Dehydration anorexia is attenuated in oxytocin-deficient mice

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Submitted 22 December 2004; accepted in final form 17 February 2005

Rinaman, Linda, Regis R. Vollmer, Joseph Karam, Donneshia Phillips, Xia Li, and Janet A. Amico. Dehydration anorexia is attenuated in oxytocin-deficient mice. Am J Physiol Regul Integr Comp Physiol 288: R1791–R1799, 2005. First published February 17, 2005; doi:10.1152/ajpregu.00860.2004.—Evidence in rats suggests that central oxytocin (OT) signaling pathways contribute to suppression of food intake during dehydration (i.e., dehydration anorexia). The present study examined water deprivation-induced dehydration anorexia in wild-type and OT −/− mice. Mice were deprived of food alone (fasted, euhydrated) or were deprived of both food and water (fasted, dehydrated) for 18 h overnight. Fasted wild-type mice consumed significantly less chow during a 60-min refeeding period when dehydrated compared with their intake when euhydrated. Conversely, fasting-induced food intake was slightly but not significantly suppressed by dehydration in OT −/− mice, evidence for attenuated dehydration anorexia. In a separate experiment, mice were deprived of water (but not food) overnight for 18 h; then they were anesthetized and perfused with fixative for immunocytochemical analysis of central Fos expression. Fos was elevated similarly in osmo- and volume-sensitive regions of the basal forebrain and hypothalamus in wild-type and OT −/− mice after water deprivation. OT-positive neurons expressed Fos in dehydrated wild-type mice, and vasopressin-positive neurons were activated to a similar extent in wild-type and OT −/− mice. Conversely, significantly fewer neurons within the hindbrain dorsal vagal complex were activated in OT −/− mice after water deprivation compared with activation in wild-type mice. These findings support the view that OT-containing projections from the hypothalamus to the hindbrain are necessary for the full expression of compensatory behavioral and physiological responses to dehydration.

Dehydration in rats increases the expression of mRNAs for c-fos, vasopressin (AVP), and oxytocin (OT) in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) (4, 5, 14, 36), and it depletes posterior pituitary stores of AVP and OT (15), while increasing their plasma concentrations (2, 14). Circulating AVP promotes renal retention of water, whereas OT promotes renal excretion of sodium in rats (40). Dehydration also inhibits gastric emptying and food intake (8, 30, 32, 34, 42), which together help limit absorption of additional osmoles into the circulation. Finally, dehydration stimulates compensatory drinking of water, when it is available; the subsequent absorption of water into the circulation helps to restore both intracellular and extracellular fluid compartments (31, 34). These adaptive and complementary neuroendocrine, physiological, and behavioral responses to dehydration help to limit its disruptive physiological impact by restoring body fluid homeostasis.

In addition to activating magnocellular PVN and SON neurons, osmotic dehydration induced acutely in rats by systemic administration of hypertonic NaCl solution activates parvocellular OT neurons in the PVN that project to sites within the CNS (38). Pharmacological studies have provided good evidence that OT acts centrally in rats to inhibit intake of solutes in solid and liquid form, including food and solutions containing NaCl and other osmolytes (33–35, 38). Parvocellular OT neurons activated during osmotic and/or volemic dehydration may contribute to central inhibitory control over food intake (i.e., dehydration anorexia), thereby helping to ensure that satiation of thirst takes precedence over feeding in dehydrated animals, even when they are metabolically deficient (43). Importantly, dehydration anorexia after a salt load in rats is attenuated by pharmacological antagonism of central OT receptors, evidence that endogenous OT signaling pathways participate in this behavioral response (23).

Similar to results in rats (7, 14), water deprivation in mice promotes body weight loss, increases plasma osmolality, decreases plasma volume, and increases Fos immunolabeling in the PVN and SON (1, 5, 22). If activation of central OT signaling pathways contributes to inhibition of food intake in mice after water deprivation, then dehydration anorexia should be attenuated in mice that lack the gene for OT (i.e., OT −/− mice) and that, therefore, lack any potential inhibitory effect of OT signaling pathways on solute intake. In the present study, we compared dehydration anorexia in OT −/− and wild-type mice, in which dehydration was induced by water deprivation concurrent with an 18-h fast (experiment 1). We also quantified and compared immunolabeling of the c-fos immediate early gene protein product, Fos, within the hypothalamic PVN and hindbrain dorsal vagal complex (DVC) in wild-type and OT −/− mice after 18 h overnight water deprivation with food available (experiment 2) to identify potential neural correlates of genotypically different behavioral responses to osmotic and volemic dehydration.

