Impaired osmoregulatory responses in rats with area postrema lesions

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Curtis, Kathleen S., Wan Huang, Alan F. Sved, Joseph G. Verbalis, and Edward M. Stricker. Impaired osmoregulatory responses in rats with area postrema lesions. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R209-R219, 1999.—Area postrema lesions (APX) in adult male rats produced a robust spontaneous intake of 0.5 M NaCl, as reported previously. The largest NaCl intakes (up to 108 ml/day) were observed when there was little incidental damage in the medial subnucleus of the nucleus of the solitary tract adjacent to the caudal and middle portions of the area postrema. Rats with discrete APX also drank substantial amounts of 0.5 M NaCl when access to saline was restricted to 7 h/day (up to 30 ml in 1 h, 48 ml in 7 h). Such large NaCl intakes stimulated considerable water ingestion and renal sodium excretion, but together these responses usually were insufficient for osmoregulation during the 7-h test period. After systemic administration of hypertonic NaCl solution, rats with APX excreted less Na⁺ in urine and secreted less vasopressin and oxytocin than control rats did. The prominent salt appetite, insufficient thirst and natriuresis in response to an ingested NaCl load, and blunted natriuresis and neurohypophysial hormone secretion in response to an injected NaCl load, all indicate that osmoregulatory responses are impaired in rats after APX.

OSMOREGREULATION INVOLVES the integrated central control of various behavioral and physiological responses to changes in the cellular or vascular fluid compartment (32). For example, increased plasma osmolality produced by an administered NaCl load in rats inhibits NaCl appetite while stimulating thirst and neurohypophysial secretion of oxytocin (OT), a natriuretic hormone, and vasopressin (VP), the antidiuretic hormone. Osmoregulation is thus achieved by the coordinated decrease in NaCl consumption and increase in water intake, complemented by renal excretion of osmoles and conservation of water in urine. Structures along the lamina terminalis in the forebrain play a key role in mediating these osmoregulatory responses (22).

Rats spontaneously increase their ingestion of concentrated NaCl solutions after lesions of the area postrema (APX; Refs. 4, 9, 14, 17, 37), a circumventricular organ in the caudal brain stem. In preliminary studies, we confirmed those observations but found that many rats with APX drank much greater volumes of 0.5 M NaCl solution than had been reported previously. Therefore, one goal of the present study was to further clarify the location and extent of the lesions in the dorsal medulla that are associated with such pronounced increases in NaCl ingestion. However, the major goal was to examine osmoregulatory responses in rats with APX, specifically, the effect of the large ingested NaCl loads on water intake and on urinary excretion of Na⁺ and water, the effect of systemic injections of hyperosmolar solutions on NaCl intake, and the effect of a systemic NaCl load on urinary excretion of Na⁺ and water and on neurohypophysial secretion of OT and VP.

METHODS

Animals. Adult male Sprague-Dawley rats (Zivic Miller, Zelienople, PA) weighing 250–450 g at the beginning of the experiment were individually housed in wire mesh cages. They had ad libitum access to laboratory chow pellets (Purina #5001) and tap water. The colony room was maintained at 22–23°C with lights on from 8 AM to 8 PM.

APX were produced by vacuum aspiration as described previously (7). Briefly, each rat was anesthetized with Equithesin (3.0 ml/kg body wt ip of a solution containing 0.98 g/dl pentobarbital sodium, 4.25 g/dl chloral hydrate, and 2.12 g/dl MgSO₄) 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. The area postrema (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 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, 1–6 wk later, the effectiveness of the lesions was assessed by one of two screening tests, described elsewhere (7): LiCl-induced conditioned taste aversion or LiCl-induced suppression of water intake elicited by water deprivation. These LiCl-evoked responses, which are robust in control rats, were absent in all rats with APX, consistent with destruction of the AP (7). Experiments began 2–3 days after these tests were completed.

In all experiments, the responses of rats with APX were contrasted with those of unoperated control rats matched in body weight. Because rats with APX weigh less than their unoperated littersmates (7), the weight-matched control rats invariably were 1–5 wk younger than rats with APX. However, the broad range of body weights and ages in rats with APX overlapped substantially with those of the control rats. In addition, the quantity of NaCl solution consumed daily by rats with APX was not related to their body weight, age, or duration of the recovery period.

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Experiment 1 assessed the spontaneous intake of NaCl solution by rats with APX. Eighty-six rats with APX had ad libitum access to 0.5 M NaCl, chow, and water. Daily intakes of 0.5 M NaCl were recorded (=1.0 ml) for 2–6 days, and mean 24-h intakes were calculated for each rat and compared with those of untreated control rats (n = 12).

