Vasopressin and oxytocin release evoked by NaCl loads are selectively blunted by area postrema lesions

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Huang, Wan, Alan F. Sved, and Edward M. Stricker. Vasopressin and oxytocin release evoked by NaCl loads are selectively blunted by area postrema lesions. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R732–R740, 2000.—The present study investigated the effect of area postrema lesions (APX) on stimulated neurohypophysial secretion of vasopressin (VP) and oxytocin (OT) in conscious rats. Blunted increases in plasma levels of both pituitary hormones were observed when rats with APX were infused intravenously with 1 M NaCl solution (2 ml/h for 6 h). In contrast, plasma VP and OT increased normally in rats with APX when equivalent increases in plasma osmolality (but not plasma Na⁺) resulted from intravenous infusion of an isosmotic solution of 1 M mannitol and 0.5 M NaCl. Furthermore, APX did not affect increases in plasma VP and OT stimulated by plasma volume deficits, nor did APX disrupt OT secretion stimulated by intravenous injection of cholecystokinin. These findings suggest that the area postrema plays an important role in mediating secretion of VP and OT in response to an NaCl load, but not in response to an isosmotic load that does not cause substantial hypernatremia, and not in response to other stimuli of neurohypophysial hormone secretion. Together with previous reports, these results suggest that APX impairs Na⁺ regulation in rats.

cholecystokinin; hypovolemia; sodium regulation; neurohypophysis; osmoregulation; posterior pituitary

RATS SHOW A ROBUST INTAKE of concentrated NaCl solutions after focal damage to the area postrema (APX), a circumventricular organ in the caudal brain stem (11, 12). This increased NaCl consumption appears to be one symptom of a general impairment of osmo- or Na⁺-regulatory mechanisms in rats with APX (12). Thus, in response to the large ingested NaCl load, rats with APX increased water ingestion, but not sufficiently for rapid osmoregulation, and they did not readily excrete in urine an NaCl load whether it had been ingested or injected intraperitoneally or subcutaneously. Furthermore, secretion of vasopressin (VP), the antidiuretic hormone, and oxytocin (OT), a natriuretic hormone, from the posterior lobe of the pituitary gland was blunted when 1 M NaCl solution was infused intravenously (2 ml/h for 2 h) (12). Another recent report (3) similarly described blunted secretion of VP by rats with APX in response to intraperitoneal injection of 0.3 M NaCl (2% body wt).

The present studies were designed to more fully evaluate neurohypophysial hormone secretion in rats with APX, initially by investigating the effects of a broad range of plasma osmolalities (P_{osmol}). The results indicated that VP and OT secretion are indeed blunted when rats with APX are continuously infused with hypertonic NaCl solution. The generality of this effect in rats with APX then was evaluated by examining VP and OT secretion in response to plasma volume deficits (38) and OT secretion in response to the peptide hormone cholecystokinin (CKC) (41). Finally, additional experiments studied the effect of APX on VP and OT secretion in response to an osmotic load provided by hypertonic mannitol mixed with hypertonic saline to determine whether the responses disrupted by APX were related to increases in P_{osmol} or, more specifically, to increases in plasma Na⁺ (P_{Na⁺}).

METHODS

Animals and preoperative maintenance. Adult male Sprague-Dawley rats (Zivic-Miller, Zelienople, PA), weighing ~300 g at the beginning of the experiments, were housed individually in wire-mesh cages. Laboratory chow pellets (type 5001, Ralston-Purina, St. Louis, MO) and water were available ad libitum, except during testing. The colony room was maintained at 22–23°C with lights on from 8 AM to 8 PM.

APX, APX were produced by vacuum aspiration with use of procedures described previously (13), except halothane was used as the anesthetic. Briefly, each rat was anesthetized, and its head was placed in a stereotaxic apparatus with the nose pointed down. A small, dorsal midline incision was made, the foramen magnum was enlarged, and the meninges were incised to expose the dorsal medulla. The area postrema (AP) was visualized through an operating microscope and aspirated through a blunted 25-gauge needle. Muscle and skin then were sutured, and a broad-spectrum antibiotic (penicillin, 30,000 U im) was administered.

