 h than that of control rats ( $P < 0.001$ ). Consequently, the test was terminated then to avoid even more severe dehydration.

Histological assessment of the brain stems from rats with APX in the present study revealed lesions that were similar in every respect to the circumscribed APX described in detail in a previous report (see Figs. 1, 2B, and 2C in Ref. 8). Destruction of AP appeared to be complete in each rat with APX. Although damage in the caudal brain stem was not confined to AP, it did not extensively invade the subadjacent nucleus of the solitary tract.

#### Experiment 2

Typically, 80–85% of daily food and water intakes were consumed during the dark period. Thus, on the day before each of the 4 test days, 24-h water intakes were much lower during overnight water deprivation ( $6.4 \pm 0.5$  ml) than daily intakes on the preceding days ( $59.6 \pm 1.8$  ml,  $P < 0.001$ ). Food intake also was significantly less on those days ( $23.0 \pm 0.4$  and  $37.3 \pm 0.7$  g, respectively,  $P < 0.001$ ). This latter change was due to a marked decrease in the amount of food consumed per bout when drinking water was withheld but no significant decrease in the number or duration of eating bouts (i.e., the rate of eating was diminished).

After overnight water deprivation, rats began to drink as soon as fluid became available, although the amounts consumed depended on which fluid was presented. In 30 min, cumulative licks were  $3,440 \pm 258$  of 0.3 M NaCl,  $1,799 \pm 219$  of 0.5 M NaCl,  $697 \pm 82$  of 0.7 M NaCl, and  $2,859 \pm 149$  of water (all  $P < 0.05$  compared with one another). The drinking bouts were of comparable lengths (18–22 min) regardless of which fluid was consumed. Thus differences in intakes reflect differences in the number and length of pauses that occurred within the drinking bouts. During the remainder of the light period, ingestion was infrequent and occurred in smaller bouts but increased conspicuously during the dark period (Fig. 6), similar to the results of *experiment 1A* (Fig. 1A). Representative strip charts shown in Fig. 7 make evident the relatively large drinking bout at the beginning of the test and the predominance of fluid ingestion during the 12-h dark period.

Analysis of drinking bouts focused on the fluid ingestion that occurred during the dark period. As indicated in Table 2, water-deprived rats drank water in 29.4 bouts that averaged 1.4 ml and occurred at intervals of 22.2 min. When given concentrated NaCl solution to drink, the rats had roughly the same number of bouts and the same interbout intervals throughout the dark period; however, the time spent drinking per bout and, therefore, the estimated volume ingested per bout were inversely related to the concentration of saline. Thus a relatively stable amount of NaCl was consumed in each drinking bout:  $>75\%$  of the bouts contained  $<2.0$  meq of  $Na^+$ , with median values of 1.1 meq regardless of NaCl concentration.

Figure 8 shows representative strip charts displaying the first ingestive episode that occurred when food and water were returned to rats after the tests. All rats that had ingested water or 0.3 M NaCl ate food without