MATERIALS AND METHODS

Animals

Male mice unable to synthesize OT (OT −/− mice) and wild-type mice, both of C57BL/6 background strain, were used for these studies. Breeding pairs of OT −/− mice, originally generated by Dr. Scott Young (44), were purchased from Jackson Laboratories (Bar Harbor, ME). Colonies were established and maintained in virus-free quarters at the University of Pittsburgh Animal Facility. Mice ranged in age from 8 to 10 mo at the time of experiments. Mice were housed in a temperature-controlled room with a 12:12-h light-dark cycle (lights on at 0700). Food (Prolab RMH 3000 5P00, LabDiet/ Purina; 0.26% sodium by weight), and water were provided ad libitum, except as noted. Mice were caged individually for the week before and during experiments. Experiments were conducted between 0900 and 1200, using protocols developed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Health Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

To identify the genotype of each mouse, DNA from a small sample of tail tissue was extracted and prepared for PCR using primers synthesized at the University of Pittsburgh (1). Pairs of primers were designed for PCR that either detected the wild-type allele (OT, 332 bp) or the mutant allele (neomycin resistance cassette, 430 bp). The primers for the wild-type allele were (forward) TCG CTC TGC CAC AGT CCG GAT TC and (reverse) TCA GTG TTC TGA GCT GCA AAC C and for the mutant allele were (forward) AGA GGC TAT TCG GCT ATG ACT G and (reverse) TTC GTC CAG ATC ATC CTG ATC. The absence of an OT DNA band and the presence of a neomycin DNA band confirmed the OT −/− genotype. The presence of an OT DNA band and the absence of a neomycin DNA band confirmed the wild-type (i.e., OT +/+ ) genotype.

Experiment 1: Effect of Water Deprivation on Food Intake After an Overnight Fast

Individually housed, experimentally naïve mice (n = 6 wild type, n = 6 OT −/− ) were acclimated to powdered diet available ad libitum for 1 wk, during which time stable daily baseline intakes were achieved. After acclimation, animals were fasted overnight for 18 h with water available in the cage. Food was removed at 1600 h and reintroduced the following morning at 1000 h. Water was removed from the cage at the time of food replacement to control for the absence of water during the feeding test in the second phase of this experiment (see below). The amount of powdered diet consumed by each euhydric mouse was recorded at 10-min intervals during the first 60 min after food was reintroduced. Mice were then returned to ad libitum access to water and powdered chow. The overnight 18-h food deprivation experiment was repeated 1 wk later using the same mice. In this iteration, however, water was unavailable during the 18-h fast to promote dehydration before the feeding test. Water also was unavailable during the 60-min feeding test to match conditions in the first phase of the experiment. The body weight of each mouse was recorded at the beginning of each 18-h deprivation period and again immediately before reintroduction of food to determine the extent of body weight loss during the 18-h fast in both genotypes and in each fasting condition (i.e., with or without water available during the fast).

Statistical analyses. Body weight and food intake values are expressed as group means ± SE. Group food intake data were analyzed by repeated-measures ANOVA to reveal significant main effects of genotype and water deprivation status on food intake over time, with subsequent pairwise comparisons within and between genotypes at each timepoint using post hoc Fishers least significant difference (LSD) protected t-tests. Differences were considered significant when P < 0.05.

Experiment 2: Water Deprivation-Induced Fos Immunolabeling

The outcome of experiment 1 revealed significant genotypic differences in the magnitude of dehydration anorexia induced by 18-h water deprivation (see RESULTS). Experiment 2 was performed to determine whether the dehydrating effects of 18-h water deprivation produce differential central neural activation in OT −/− and wild-type mice. For this purpose, individually caged male mice (n = 4 wild type, n = 4 OT −/− ) were weighed, and water was removed at 1600. Mice were not deprived of food. Additional male mice (n = 3 wild type, n = 3 OT −/− ) were weighed at 1600, but their water was not removed; these mice served as nondeprived controls for determining basal Fos immunolabeling.

At 1000 on the following day (i.e., 18 h later), mice were again weighed and then were anesthetized by intraperitoneal injection of Equithesin (4 mg/kg sodium pentobarbital, 17 mg/kg chloral hydrate, and 21.3 mg/ml magnesium sulfate dissolved in water containing 44% propylene glycol and 10% ethanol). Cardiac blood samples were collected into heparinized Vacutainer tubes and spun in a refrigerated centrifuge to isolate plasma in which protein concentrations were determined using a refractometer. Immediately after blood sampling, mice were perfused transcardially with 0.15 M NaCl followed by fixative (0.1 M sodium phosphate buffer containing 4% paraformaldehyde, 1.4% lysine and 0.2% sodium metaperiodate) (19). Fixed brains were removed; these mice served as nondeprived controls for determining the absence of water during the feeding test in the second phase of this experiment (see RESULTS).