Experiment 2 assessed the effects of NaCl consumption by rats with APX on water intake and urinary excretion of Na+ and water. Because rats with APX consume saline primarily in the dark portion of the dark-light cycle, when food also is ingested (28), we used a protocol that avoids the effects of food consumption on water intake and urinary excretion. Access to 0.5 M NaCl was restricted to 7 h/day, food was available during the remaining 17 h, and water was available continuously. This protocol has been used to study several other models of salt appetite in rats (23, 27, 29, 34).

In experiment 2A, all 86 rats with APX used in experiment 1 were placed on the schedule of restricted access to NaCl solution for 3–4 days, during which intakes of 0.5 M NaCl and water were recorded (=1.0 ml) at 5, 10, 15, 30, 45, and 60 min and then hourly for 7 h/day. NaCl intake by individual rats was consistent over time, thus data from only the final day of testing are presented. Urine was collected hourly from funnel tubes attached beneath the cages and its volume and Na+ concentration (Na\textsuperscript{+}) in meq/l were determined by a Na+-sensitive electrode (Beckman Electrolyte Analyzer II; Beckman Instruments, Fullerton, CA). The amount of Na+ excreted in urine was calculated by multiplying the [Na\textsuperscript{+}] of urine by its volume. The results were compared with those of untreated control rats (n = 12).

Plasma osmolality (P\textsubscript{osmol}, in mosmol/kgH\textsubscript{2}O) was estimated by a formula used previously for this purpose (27)

\[ P_{\text{osmol}} = 1,000 \times \frac{[(0.67)(\text{body wt})(0.3)] + [(2)(\text{Na}_{\text{in}} - \text{Na}_{\text{out}})]}{[(0.67)(\text{body wt})] + [\text{H}_2\text{O}_{\text{in}} - \text{H}_2\text{O}_{\text{out}}]} \]

where Na\textsubscript{in} is the amount of Na\textsuperscript{+} ingested in milliequivalents, Na\textsubscript{out} is the amount of Na\textsuperscript{+} excreted in urine in milliequivalents, H\textsubscript{2}O\textsubscript{in} is the volume of fluid consumed in milliliters, and H\textsubscript{2}O\textsubscript{out} is urine volume in milliliters. An initial P\textsubscript{osmol} of 300 mosmol/kgH\textsubscript{2}O and body water of 67% body wt were assumed.

In experiment 2B, the effect of systemically administered hyperosmolar solutions on NaCl consumption by rats with APX was assessed to determine whether NaCl intake was inhibited, as occurs in other models of NaCl appetite (3, 27, 30). After completion of experiment 2A, two separate subgroups of the 86 rats with APX were kept on the same schedule of restricted access and tested further. One group (n = 11) was injected with 2 ml ip of 2 M NaCl just before the drinking test, and the other group (n = 10) similarly received an equiosmotic load of 4 ml of 2 M sorbitol solution. The saline and water intakes of both groups were recorded hourly for 7 h and compared with their intakes in experiment 2A, when no injections were given.

Experiment 3 assessed renal and endocrine responses to an administered NaCl load in rats with APX. In experiment 3A, the effect of a systemic injection of hypertonic NaCl solution on urinary Na\textsuperscript{+} and water excretion was examined. A third subgroup (n = 25) of the 86 rats with APX used in experiment 1 were injected with hypertonic NaCl saline (2 ml of 2 M NaCl ip or sc) just before a 3-h period during which no drinking fluids were available. Urine was collected hourly, and its volume and [Na\textsuperscript{+}] were compared with those of control rats matched in body weight (n = 13) and treated identically.

Experiment 3B determined the effects of hypertonic NaCl solution, infused intravenously, on neurohypophysial secretion of OT and VP by rats with APX. Separate groups of rats with APX (n = 10) and control rats matched in body weight (n = 17), not used previously, were used in this experiment. One day before testing, all rats were anesthetized with halothane; then the femoral vein was cannulated with polyvinyl tubing (0.98 mm ID, 0.965 mm OD; Biolab, Lake Havasu, AZ), and the femoral artery was cannulated with PE-50 tubing filled with heparinized saline (Clay Adams, Parsippany, NJ). The free ends of the catheters were guided subcutaneously along the back to exit between the scapulae. On the test day, catheters were extended through the top of the cage, and the venous catheter was connected to an infusion pump. Water and food were removed from the cages, and then rats received a continuous infusion (2 ml/h iv) of 1 M NaCl solution for 2 h. Blood samples (2 ml), taken from the arterial catheter just before the infusion started and 2 h later, were collected into chilled tubes containing EDTA (Vacutainer; Becton Dickinson, Franklin Lake, NJ); the baseline samples were replaced by equal volumes of prewarmed isotonic saline. After centrifugation, P\textsubscript{osmol} was measured from 50-ml aliquots by freezing point depression using a micro-Osmette osmometer (Precision Systems, Natick, MA).