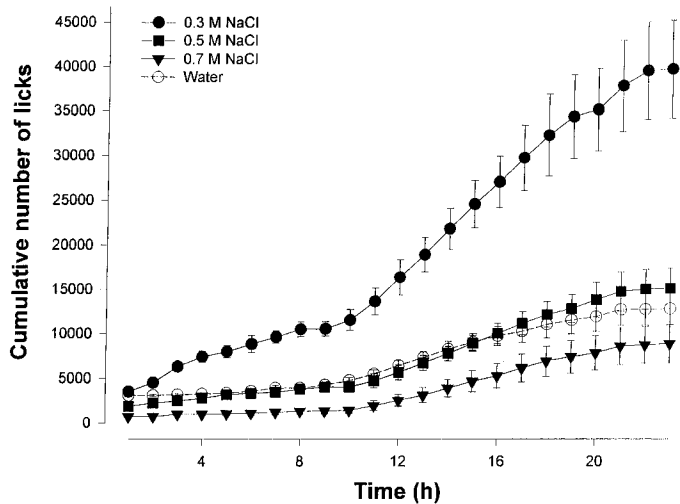


Fig. 6. Mean cumulative intakes, in total licks, of 0.3, 0.5, or 0.7 M NaCl solution or water by rats after overnight water deprivation ( $n = 5/\text{group}$ ). Although the intakes of each rat were monitored every 6 s, hourly intakes are presented for purposes of convenience. Intakes in milliliters can be estimated by dividing these values by 160. Those values resemble the relevant volumes consumed by comparably treated rats, shown in Fig. 1A. At 23 h, statistically reliable differences were observed between intake of 0.3 M NaCl and intake of the other three fluids (all  $P < 0.05$ ). The dark period began  $\sim 10$  h after onset of the 23-h observation period and lasted 12 h.

first ingesting water, whereas all rats that had consumed 0.5 or 0.7 M NaCl drank water first. Rats in the two latter groups drank water in large bouts that lasted  $\sim 6$  min and provided  $\sim 10$  ml and then began to eat within 25 s after they stopped drinking. Subsequently, they alternated bouts of eating and drinking before the ingestive episode ceased. Rats ate comparable amounts of food during this episode regardless of what had been the test fluid but drank much more water if they had consumed 0.5 or 0.7 M NaCl during the test (Table 3; both  $P < 0.001$ ).

## DISCUSSION

During a forced period of water deprivation, rats excrete osmolytes and thereby blunt the increase in  $P_{\text{osmol}}$  (19). When again allowed access to drinking water, they consume water rapidly and, in doing so, restore body fluid osmolality to normal. In the present series of experiments, water-deprived rats were given 0.3 or 0.5 M NaCl solution to drink instead of water. Rather than avoid these concentrated solutions and the consequent increase in plasma osmolality, the water-deprived rats ingested very large amounts of each fluid during 24-h tests. Mean intake volumes of 200 ml of 0.3 M NaCl and 102 ml of 0.5 M NaCl contain 60 and 51 meq of  $\text{Na}^+$ , respectively, which are more than five times the amount of  $\text{Na}^+$  normally present in the extracellular fluid of adult rats. These results confirm and extend Adolph's (1) finding that water-deprived rats consume large quantities of concentrated NaCl solutions when drinking water is not available.

## Osmoregulation by Water-Deprived Rats

Rats ingesting 0.3 M NaCl corrected their dehydration, despite consuming a prodigious NaCl load. They osmoregulated by excreting the ingested load in urine that was more concentrated than the drinking fluid and was  $\sim 20$  ml smaller in volume. Although the excreted urine was not sufficiently concentrated to restore estimated  $P_{\text{osmol}}$  to normal by 7 h of the test, rats continued to drink the hypertonic fluid in large volumes. The more fluid they consumed, the more concentrated urine they excreted and, therefore, the less concentrated their urine had to be to conserve enough urinary water for osmoregulation. Ultimately, rats ingested so much fluid that estimated  $P_{\text{osmol}}$  was restored to normal, even though  $U_{\text{Na}}$  was not much more than 300 meq  $\text{Na}^+/\text{l}$  during the last 17 h of the test.

The  $U_{\text{Na}}$  from rats that drank 0.5 M NaCl usually was comparable to that from rats that drank 0.3 M NaCl during the first 2 h of the drinking test. Thereafter, rats that consumed 0.3 M NaCl maintained  $U_{\text{Na}}$  at concentrations above that of the ingested fluid, whereas rats that drank 0.5 M NaCl did not sustain  $U_{\text{Na}}$  at levels above the  $\text{Na}^+$  concentration of the drinking fluid. Thus it is possible that rats adaptively limited intake of 0.5 M NaCl to avoid more severe dehydration.

