Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10380–10384, November 1993
Neurobiology

Central oxytocin inhibition of salt appetite in rats: Evidence for differential sensing of plasma sodium and osmolality

(ricin A toxin/mannitol/osmo- and Na^+ receptors/thirst/hypovolemia)

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Communicated by Eliot Stellar, July 28, 1993

ABSTRACT **Sodium chloride ingestion is stimulated during conditions of sodium deficiency to maintain body fluid and electrolyte balance. Recent studies have indicated that salt appetite in rats is often inversely related to peripheral and central secretion of the hormone oxytocin (OT). We studied the potential role of central OT on salt and water ingestion by treating rats intracerebroventricularly with OT conjugated to the A chain of the plant cytotoxin ricin (rAOT) to produce a chronic selective inactivation of brain cells containing OT-receptive elements. The rats treated with rAOT and control rats treated with the ricin A chain alone were given 5-hr two-bottle (water and 0.5 M NaCl) drinking tests 30 min after they were made hyperosmolar by injections of hypertonic (2 M) mannitol solution, which elevated plasma osmolality but reduced plasma Na^+ concentration. In the control rats only water intake was stimulated in response to the induced hyperosmolality, but in the rAOT-treated rats hypertonic mannitol caused a robust salt appetite as well as thirst. Analogous results were obtained in rats treated with two different OT-receptor antagonists prior to induction of hyperosmolality with mannitol. In contrast to these results, when hyperosmolality was induced by administration of equivalently hypertonic (1 M) NaCl, which elevated both plasma osmolality and plasma Na^+ concentration, only water intake but not salt intake was stimulated in both control and OT-receptor antagonist-treated rats. When salt appetite was stimulated by the physiological stimulus of polyethylene glycol-induced hypovolemia, hypertonic mannitol similarly inhibited salt ingestion in control animals but not in rAOT-treated rats, whereas hypertonic NaCl inhibited subsequent salt ingestion in both groups. These results suggest that salt appetite is regulated by both Na^+- and osmolality-sensing mechanisms in rats. In addition, they indicate that central OT likely mediates a significant component of osmolality-related inhibition of salt appetite but does not appear to be essential for Na^+-related inhibition of this important homeostatic behavior.**

Salt appetite is a vital homeostatic behavior in many species. The ingestion of salt in response to a perceived body deficit of Na^+ is analogous to the ingestion of water by dehydrated animals. Together these two behaviors promote maintenance of body fluid and electrolyte balance within very narrow limits. In rats, ingestion of concentrated NaCl solutions can be induced by diverse experimental manipulations. However, regardless of the paradigm employed, NaCl ingestion is generally most prominent when pituitary oxytocin (OT) release is either low or at basal levels and is almost always inhibited when OT release is stimulated (1). Since central, but not peripheral, administration of OT can inhibit salt appetite in rats, it has been proposed that OT pathways projecting within the brain rather than those projecting to the pituitary underlie this inhibition (1–3). In support of this hypothesis, several treatments known to evoke coactivation of both central- and pituitary-projecting OT cells, such as administration of naloxone and angiotensin II, have been shown to cause an inhibition of stimulated salt appetite, and this inhibition can be eliminated by central treatment with specific OT-receptor antagonists (3, 4).

The present experiments extend this line of investigation by utilizing recent advances in selective cytotoxin methodology to study salt appetite. This methodology involves conjugation of the toxic A chain of the plant cytotoxin ricin (rA) to a ligand that binds to a specific target cell and is translocated into the cell. The complex then inhibits protein synthesis by enzymatically inactivating the elongation factor 2 binding site of the 60S ribosomal subunit, resulting in cell death (5). When the rA is unconjugated, access to the cell interior is prevented and no toxicity is exerted, producing an appropriate control treatment (6, 7). By using a conjugate of rA coupled to OT (rAOT), this technique allowed us to investigate salt appetite in rats under conditions in which brain cells bearing OT receptors were functionally inactivated on a chronic basis but OT secretion was unimpaired. In selecting a treatment to stimulate OT neurons, we chose hyperosmolality, since acute increases in plasma osmolality are well known to stimulate both pituitary and brain OT secretion (8, 9). However, instead of using only hypertonic NaCl solutions, which are most commonly used experimentally to elevate plasma osmolality, our studies also used hypertonic solutions of mannitol to evaluate the effects of hyperosmolality independently of concurrent increases in plasma Na^+ concentration.