Histological analysis. 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 in 33-µm sections along the rostrocaudal extent corresponding to the AP. Sections were mounted and stained for Nissl substance either with neutral red or cresyl violet.

Brain stems from all 86 rats with APX in experiments 1 and 2 were examined for completeness of APX and extent of the lesions. APX were evaluated at three levels corresponding to the rostral, middle, and caudal portions of the AP (~50, 200, and 350 µm caudal to obex, respectively). Damage to subadjacent structures including the nucleus of the solitary tract (NTS), the dorsal motor nucleus of the vagus (DMV), and the hypoglosus nucleus (HGN) was noted. Lesions were scored at each level using a scale of 0 to 3, in steps of 0.5, where 0 indicated a discrete lesion confined to AP and 3 indicated extensive damage to adjacent structures. In a further attempt to determine how variability in lesion-induced damage was related to the magnitude of NaCl intake, camera lucida drawings were made of the brain stem sections from the 12 rats with APX that had the largest 24-h NaCl intakes in experiment 1 and from the six rats with APX that had the smallest intakes.

Brain stems from the 10 rats with APX in experiment 3 also were examined for completeness and extent of lesions, but the quantitative scale described above was not used.

Statistical analysis. Using SigmaStat software (Jandel Scientific, San Rafael, CA), regression lines and correlation coefficients (r) were computed from individual values by the method of least squares. Means and SE were computed from group values. Statistical significance was determined by χ² analysis, by Student’s t-test (or, when variability was large, by Mann-Whitney rank sum test), or by two-way repeated measures ANOVA with planned comparisons made using the Student-Newman-Keuls method.

**RESULTS**

Experiment 1. Histological examination of the brain stems from 86 rats with APX showed that the lesions always were complete (with the exception of 1 rat that had <10% of AP remaining). Figure 1 shows photomicrographs of a series of brain stem sections corresponding
to the caudal, middle, and rostral AP from a control rat and from a rat with APX. Fifty-seven of 86 rats with APX had little damage to the NTS subadjacent to caudal and middle portions of the AP. The other 29 rats with APX had more substantial damage to those portions of the NTS, which often extended to include the dorsomedial aspects of the DMV and HGN. Damage in regions subadjacent to rostral AP occurred in most rats and was nondifferentiating. Thus these animals were divided into two groups on the basis of their summed scores indicating damage to the areas subadjacent to caudal and middle AP. Group A included rats with APX (n = 57) in which the summed score of lesion-induced damage in these areas was $\geq 1.5$, and group B included rats with APX (n = 29) in which the summed score of lesion-induced damage in these areas was $\geq 2.0$.

Mean daily intakes of 0.5 M NaCl by rats with APX were highly variable (range = 1–108 ml). However, the intakes of individual rats varied little from day to day, so calculated mean values closely approximated actual daily intakes. Those NaCl intakes bore no apparent relation either to preoperative body weight or to the postoperative time when experiments began, but they were closely related to lesion size and location (Table 1). Relatively discrete APX were associated with large 24-h NaCl intakes, whereas APX that caused more extensive damage to structures subadjacent to caudal and middle AP were associated with small intakes ($r = -0.54, P < 0.001$). Damage in regions subadjacent to rostral AP was unrelated to NaCl consumption.

Figure 2 shows camera lucida drawings of brain stem sections from an intact rat (Fig. 2A), the rat with APX from group A with the most discrete lesion (Fig. 2B), the rat with APX from group A with the most extensive lesion (Fig. 2C), and a representative rat with APX from group B (Fig. 2D). These three rats with APX drank 108, 63, and 2 ml/day, respectively. The drawings illustrate that APX in rats that consumed larger amounts of saline tended to spare the medial sub-

Table 1. Percentage of rats with APX having at least some lesion-induced damage to brain stem structures subadjacent to caudal, middle, or rostral portion of AP

<table>
<thead>
<tr>
<th>NaCl Intake, ml</th>
<th>n</th>
<th>Caudal</th>
<th>Middle</th>
<th>Rostral</th>
<th>C + M</th>
<th>C + M + R</th>
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<td>&gt;70</td>
<td>7</td>
<td>0</td>
<td>14</td>
<td>71</td>
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<td>0</td>
<td>70</td>
<td>90</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>31–50</td>
<td>24</td>
<td>29</td>
<td>63</td>
<td>83</td>
<td>58</td>
<td>71</td>
</tr>
<tr>
<td>10–30</td>
<td>31</td>
<td>23</td>
<td>90</td>
<td>94</td>
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These 86 rats are grouped according to their mean daily intake of 0.5 M NaCl. Incidental damage was defined as a score of $\leq 0.5$ on the rating scale used to evaluate the lesions. APX, area postrema lesions; C, caudal; M, middle; R, rostral.
nucleus of the NTS (mNTS) subadjacent to caudal and middle AP regardless of damage incurred in other adjacent structures.