As expected (13), APX usually caused a long period of hypophagia and body weight loss. Some rats required sweetened liquid foods for several weeks to encourage eating. When the rats recovered their preoperative body weights, ~6 wk later, the effectiveness of the lesions was confirmed by the failure of LiCl to suppress water intake elicited by water deprivation (13). Experiments began ~3 days after this screening test was completed.

Infusion of 1 M NaCl. One day before the experiments, rats were anesthetized with Equithesin (3 ml/kg body wt ip of a solution containing 0.98 g/dl pentobarbital sodium, 4.25 g/dl chlorhydrate, and 2.12 g/dl MgSO₄). Then one catheter (PE-50 tubing, Clay Adams, Parsippany, NJ) was placed into the right femoral artery for withdrawal of blood samples, and
another catheter (polyvinyl tubing with 0.58 mm ID, 0.965 mm OD; Biolab, Lake Havasu, AZ) was placed into the right femoral vein for infusion of hyposmotic solutions. The free ends of the catheters were guided subcutaneously along the back to exit between the scapulae, whereupon the catheters were encased in a steel spring (to prevent them from being damaged) and extended through the top of the cage.

Two groups of animals, rats with APX (n = 9) and body weight-matched control rats (n = 12), were studied. On the day of experiments, water and food were removed from each cage and the venous catheter was connected to an infusion pump (Harvard Apparatus, S. Natick, MA) for administration of isotonic saline (2 ml/h). After a 1-h control period, the administered fluid was switched and 1 M NaCl was infused continuously for 6 h (2 ml/h iv). Blood samples were obtained from the arterial catheter before the infusion of hypertonic saline started and then at 2-h intervals for 6 h. The arterial line was flushed with isotonic saline after each blood sampling. The blood samples were collected into chilled tubes containing EDTA (Vacutainer, Becton Dickinson, Franklin Lake, NJ) and were immediately centrifuged (1,100 g for 8–10 min at 4°C). The baseline sample (~1.5 ml) was replaced by an equal volume of prewarmed isotonic saline and returned to the rats. P_prot was determined by an Na sensitive electrode (Electrolyte Analyzer II, Beckman Instruments, Fullerton, CA) in a subset of animals. The remaining plasma was frozen at ~70°C for RIA of VP and OT.

Pilot studies indicated that APX did not impair urinary excretion when this dose of 1 M NaCl was infused intravenously, perhaps because it raised arterial blood pressure by ~20 mmHg in rats with APX but not at all in control animals and, thereby, caused a pressure natriuresis that overcame the expected impairments in excreting the NaCl load. In any case, comparable increases in P_prot were observed in both groups of rats in response to this treatment with hypertonic saline.

Injection of CCK. Rats with APX and control rats (n = 5 and 6, respectively), not used in previous experiments, were injected with CCK. Catheters were implanted as described above, except halothane was used instead of Equithesin. The injections of CCK (10 µg/kg iv, 1 ml/kg) were administered ~6 h after animals regained consciousness from the anesthetic. Blood samples (1 ml) were taken 30 min before and 5 min after injections of CCK, and this volume was not replaced. The samples were collected into chilled tubes containing EDTA and centrifuged immediately (1,100 g for 8–10 min at 4°C), and plasma was removed and frozen at ~70°C for RIA of OT.

Hypovolemia. A gradual reduction in plasma volume without substantial changes in systemic blood pressure was induced in rats with APX (n = 6) and control rats (n = 8) by injection of a 30% solution of polyethylene glycol (PEG; Carbowax, Compound 20-M, Fisher Scientific, Pittsburgh, PA) in isotonic saline (wt/wt, 5 ml sc) (29, 32). None of these PEG-treated rats had received hypertonic NaCl solution, but 11 of them (6 rats with APX and 5 control rats) had received CCK on the previous day.

Blood samples (1.5 ml) were taken from the arterial catheters one to three times during a 9-h period after PEG treatment. Baseline values were not obtained to reduce the number of blood samples in these hypovolemic rats; however, numerous observations in this laboratory have shown that plasma protein concentrations (P_prot) in untreated control rats are ~6.1 g/dl, and APX apparently does not affect those values (3). The samples were centrifuged immediately (1,100 g for 8–10 min at 4°C), and erythrocytes were resuspended in warmed isotonic saline and returned to the rats. P_prot was measured by refractometry and used to estimate plasma volume deficits (32), with the assumption of basal values of 6.1 g/dl and correction for the loss of protein in prior blood samples. The remaining plasma was frozen at ~70°C for RIA of VP and OT.