Neurohypophyseal secretion of VP and OT is sensitive to 1–2% increases in  $P_{\text{osmol}}$  (12, 24). Similarly, renal mechanisms for voiding an NaCl load in concentrated urine are exquisitely responsive to small changes in VP and OT (18, 29). Secretion of VP and OT is controlled by the extent of plasma hyperosmolality, which, in the present experiments, was largely influenced by how much of the concentrated NaCl solution the rats drank. After 7 h, estimated  $P_{\text{osmol}}$  was elevated to levels above those expected to stimulate secretion of both hormones. Those  $P_{\text{osmol}}$  (and measured  $P_{\text{Na}}$ ) were similar whether rats drank 0.3 or 0.5 M NaCl, and their plasma levels of VP and OT likely were similar then too. Thus it is of interest that the  $U_{\text{Na}}$  of the two groups was so different. It seems probable that the  $U_{\text{Na}}$  from rats that drank 0.3 M NaCl was lower than  $U_{\text{Na}}$  from rats that drank 0.5 M NaCl owing to the much greater volumes of saline they consumed and the much greater volumes of urine they excreted in a solute diuresis.

When water-deprived rats were further challenged with an injected NaCl load, the animals did not increase their fluid intakes, despite a presumed increase in thirst. Instead, rats given 0.3 or 0.5 M NaCl to drink reduced their intake at first, although thereafter they drank as much saline as animals not given HS. These HS-treated rats had much higher  $U_{\text{Na}}$  than did their counterparts not given HS, perhaps reflecting greater levels of plasma VP and OT, resulting from higher  $P_{\text{osmol}}$ . By excreting more concentrated urine in volumes similar to those ingested, these rats not only eliminated the 4 meq of  $\text{Na}^+$  from the injected load but additionally excreted more than normal amounts of the