Rats with APX in group A usually drank much more saline in 24 h than did rats with APX in group B (42.2 ± 3.3 and 15.9 ± 2.4 ml, respectively; t = 699.0, P < 0.001). As shown in Fig. 3, all 17 rats that drank >50 ml/day were in group A (χ² = 27.19, P < 0.001). In contrast, of the 14 rats that consumed ≤10 ml/day, 13 were in group B and only 1 was in group A. Saline intakes by these latter rats were comparable to those ingested by intact control rats (4.1 ± 1.5 ml/day).

Experiment 2A. The consumption of 0.5 M NaCl by the 86 rats with APX during the 7-h tests also was variable (range = 0–48 ml) but was highly correlated with their 24-h intakes of saline (Fig. 3; r = 0.83, P < 0.001). These 7-h saline intakes also were inversely correlated with damage in the mNTS subadjacent to caudal and middle AP (r = −0.53, P < 0.001). Thus, as shown in Fig. 3, 29 of 33 rats with APX that drank ≥20 ml of saline in 7 h were in group A and only 4 were in group B. In contrast, 12 of 17 rats with APX that consumed ≤8 ml of saline in 7 h were in group B and only 5 were in group A (χ² = 17.63, P < 0.001).

Figure 4 shows the mean 7-h intakes of 0.5 M NaCl and water by rats with APX in group A and in group B. Significant effects of group [F(2,95) = 33.1, P < 0.001], time [F(6,95) = 16.9, P < 0.001], and group × time interaction [F(12,685) = 10.1, P < 0.001] in the saline intakes were observed. Planned comparisons revealed that 7-h intakes of saline by rats with APX in group A (22.1 ± 1.6 ml) were significantly greater than those by rats with APX in group B (10.2 ± 1.4 ml, P < 0.001), and both of these groups drank more saline in 7 h than did intact control rats (0.2 ± 0.1 ml; both P values <0.001). Ingestion of 0.5 M NaCl occurred primarily during the first hour of the test and slowed during subsequent
hours (Fig. 4). Rats with APX in group A drank significantly more saline during the first hour of the test period than did either rats with APX in group B or intact control rats (13.5 ± 0.8, 7.4 ± 1.1, 0.0 ± 0.0 ml, respectively; both P values, 0.001). Thirty-one of 35 rats with APX that drank the most saline in the first hour (range 5–13–30 ml) were in group A, whereas only 4 were in group B.

Figure 4 also shows that NaCl intake by rats with APX began during the first few minutes of the test period and invariably preceded water intake, which began 10–15 min later. As shown in Fig. 5, the volumes of 0.5 M NaCl and water consumed in 7 h were significantly correlated with one another in group A (r = 0.73, P < 0.001) and in group B (r = 0.75, P < 0.001). However, only 1 of 52 rats in group A and 2 of 18 rats in group B consumed sufficient water to dilute the ingested saline to a fluid mixture that approximated isotonicity (indicated by diagonal line in Fig. 5), whereas the others consumed a mixture that was quite hypertonic. Note that in this analysis and the following ones relating NaCl intake with urinary excretion, 16 rats with APX (5 in group A, 11 in group B) were excluded from consideration to ensure that their low responses (e.g., saline intakes <4 meq Na⁺) during the 7-h test would not exaggerate the correlation.

Urinary excretion of Na⁺ during the 7-h test varied in proportion to the magnitude of the NaCl intake by rats with APX in group A (r = 0.96, n = 52, P < 0.001) and in group B (r = 0.92, n = 18, P < 0.001). Thus rats with APX in group A excreted much more Na⁺ in urine than did rats in group B (8.35 ± 0.64 and 3.56 ± 0.51 meq, respectively; t = 389.0, P < 0.001). However, regardless of how much NaCl was consumed, 74.3% of the ingested Na⁺ usually was excreted in urine by the end of the 7-h test (Fig. 6, top). Similarly, urine volume varied in proportion to ingested fluid volume (group A: r = 0.91, n = 52, P < 0.001; group B: r = 0.85, n = 18, P < 0.001), and 81.6% of the fluids consumed were excreted in
urine by the end of the test. As shown in Fig. 6, bottom, urinary [Na\(^+\)] varied in proportion to, but usually was less concentrated than, the [Na\(^+\)] of the ingested fluid mixture (i.e., ingested Na\(^+\), in meq, divided by the summed volume of saline and water consumed, in liters; group A: r = 0.91, n = 52, P < 0.001; group B: r = 0.80, n = 18, P < 0.001).