For immunocytochemistry, sections were rinsed in 0.1 M sodium phosphate buffer (pH 7.2), treated for 30 min in buffered 1% sodium borohydride (Sigma, St. Louis, MO), rinsed, treated for 20 min in buffered 0.3% H2O2, and rinsed again. Primary and secondary antisera were diluted in buffer containing 0.3% Triton X-100 and 1% normal donkey serum. Rinses were performed in multiple changes of buffer over 30 min. The Fos antiserum used in this study was generated in rabbits immunized with amino acids 4–17 of synthetic Fos protein; its specificity has been reported (28). Tissue sections were incubated for 48 h at 4°C in rabbit anti-Fos (1:50,000), rinsed, and then incubated for 1 h at room temperature in biotinylated donkey anti-rabbit IgG (1:600; Jackson ImmunoResearch Laboratories, West Grove, PA). After being rinsed, sections were processed using Vectastain Elite avidin-biotin immunoperoxidase reagents (Vector Laboratories, Burlington, CA). Blue-black nuclear Fos immunolabeling was generated in a solution of diaminobenzidine (DAB), nickel sulfate, and H2O2 in 0.1 M sodium acetate buffer (pH = 6.0).

One set of Fos-labeled forebrain sections from wild-type and OT −/− mice was subsequently processed for dual immunolocalization of AVP using rabbit anti-AVP primary antiserum (1:20,000; Peninsula Laboratories), biotinylated donkey anti-rabbit IgG (1:600), Vectastain Elite avidin-biotin immunoperoxidase reagents, and a nonimmunogen-DAB reaction with H2O2 in Trizma buffer (pH = 7.5) to produce a brown cytoplasmic AVP immunolabel. A serially adjacent set of Fos-labeled forebrain sections from wild-type mice was processed similarly for localization of cytoplasmic OT using rabbit anti-OT antiserum (1:20,000; Peninsula Laboratories). Single- or double-immunostained sections were mounted onto SuperFrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA), dehydrated, cleared in graded ethanol and xylene, and coverslipped using Histomount (VWR). To help determine the anatomic localization of Fos-positive nuclei, an adjacent set of tissue sections from each mouse was mounted onto slides, stained for Nissl substance with cresyl violet, and coverslipped for microscopic analysis. Patterns of treatment-induced increases in Fos immunolabeling were first evaluated qualitatively by microscopic inspection of immunolabeled brainstem and forebrain sections, with reference to adjacent Nissl-stained sections as necessary.

Quantitative analysis of hypothalamic activation. Fos labeling in AVP-positive PVN neurons was quantified in double-labeled sections from wild-type and OT −/− mice. For this purpose, two sections spaced 160 μm apart and judged to contain the largest number of AVP-positive PVN neurons relative to sections located more rostrally or caudally were selected for analysis in each animal. AVP-positive PVN neurons were identified and counted bilaterally based on their content of brown cytoplasmic immunolabel and a visible nucleus. AVP neurons were considered Fos-positive (i.e., activated) when their nucleus contained visible blue-black immunoreactivity, regardless of intensity, and Fos-negative when their nucleus lacked visible blue-black immunolabel. Average AVP cell counts per PVN (i.e., bilateral PVN counts across two sections per mouse, divided by 4) and the proportion of AVP neurons activated to express Fos were averaged by genotype and treatment groups, with group values expressed as means ± SE. A similar approach was used to determine the number of OT-immunopositive PVN neurons in wild-type mice and the proportion activated to express Fos. For this purpose, the number of
Wild-type mice were compared using a one-way ANOVA, with genotype (wild type vs. OT -/-) and treatment (water deprived vs. nondeprived) as independent variables. When F values indicated significant overall main effects of genotype or treatment group, post hoc pairwise comparisons of interest were made using Fishers LSD protected t-tests. Potential differences in the number of AVP neurons counted or in the proportion activated to express Fos were considered statistically significant when P < 0.05. Counts of AVP-positive neurons and the proportion activated to express Fos in wild-type mice were compared using a t-test, with treatment group (water deprived vs. nondeprived) as the independent variable. Potential differences in the number of OT neurons counted or in the proportion activated to express Fos were considered significant when P < 0.05.