Infusion of 1 M mannitol and 0.5 M NaCl. Rats with APX and control rats (n = 9 in each group), not used previously, were studied using the same protocol used to investigate the effects of 1 M NaCl, described above, except a 50:50 mixture of 1 M mannitol-0.5 M NaCl was infused instead of saline.

This mixed hyposmotic solution was selected, because 1) it is equiosmotic with 1 M NaCl solution and, therefore, should cause a comparable increase in P_prot, 2) the effect of 0.5 M NaCl to increase P_prot is moderated by the effect of 1 M mannitol to cause osmotic movement of water from cells, and 3) the presence of 0.5 M NaCl in the infusate prevents hyponatremia that otherwise is caused by administration of hypertonic mannitol solution. As with infusion of 1 M NaCl (see above), pilot studies indicated that intravenous infusion of this mixed hyposmotic solution raised arterial blood pressure by ~20 mmHg in rats with APX but not at all in control animals and that comparable increases in P_prot were observed in both groups of rats.

One week after receiving the mixed hyposmotic solution, three rats with APX were studied again, this time with 1 M NaCl (as described above).

Finally, the same protocol was used again to study two other groups of control rats (n = 7 in each group) given the same infusions described above of 1 M NaCl or the mixed hyposmotic solution. Blood samples were taken at 2, 4, and 6 h during the infusions and analyzed in the same RIA for VP and OT.

Assays of VP and OT. For measurement of plasma levels of VP (PVP) and OT (POT), plasma samples were extracted using C18 Sep-Pak Vac cartridges (1 ml, 50 mg; Waters, Milford, MA), as described previously (28). VP and OT were measured by RIA in separate aliquots of this extract. Samples were assayed in duplicate for OT but not for VP (because there was insufficient sample). The assay sensitivity for VP was 2.5 pg/ml, and for OT it was 6.8 pg/ml. Samples from rats with APX were assayed at the same time as blood from their control animals. Occasionally, inadequate sample sizes prevented the assays of VP and OT in a single sample.

Histological analysis. After completion of testing, rats with APX were anesthetized with an overdose of urethan and perfused intracardially with 0.15% buffered Formalin solution. Brain stems were removed and stored in 10% Formalin until they were cut in 50-µm sections along the rostral-caudal extent corresponding to the AP. Sections were mounted, stained for Nissl substance with cresyl violet, and examined microscopically to determine the completeness and extent of APX.

Statistical analysis. The effects of intravenous infusion of hypertonic saline or the mixed hyposmotic solution, of subcutaneous PEG treatment, of intravenous injection of CCK, and of APX were evaluated by two-way ANOVA with repeated measures in the time parameter. The error terms and degrees of freedom from the ANOVA were used in t-tests to compare treatment values with baseline values within groups. Comparisons between groups at different points in time were made with one-way ANOVA followed by the Bonferroni adjusted t-test. Means ± SE were computed from group
values. Effects of APX on PVP and POT, expressed as a function of Posmol and presented in scatter plots, were evaluated by $\chi^2$ analysis. $P < 0.05$ was considered to be statistically significant.

RESULTS

Infusion of 1 M NaCl. Infusion of 1 M NaCl solution into conscious control rats increased $P_{\text{osmol}}$ progressively, as intended. Specifically, $P_{\text{osmol}}$ increased from baseline values of 291 ± 1 to 317 ± 1 mosmol/kg after 2 h of infusion and to 330 ± 2 mosmol/kg after 6 h (Fig. 1). Significant increases in $P_{\text{VP}}$ and $P_{\text{OT}}$ paralleled the induced hyperosmolality. Thus, $P_{\text{VP}}$ increased from baseline values of 3 ± 1 to 73 ± 9 pg VP/ml after 6 h of infusion, whereas $P_{\text{OT}}$ increased from 11 ± 3 to 175 ± 13 pg OT/ml (Fig. 2, A and B).