The intakes and urinary excretion of Na\(^+\) and water were used to estimate Posmol in rats with APX. At 7 h the increase in estimated Posmol was significantly greater in group A rats (11.8 ± 1.2 mosmol/kgH\(_2\)O, ~4% of basal values) than in group B rats (5.9 ± 1.4 mosmol/kgH\(_2\)O, ~2% of basal values; t = 489.0, P < 0.001). Thirty-nine of 86 rats with APX had an increase in estimated Posmol >9 mosmol/kgH\(_2\)O at 7 h. In general, these 39 rats either had very large NaCl intakes, relatively low water intakes and urinary [Na\(^+\)], or both. For example, 15 of them, all from group A, were among the 16 rats with APX that had the largest saline intakes (range = 26–48 ml). In contrast, the seven rats that consumed the smallest volumes of 0.5 M NaCl in 7 h (n = 5 in group A, 2 in group B; range = 9–15 ml) all drank relatively little water and had urinary [Na\(^+\)] that were lower than the [Na\(^+\)] of the ingested fluid mixture.

Conversely, 18 rats with APX had large 0.5 M NaCl intakes (n = 13 in group A, 5 in group B; range = 17–31 ml) yet did not have an increase of >9 mosmol/kgH\(_2\)O in estimated Posmol at 7 h. Four of them drank water in unusually large amounts (range = 32–37 ml) relative to their saline intakes (range = 18–20 ml). The other 14 rats drank water in amounts that were smaller than or approximately equal to their saline intakes, but they were among the minority of animals whose urinary [Na\(^+\)] was higher than the [Na\(^+\)] of the ingested fluid mixture (see Fig. 6, bottom). Thus rats with APX did osmoregulate well despite large NaCl intakes when their water intakes or urinary [Na\(^+\)] were relatively high.

No apparent histological differences among rats with APX were found to differentiate the animals that failed to osmoregulate well during the 7-h test period from those that did osmoregulate well.

Experiment 2B. Figure 7, top, shows the mean ± SE intakes of 0.5 M NaCl by 11 rats with APX (all in group A) after injection of 2 ml ip of 2 M NaCl and in the noninjected baseline condition. Statistically significant effects of group [F(1,10) = 23.45; P < 0.001] and time [F(6,60) = 9.38; P < 0.001] were observed, whereas the group × time interaction [F(10,153) = 2.25] was not significant. Planned comparisons revealed that intakes of saline during the first hour by rats with APX after 2 M NaCl treatment (5.2 ± 1.1 ml) were significantly less than those in the baseline condition (12.7 ± 1.0 ml; P < 0.001).

Similar effects on 0.5 M NaCl intake were observed when 10 rats with APX (all in group A) were injected with 4 ml ip of 2 M sorbitol (Fig. 7, bottom). Statistically significant effects of group [F(1,9) = 16.80; P < 0.01] and time [F(6,9) = 8.74; P < 0.01] were observed, whereas the group × time interaction [F(9,139) = 0.45] was not significant. Planned comparisons revealed that intakes of saline during the first hour by rats with APX after 2 M sorbitol treatment (7.3 ± 2.0 ml) were significantly smaller than those in the baseline condition (15.6 ± 1.8 ml; P < 0.05).

Water intakes by both groups 1 h after the injections were not significantly different from their baseline water intakes (19.7 ± 1.2 ml after 2 M NaCl vs. baseline intake of 16.5 ± 1.6 ml; 14.7 ± 2.7 ml after 4 M sorbitol vs. baseline intake of 13.8 ± 2.4 ml). Note that both groups reduced 1-h saline intakes by amounts approximating the 8.0 mosmol contents of the injected solutions. This reduction persisted throughout the 7-h test (12.5 ± 2.5 ml after 2 M NaCl vs. baseline intake of 23.9 ± 3.7 ml; 16.4 ± 4.6 ml after 2 M sorbitol vs. baseline values of 24.9 ± 3.9 ml), and the cumulative intakes of 0.5 M NaCl were comparable during hours 1-7 of the test (i.e., after the first hour, the lines in Fig. 7 roughly parallel one another regardless of whether or not the rats received an injection and regardless of which hyperosmotic solution was injected). Cumulative 7-h water intakes by both groups were not significantly different than those in the baseline condition (26.2 ± 2.1 ml after 2 M NaCl vs. baseline intake of 21.8 ± 1.8 ml; 19.5 ± 3.3 ml after 2 M sorbitol vs. baseline intake of 20.4 ± 3.4 ml).