Quantitative analysis of hindbrain DVC activation. A separate quantitative analysis of water deprivation-induced increases in Fos immunolabeling was conducted in the hindbrain DVC, comprising the area postrema (AP), nucleus of the solitary tract (NST), and dorsal motor nucleus of the vagus (DMV). Fos-positive nuclei in the AP, NST, and DMV were counted in three hindbrain tissue sections spaced by 160 μm through the rostrocaudal extent of the AP in each mouse. Cells were considered Fos-positive when their nuclei contained visible blue-black immunoreactivity, regardless of intensity. The absolute number of Fos-positive cells within the cytoarchitectural boundaries of the AP, NST, and DMV was totaled in each animal across the three tissue sections. Regional values then were averaged across animals within each genotype and treatment group, with final values expressed as means ± SE.

Statistical analyses of DVC activation. Potential group differences in regional Fos counts were tested for statistical significance by using separate two-way ANOVAs for each DVC subregion (i.e., AP, NST, DMV), with mouse genotype (OT +/+ vs. OT -/-) and treatment group (water deprived vs. nondeprived) as independent variables. When F values indicated significant overall main effects of genotype and/or treatment group on Fos counts, post hoc pairwise comparisons of interest were made using Fishers LSD protected t-tests. Differences were considered significant when P < 0.05.

### RESULTS

**Experiment 1: Effect of Water Deprivation on Food Intake After an Overnight Fast**

Overnight 18-h fasting produced body weight losses of ~10% in OT -/- and wild-type mice, with no significant effect of either genotype or water availability on the degree of body weight loss (Table 1). When water was available during the 18-h fast, mice of both genotypes consumed ~4% of their fasted body weight in powdered chow during the subsequent 1-h refeeding period (i.e., 1.25 ± 0.16 g average cumulative intake for wild-type mice; 1.24 ± 0.16 g average cumulative intake for OT -/- mice; Fig. 1). There was no significant effect of genotype on food intake at any time point when water was available during the 18-h fast (Fig. 1; compare upper two lines).

Repeated-measures ANOVA of the effects of hydration status, time, and genotype on food intake revealed significant main effects of hydration status [F(1,23) = 11.73; P < 0.001], time [F(5,115) = 9.32; P < 0.001], and genotype [F(1,23) = 6.41; P = 0.019], as well as significant interaction effects of hydration status × time [F(5,115) = 6.18; P < 0.001], hydration status × genotype [F(1,23) = 6.50; P = 0.020], time × genotype [F(5,115) = 2.86; P = 0.017], and hydration status × time × genotype [F(5,115) = 2.46; P = 0.032]. The difference between genotypes was apparent in dehydrated OT -/- mice (*P* = 0.16 g average cumulative intake for OT -/- mice; Fig. 1). There was no significant effect of genotype on food intake at any time point when water was available during the 18-h fast (Fig. 1; compare upper two lines).

### Table 1. Effects of 18-h food and/or water deprivation on body weight

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Genotype, n</th>
<th>BW Before, g</th>
<th>BW After, g</th>
<th>BW Loss, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food-deprived, water available (experiment 1)</td>
<td>wild-type (6)</td>
<td>32.6 ± 1.3</td>
<td>29.4 ± 1.2</td>
<td>9.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>OT -/- (6)</td>
<td>32.7 ± 0.8</td>
<td>29.8 ± 0.8</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td>Food and water-deprived (experiment 1)</td>
<td>wild-type (6)</td>
<td>32.0 ± 1.3</td>
<td>28.5 ± 1.2</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>OT -/- (6)</td>
<td>32.8 ± 0.9</td>
<td>29.4 ± 0.7</td>
<td>10.3 ± 0.5</td>
</tr>
<tr>
<td>Water-deprived, food available (experiment 2)</td>
<td>wild-type (4)</td>
<td>36.1 ± 1.8</td>
<td>33.7 ± 1.8</td>
<td>6.7 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>OT -/- (4)</td>
<td>36.2 ± 2.3</td>
<td>33.9 ± 2.0</td>
<td>6.3 ± 0.61</td>
</tr>
<tr>
<td>No food or water deprivation (experiment 2)</td>
<td>wild-type (3)</td>
<td>31.7 ± 0.5</td>
<td>31.8 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>OT -/- (3)</td>
<td>33.1 ± 1.0</td>
<td>33.4 ± 0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. BW, body weight.
and time \[F(5,120) = 40.0; P < 0.0001\], and a significant interaction between these two factors \[F(5,120) = 3.8, P = 0.003\]. ANOVA revealed no significant main effect of genotype \[F(1,23) = 0.6; P = 0.45\] on food intake and no significant interaction between genotype and time \[F(5,120) = 0.46, P = 0.8\]. However, there was a significant interaction between genotype and hydration status \[F(1,72) = 14.7; P < 0.001\] on food intake.