The NaCl load also increased $P_{\text{osmol}}$ progressively in rats with APX, from baseline values of 288 ± 2 to 316 ± 4 mosmol/kg after 2 h of infusion and to 331 ± 3 mosmol/kg after 6 h (Fig. 1). Parallel increments in $P_{\text{Na}}$ also were observed (from 138 ± 1 meq/l at baseline to 151 ± 2 meq/l at 6 h, $n = 6$). However, although the induced increases in $P_{\text{osmol}}$ did not differ significantly from those in the control rats (Fig. 1), neurohypophysial secretion of VP and OT was blunted significantly in rats with APX (although baseline values did not differ; Fig. 2, A and B). Thus $P_{\text{VP}}$ increased from 5 ± 1 to only 30 ± 5 pg VP/ml after 6 h of infusion, and $P_{\text{OT}}$ increased from 17 ± 4 to 63 ± 13 pg OT/ml (Fig. 2, A and B).

The mean ± SE values, shown in Fig. 1 and Fig. 2, A and B, obscure variability within groups; thus Fig. 2, C and D then were replotted as a function of associated Posmol. Symbols represent individual animals at specified times. Crosshatched symbols represent values from 3 rats with APX that were given hypertonic saline 1 wk after receiving an equiosmotic mixture of 1 M mannitol and 0.5 M NaCl solution.
and D, presents $P_{\text{VP}}$ and $P_{\text{OT}}$ from individual rats, plotted as a function of the associated $P_{\text{osmol}}$ at the three time points. Figure 2, C and D, clearly indicates that $P_{\text{VP}}$ and $P_{\text{OT}}$ were largely segregated according to group; values from rats with APX were much smaller than values from control rats ($\chi^2 = 23.9$ and 32.3, respectively, both $P < 0.001$), a difference that was most pronounced when $P_{\text{osmol}}$ was high.

Injection of CCK. In control rats, injection of CCK (10 µg/kg iv) increased $P_{\text{OT}}$ from baseline values of 17 ± 3 to 32 ± 5 pg/ml within 5 min ($P < 0.05$). That effect did not differ significantly from the effect of CCK treatment in rats with APX; $P_{\text{OT}}$ increased from 17 ± 2 to 38 ± 5 pg/ml ($P < 0.05$).

PEG-induced hypovolemia. Injection of 30% PEG solution produced a gradual elevation of $P_{\text{Prot}}$, as expected (32). Values of 9–10 g/dl were observed 9 h after PEG treatment, which suggest 33–40% decreases in plasma volume at that time. The induced changes in $P_{\text{Prot}}$ did not differ significantly in rats with APX and in control animals (Fig. 3).

Figure 4, A and B, indicates that mean values of $P_{\text{VP}}$ and $P_{\text{OT}}$ also increased progressively as a function of time, in parallel with the elevation in $P_{\text{Prot}}$, and that APX had no significant effects on these values. Similarly, comparable values of $P_{\text{VP}}$ and $P_{\text{OT}}$ were seen in PEG-treated rats with APX and control rats when data from individual animals were plotted as a function of the associated $P_{\text{Prot}}$ (Fig. 4, C and D).

Infusion of 1 M mannitol and 0.5 M NaCl. Infusion of a mixed solution of 1 M mannitol and 0.5 M NaCl elevated $P_{\text{osmol}}$ comparably in rats with APX and in control rats.
control animals (Fig. 5) to values resembling those observed when an equiosmotic solution of 1 M NaCl was infused (Fig. 1). In contrast, PNa after administration of the mixed hyperosmotic solution were much lower (143 ± 1 meq/l at 6 h) than those observed after treatment with hypertonic saline (P < 0.05), as expected.

Mean values of PVP and POT increased with time as the mixed hyperosmotic solution was infused in control rats (Fig. 6, A and B). However, APX did not blunt VP or OT secretion in response to the mixed hyperosmotic solution (Fig. 6, A and B), in contrast to its effects when 1 M NaCl was given (Fig. 2, A and B). Similarly, comparable values of PVP and POT were seen in rats with APX and control rats in response to the mixed hyperosmotic solution when data from individual animals were plotted as a function of the associated Posmol (Fig. 6, C and D). In contrast, PVP and POT from the three rats with APX that received hypertonic saline 1 wk later did show blunted hormone secretion in response to this treatment, as in the other group of rats with APX (Fig. 2, A and B).

In control rats the increases in POT stimulated by infusion of the mixed hyperosmotic solution (Fig. 6, A and C) appeared to be smaller than those stimulated by infusion of hypertonic saline (Fig. 2, A and C). Although this difference did not reach statistical significance, six additional rats were infused with the two solutions to further assess this comparison. Again, no statistically significant differences were found when POT was plotted as a function of Posmol (data not shown). The same was true when PVP was plotted as a function of Posmol.