MATERIALS AND METHODS

Animal Preparation. Adult male rats (300–350 g) of the Sprague–Dawley strain (Zivic–Miller) were caged individually in a temperature-controlled room (22°C) on a light/dark 12/12-hr schedule. For at least 5 days prior to surgery, all rats were allowed ad libitum access to pelleted food (Wayne Lab Blox, Chicago), tap water, and 0.5 M NaCl solution. On the day of surgery, intracerebroventricular (i.c.v.) cannulae were implanted in the right lateral ventricle by using standard stereotaxic procedures and equithesin anesthesia (0.33 ml i.p. per 100 g of body weight of a solution containing pentobarbital sodium at 0.98 g/dl, chloral hydrate at 4.25 g/dl, and $MgSO_4$ at 2.12 g/dl) as described (3). For studies involving rA

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Abbreviations: i.c.v., intracerebroventricular; CSF, cerebrospinal fluid; aCSF, artificial CSF; rA, ricin A chain; OT, oxytocin; LHRH, luteinizing hormone-releasing hormone; rAOT and rALHRH, rA conjugated to OT and LHRH; OVT, OT-antagonist ornithine vasotocin; AVP, arginine vasopressin; PEG, polyethylene glycol; osm, osmolal; mosmol, milliosmole.

conjugates, rats were treated during surgery with i.c.v. injections of rAOT [5 μg per rat in 5 μl of artificial cerebrospinal fluid (aCSF)] or with control injections of either unconjugated rA [5 μg per rat in 5 μl of aCSF] or rA conjugated to luteinizing hormone-releasing hormone (rALHRH; 5 μg per rat in 5 μl of aCSF). Since such treatments often were associated with a transient loss of body weight, all ricin-treated rats were allowed to recover from surgery for at least 7 days until the original body weight was reattained and a 3-day period of consecutive weight gain had been achieved. Some of the animals also had jugular venous catheters surgically implanted for repeated blood sampling; these were inserted after recovery from the i.c.v. surgery and at least 48 hr prior to further experimentation. For studies of reversible blockade of central receptors, separate groups of rats were surgically implanted with i.c.v. cannulae as described above, but no central injections were made at the time of surgery. These animals also were allowed to recover for 7 days prior to experimentation, with free access to chow, water, and 0.5 M NaCl in the interim. An additional group of rats had indwelling cannulae implanted stereotactically into the cisterna magna rather than the lateral ventricle to allow CSF sampling and measurement of CSF Na^+ concentrations. All subsequent experiments were conducted between noon and 1700 on the day of study.

rA Conjugate Studies. The efficacy of the rAOT treatment was evaluated functionally prior to experimentation by verifying the absence of grooming behaviors normally seen in response to central injections of OT (10). OT (Sigma) was injected i.c.v. at a dose of 10 μg per rat in 5 μl of aCSF, and only those rAOT-treated rats who failed to groom within 15 min of OT administration were included in subsequent studies. Initial experiments were conducted to characterize the physiological and behavioral effects produced by administration of hypertonic mannitol in rAOT-treated (n = 9) or rA-treated rats (n = 5). Food and fluids were removed 1 hr prior to injection with hypertonic mannitol i.p. (2 ml of 2 M D-mannitol; Fisher Scientific). Thirty minutes later rats were allowed access to both water and 0.5 M NaCl. Subsequent fluid intakes were measured hourly for 5 hr. When rats did not drink during the test period, fluid intake was assumed to be equivalent to the accuracy of the measurement (± 0.25 ml). Blood samples (1 ml) were withdrawn both immediately before and 30 min after the injection of mannitol; additional blood samples were taken at 2 hr and 5 hr after mannitol administration. Plasma was separated and analyzed for measurement of osmolality (Advanced Instruments 3C2 osmometer) and Na^+ concentration (Beckman electrolyte 2 analyzer). Additional groups of 8 rA- and 8 rAOT-treated rats with indwelling jugular catheters were given i.p. injections of 2 M mannitol, as above, and blood samples (1 ml) were withdrawn 30 min after the mannitol treatment for measurement of plasma OT concentration by radioimmunoassay as described (1). Rats with indwelling cisterna magna cannulae (n = 6) had CSF samples (50 μl) drawn before and 30 min after injection of the same dose of hypertonic mannitol for measurement of CSF Na^+ concentration (Beckman electrolyte 2 analyzer).