Follow-up ANOVAs within each genotype group revealed a significant main effect of hydration state on fasting-induced food intake in wild-type mice \[F(1,11) = 14.77; P = 0.003\]. Wild-type mice ate significantly less powdered chow at each 10-min timepoint during the refeeding period when water was unavailable during the fast (i.e., dehydrated state), compared with their intake when water was available during the fast (i.e., euhydrated state) \((P < 0.05\) at each timepoint; Fig. 1). By the end of the 60-min feeding period, dehydrated wild-type mice had consumed an average of 0.69 ± 0.09 g powdered chow, a significant 45% reduction compared with their intake when euhydrated (Fig. 1). Thus significant dehydration anorexia was evident in wild-type mice.

Conversely, ANOVA failed to reveal a significant main effect of hydration state on food intake in OT \(-/-\) mice \([F(1,11) = 2.17, P = 0.171]\). A trend toward reduced food intake was apparent in dehydrated OT \(-/-\) mice when water was not available during the 18-h fast compared with their food intake when euhydrated, but differences in food intake between the two hydration states did not achieve statistical significance at any timepoint (Fig. 1). By the end of the 60-min feeding period, dehydrated OT \(-/-\) mice had consumed an average of 0.91 ± 0.15 g of powdered chow, representing a nonsignificant 27% reduction compared with their intake when euhydrated (Fig. 1). Thus the anorectic effect of dehydration was significantly attenuated in OT \(-/-\) mice compared with wild-type mice.

When the extent of dehydration-induced feeding suppression was compared directly between wild-type and OT \(-/-\) mice (Fig. 2), ANOVA revealed a significant main effect of genotype on the extent of feeding suppression during the dehydrated state \([F(1,11) = 6.45, P = 0.029]\). Post hoc t-tests revealed that the genotypic difference in feeding suppression was significant at 10-, 30-, 40-, 50-, and 60-min timepoints, with wild-type mice manifesting more dehydration anorexia (i.e., greater feeding suppression) compared with OT \(-/-\) mice (Fig. 2).

**Experiment 2: Water Deprivation-Induced Increases in Fos Immunolabeling**

Physiological effects of 18-h water deprivation. Wild-type mice lost 6.7 ± 0.4% and OT \(-/-\) mice lost 6.3 ± 0.6% of their initial body weight during overnight water deprivation with food available (\(n = 4\) per genotype; Table 1). These body weight losses were not significantly different between genotypes \((P = 0.48)\). Plasma protein concentrations were elevated by ∼17% and 18.5% in fluid-deprived wild-type and OT \(-/-\) mice \((5.29 ± 0.24 and 4.98 ± 0.18 \text{ g/dl}, \text{respectively})\) compared with plasma protein concentrations in nondeprived euhydrated controls \((4.53 ± 0.15 and 4.20 ± 0.28 \text{ g/dl}, \text{respectively})\). There were no genotypic differences in plasma protein concentrations in either hydration state \((P > 0.05\) for each comparison).

Effects of 18-h water deprivation on central Fos expression. In euhydrated wild-type and OT \(-/-\) control mice, Fos-positive nuclei were sparse within the hypothalamus, basal and limbic forebrain (not shown) and DVC (Fig. 3A). Quantitative analysis revealed no significant genotypic differences in the number of AVP-immunopositive neurons counted within the PVN in euhydrated controls \((88.3 ± 11.4\) in wild-type vs. 94.5 ± 12.2 in OT \(-/-\) mice; difference not significant), or in the low proportions of AVP neurons expressing nuclear Fos immunolabeling (Fig. 4). There also were no significant genotypic differences in the number of Fos-positive nuclei counted within each DVC subregion in euhydrated control mice (Fig. 5).