**Fig. 5.** Effect of infusing a mixed solution of 1 M mannitol (Mann) and 0.5 M NaCl (2 ml/h iv) on Posmol in rats with APX (n = 9) and control rats (n = 9) as a function of time. Infusion was started at 0 h, and Posmol increased with time of infusion in both groups (both P < 0.01). No statistically significant differences between groups were observed. Values are means ± SE.

**Fig. 6.** Effect of infusing a mixed solution of 1 M mannitol and 0.5 M NaCl (2 ml/h iv) on plasma OT (A) and plasma VP (B) in rats with APX (n = 9) and control rats (n = 9) as a function of time. Posmol in these animals are shown in Fig. 5. Secretions of VP and OT were not blunted in rats with APX. Values are means ± SE. Stimulated values of plasma OT (C) and plasma VP (D) then were replotted as a function of associated Posmol in these animals. Symbols represent individual animals at specified times.
Relation between PVP and POT. Figure 7 displays individual values of POT plotted as a function of the associated PVP in the same blood sample when rats were given 1 M NaCl solution, the mixed hyposmotic solution, or PEG treatment. The results indicate that the relation between these variables was similar in rats with APX and in control rats regardless of the treatment, the time of blood sampling, or the induced increase in P\textsubscript{osmol} (after infusion of hypertonic saline or the mixed hyposmotic solution) or in P\textsubscript{prot} (after PEG treatment). Thus the increases in PVP and POT were blunted equivalently by APX when 1 M NaCl solution was given. The ratio of POT to PVP was \(~2:1\) when P\textsubscript{osmol} was elevated, whereas it was only \(~1:1\) when rats were hypovolemic, as noted previously (37).

Histology. Histological assessment of the brain stems from rats with APX in the present study revealed lesions similar to the large APX reported previously by this laboratory (12). Destruction of the AP appeared to be complete in each rat with APX. However, damage in the caudal brain stem was not confined to the AP. Instead, it often invaded portions of the subadjacent nucleus of the solitary tract (NST) and occasionally extended to the dorsal motor nucleus of the vagus and the hypoglossal nucleus.

Following convention, all brain-damaged animals were referred to as “rats with APX” throughout this report. However, it should be borne in mind that the critical damage may include the medial subnucleus of the NST, a site where neurons that alter discharge rate in response to increases in plasma Na\textsuperscript{+} have been noted (20).

DISCUSSION

Three main findings derive from these experiments on the neurohypophysial secretion of VP and OT in conscious rats with APX. First, APX markedly impaired the increases in PVP and POT that were stimulated by intravenous infusion of hypertonic NaCl solution. Second, APX did not affect the increase in POT that was evoked by intravenous injection of CCK or the increases in PVP and POT that were elicited by hypovolemia. Third, APX did not affect the increases in PVP and POT stimulated by intravenous infusion of a hyposmotic solution that did not cause substantial hypernatremia. These findings suggest that APX in rats selectively blunts VP and OT secretion evoked by NaCl loads in association with hypernatremia.

It has long been known that an infused NaCl load stimulates secretion of the antidiuretic hormone VP (43). More recently, it has been shown that administration of hypertonic NaCl solution also elicits OT secretion in rats (8, 38) and that increased POT serves the useful function of promoting urinary Na\textsuperscript{+} excretion after an NaCl load (22, 40). These effects of hypertonic saline on secretion of neurohypophysial hormones are abolished by surgical destruction of the organum vasculosum of the lamina terminalis and the nucleus medianus, the location of forebrain osmoreceptors (18, 25).

The present experiments indicate that surgical destruction of the AP in the caudal brain stem also impairs neurohypophysial secretion of VP and OT in response to an infused NaCl load. The effect was seen over a broad range of P\textsubscript{osmol} values in 12 rats with APX, when blood samples were obtained at multiple time points during continuous intravenous infusion of hypertonic saline.