Experiment 3A. Rats with APX excreted similar volumes and amounts of Na\(^+\) in urine during the 3-h period after systemic injection of 2 ml of 2 M NaCl solution, regardless of lesion size (n = 15 in group A, 10 in group B) or route of administration (n = 19 injected...
intraperitoneally, 6 injected subcutaneously). Thus these data were combined for analysis and comparison with the results obtained when the same injections were given to 13 control rats (n = 6 injected intraperitoneally, 7 injected subcutaneously). Figure 8 shows that control rats excreted significantly more volume and Na⁺ in urine than did rats with APX (respectively, 8.9 ± 0.4 and 6.3 ± 0.4 ml, t = 4.50; 2.7 ± 0.1 and 1.9 ± 0.1 meq, t = 4.43; both P values < 0.001).

Figure 8 also shows that urine Na⁺ excretion was highly correlated with urine volume in all rats (r = 0.94, n = 38, P < 0.001). Not shown are the separate regression lines for rats with APX (r = 0.96, P < 0.001) and for control rats (r = 0.71, P < 0.01), which were not significantly different from one another. The integrated regression line for the two groups had a slope of 313 meq/l, which is the mean [Na⁺] of urine excreted by these animals.

Experiment 3B. Comparably low plasma levels of VP (PVP, range 1–8 pg/ml) and of OT (POT, 2–25 pg/ml), and comparable P-osmol (285–297 mosmol/kgH₂O) were observed in baseline blood samples from rats with APX and control animals. As expected, each value was observed in baseline blood samples from rats with APX and control animals. As expected, each value was increased in all rats in both groups when they received an intravenous infusion of 1 M NaCl (2 ml/h for 2 h), although there was considerable within-group variability (rats with APX: P-osmol = 320 ± 4 mosmol/kgH₂O, PVP = 15 ± 2 pg/ml, POT = 39 ± 7 pg/ml; control rats: P-osmol = 314 ± 2 mosmol/kgH₂O, PVP = 20 ± 2 pg/ml, POT = 53 ± 5 pg/ml). When the increases in POT and PVP were expressed as a function of the increase in P-osmol in the same samples, blood levels of both hormones were revealed to have increased significantly less in rats with APX than in control rats (both P values < 0.05; Fig. 9).

Of the 10 rats with APX that were used in this experiment, only 1 had discrete APX, whereas the brain stem damage in the other 9 animals was more extensive and included relatively large portions of the subadjacent NTS as well as the DMV and HGN.

DISCUSSION

The AP has long been suspected of playing a role in body water and sodium homeostasis. Especially striking in this regard is the marked increase in the ingestion of concentrated NaCl solutions by rats after APX (4, 9, 14, 17, 37). The present series of studies sought to examine the basis of this robust drinking and, additionally, to examine the effect of NaCl loads on various osmoregulatory responses in rats with APX. The results of these experiments clarified the specific location and extent of the lesions in the dorsal medulla that are associated with pronounced increases in NaCl ingestion and also identified more subtle dysfunctions of rats with APX in their water drinking, urinary excretion, and neurohypophysial hormone secretion responses to NaCl loads. Each of these issues will now be considered, in turn.

In 1981, Contreras and Stetson (4) first reported that rats consume relatively large amounts of concentrated NaCl solution after lesions of the caudal brain stem focused on the AP. They found that 18 rats with APX drank ~7 ml of 0.5 M NaCl (3.5 meq Na⁺) daily in two-bottle tests with water and ~46 ml (13.8 meq Na⁺) when given 0.3 M NaCl instead of the more concentrated NaCl solution. Similar 24-h NaCl intakes by rats with APX have been reported subsequently: 7 rats drank ~15 ml of 0.5 M NaCl (7.5 meq Na⁺) (14), 4 rats drank ~55 ml of 0.3 M NaCl (16.5 meq Na⁺) (17), and 10 rats drank ~50 ml of 0.15 M NaCl (7.5 meq Na⁺) (38). In the present study, many rats with APX consumed much larger amounts of saline. Mean daily intake of 0.5 M NaCl by the 86 rats with APX in
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The AP is known to send a prominent neural projection to the mNTS (5, 25), at least a portion of which appears to inhibit NTS neurons (16). The mNTS, in turn, projects to areas in the forebrain and hypothalamus that are known to be important in control of body fluid homeostasis and ingestive behavior (33). In this regard, neurologically intact rats typically do not consume concentrated NaCl solutions in large amounts, as if saline intake normally was constrained by tonic central inhibition. It is therefore possible that the AP provides an inhibitory influence on mNTS neurons that inhibit areas in the forebrain and/or hypothalamus that contribute to the inhibition of NaCl intake. Thus, the larger NaCl intakes by rats with discrete APX may reflect release of mNTS from inhibition by the AP, whereas more extensive APX that additionally damage the mNTS may prevent large NaCl intakes by disrupting this putative disinhibition. Further investigation is required to evaluate this hypothesis and other speculation concerning the basis of the marked NaCl intake that occurs after discrete APX in rats.