After 18-h water deprivation with food available, Fos immunolabeling was markedly increased in osmo- and volume-sensitive forebrain and hypothalamic regions [i.e., subformical organ (SFO), median preoptic nucleus (MnPO), organum vasculosum of the lamina terminalis (OVLT), PVN, and SON] in wild-type and OT \(-/-\) mice (Fig. 6), with no apparent genotypic differences in dehydration-induced neural activation in any forebrain region. The apparent majority of AVP-positive neurons in the PVN and SON were activated to express Fos in wild-type and OT \(-/-\) mice (Fig. 7, top). Quantitative analysis of AVP immunolabeling in wild-type and OT \(-/-\) mice revealed no effect of water deprivation on the total number of AVP-positive neurons counted within the PVN \((93.5 ± 6.3\) in wild-type mice vs. 92.75 ± 13.8 in OT \(-/-\) mice, similar to euhydrated controls) but revealed a significant effect of water deprivation to markedly increase the proportion of AVP-positive PVN neurons activated to express Fos in mice of both genotypes (Figs. 4 and 7). There was no genotypic difference in the extent of AVP neural activation in water-deprived mice (Fig. 4).
As expected, there was no significant effect of hydrational status on the number of OT-immunopositive neurons counted within the PVN (86.8 ± 5.7 in water-deprived wild-type mice vs. 78.8 ± 11.8 in euhydrated wild-type mice; difference not significant). The proportion of OT-positive neurons expressing Fos in wild-type mice was significantly greater after water deprivation (18.5 ± 3.1%, n = 4) compared with OT activation in euhydrated controls (0.8 ± 0.8%, n = 3; P < 0.001 between treatment groups). Thus, although overnight water deprivation produced significant activation of OT-positive neurons in wild-type mice, the proportion of OT neurons activated (i.e., ~18.5%) was significantly less (P < 0.001) than the proportion of AVP-positive neurons activated in the same mice (i.e., ~60%; Fig. 5).

Within the hindbrain, there was no significant effect of either genotype or hydrational state on Fos expression within the DMV (Fig. 6). Conversely, overnight water deprivation significantly increased Fos expression within the NST and AP in both wild-type and OT −/− mice (P < 0.05 within each subregion compared with activation in euhydrated controls; Figs. 4 and 6). The magnitude of increased Fos immunolabeling within NST and AP was significantly different between

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**Fig. 3.** Fos immunolabeling in representative sections through the caudal dorsomedial medulla in a euhydrated control OT −/− mouse (A), an 18-h water-deprived OT −/− mouse (B), and an 18-h water-deprived wild-type mouse (C). A: AP, area postrema; DMV, dorsal motor nucleus of the vagus; NST, nucleus of the solitary tract; cc, central canal.

**Fig. 4.** The proportion (%) of vasopressin (AVP)-immunopositive PVN neurons activated to express Fos is shown in euhydrated and dehydrated wild-type and OT −/− mice. Similar low proportions of AVP-positive cells expressed Fos in nondeprived euhydrated control mice (n = 3 per genotype). Overnight (18 h) water deprivation promoted significantly increased activation of AVP neurons in wild-type and OT −/− mice (n = 4 per genotype), with no effect of genotype on the proportion of AVP neurons activated. Bars with different letters (i.e., a, b) are significantly different (P < 0.05). See text for total AVP cell counts in each group.

**Fig. 5.** Counts of Fos-positive neurons in three subdivisions of the dorsal vagal complex in wild-type and OT −/− mice. Similar and relatively low numbers of Fos-positive cells were counted in the DMV, NST, and AP in nondeprived euhydrated control mice (n = 3 per genotype). Overnight (18 h) water deprivation promoted significantly increased Fos expression within the NST and AP (n = 4 per genotype), with significantly greater increases observed in wild-type mice compared with OT −/− mice. Within each subregion, bars with different letters (i.e., a, b, c) are significantly different (P < 0.05).
genotypes (Figs. 3, B and C and Fig. 5). Approximately 31% fewer NST neurons ($P < 0.05$) and 57% fewer AP neurons ($P < 0.05$) were activated in OT/H11021/H11021 mice after 18-h water deprivation compared with NST and AP activation in water-deprived wild-type mice (Fig. 5).

**DISCUSSION**

The present study revealed a significant attenuation of dehydration anorexia in OT/H11021/H11021 mice, accompanied by attenuated Fos expression in the hindbrain DVC. As discussed further below, we speculate that OT-containing inputs to the DVC are an important component of the central neural circuits that mediate dehydration anorexia in mice after water deprivation, consistent with previous findings in adult and developing rats, in which dehydration was induced acutely by a systemic salt load.

**Physiological effects of food and water deprivation.** Wild-type and OT/H11021/H11021 mice lost similar amounts of body weight during an 18-h fast. Body weight loss was somewhat greater in both genotypes when mice were water-deprived during the fast, although the effect of water availability on fasting-induced weight loss did not reach statistical significance in either group. Mice underwent a smaller reduction of body weight when they were deprived overnight of water but not food, with no significant difference between genotypes. These findings suggest that the gross physiological effects of food and water deprivation were not significantly different between genotypes. Additional support for this conclusion comes from the finding that plasma protein concentrations were significantly and similarly elevated in wild-type and OT/H11021/H11021 mice after water deprivation. Plasma protein concentrations increase as blood volume decreases; thus these data indicate that 18 h of water deprivation with food available induced a similar degree of hypovolemia in both genotypes. A previous report from our group (1) included results from additional measures of osmotic and volemic dehydration (e.g., plasma osmolality, plasma sodium, hematocrit) in wild-type and OT/H11021/H11021 mice after overnight 18-h fluid deprivation with food available, as in the current experiment 2. That study revealed significant fluid deprivation-induced volemic and osmotic dehydration, with no effect of genotype (1). The same deprivation protocol in the present study produced strikingly similar body weight losses in wild-type and OT/H11021/H11021 mice (i.e., $-6.5\%$). These results also are consistent with the increased plasma osmolality and re-
duced body weight reported in mice after 12 h (5) or 24 h of water deprivation (22).