The selectivity of this blunting effect of APX on VP and OT secretion stimulated by hypertonic saline was evaluated by using three other treatments that produce different stimuli for neurohypophysial hormone secretion. One treatment known to cause secretion of OT in rats, although not VP, is systemic administration of CCK (41). This effect appears to be mediated by gastric vagal afferents that project to the NST in the brain stem and then to hypothalamic magnocellular neurons, inasmuch as CCK-induced OT secretion is abolished by gastric vagotomy (41) and by surgical destruction of the NST (19). The present findings indicate that OT secretion after CCK treatment in rats with APX was comparable to that in control animals, in contrast to the effects of hypertonic NaCl solution. Although APX were not confined to the AP but invaded the subadjacent NST, evidently this additional damage was not large enough or appropriately placed within the NST to disrupt OT secretion in response to CCK. In contrast, Carter and Lightman (10) found that APX did blunt OT secretion by rats in response to 100 µg/kg of CCK (10 times the dose employed in the present experiments), and perhaps the brain stem lesions in their study did produce more extensive incidental damage to the NST.

A second treatment, known to elicit secretion of VP and OT in rats, involves subcutaneous injection of 30% PEG solution, which leaches protein-free isosmotic plasma fluid from the circulation into the local interstitium and, thereby, induces hypovolemia (32). In consequence, VP and OT are known to be secreted exponentially in association with progressive plasma volume...
deficits (15, 37, 38). The present experiments indicated that hypovolemic stimulation of VP and OT secretion was not impaired in rats with APX when the induced plasma volume deficits were ~10%-40%. Individual values of PVP or POT plotted as a function of PProt from PEG-treated rats with APX were intermingled with those from PEG-treated control rats throughout this broad range of volume deficits (Fig. 4, C and D), in contrast to the segregation of values seen when hypertonic saline provided the stimulus for VP and OT secretion (Fig. 2, C and D).

Finally, in assessing the specificity of the disruption by APX of neurohypophysial hormone secretion in response to hypertonic NaCl solution, we also determined whether comparable impairments in VP and OT secretion would be seen when rats with APX were infused with a hyperosmotic solution that did not cause substantial hypnaetraemia. In the control animals the mixed hyperosmotic solution stimulated increases in PVP and POT, as expected (24, 27, 43). It was therefore striking that, after treatment with the mixed hyperosmotic solution, rats with APX did not show blunted increases in PVP and POT. Furthermore, the increases in PVP and POT stimulated by hypertonic saline were reduced equivalently by APX, indicating that the lesions did not affect secretion of those peptide hormones differentially. These results suggest that the AP plays an important role in mediating the secretion of both neurohypophysial hormones in response to stimulation by plasma hyperosmolarity only when associated with hypnaetraemia.

Treatments that increase P-osmol without substantial hypnaetraemia (e.g., systemic administration of hypertonic solution of mannitol) have several prominent effects that closely resemble those observed after treatment that increase P-osmol and P-Na (e.g., systemic administration of hypertonic NaCl solution). For example, both treatments evoke increases in secretion of VP and OT (Figs. 2 and 6) (24) and in water intake (16, 24). However, different effects of the two treatments occurred when central OT receptors in rats were pharmacologically blocked or destroyed; the inhibitory effect of hypertonic mannitol on an induced NaCl appetite then was prevented, whereas the inhibitory effect of equiosmotic hypertonic NaCl solution on NaCl appetite was not affected (6). The present experiments provide another example of brain damage revealing differential effects of these hypertonic solutions in rats, this time with respect to the control of VP and OT secretion.

Given the common conceptualization of cerebral osmoreceptors as responsive to the effective osmolality of extracellular fluid (27), the two equiosmotic hypertonic solutions used in the present investigations should be equally potent in causing osmoreceptors to communicate with the AP. Therefore, reducing the volume of osmoreceptor cells. Thus it is unclear how damage to a remote brain site could produce the differential effects on neurohypophysial hormone secretion that were observed. One possibility is that hypertonic saline somehow signals the forebrain osmoreceptors that another input to those cells is required for full expression of the stimulus for VP and OT secretion. In light of the present results, this input might come from Na+ receptors located in the AP or in a visceral site that signals the brain via the AP, which then communicate this complex message via polysynaptic projections to the magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei (30). Although such speculation remains to be evaluated, it is consistent with previous reports that AP neurons are responsive to hypertonic NaCl applied directly (1, 5), that the AP may receive afferent information regarding the presence of hypertonic NaCl (but not of hyperosmotic solutions generally) in the hepatic portal vein (2, 26), and that these neural signals may affect neurohypophysial secretion (4, 9) as well as NaCl intake (39).