OT-Receptor Antagonist Studies. To verify that the effects of rAOT-treatment on salt appetite were not due to nonspecific actions of the conjugate, we utilized a different methodology to pharmacologically block central OT receptors and repeated the drinking tests. Rats without any prior treatments had food and fluids removed 1 hr before experimentation and were given i.c.v. injections of either aCSF (5 μl i.c.v.; n = 12) or the OT antagonist [d(CH_2)$_5$,Tyr(Me)^2,Orn^8]-vasotocin (OVT, Peninsula Laboratories; 10 μg per rat in 5 μl of aCSF i.c.v.; n = 12) or were given i.p. injections of the cyclic hexapeptide OT antagonist cyclo-(L-Pro-D-2-naphthylalanine-L-Ile-D-pipecolic acid-L-pipecolic acid-D-His) (L-366,948, Merck Sharpe & Dohme; 1.0 mg/kg of body weight in 1.5 ml of 0.15 M NaCl; n = 12). These OT-receptor antagonists have been documented to block a variety of central actions of OT (3, 4, 11–14). Thirty minutes after these treatments, the rats were given i.p. injections of either hypertonic mannitol (2 ml of a 2 M solution) or equimolar amounts of hypertonic NaCl (2 ml of a 1 M solution), and the water and 0.5 M NaCl drinking solutions were returned 30 min later. Fluid intakes were measured hourly for 5 hr as before.

Hypovolemia Studies. Additional rAOT-treated rats were used to examine the effects of mannitol-induced hyperosmolality under more physiological conditions of stimulated salt appetite. For this we chose a model of prolonged hypovolemia induced by subcutaneous administration of the colloid polyethylene glycol (PEG), a treatment that produces intravascular volume deficits by redistribution of extracellular fluid and thereby elicits a robust salt appetite in the absence of a negative Na^+ balance (15). Groups of rats treated previously with either rAOT (n = 16), rA (n = 7), rALHRH (n = 5), or aCSF (n = 6) were anesthetized briefly with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) and given subcutaneous injections of 5 ml of PEG solution [30% (wt/wt) in 0.15 M NaCl; PEG 20-M, Carbowax, Fisher Scientific] into the back (16), after which the animals were returned to their home cages and deprived of food and fluids for 24 hr. All rats were then injected with hypertonic mannitol i.p. (2 ml of a 2 M solution) and 30 min later were given access to water and 0.5 M NaCl in a two-bottle 5-hr testing paradigm as before. Another group of rA-treated (n = 5) and rAOT-treated (n = 5) rats were similarly injected with PEG, and their 5-hr intakes of water and 0.5 M NaCl were measured 24 hr later after i.p. injection of hypertonic NaCl (2 ml of a 1 M solution) instead of hypertonic mannitol.

Statistical Analyses. For comparison of total 5-hr fluid intakes, data were analyzed by using one-way ANOVA across all groups with post-hoc comparison via Tukey's protected t test where appropriate. For comparison of multiple groups at different time points, data were analyzed by two-way ANOVA using time as the repeated variable and group as the independent variable, with post-hoc comparisons at individual time points via Tukey's protected t test where appropriate.