**Fasting-induced food intake in euhydration and dehydrated mice.** When water was available during the 18-h overnight fast, both wild-type and OT−/− mice consumed ~4% of their fasted body weight during the subsequent 60-min refeeding period. Thus there was no effect of genotype on compensatory feeding after an overnight fast in euhydrated mice. However, a significant effect of genotype was revealed when mice began the feeding session in a dehydrated state after combined food and water deprivation. Dehydration significantly inhibited fasting-induced food intake at every timepoint examined in wild-type mice but not in OT−/− mice. The nonsignificant trend toward reduced food intake in OT−/− mice after overnight water deprivation likely reflects the residual contribution of other neurochemical signaling pathways that support dehydration anorexia, although apparently such pathways are insufficient to support the full anorectic effect in the absence of OT signaling.

**Effect of water deprivation to increase forebrain Fos immunolabeling.** Qualitative observations did not reveal any differences in forebrain Fos labeling patterns in wild-type and OT−/− mice after 18-h water deprivation. In both genotypes, water deprivation was associated with elevated Fos immunolabeling in osmo- and volume-sensitive forebrain regions, including the SFO, MnPO, OVLT, PVN, and SON. Activated PVN and SON neurons in wild-type mice included those that were AVP- and OT-immunopositive, consistent with results in rats (7) and outbred white-footed mice (5) after water deprivation. Results from the present study indicated that 18 h of water deprivation with food available activated ~18.5% of OT-positive neurons in wild-type mice, whereas less than 1% of OT neurons expressed Fos in euhydrated controls. OT immunolabeling is absent in OT−/− mice; thus, to determine whether water deprivation produced differential activation of hypothalamic neurons in wild-type and OT−/− mice, a quantitative assessment of Fos expression in AVP-positive PVN neurons was conducted. Very few AVP neurons were Fos-positive in euhydrated wild-type or OT−/− mice, whereas water deprivation activated ~60% of AVP-positive neurons in mice of both genotypes. These findings suggest that the diminished ability of water deprivation to inhibit food intake in OT−/− mice cannot be accounted for by a generally diminished ability of dehydration to recruit hypothalamic neurons in these mice.

Approximately 60% of AVP neurons were activated in wild-type mice after 18-h water deprivation, whereas just under 20% of OT neurons were activated in the same mice. The reasons for differential activation of AVP and OT neurons in the PVN after water deprivation are unclear, but these results are consistent with a report in female rats in which 24 h of water deprivation activated ~75% of AVP-positive neurons and ~25% of OT-positive neurons (7). It is unclear whether activated OT- and AVP-positive PVN neurons in wild-type mice include both magnocellular and parvocellular populations. As discussed further below, we hypothesize that OT neurons activated by water deprivation in wild-type mice include those that project to the caudal medulla, although we do not yet have direct evidence to support this hypothesis.

**Effect of water deprivation to increase hindbrain Fos immunolabeling.** In contrast to the apparently similar effect of water deprivation to activate hypothalamic neurons in wild-type and OT−/− mice, water deprivation activated significantly fewer DVC neurons in OT−/− mice compared with activation in wild-type mice. This differential effect was confined to the NTS and the AP; water deprivation did not significantly alter DMV Fos immunolabeling in either genotype. Thus water deprivation-induced Fos expression in the NST and AP was blunted in OT−/− mice compared with wild-type mice, despite apparently equivalent effects of water deprivation on body weight loss, plasma protein concentrations, and activation of hypothalamic neurons in both genotypes. These findings are consistent with the hypothesis that the attenuated ability of dehydration to inhibit food intake in OT−/− mice is functionally associated with attenuated DVC neural activation. Viscerosensory signals from the gastrointestinal tract that are relayed through the DVC play an important role in the ability of water deprivation to inhibit food intake in rats (30). Hydration signals from the gut are relayed from the DVC to the hypothalamus and other brain regions that contribute to the control of eating, drinking, and pituitary hormone secretion (6, 13, 16, 17). Results from the present study suggest that, in the absence of central OT signaling pathways, sensory inputs to the DVC that provide information about gut hydration are insufficient for the normal manifestation of dehydration anorexia in water-deprived mice.