Rats with APX in group A drank very large volumes of 0.5 M NaCl during the first 15 min of the 7-h test. Regardless of why they initiated drinking, this observation suggests that APX had impaired an early inhibition of further salt consumption, a usual consequence of drinking concentrated NaCl solution. One such inhibitory signal has been associated with gastric distension (26). In this regard, rats with APX appear to be insensitive to the moderate gastric distension that occurs early in an episode of rapid fluid or food ingestion. For example, rats with APX have very large ingestive episodes when consuming powdered chow, water, and 0.5 M NaCl solution ad libitum (28); they also drink unusually large amounts of dilute sucrose solution when deprived of food overnight (8). Similar results are seen in rats after systemic injection of the neurotoxin capsaicin is used to destroy vagal afferent fibers (6), a portion of which project to the AP (19). Thus, the consumption of large volumes of 0.5 M NaCl by rats with APX during the first 15 min of the test period may result, in part, from blunted inhibitory feedback normally associated with gastric distension. However, impaired detection of inhibitory signals of gastric distension, if true, cannot be the sole basis for the reduced NaCl intake by rats with APX later in the 7-h test because much of the ingested fluid had been excreted in urine by then.

In addition to detecting neural signals from the periphery, the AP is a circumventricular organ that may participate in the central control of salt appetite by detecting some blood-borne factor that inhibits NaCl intake. In this regard, AP neurons respond to NaCl administered systemically (21), and elevated PNa inhibits NaCl intake by rats with an experimentally induced
salt appetite (3, 27, 30). Nonetheless, rats with APX apparently remain responsive to substantial increases in $P_{Na}$ because their saline intakes were suppressed after treatment with 2 M NaCl solution, which was given in a dose that increases $P_{Na}$ markedly. Similar effects were obtained with injections of 2 M sorbitol, which indicates that the inhibitory signal may relate to hyperosmolality of body fluids rather than to hypernatremia per se (3). On the other hand, it remains possible that rats with APX are less sensitive to inhibitory effects of modest hyperosmolality, which occur early in the initial episode of 0.5 M NaCl drinking, and that this putative dysfunction then contributes to the rapid drinking of saline.

The large NaCl load consumed rapidly by rats with APX in group A disturbs their osmotic balance, much like the effects of an experimentally administered NaCl load. In addition to inhibition of salt appetite in rats after systemic injection of hypertonic saline, other counter-regulatory responses occur, such as water intake (to dilute the NaCl load), pituitary VP secretion (to conserve urinary water), and pituitary OT secretion (to excrete the NaCl load in urine). A major goal of the present experiments was to evaluate those responses, and the restricted access test was chosen to facilitate observations of the NaCl ingestion and its secondary effects on water intake and on urinary Na$^+$ and water excretion.

In other models of salt appetite that have been studied using this protocol, when rats consumed 0.5 M NaCl solution first they then drank water promptly and in sufficient volumes to dilute to isosmotic the amount of ingested NaCl that was retained (27, 29, 34). Rats with APX also drank water soon after an initial bout of saline intake, but in volumes that often were not large enough to reduce the elevated $P_{osmol}$ of body fluids to isosmotic. Thus, at the end of the 7-h test period, 39 of 86 rats with APX had an increase in estimated $P_{osmol} \geq 3\%$ above basal osmolalities (i.e., $\geq 9$ mosmol/kgH$_2$O). Because a 1–2% increase in $P_{osmol}$ is the documented threshold for the stimulation of water intake in rats (10), increases in estimated $P_{osmol} \geq 3\%$ imply impairments in osmoregulatory thirst. These results suggest that in rats with APX, whether in group A or in group B (i.e., whether or not APX were discrete), water intake is prominent when $P_{osmol}$ is increasing rapidly soon after a large NaCl load is consumed (or injected; Ref. 8); however, rats with APX may be less sensitive to modest increases in $P_{osmol}$ that exceed the threshold for thirst in control rats such as occurs during the last few hours of the 7-h test period. Inasmuch as rats with APX are normonatremic under standard maintenance conditions (8), the failure of many of the present rats with APX to drink enough water to reduce estimated $P_{osmol}$ below 9 mosmol/kgH$_2$O during a 7-h test period likely reflects delayed osmoregulatory responses rather than completely impaired osmoregulation.