RESULTS

Effects of Chronic OT-Receptor Inactivation on Saline and Water Intake. Treatment with rAOT or rA was not by itself associated with spontaneous baseline ingestion of 0.5 M NaCl during the 7-day postsurgical recovery period. After administration of hypertonic mannitol, the rA-treated control rats exhibited a significantly increased water intake (8.2 ± 0.6 ml) appropriate to their increased plasma osmolality but did not ingest appreciable amounts of 0.5 M NaCl (≤ 0.25 ml; Fig. 1 *A* and *B*). In striking contrast, rAOT-treated rats drank relatively large amounts of 0.5 M NaCl after the administration of hypertonic mannitol (6.4 ± 0.9 ml), which were equivalent to their water intakes (7.0 ± 1.0 ml); the stimulated salt ingestion continued throughout the first 3 hr after mannitol treatment and paralleled the pattern of stimulated water ingestion during this time (Fig. 1 *A* and *B*). In both groups mannitol treatment resulted initially in a rapid and significant rise in plasma osmolality [rA = 301 ± 2 to 314 ± 2 mosmol/kg of H_2O—i.e., milliosmolal (mosm)—30 min after injection; rAOT = 290 ± 6 to 324 ± 5 mosmol/kg of H_2O—i.e., mosm—30 min after injection; Fig. 1*C*] and a compensatory decline in plasma Na^+ concentration secondary to osmotically induced shifts of water out of the intracellular compartment [rA = 144 ± 1 to 134 ± 3 mmol/liter (mM) 30 min after injection; rAOT = 140 ± 1 to 132 ± 3 mmol/liter (mM) 30 min after injection; Fig. 1*D*]. Measurement of CSF Na^+ concen-

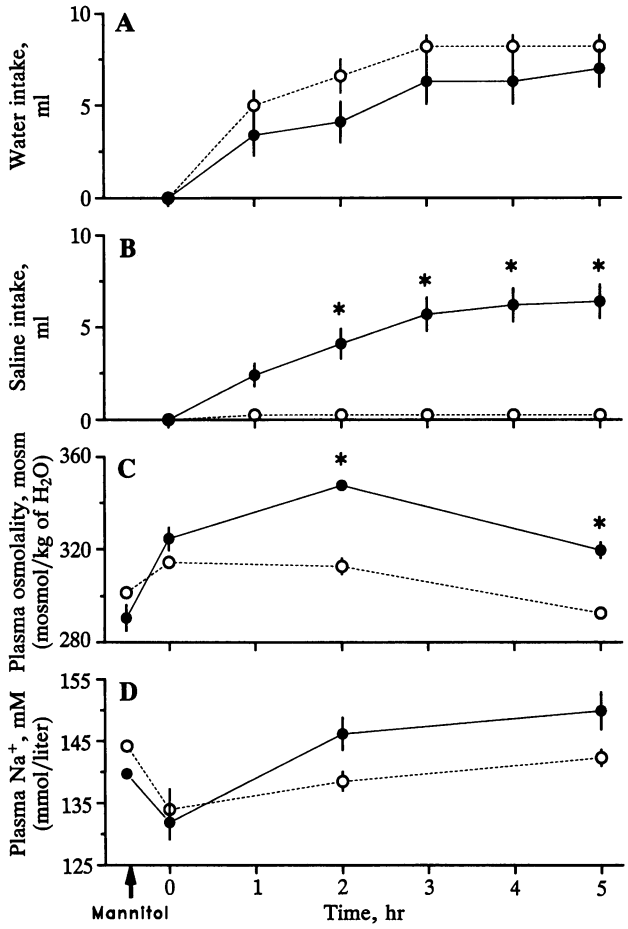

FIG. 1. Cumulative intakes of water (*A*) and 0.5 M NaCl (*B*) solution by rats during two-bottle drinking tests that began 30 min after administration of hypertonic mannitol (arrow). Rats were treated 7 days prior to mannitol administration with i.c.v. injections of either rA (○) (5 μg per rat; $n = 5$) or rAOT (●) (5 μg per rat; $n = 9$). Blood samples were drawn before and at 30 min, 2.5 hr, and 5.5 hr after the mannitol administration for measurement of plasma osmolality (*C*) and plasma Na^+ concentration (*D*). All values are expressed as means ± SEM. *, $P < 0.01$ compared with rA-treated rats.