**Potential role of OT inputs to the DVC.** Before the present study, evidence to support a role for central OT in dehydration anorexia has come primarily from short-term experiments in rats in which osmotic dehydration is induced by systemic administration of hypertonic solutions, and OT signaling is manipulated by central administration of OT receptor agonists and antagonists. Such experiments have demonstrated that OT-containing projections from the PVN to the DVC provide a tonic hypothalamic inhibition of vagally mediated gastric motility and emptying (8, 29), that this inhibitory influence is amplified during osmotic dehydration (9), and that OT receptor blockade attenuates both dehydration-induced inhibition of gastric emptying and dehydration anorexia (23).

Inhibition of vagally mediated gastric emptying by dehydration is associated with increased Fos immunolabeling within the hindbrain DVC in rats (3, 28). However, medial subregions of the NST that are activated to express Fos in adult rats after acute osmotic dehydration are not activated in 2-day-old rats (28), and 2-day-old rats do not display dehydration anorexia (3). Further, the progressive developmental ingrowth of OT-immunopositive fibers within the rat DVC is temporally correlated with the postnatal emergence of dehydration-induced Fos within the DVC, and both are correlated with the postnatal emergence of dehydration anorexia (3, 26, 28). Thus the inhibitory effect of osmotic dehydration on food intake emerges gradually during postnatal development in rats and closely coincides with the maturation of OT-containing neural projections from the PVN to the DVC. Further, centrally administered OT inhibits intake of food and NaCl solutions in adult rats (24, 33, 39). Considered together with results from the present study, it seems reasonable to hypothesize that OT signaling pathways from the PVN to the DVC recruit neural circuits that contribute importantly to dehydration anorexia both in rats and in mice.
In addition to a potential role in gastrointestinal functions, OT inputs to the DVC have been implicated in cardiovascular regulation (12, 20, 21, 37). It is possible that documented differences between wild-type and OT−/− mice in cardiovascular functions (21) contribute to the behavioral and Fos expression differences observed in the present study. We previously reported that male OT−/− mice consume significantly greater volumes of NaCl solution than wild-type cohorts after fluid deprivation, despite genotypically equivalent hyperosmolality and hypovolemia (1). OT−/− mice also consume significantly more NaCl solution than wild-type mice after being subjected to the mild stress of a novel environment (25), suggesting that OT signaling pathways normally provide inhibitory control over NaCl intake in both situations. Further evidence that OT signaling pathways inhibit solute intake in mice comes from a recent report that OT mRNA levels in the PVN are inversely correlated with behavioral expression of sodium appetite, such that higher OT mRNA levels are associated with reduced NaCl intake, and vice versa (10). Thus endogenous OT may exert an inhibitory influence over NaCl ingestion in mice, consistent with the effects of exogenously administered OT and OT receptor blockade on NaCl and food intake in rats (23, 24, 39). An inhibitory effect of central OT on solute intake is consistent with the greater magnitude of dehydration anorexia observed in wild-type mice compared with OT−/− mice in the present study. The congenital absence of OT signaling pathways had no significant effect on baseline or fasting-induced food intake in euhydrated OT−/− mice. A previous study revealed that the anorexigenic effect of systemically administered cholecystokinin octapeptide (CCK) is similar in OT−/− and wild-type mice and that there is no genotypic difference in the ability of CCK to activate forebrain or DVC Fos expression (18). Thus, in contrast to the apparent role of endogenous OT in facilitating anorexia and DVC Fos expression in mice after overnight water deprivation, the absence of OT signaling pathways does not significantly affect baseline food intake, CCK-induced anorexia, or CCK-induced Fos expression. Indeed, the hypothalamus and the entire brain are unnecessary for the behavioral effects of systemic CCK in adult decerebrate rats (11), and CCK-induced DVC Fos expression and anorexia in neonatal rats appear to occur independent of hypothalamic involvement (27). Conversely, findings from the present study suggest that hypothalamic OT-containing projections from the PVN to the caudal brainstem are necessary for the full expression of compensatory behavioral and physiological responses to dehydration induced by water deprivation.

ACKNOWLEDGMENTS

We thank Dr. Hou-ming Cai for technical help with the animal colony.

GRANTS

This research was supported by National Institutes of Health Research Grants MH-59911 (L. Rinaman), HD-37268 (J. A. Amico), and HD-44898 (J. A. Amico).

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