Mice deficient in oxytocin manifest increased saline consumption following overnight fluid deprivation

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Received 23 April 2001; accepted in final form 19 June 2001

Mice deficient in oxytocin manifest increased saline consumption following overnight fluid deprivation. Am J Physiol Regulatory Integrative Comp Physiol 281: R1368–R1373, 2001.—Male mice (9–13 mo of age) in which the gene for oxytocin (OT) had been deleted (OT −/−) were administered 0.5 M sodium chloride (NaCl) solution or tap water as a two-bottle choice test following overnight fluid deprivation (1600 to 1000 the following day). Compared with wild-type cohorts (OT +/+), OT-deficient mice ingested sevenfold greater amounts of saline in the first hour following reintroduction of fluids, P < 0.001, and fourfold greater amounts at the end of 6 h, P < 0.02. No significant difference in total water ingested was noted between the two genotypes at the end of either 1 or 6 h. If food deprivation accompanied the overnight fluid deprivation and food was reintroduced 1 h after the reintroduction of both water and saline, OT −/− mice still ingested greater amounts of saline, but not water, than OT +/+ mice at both 1 h, P < 0.001, and 6 h, P < 0.02. No differences were noted between genotypes in the daily intake of 0.5 M NaCl solution or water during a 3-day observation period before the overnight fluid deprivation. The volume of saline consumed in each 24-h observation period represented about one-tenth of the total fluids ingested in each genotype. We conclude that OT −/− mice display an enhanced salt appetite compared with OT +/+ mice when fluid deprived overnight. The salt appetite was only apparent in the presence of a perturbation such as fluid deprivation, which predisposes the animal to moderate hypovolemia. The observations support an inhibitory role for OT in the control of sodium appetite in mice.

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direct approach is to study the effects of OT in a model in which OT is absent. Mice in which the gene for OT has been deleted (OT /–/ or null mice) provide a novel and direct means of assessing the effects of OT deficiency with salt appetite in the laboratory rodent because OT is not present during any stage of life. We report that consumption of 0.5 M NaCl solution is greater in OT null mice vs. wild-type cohorts after overnight fluid deprivation, a stimulus that predisposes the animal to modest volume depletion, and hence should stimulate sodium appetite. The observations suggest an inhibitory role for OT in the control of salt appetite in the mouse.

MATERIALS AND METHODS

Animals

Male wild-type (OT +/+ ) and null (OT /–/ ) mice of C57BL/6 background were used for these studies. The OT /–/ mice were generated by Dr. Scott Young, National Institute of Mental Health (21), and breeding pairs were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were bred and housed for this study in the viral-free quarters of the University of Pittsburgh Animal Facility under a 12:12-h light-dark cycle (lights on at 0700). Animals used for the studies were from the F3 generation and ranged in age from 9 to 13 mo. The mean weight of the OT /–/ mice, 31.8 ± 1.4 g, did not differ from that of the OT +/+ mice, 33.9 ± 1.2 g. Mice were housed in standard suspended or shoe-box cages in groups of up to five animals per cage with free access to water and food (Prolab RMH 3000 5P00, LabDiet/Purina; 0.26% sodium by weight). The studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

OT /–/ mice are unable to nurse young because of an inability to eject milk (21). Therefore, we use heterozygote (OT /–/+ ) female and male OT /–/ mice for breeding in our colony. For mating, 2-mo-old OT /–/+ females (1 to 3) were placed in a cage with one OT /–/ male. After 16 days, mice were checked daily for pregnancy. When parturition seemed imminent, each female was removed to an individual cage for delivery. Mice nursed their pups for ~24–26 days. After weaning, dams were again housed in groups of up to five per cage.

To identify the genotype of the mice, DNA from ~1 cm of mouse tail was extracted and prepared for PCR using adaptations to methods previously published (21). Pairs of primers were designed for PCR that detected either the wild-type allele (OT, 332 bp) or the mutant allele (neomycin resistance cassette, 430 bp). The primer pairs for the wild-type allele were (forward) TCG TCT TCG CAC AGT CCG GAT TC and (reverse) TCA GTG TTC TGA GCT GCA AAC C, and for the mutant allele, they were (forward) AGA GGC TAT TCG GCT ATG ACT G and (reverse) TTC GTC CAG ATC ATC CTG ATC. Primers were synthesized at the University of Pittsburgh sequence facility.

Experimental Protocols

Protocol 1. Observations were made in six OT +/+ and six OT /–/ mice. For testing, animals were removed from group housing and acclimated to single housing for a week before the test day. Because the test day involved a two-bottle choice test between tap water or 0.5 M (2.9%) NaCl solution (prepared by dissolving 8.77 g of NaCl in 300 ml tap water), animals were acclimated to both solutions daily for 3 days before the test day. The amount of each liquid consumed was recorded daily.