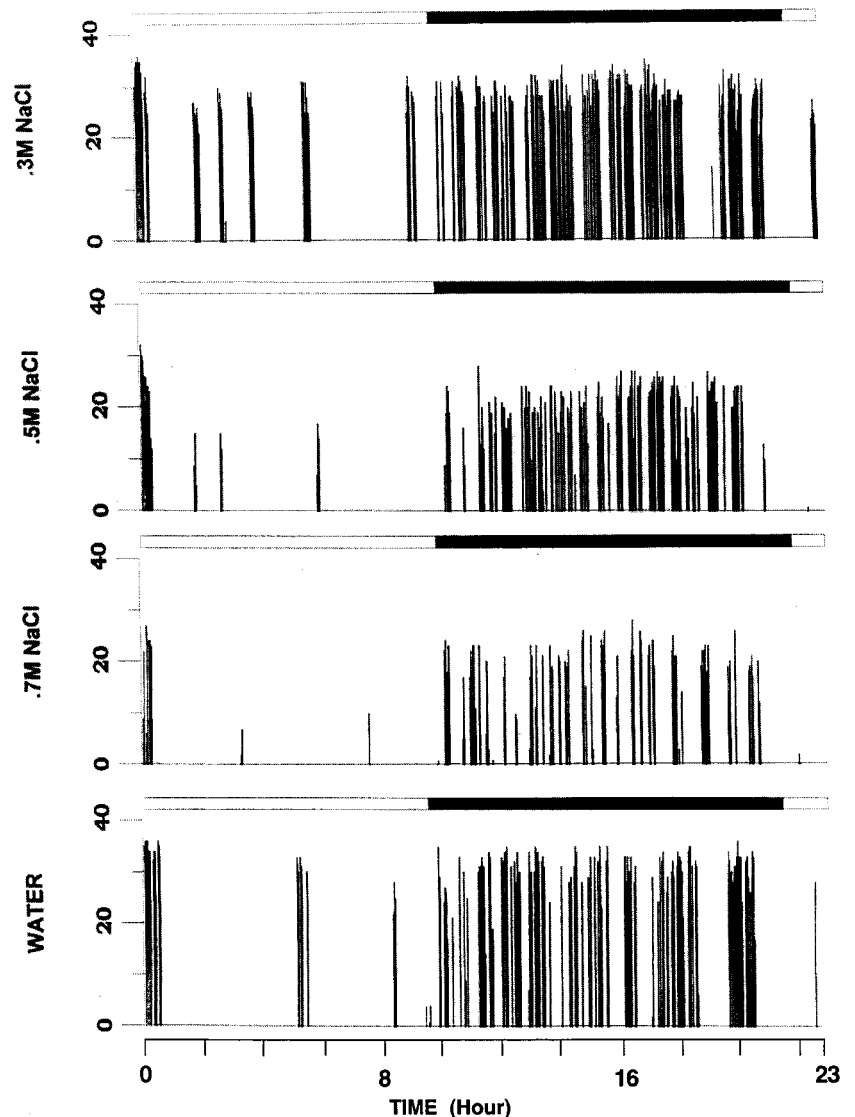


Fig. 7. Incidence of drinking bouts when 0.3 M, 0.5 M, or 0.7 M NaCl solution or water was consumed by rats during the 23-h period after overnight water deprivation. Representative strip charts highlight the burst of drinking at the beginning of the test and the predominance of fluid ingestion during the 12-h dark period (solid horizontal bar). The *x*-axis is blocked in 6-s bins; the *y*-axis indicates licks per 6-s bin.

ingested NaCl load. In fact, HS-treated rats drinking 0.5 M NaCl appeared to osmoregulate much better in 24 h than did rats that were not pretreated with HS.

Rats with APX consumed much more 0.5 M NaCl daily than control rats did when drinking water was

available, as reported previously (7, 8). Those NaCl intakes were associated with focal damage to the AP (7, 8, 13) that appears to disrupt postingestive feedback inhibition of NaCl intake (8, 21). In *experiment 1C*, when animals were deprived of water, rats with APX

Table 2. *Intakes, bout frequencies, and bout sizes of water-deprived rats ingesting water or 0.3, 0.5, or 0.7 M NaCl*

	Water	NaCl		
		0.3 M	0.5 M	0.7 M
Intake, ml/23 h	66.5 ± 9.8	194.1 ± 27.0	125.9 ± 11.4	65.5 ± 8.5
No. of bouts				
Day	7.4 ± 0.8	7.0 ± 0.8	6.8 ± 1.4	6.2 ± 1.4
Night	29.4 ± 2.1	27.4 ± 2.1	31.0 ± 3.4	27.2 ± 4.1
IBI (night), min	22.2 ± 1.8	18.6 ± 2.2	18.4 ± 3.1	23.7 ± 4.2
Bout duration (night), min	3.4 ± 0.7*	6.7 ± 0.8	5.0 ± 0.7*	4.0 ± 0.8*
Intake during bout (night), ml	1.4 ± 0.2*	4.8 ± 0.6	3.4 ± 0.7*†	2.2 ± 0.3*
Na <sup>+</sup> intake during bout (night), mEq	0.0 ± 0.0	1.4 ± 0.2	1.7 ± 0.4	1.5 ± 0.2

Values are means ± SE. IBI, interbout interval. Rats were deprived of water, but not food, overnight; then food was removed, and they were given one of the four fluids to drink for 23 h. Tests were conducted at weekly intervals. The same 5 rats were used in each test. Rats drinking water at night were not dehydrated then, unlike rats drinking concentrated NaCl solution. \**P* < 0.05 vs. 0.3 M NaCl; †*P* < 0.05 vs. water.