trations 30 min after administration of hypertonic mannitol to a separate group of rats with indwelling cannulae in the cisterna magna showed a significant increase of CSF Na^+ concentration [basal CSF Na^+ = 152 ± 1 mmol/liter (mM) and CSF Na^+ 30 min after mannitol = 155 ± 1 mmol/liter (mM); $P < 0.01$], despite the decrease in plasma Na^+ concentration at the same time point (Fig. 1*D*). In rA-treated rats, plasma osmolality then declined gradually toward basal values as a result of stimulated water intake. In rAOT-treated rats, plasma Na^+ concentration normalized more rapidly as a result of their intake of 0.5 M NaCl, but this caused a further increase in their plasma osmolality to very high levels (Fig. 1 *C* and *D*). Radioimmunoassay of OT concentrations (8) in plasma taken from additional rA- and rAOT-treated rats 30 min after hypertonic mannitol administration revealed that plasma OT concentrations were elevated to equivalent degrees in both groups at this time point (basal plasma OT = 9.8 ± 2.1 pg/ml, $n = 8$; after mannitol treatment, rA-treated plasma OT = 26.8 ± 8.6 pg/ml, $n = 7$, and rAOT-treated plasma OT = 34.3 ± 12.9 pg/ml, $n = 8$), confirming equivalent osmotic stimulation of pituitary-projecting OT magnocellular neurons in both groups.

Effects of OT Receptor Blockade on Saline and Water Intake. Total 5-hr intakes of water and 0.5 M NaCl by rats treated with either of two OT-receptor antagonists and then given hypertonic mannitol or hypertonic NaCl are shown in Fig. 2. The results using both antagonists and hypertonic mannitol were similar to those seen in the rats that received rAOT. In control (aCSF-treated) rats, mannitol stimulated intake of water (5.5 ± 0.4 ml) but not 0.5 M NaCl (≤ 0.25 ml), whereas rats treated with either OVT or L-366,948 drank significant amounts of 0.5 M NaCl (2.8 ± 0.5 ml and 3.6 ± 0.3 ml, respectively) in addition to water (5.3 ± 0.6 ml and 4.8 ± 0.5 ml, respectively). However, when rats treated with the same OT-receptor antagonists were given equimolar injections of hypertonic NaCl rather than hypertonic mannitol, their intakes resembled those of the control animals treated with aCSF in that only pronounced thirst was manifested (5–7 ml) without any stimulated NaCl intake (≤ 0.25 ml in all groups).

Effects of Chronic OT-Receptor Inactivation on Hyperosmolar Inhibition of Hypovolemia-Stimulated Salt Appetite. Cumulative 5-hr intakes of water and 0.5 M NaCl by PEG-treated rats after administration of hypertonic mannitol are shown in Table 1. Rats treated with rAOT had significantly higher 0.5 M NaCl intakes than those treated with nonconjugated rA, whereas their water intakes were equivalent. The 0.5 M NaCl intake of the rAOT-treated rats (9.6 ± 1.4 ml) was comparable to that of normal rats treated with this dose of PEG under similar conditions in recent studies from our laboratories (6–7 ml; ref. 3). In additional control groups of rats treated with i.c.v. injections of either aCSF or rALHRH, mannitol abolished hypovolemia-stimulated 0.5 M NaCl ingestion but spared the thirst response, analogous to the rA-treated rats (Table 1). In contrast, injecting rA- and rAOT-treated hypovolemic rats with hypertonic NaCl rather than mannitol eliminated NaCl intake (≤ 0.25 ml in both rA- and rAOT-treated rats) and stimulated only water intake in both groups (rA = 11.4 ± 0.6 ml and rAOT = 11.6 ± 1.7 ml),

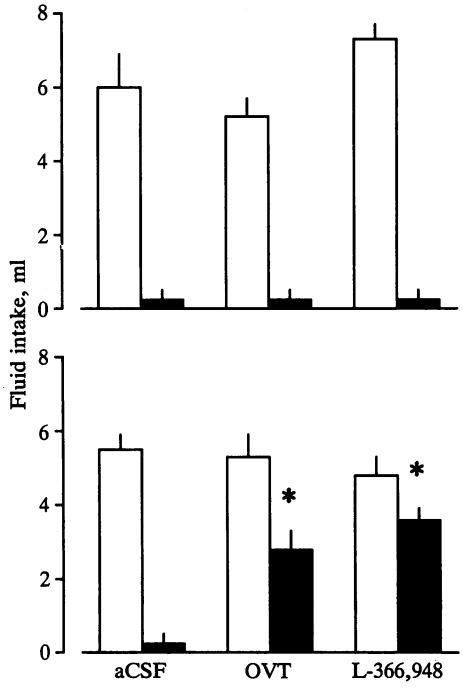

FIG. 2. Total 5-hr cumulative intakes of water (open bars) and 0.5 M NaCl (solid bars) by rats treated with aCSF ($n = 12$) or different OT-receptor antagonists (OVT, $n = 12$; L-366,948, $n = 12$) during a two-bottle drinking test that began 30 min after i.p. injection of hypertonic solutions of either NaCl (2 ml of 1 M i.p.) (*Upper*) or mannitol (2 ml of 2 M i.p.) (*Lower*). All values are expressed as means ± SEM. *, $P < 0.01$ compared with control intakes (aCSF).