The evening preceding the test, both bottles were removed from the cage at 1600, and both were reintroduced simultaneously at 1000 the following day. The volume of ingested water and saline was recorded in the first hour immediately following reintroduction of fluids and for the ensuing 6 h. Liquids, which were freshly prepared daily, were supplied in graduated bottles calibrated in 0.1-ml increments.

Protocol 2. After 1 wk, animals used for protocol 1 were restudied, and an additional three animals were added to the OT +/+ group (total n = 9) and two animals were added to the OT /–/ group (total n = 8). The protocol was exactly the same as protocol 1 except that, in addition to the fluids, animal chow was also removed at 1600. Both fluids, but not food, were reintroduced simultaneously at 1000 the following day. The fluids consumed during the first hour were recorded. At 1100, food was reintroduced, and the fluid intakes were recorded again at 6 h.

Protocol 3. This experiment was done to determine if both genotypes achieved an equivalent hypovolemic stimulus following overnight fluid deprivation. After 2 to 3 wk, animals studied in protocols 1 and 2 were restudied. The design was identical to protocol 1, except at the maximum point of dehydration, when the animals would normally be reintroduced to fluids, mice were weighed, anesthetized with 0.3 ml Equithesin (a mixture of 8.5 g chloral hydrate, 4.25 g MgSO4·7H2O, and 1.96 g pentobarbital sodium in 200 ml water), and killed, and blood was obtained to measure hematocrit and plasma sodium. The difference in body weight before removal of fluids minus the weight at the end of fluid deprivation was calculated and expressed as a percentage of total body weight.

Statistical Analysis

Data are presented as means ± SE. Daily water and saline intakes during the acclimation period were analyzed by ANOVA for repeated measures. The fluid intakes after water deprivation as well as the weights, plasma sodium, and hematocrits of wild-type vs. null mice were analyzed by two-tailed t-test. Significance was set at P < 0.05.

RESULTS

During acclimation to the water/saline choice paradigm, the mean 24-h intake of water or saline solution was not significantly different between OT /–/ and OT +/+ mice (Fig. 1). However, after overnight fluid deprivation, the amount of 0.5 M NaCl solution ingested was sevenfold higher in the OT /–/ than OT +/+ mice in the first hour after reintroduction of fluids, P < 0.001, and fourfold greater at the end of 6 h, P < 0.02, two-tailed t-test (Fig. 2). No significant differences in total water ingested were noted between the two groups at the end of either 1 or 6 h (Fig. 2).

To be certain that differences in saline consumption were not dependent on the amount of food consumed during the period of overnight fluid deprivation, the experiment was repeated with a modified protocol with both food and fluids withheld. Saline solution and tap water were reintroduced the next day at 1000. Similar to the first experiment, OT /–/ mice still ingested a greater amount of saline, but not water, in the first hour, P < 0.001, compared with OT +/+ mice. Furthermore, the differences persisted over the ensuing 5 h.
We report that male mice deficient in OT manifest an enhanced consumption of NaCl-containing solutions compared with their wild-type cohorts. The salt appetite is apparent after overnight fluid deprivation, which is known to produce a moderate volume depletion. These findings in the laboratory mouse complement and enhance previous reports that OT inhibits salt intake in the laboratory rat.

The OT -/- animals, and not the OT +/+ animals, developed a salt appetite during the overnight water deprivation. The expression of this salt appetite occurred when overnight fluid-deprived animals were given a choice between 0.5 M NaCl and water. The intake of 0.5 M NaCl was markedly increased in OT -/- mice, being sevenfold greater than OT +/+ animals in the 1 h after animals were given access to the fluids. These differences in saline consumption following fluid deprivation could not be accounted for by size differences between the genotypes (e.g., larger animals may consume more fluids) because the weights of the paired cohorts were not different.

Overnight fluid deprivation provides a hypovolemic stimulus (8). We determined if both genotypes achieved equivalent hypovolemia following overnight fluid deprivation. Both genotypes lost similar amounts of body weight following overnight fluid deprivation. The hematocrits, which indirectly measure the degree of hemoconcentration and hypovolemia, were identical between genotypes. Thus differences in hypovolemic stimuli do not account for the greater salt appetite in OT -/- mice. Plasma sodium, which is the major determinant of plasma osmolality, did not differ significantly between genotypes following fluid deprivation. Our experiments also indicate that the stimuli for thirst were equivalent in the OT -/- and OT +/+ mice because the two genotypes drank equivalent amounts of water after overnight fluid deprivation. This occurred regardless of whether the water was reintroduced as a two-bottle choice test with saline or as the only fluid.