Hyperosmolar body fluids can be diluted to isotonicity most rapidly by water consumption, but excretion of Na$^+$ in concentrated urine also contributes importantly to osmoregulation. In fact, rats with APX always excreted Na$^+$ in concentrated urine after consuming large amounts of 0.5 M NaCl solution. However, their urine was usually less concentrated than the ingested fluid mixture, regardless of the amount of NaCl consumed, and they excreted only ~70% of the NaCl load in 7 h. Neurologically intact rats that had been deprived of dietary Na$^+$ for 8 days similarly consumed and retained large amounts of 0.5 M NaCl in a 7-h test (29), although Na$^+$-deprived rats have elevated plasma levels of angiotensin and aldosterone that probably are responsible both for their NaCl appetite and their urinary Na$^+$ retention (31). Rats with APX, in contrast, drank NaCl solution and retained Na$^+$ in urine without apparent activation of the renin-angiotensin-aldosterone system (17). Moreover, when a fixed NaCl load was administered intraperitoneally, the total excretion of Na$^+$ in urine was much larger in control animals than in either group of rats with APX. These findings suggest that the natriuretic response of rats is blunted by APX, even when the lesions are not discrete (unlike the enhanced NaCl intake, which required discrete APX).

In response to an NaCl load administered intravenously, both $P_{VP}$ and $P_{OT}$ were found to be significantly lower in rats with APX than in control animals (see also Refs. 1 and 15). Although attenuated, $P_{VP}$ nonetheless exceeded the levels required for maximal antidiuresis, and thus little effect on urinary water conservation would be expected. In contrast, $P_{OT}$ did not exceed the levels required for maximal natriuresis (35), so the observed attenuation in $P_{OT}$ would be expected to impair urinary Na$^+$ excretion (13). Thus the marked elevations of estimated $P_{osmol}$ within the 7-h test period by rats with APX likely result not only from insufficient water intake in response to the large ingested NaCl load but also from a diminished contribution of neurohypophysial OT to urinary Na$^+$ excretion.

These impairments in consumption of water intake and in excretion of Na$^+$ in urine did not always coexist in rats with APX. Some rats did drink relatively large amounts of water, and some rats did excrete urine with a relatively high [Na$^+$]. Not surprisingly, those were the rats in which estimated $P_{osmol}$ were relatively low at the end of the 7-h test period despite a large initial NaCl load. Conversely, estimated $P_{osmol}$ was very high in some rats with APX despite smaller intakes of 0.5 M NaCl, probably the result of low water intakes and/or low urinary Na$^+$ excretion. Although subtle differences in the size or location of APX may account for the sparing of some osmoregulatory responses but not others, these findings suggest that distinct mechanisms within the AP mediate the different components of osmoregulation.

In summary, rats with APX that had little damage to the mNTS subadjacent to caudal and middle AP drank remarkably large volumes of 0.5 M NaCl when it was available ad libitum and also when access was restricted to 7 h/day. In response to the hyperosmolality of body fluids resulting from this ingested NaCl load, the rats decreased subsequent NaCl intake, increased water intake, and conserved water in urine while excreting the NaCl load. However, collectively these
adaptive responses often were insufficient for osmoregulation during the 7-h test period, even when NaCl intakes were relatively modest. Regardless of whether or not the lesions were discrete, rats with APX appropriately reduced their NaCl intakes when an osmotic load was injected intraperitoneally, but urinary Na⁺ excretion and neurohypophysial secretion of VP and OT in response to an administered NaCl load invariably were impaired. Thus excessive intake of concentrated NaCl solutions, which evidently require relatively discrete APX, is not the only disruptive effect of APX on body fluid homeostasis in rats; significant impairments in some associated osmoregulatory responses, which do not require such discrete APX, also may be observed. Further work is needed to identify the role of the AP in specific central neural pathways that mediate these behavioral and physiological contributions to osmoregulation.

Perspectives

The central control of body fluid homeostasis is complex and involves forebrain structures as well as the caudal brain stem structures emphasized in this report. Specifically, the ventral portion of nucleus medius, just dorsal to the organum vasculosum of the lamina terminalis and ventral to the anterior commissure, is thought to be an integration site for osmoregulation because its damage eliminates water intake and neurohypophysial secretion of VP and osmocin when the same responses to hyponatremia (12, 18). Lesions of the nucleus medius also produce spontaneous NaCl intake by rats (11). Thus the AP and nucleus medius both may provide tonic inhibition to the central control of NaCl intake and more generally participate in mediating the various adaptive responses that are stimulated by an NaCl load.

Separate Na⁺ and osmoregulatory signals appear to inhibit salt appetite in rats, perhaps by involving centrally released OT and atrial natriuretic peptide (ANP) as neurotransmitters (2, 3). Both hypertenaemia and gastric distension are effective stimuli of central OT secretion (20), as are other treatments that inhibit NaCl intake by rats (31), whereas little is known about the stimuli for central release of ANP. Further work is needed to differentiate between the neural systems that control body fluid osmolality and those that control extracellular Na⁺ and to clarify the roles of central ANP and OT in mediating the influence of the AP and nucleus medius on osmoregulation and NaCl ingestion in rats.

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REFERENCES