Table 1. Cumulative 5-hr intakes of water and 0.5 M NaCl by rats in two-bottle drinking tests that began 24 hr after PEG treatment and 30 min after administration of hypertonic mannitol i.p.

Prior treatment	*n*	Cumulative fluid intakes, ml	
		H_2O	0.5 M NaCl
aCSF	6	18.3 ± 4.4	0.2 ± 0.2
rA	7	15.6 ± 5.4	0.3 ± 0.2
rALHRH	5	23.4 ± 6.0	0.8 ± 0.6
rAOT	16	14.2 ± 3.7	9.6 ± 1.4*

Values are means ± SEM.
*$P < 0.01$, compared to all control groups (aCSF, rA, and rALHRH).

analogous to the results in OT-receptor antagonist-treated rats (Fig. 2).

DISCUSSION

Whether the brain primarily senses osmolality or Na^+ concentration in regulating body fluid and electrolyte homeostasis has remained a controversial question since Verney's proposal that hypothalamic osmoreceptors regulate pituitary vasopressin [arginine vasopressin (AVP)] secretion. Verney's theory was based on evidence that AVP secretion was elicited by hyperosmolar solutions of impermeant solutes such as sucrose or NaCl, which cannot readily penetrate cells, but not by hyperosmolar solutions of solutes that penetrate cells freely, such as urea and dextrose (17). He concluded that osmoreceptive mechanisms primarily regulated AVP secretion in response to cellular dehydration and shrinkage induced by an increased osmotic gradient across the cell membrane regardless of the nature of the impermeant solute. However, Andersson *et al.* (18, 19) subsequently provided evidence suggesting that sensors within the brain also influenced water and electrolyte homeostasis by detecting changes in the Na^+ concentration of CSF. Our studies indicate that both osmolality- and Na^+-sensing mechanisms participate in the control of NaCl intake by rats. Mannitol-induced hyperosmolality caused an osmoregulatory water intake with absent or inhibited saline intake in normal and hypovolemic rats, but when central OT receptors were inactivated by rAOT treatment or blocked by central administration of OT-receptor antagonists, the same degree of hyperosmolality no longer was effective in inhibiting saline ingestion. The physiological importance and behavioral specificity of this OT-mediated inhibition of salt appetite for maintenance of body fluid homeostasis can be best appreciated by the observation that rats in which these pathways were blocked continued to drink concentrated NaCl solution as their plasma Na^+ concentrations increased toward normal, despite the fact that this behavior caused large increases of plasma osmolality into pathological ranges. In contrast, the inhibitory effects on salt appetite of hypertonic NaCl injected systemically were completely unaffected by treatments that impaired central OT receptors. These results can be best explained by a simultaneous participation of both osmoreceptors and Na^+ receptors in the control of water and NaCl intake: hyperosmolality induced by any osmotically active solute causes stimulation of central OT pathways leading to an inhibition of salt intake, whereas blocking central OT function prevents this osmotic inhibition of NaCl ingestion but cannot block a parallel, but separate, inhibition produced by high plasma Na^+ levels. This latter inhibitory stimulus must therefore be detected by separate Na^+-sensing mechanisms that do not require central OT pathways.

Our results additionally indicate that mannitol, an agent previously associated only with the production of hyperosmolality and induced water intake, also appears to produce a heretofore unsuspected excitatory stimulus that can cause a potent activation of salt appetite in rats. Because mannitol administration decreases plasma Na^+ concentration while increasing plasma osmolality, it seems reasonable to consider the possibility that hyponatremia may constitute a significant component of this induced salt appetite. This possibility is similarly supported by the markedly disparate results observed when hypertonic NaCl was used instead of hypertonic mannitol to induce hyperosmolality. However, since hyponatremia induced by acute water loading does not stimulate ingestion of NaCl solution by rats (20), the stimulation of salt appetite by hypertonic mannitol administration observed in rAOT- or OT-receptor antagonist-treated rats must be more complex than merely the isolated excitation of a putative Na^+ receptor and possibly requires the simultaneous presence of a generalized stimulus to fluid ingestion such as hyperosmolality or hypovolemia.