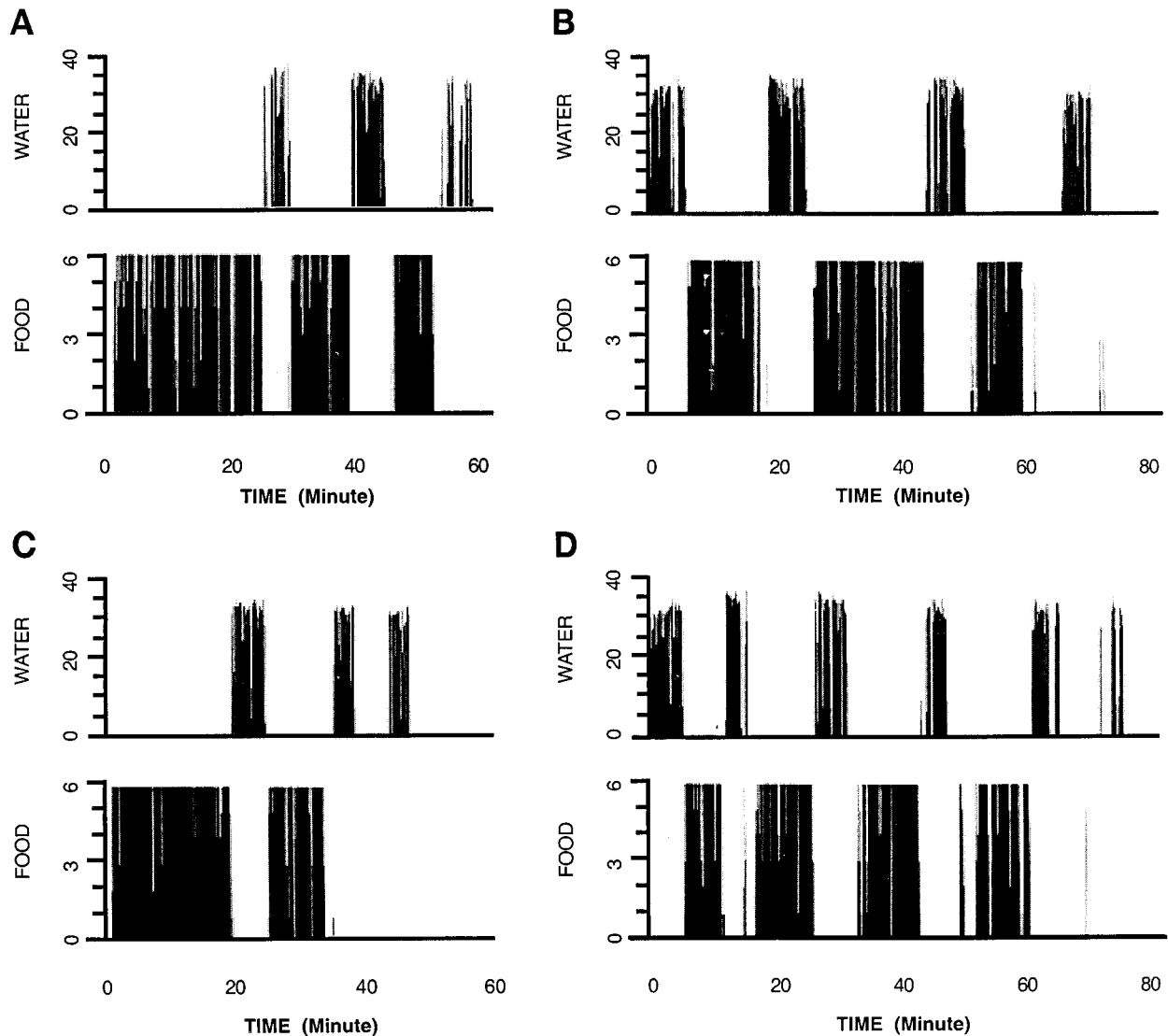


Fig. 8. Incidence of eating and drinking bouts during the first ingestive episode that occurred when food and water were returned to rats after test days during which only water (A), 0.5 M NaCl (B), 0.3 M NaCl (C), or 0.7 M NaCl (D) was available. Representative strip charts indicate that food was consumed immediately when rats had drunk 0.3 M NaCl or water during the test, whereas water was consumed before food when rats had drunk 0.5 or 0.7 M NaCl. The x-axis is blocked in 6-s bins; the y-axis indicates the number of licks of water and seconds of eating per 6-s bin for each animal. The size of the bouts is indicated by the thickness of the lines.

still drank concentrated NaCl solution in much larger volumes than control rats did. These intakes were especially striking when 0.5 M NaCl was the drinking fluid, because saline consumption persisted, despite severe body fluid hyperosmolality. Previous experiments have suggested that rats with APX can detect such hyperosmolality (8). Nonetheless, the inhibition of NaCl intake ordinarily associated with body fluid hyperosmolality apparently is disrupted in these animals, as indicated by their very large intakes of NaCl solution during the first 7 h of the tests.