To test for the possibility that the increased consumption of NaCl by OT -/- mice might reflect a compensatory response secondary to a difference in food consumed during the period of fluid deprivation, we retested the animals with food being withdrawn at the same time as fluids. In this experiment, saline and water were reintroduced simultaneously without food. OT -/- animals ingested 2.9-fold greater amounts of saline solution compared with wild-type cohorts in 1 h. The food deprivation was associated with a reduction in fluid intakes (both water and saline) in all animals compared with the experiment in which only fluids were withheld overnight.

A small voluntary ingestion of salt solution was observed in mice of both genotypes when animals were given ad libitum access to 0.5 M NaCl solution and water. The 24-h consumptions of salt solution were not different between the two genotypes, and the volume of 0.5 M NaCl solution consumed represented approximately one-tenth of the total volume of fluids ingested. The altered sodium appetite for 0.5 M NaCl solution in the OT -/- animals was not apparent in the absence of a perturbation in the availability of fluids.

The model chosen for this experiment was the OT-deficient laboratory mouse. Most of the behavioral studies done to date in the OT-deficient mouse have focused on the reproductive related behaviors of the animal because of the well-known effects of OT at...
parturition (e.g., myometrial contractility) and during lactation (e.g., milk ejection). Although an OT-deficient mouse is able to deliver its young and produce milk (a prolactin-dependent function), the animal is unable to eject milk (21), which is an OT-dependent function (5). OT is believed to have multiple functions in the brain, some of which are not related to reproduction. Proposed central actions of OT include the onset of maternal behavior (9), penile erection (2), yawning (2), grooming (10), regulation of adrenocorticotropin secretion (1), control of gastric motility (7), and inhibition of salt (18) and food (19) intake. Our findings in this study confirm prior studies in the rat and suggest an inhibitory role for OT with salt intake in the laboratory mouse. Although our experiments were conducted in a laboratory setting, the duration of fluid deprivation induced in this study may also be encountered in feral mice that have limited access to water.

The findings in this study suggest that due to the absence of OT in the “knockout” animals, fluid depri-

![Fig. 2. Oral intake of 0.5 M NaCl solution and tap water in male OT null mice (OT^+/−) vs. wild-type cohorts (OT^+/+) following overnight fluid deprivation. Animals had access to food during the period of fluid deprivation. The volume of each ingested fluid was recorded at 1 (a) and 6 (b) h after its reintroduction. OT^−/− mice ingested greater amounts of saline solution, but not water, than wild-type cohorts (**P < 0.001; *P < 0.02, 2-tailed t-test).]
Oxytocin-deficient mice and saline consumption

Fig. 3. Oral intake of 0.5 M NaCl solution and tap water in male OT null mice (OT−/−) or wild-type cohorts (OT+/+) following overnight fluid and food deprivation. The volume of each ingested fluid was recorded at 1 (a) and 6 (b) h after its reintroduction. OT−/− mice ingested greater amounts of saline solution, but not water, than wild-type cohorts (**P < 0.001; *P < 0.02, 2-tailed t-test).

Discussion

Activation results in a marked sodium appetite. This sodium appetite is not expressed in wild-type animals, perhaps due to activation of central OT systems that inhibit the sodium appetite. Increased osmolality secondary to water deprivation would serve as the stimulus to activate central OT systems in the wild-type animals. Consistent with studies in rats, elevated central OT would inhibit sodium appetite. A system that may be involved in the development of the sodium appetite may be the renin-angiotensin system in response to the hypovolemia produced by water deprivation.

OT−/− mice may be unable to suppress the sodium appetite due to increased angiotensin formation. Future studies to test this possibility are underway.
In general, sodium appetite is viewed mainly as a stimulatory behavior (e.g., a sodium appetite does not exist unless the appropriate conditions exist to stimulate this behavior). The fact that OT plays an important inhibitory role in the control of sodium appetite provides a basis for viewing the maintenance of sodium balance in a different perspective. Salt appetite plays a role in disease states such as hypertension. Although studies in mice cannot be directly extrapolated to humans, recent studies in humans have identified an inverse correlation between OT and blood pressure (6). Confirming the role of OT in sodium appetite may have relevance to our understanding of conditions associated with sodium appetite (the marked increase in sodium appetite often seen in patients with adrenal insufficiency or in certain subsets of patients with hypertension). In addition, patients with hypertension or congestive heart failure often have difficulty adhering to salt restriction. The possibility that OT is a specific signal for inhibition of sodium appetite suggests that it or an analog may aid in the achievement of adequate sodium restriction in certain clinical settings.

The authors acknowledge the expert technical assistance of X. Li in the performance of these studies.

Studies were supported by National Institutes of Health Grant HD-37268 (J. A. Amico) and funds from the Department of Veterans Affairs Merit Review Award (J. A. Amico).

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