Previous reports have shown that lowering CSF Na^+ concentration by i.c.v. mannitol administration can stimulate salt appetite in sheep and goats despite elevation of total CSF osmolality (21, 22). However, reduction of CSF Na^+ concentration appears to be an insufficient stimulus by itself for saline ingestion in the rat (23). In our studies systemic mannitol administration caused NaCl ingestion in rats with impaired central OT function despite an increased CSF Na^+ concentration as a result of osmotic withdrawal of water from the brain. Consequently, it would appear that receptor(s) responsible for monitoring blood Na^+ concentrations in rats must lie outside the blood–brain barrier. Under these circumstances it is likely that systemic mannitol is perceived primarily as an osmotic stimulus in intact rats and inhibits NaCl ingestion by activating centrally projecting OT pathways. The concurrent pituitary release of OT stimulated by mannitol would serve the added purpose of ridding the body of excess Na^+ by virtue of its natriuretic properties (24). These complementary central and peripheral OT effects on solute ingestion and excretion in combination with the well-known antidiuretic effects of osmotically stimulated pituitary AVP secretion would therefore allow more efficient regulation of osmotic homeostasis (25). However, when central OT pathways are either destroyed or pharmacologically blocked, the systemic hyponatremia induced by hypertonic mannitol acts instead as an unopposed signal of Na^+ depletion and stimulates salt appetite inappropriately. In contrast, when hyperosmolality is accompanied by hypernatremia, as when hypertonic NaCl is administered, salt appetite does not appear even in the absence of central OT activity. Presumably this results both from an absence of any stimulatory drive to NaCl ingestion as well as an active inhibition of salt appetite mediated by Na^+-receptive mechanisms outside the blood–brain barrier.

Because of the ventricular route of administration of both rAOT and the OT-receptor antagonists in these studies, it is impossible to propose a specific pathway or site at which central OT may exert its inhibitory effects on NaCl ingestion. The use of i.c.v. injections of rAOT in these studies likely produced widespread lesions of OT-receptive cells throughout the brain, although the degree of penetration of the rAOT conjugates from the ventricular system into brain parenchyma is not known. This question will require anatomical studies to evaluate the location, extent, and specificity of the cellular lesions produced by central administration of rA-peptide conjugates, along with physiological and behavioral studies using site-specific injections of the conjugates into brain areas with high concentrations of OT receptors.

We have previously proposed that activation of central OT pathways constitutes an important inhibitory component in the control of salt appetite, since treatments known to stimulate parvocellular OT cells abolish or attenuate stimulated NaCl ingestion (2), effects which can be diminished by

temporary OT-receptor blockade (3, 4). The present studies showed more substantial effects on NaCl ingestion following irreversible inactivation of cells bearing OT-receptive elements. These results likely reflect a fundamental difference between the two methodological approaches aimed at impairing central OT function. Central injections of receptor antagonists can effectively block activation of specific receptors, but they allow the receptor-bearing cells to respond to other afferent signals. In contrast, administration of rA-peptide conjugates allows the toxin to gain access to cells with receptors to the conjugated peptide, but once internalized, the toxin inactivates a broader range of cellular functions sometimes leading to cell death (5–7). Consequently, use of rA-peptide conjugates *in vivo* allows inactivation and/or destruction of whole neuronal systems by using peptide receptors to gain entry into chemically specific cells, thereby constituting a powerful technique to eliminate entire regulatory systems rather than just individual receptor-mediated functions. As demonstrated by these studies, this new methodology is particularly well-suited for studies of complex regulatory behaviors by virtue of its ability to eliminate chemically specific neuronal networks while leaving other brain functions intact. This technique should allow more detailed analyses of the peptidergic systems involved with the control of specific brain functions.