Surgical destruction of osmoreceptors in the organum vasculosum of the lamina terminalis and nucleus medianus eliminates secretion of VP and OT induced by administered NaCl loads (18, 25) but does not disrupt the inhibition of food or NaCl intake (17, 44). Thus additional receptors of functional significance must exist elsewhere than these structures. As a circumventricular organ lacking a blood-brain barrier, the AP is in a privileged position to monitor changes in the blood while also receiving neural information from the viscera (7). It seems plausible that selective Na+ receptors in the periphery, in the AP, or in both sites subserve Na+ regulation.

Previously, APX have been reported to attenuate VP secretion in response to hypertonic NaCl (3, 23), consistent with the present results. Although Honda et al. (21) reported that an induced OT secretion was not blunted by APX, that study was conducted in rats that were anesthetized rather than conscious. In each of these previous investigations, a single small dose of hypertonic saline was injected intraperitoneally; this procedure produces a transient spike in P-osmol (38), rather than the steady increase that is observed when saline is infused (Fig. 1), and it likely confounds the investigation of OT secretion by introducing pain as an additional variable (42). Recent work from our laboratories (12) in which 1 M NaCl was infused intravenously (2 ml/h for 2 h) produced results consistent with the more extensive findings now reported.

Arima et al. (3) also reported that APX caused a small but statistically significant attenuation in the increased PVP of rats given an intraperitoneal PEG treatment that produced only ~11% plasma volume deficits (i.e., PProt increased from 6.3 to 7.1 g/dl). In the present experiment, there were too few observations of PVP at such modest hypovolemia to allow direct comparison of the two sets of results. Although further work is needed to clarify the effect of APX in mediating VP secretion in rats when plasma volume deficits are very small, the present findings indicate that APX do not affect the secretion of VP or OT in rats when plasma volume deficits are more substantial. Skoog et al. (31) similarly reported that the increased PVP that occurs in response to serial hemorrhage is not affected by APX in rats.

To summarize, APX in rats markedly blunted neurohypophysial hormone secretion in response to a range
of NaCl loads infused intravenously. However, this effect of APX was not seen when rats were given equiosmotic loads that produced comparable increases in $P_{\text{osmol}}$ without substantial hypernatremia. Similarly, APX did not affect the increase in $P_{\text{OT}}$ that was elicited by CCK injected intravenously or the increases in $P_{\text{Na}}$ and $P_{\text{OT}}$ that were stimulated by a range of isosmotic plasma volume deficits. These results suggest the AP plays a specific role in mediating neurohypophysial hormone secretion in response to hypernatremia and, additionally, provide further evidence that the overlapping controls of $Na^+$ regulation and osmoregulation can be separated.

**Perspectives**

Administration of an NaCl load stimulates multiple adaptive responses in animals that serve to buffer the induced increases in $P_{\text{Na}}$ and $P_{\text{osmol}}$. In addition to antidiuresis and natriuresis, two behavioral responses occur that also are adaptive. One, increased thirst, is a familiar consequence of increased $P_{\text{osmol}}$. Less well understood but also reliable is a reduction of NaCl intake in rats (6, 33). It is noteworthy that APX impairs each of these physiological and behavioral responses. The present report, demonstrating that VP and OT secretion are disrupted by APX, may explain the impairments in urinary water conservation and $Na^+$ excretion that occur when rats with APX ingest a large NaCl load (12). In addition, although rats with APX drink more water than control rats do in response to an injected NaCl load (14), they do not drink enough water to osmoregulate rapidly when the NaCl load is consumed (12). Similarly, rats with APX do not drink 0.5 M NaCl ad libitum in very small bouts (34), as neurologically intact control rats do when they have a pronounced NaCl appetite (35, 36). These findings suggest that rats with APX have a general impairment in $Na^+$ regulation, rather than specific impairments in VP and OT secretion, in urinary $Na^+$ excretion, and in ingestion of water and saline. For example, each of these separate dysfunctions may have in common an impaired ability of rats with APX to sense a systemic or gastric NaCl load. This hypothesis remains to be evaluated in further experiments determining the complex role of the AP in body fluid homeostasis.

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