Rats with APX also showed marked impairments in urinary water conservation. This was evident even during ingestion of 0.3 M NaCl, when rats were able to osmoregulate, because their  $U_{Na}$  was significantly lower than those of intact rats excreting similarly large

Table 3. Food and water intakes during the first ingestive episode after the test day

Test Fluid	Food, g	Water, ml
Water	$8.1 \pm 0.6$	$11.7 \pm 1.6$
NaCl		
0.3 M	$5.9 \pm 0.5$	$13.4 \pm 1.1$
0.5 M	$8.5 \pm 0.3$	$35.8 \pm 3.5^*$
0.7 M	$9.3 \pm 0.3$	$38.0 \pm 2.8^*$

Values are means  $\pm$  SE estimated from time spent eating and licks of fluid. Rats were deprived of water, but not food, overnight; then food was removed, and they were given one of the four fluids to drink for 23 h. Then the test fluids were removed, and food and water were returned. Tests were conducted at weekly intervals. The same 5 rats were used in each test. \* $P < 0.001$  vs. water.

volumes of urine. More striking was the relatively low  $U_{Na}$  observed when rats with APX consumed 0.5 M NaCl solution. In contrast to intact rats, rats with APX could not concentrate urine to levels above those excreted when they drank 0.3 M NaCl, perhaps because secretion of VP and OT was blunted (4, 15). Thus, because they drank too much 0.5 M NaCl and excreted too little of the ingested NaCl load in urine, rats with APX seemed to osmoregulate much less well than control rats under these testing conditions.

#### *Analysis of Saline Drinking Bouts*

Dehydrated rats are thirsty and likely will begin to drink whatever fluid is available to them. When the drinking fluid is water, its consumption by thirsty animals has been shown to cease well before the fluid is absorbed into the circulation and eliminates the excitatory signal for thirst (2, 26). When the drinking fluid is concentrated NaCl solution, rats similarly may obtain temporary satiation before they experience the postabsorptive increase in  $P_{osmol}$  that occurs as a consequence of consuming hypertonic solutions.

Two possible early signals of satiety have been proposed. First, a rapid inhibition of thirst (and VP secretion) has been attributed to some aspect of the act of drinking, perhaps swallowing, that allows fluid passing through the oropharynx to be metered (2, 26). Compelling support for this hypothesis are findings that water intake and plasma VP levels diminished rapidly and remained low for 15–20 min in dehydrated dogs equipped with a gastric fistula through which ingested water drained from the stomach (26). Furthermore, and of direct relevance to the present experiments, rapid inhibitory effects on intake and plasma VP also occurred when water-deprived dogs were given hypertonic NaCl solution to drink instead of water (3). Rehydration did not result from fluid consumption in either case, yet fluid intake and VP secretion were temporarily inhibited nonetheless.

Such rapid but temporary satiety may have occurred when the water-deprived rats in the present study drank water or concentrated NaCl solution. However, this putative early effect cannot be the only signal that controls fluid ingestion in these conditions, because rats did not drink in bouts of similar size when consuming the three concentrated NaCl solutions. If it is assumed that licks were proportional to swallows and to volumes consumed, these findings are not consistent with hypotheses that thirsty rats stop drinking after they have swallowed a certain number of times. A recent study of VP secretion in rats has led to the same conclusion (16).

A second early signal to inhibit drinking behavior might occur when ingested water enters the stomach, small intestines, or hepatic portal vein well before the fluid could have a substantial impact on the osmolality of blood sensed by osmoreceptors located in basal forebrain. Consistent with this suggestion are findings that destruction of vagal sensory afferents by systemic treatment with the neurotoxin capsaicin produced a

marked overconsumption of water by thirsty rats (9). Similar results were obtained in rats when lesions destroyed the vagal projection sites in AP and subadjacent nucleus tractus solitarius (11, 21). Thus relatively small volumes of ingested water may provide inhibitory signals from the viscera that anticipate satiety and limit further drinking before rehydration of body fluids has occurred.