The authors thank Marge Altvater for secretarial assistance, Darina Sipula for technical assistance, and Dr. Tibor Ivanyi for assistance with the measurement of CSF Na^+ concentrations. We are indebted to Dr. R. M. Freidinger, Department of Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories for the generous supply of L-366,948. These studies were supported by Research Grants MH-25140, DK-38094, and HD-25373 from the National Institutes of Health and a grant from the American Heart Association.

1. Stricker, E. M. & Verbalis, J. G. (1990) in *Handbook of Behavioral Neurobiology 10: Neurobiology of Food and Fluid Intake*, ed. Stricker, E. M. (Plenum, New York), pp. 387–419.
2. Stricker, E. M. & Verbalis, J. G. (1987) *Behav. Neurosci.* **101**, 560–567.
3. Blackburn, R. E., Stricker, E. M. & Verbalis, J. G. (1992) *Neuroendocrinology* **56**, 255–263.
4. Blackburn, R. E., Demko, A. D., Hoffman, G. E., Stricker, E. M. & Verbalis, J. G. (1992) *Am. J. Physiol.* **263**, R1347–R1353.
5. Vitetta, E. S., Fulton, R. J., May, R. D., Till, M. & Uhr, J. W. (1987) *Science* **238**, 1098–1104.
6. Samson, W. K., Martin, L., Mogg, R. J. & Fulton, R. J. (1990) *Endocrinology* **126**, 1610–1617.
7. Samson, W. K., Alexander, B. D., Skala, K. D., Huang, F. L. S. & Fulton, R. J. (1992) *Can. J. Physiol. Pharmacol.* **30**, 773–778.
8. Stricker, E. M. & Verbalis, J. G. (1986) *Am. J. Physiol.* **250**, R267–R275.
9. Landgraf, R., Neumann, I. & Schwarzberg, H. (1988) *Brain Res.* **457**, 219–225.
10. Pederson, C. A., Caldwell, J. D., Drago, F., Noonan, L. R., Peterson, G., Hood, L. E. & Prange, A. J., Jr. (1986) *Ann. N.Y. Acad. Sci.* **578**, 245–256.
11. Stivers, J. A., Kaltwasser, M. T., Hill, P. S., Hruby, V. J. & Crawley, J. N. (1988) *Peptides* **9**, 223–231.
12. Caldwell, J. D., Barakat, A. S., Smith, D. D., Hruby, V. J. & Pederson, C. A. (1990) *Brain Res.* **512**, 291–296.
13. Olson, B. R., Drutarosky, M. D., Stricker, E. M. & Verbalis, J. G. (1991) *Am. J. Physiol.* **260**, R448–R452.
14. Flanagan, L. M., Olson, B. R., Sved, A. F., Verbalis, J. G. & Stricker, E. M. (1992) *Brain Res.* **578**, 256–260.
15. Stricker, E. M. (1981) *J. Comp. Physiol. Psychol.* **95**, 1–25.
16. Stricker, E. M. (1966) *Am. J. Physiol.* **211**, R232–R238.
17. Verney, E. B. (1947) *Proc. R. Soc. London B* **135**, 25–106.
18. Andersson, B. (1971) *Am. Sci.* **59**, 408–415.
19. Andersson, B. (1977) *Annu. Rev. Physiol.* **39**, 185–200.
20. Stricker, E. M. & Wolf, G. (1966) *J. Comp. Physiol. Psychol.* **62**, 275–279.
21. McKinley, M. J., Denton, D. A. & Weisinger, R. S. (1978) *Brain Res.* **141**, 89–103.
22. Denton, D. A. (1991) in *Thirst: Physiological and Psychological Aspects, Human Nutrition Reviews*, eds. Ramsay, D. J. & Booth, D. A. (Springer, London), pp. 131–146.
23. Denton, D. A., McKinley, M. J., Nelson, J. F., Osborne, P., Simpson, J., Tarjan, E. & Weisinger, R. S. (1984) *J. Physiol. (Paris)* **79**, 499–504.
24. Verbalis, J. G., Mangione, M. P. & Stricker, E. M. (1991) *Endocrinology* **128**, 1317–1322.
25. Verbalis, J. G., Blackburn, R. E., Olson, B. R. & Stricker, E. M. (1993) *Regul. Pept.* **45**, 149–154.