Because most drinking in the present study occurred in the dark period, the analyses of the temporal and quantitative properties of drinking bouts focused on the intakes that occurred then. The size of those bouts was inversely related to the concentration of the NaCl solution. When the mean bout sizes (estimated in ml) of the three groups were multiplied by the concentration of the ingested NaCl solution, remarkably similar amounts of  $Na^+$  (in meq) were ingested per bout by the three groups. Estimated median intakes of  $\sim 1.1$  meq  $Na^+$  resemble the size of saline bouts in studies of rats with a salt appetite using testing conditions in which 0.5 M NaCl and water were available (22, 23). These observations suggest that, in each case, ongoing NaCl ingestion may have been limited by putative visceral osmo- or  $Na^+$  receptors that sense the early consequences of drinking concentrated NaCl solution (26). Although it is also possible that the inverse relation between the intake and concentration of NaCl solution simply reflects palatability of the fluids (5), that hypothesis seems unlikely, because the animals drank for at least several minutes. Whatever the origin of bout termination, the finding that interbout intervals of  $\sim 20$  min were relatively stable throughout the dark period suggests that the inhibitory effects were temporary and that the animals did not learn to avoid the saline solution, even though the initial postabsorptive effect of ingesting concentrated NaCl solution is to increase body fluid osmolality. The failure of rats to learn under these testing conditions should not be surprising given the animals' long experience that fluid consumption always is satiating.

Another noteworthy observation was that rats had similar numbers of drinking bouts during the dark regardless of whether they drank water or concentrated NaCl solution. These results were surprising, because rats that drank 0.5 or 0.7 M NaCl were dehydrated and presumably thirsty throughout the dark period, whereas rats that drank water probably had been rehydrated before the beginning of the dark period. These findings suggest that the onset of drinking during the dark period is influenced, at least in part, by circadian factors independent of thirst.

Another observation of interest was made on the day after the test, when food and water were returned to the cages. Rats that had consumed water or 0.3 M NaCl began to eat food immediately, whereas rats that had ingested 0.5 or 0.7 M NaCl first drank water before they ate. These findings support the conclusion that rats consuming 0.3 M NaCl were rehydrated by the end of the test period, whereas rats consuming 0.5 or 0.7 M NaCl solutions were not. Furthermore, the latter two groups usually began eating soon after their consump-

tion of water had ended; these observations suggest that the inhibition of food intake (i.e., dehydration anorexia) can be relieved by some early consequence of drinking water that precedes complete rehydration (30). As with the control of bout size, this anticipatory satiety signal may have resulted from feedback related to the postgastric detection of consumed water in the intestines, hepatic portal vein, or liver.

### Summary and Conclusions

Experimental studies of osmoregulation traditionally examine the behavioral and physiological responses of animals to an NaCl load that had been determined and administered by the investigator. In the present experiments, the magnitude of the NaCl load instead was a consequence of the amount of concentrated NaCl solution the water-deprived rats drank. Under these circumstances, rats consumed large volumes when the drinking fluid was not too concentrated (e.g., 0.3 M NaCl), even though the ingestion of hypertonic fluid initially aggravated dehydration. The large NaCl intakes caused a solute diuresis, which precluded the strategy of excreting relatively small volumes of maximally concentrated urine normally seen during dehydration. Instead, as shown in *experiment 1*, rats drinking 0.3 M NaCl excreted relatively large volumes of urine with the lowest  $U_{Na}$  that still allowed sufficient urinary water conservation for osmoregulation. When the drinking fluid was very concentrated (e.g., 0.5 M NaCl), however, large intakes were counterproductive to osmoregulation. The ingested NaCl load not only markedly raised estimated  $P_{osmol}$ , but it also caused a solute diuresis that compromised urinary water conservation under conditions in which rats could not easily sustain  $U_{Na}$  above the concentration of the ingested fluid. Consequently, dehydration was aggravated in proportion to how much fluid was consumed, and appropriately rats drank much less 0.5 M NaCl than 0.3 M NaCl solution. As shown in *experiment 2*, rats adaptively limited their NaCl intake by drinking not less frequently but in relatively small bouts, as if in response to an inhibitory signal associated with the detection of the concentrated fluid by putative visceral osmo- or  $Na^+$  receptors. The apparent impairment in the ability of rats with APX to respond to signals inhibiting NaCl intake and the severe body fluid hyperosmolality as a consequence of the large self-administered NaCl loads make clear the adaptive significance of such inhibition in control rats.

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