Oxytocin gene deletion mice overconsume palatable sucrose solution but not palatable lipid emulsions

J. A. Miedlar,1 L. Rinaman,2 R. R. Vollmer,1 and J. A. Amico1,3

Departments of 1Pharmaceutical Sciences, 2Neuroscience, and 3Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Submitted 4 April 2007; accepted in final form 21 June 2007

Miedlar JA, Rinaman L, Vollmer RR, Amico JA. Oxytocin gene deletion mice overconsume palatable sucrose solution but not palatable lipid emulsions. Am J Physiol Regul Integr Comp Physiol 293: R1063–R1068, 2007. First published June 27, 2007; doi:10.1152/ajpregu.00228.2007.—We previously reported that oxytocin knockout (OT KO) mice display markedly enhanced intake of sweet and nonsweet carbohydrate solutions compared with intake by wild-type (WT) mice of the same background strain. The present study was conducted to determine whether OT KO mice demonstrate enhanced intake of Intralipid, a palatable lipid emulsion. Male or female mice of both genotypes that were naive to the test solution were given continuous two-bottle access to Intralipid and water with food available ad libitum for 3 days. Throughout the experiment, mice of both genotypes showed a marked preference for Intralipid over water. On the 1st day, OT KO mice displayed twofold greater preference and consumed nearly twice as much Intralipid compared with WT cohorts. However, on subsequent days of exposure, Intralipid preference and intake did not differ between genotypes over a range of lipid concentrations presented in descending or ascending order. Daily and hourly measures of lipid vs. sucrose intake confirmed that OT KO mice consumed more sucrose solution, but not lipid emulsion, than WT mice. During ad libitum access to Intralipid, both genotypes consumed significantly more calories from the emulsion as concentration increased. Both genotypes maintained consistent total daily caloric intake (lipid plus chow) and compensated by decreasing chow intake over the course of the study. These findings, coupled with prior reports from our laboratory, support the view that OT signaling pathways participate in limiting intake of palatable carbohydrate-containing solutions, but do not appear to play a role in limiting intake of Intralipid.

Our laboratory previously reported that oxytocin (OT) signaling pathways modulate intake of sweet and nonsweet carbohydrate-containing solutions in mice. Compared with wild-type (WT) mice of the same background strain, male and female OT gene knockout (OT KO) mice consume larger volumes of sucrose or saccharin solutions during initial and sustained exposure (2, 3). OT KO mice also consume greater amounts of palatable but nonsweet carbohydrate solutions (i.e., Polycose and cornstarch) compared with WT cohorts (9). Although WT mice eventually develop a strong preference for sucrose or saccharin solution over water, initial intake is attenuated compared with OT KO mice (2). Furthermore, WT mice reach a plateau in daily sucrose or saccharin intake that is significantly less than the daily intake by OT KO mice (2, 3). Thus the genetic absence of OT in male and female mice enhances their initial and sustained daily consumption of sweet and nonsweet carbohydrate-containing solutions.

Given the exaggerated intake of palatable carbohydrate solutions by OT KO compared with WT mice, the present study was designed to determine whether OT KO mice also display enhanced intake of a palatable fat-containing solution. Mice and rats readily ingest Intralipid, a commercial nonsweetened stable soybean oil emulsion that is useful for studies requiring an easily quantifiable and palatable liquid source of fat (4, 5, 8, 9). If OT signaling pathways play a special role in limiting intake of palatable carbohydrate-containing solutions, then intake of fat (Intralipid emulsions) should be similar between genotypes. A recent study in which male mice were exposed to a 4% Intralipid emulsion did not reveal differences in intake between OT KO and WT mice (9). The present study was designed to extend these initial observations by monitoring daily fluid intake in male and female OT KO and WT mice during novel (first-time) exposure to Intralipid emulsion, and to assess intake during reexposure to Intralipid at a range of concentrations. Intralipid was provided ad libitum, along with water and laboratory chow. Caloric intake of both food and fluids was calculated daily. We also compared the hourly intake of Intralipid and water with the hourly intake of sucrose and water (in separate tests) during a 6-h evening period corresponding to the transition from day to night (i.e., 2 h before lights out until 4 h after lights out).

MATERIALS AND METHODS

Animals. OT KO and WT mice were bred and housed in a viral-free facility at the University of Pittsburgh. Founder mice for the breeding colony were purchased from Jackson Laboratories and were derived from mice originally generated by Dr. Scott Young (10). Each experiment was performed in either male (F10 generation, 5–6 mo of age at the start of the experiment) or female mice (F10 generation, 13–16 mo of age, and F11 generation, 5 mo of age at the start of the experiments).

Animals were genotyped by extracting DNA from a small tail sample collected at the time of weaning. The sample was then prepared for polymerase chain reaction using methods previously described in Refs. 1 and 10. Animals were housed in a temperature- and light-controlled room (12 h dark: 12 h light, lights on at 0700). Mice were transferred from group housing (4–5 per cage) to single housing 72 h before beginning experimental manipulations and were maintained in single housing throughout the remainder of the study. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Mice were tested as paired cohorts, according to the transition from day to night (i.e., 2 h before lights out until 4 h after lights out).
to genotype and treatment, with eight animals in each group. Body weights were recorded at the beginning and end of each experiment.

Liquid and food. Intralipid (20%, Fresenius Kabi, Uppsala, Sweden) was stored at 4°C and was freshly diluted each day with tap water. Each 100 ml of a 20% stock emulsion contains 20 g of purified soybean oil, 1.2 g of purified egg phospholipids, 2.25 g of glycerol, and water. The caloric content of the Intralipid solutions used in this study are as follows: 10%, 1 kcal/ml; 8.2%, 0.82 kcal/ml; 5%, 0.5 kcal/ml; 4.1%, 0.4 kcal/ml; 2.5%, 0.25 kcal/ml; 2.1%, 0.21 kcal/ml; 1%, 0.1 kcal/ml; and 0.5%, 0.05 kcal/ml. Water and test solution were presented simultaneously to mice (available ad libitum) via two stainless steel drinking spouts affixed to graduated cylinders calibrated in 0.1-ml increments. The position of water and test solution remained constant during each experiment.

Standard pelleted rodent chow (Prolab RMH 3000/Purina; 1% sucrose, 0.1% glucose, 0.2% fructose, 0% lactose by weight; 5% crude fat; 4.1 kcal/g) was available ad libitum during and between experiments. In experiment 4, chow was presented in powdered form to facilitate quantification of daily food intake.

Experiment 1. Daily intake of 10% Intralipid emulsion in male mice during first-time exposure. To determine whether OT KO and WT mice differ in their initial or sustained daily intake of lipid-rich emulsion, male mice (8 OT KO and 8 WT) were given two-bottle access to water and 10% Intralipid available ad libitum for 3 days. Volumes of water and Intralipid consumption were recorded daily at 1000. Intralipid preference scores were calculated each day by dividing Intralipid intake by total fluid intake. On the 3rd day of exposure, hourly intakes of each fluid were recorded from 1700 to 2300 (i.e., from 2 h before lights out until 4 h after lights out). This time period was selected based on our previous experience that this time corresponds to the maximal rate of palatable fluid intake.

We previously reported that OT KO mice consume significantly more 10% sucrose solution than WT mice (2, 3), and we wanted to confirm that finding in the present study and also compare sucrose intake to Intralipid intake. For this purpose, the experiment 1 Intralipid intake protocol was repeated in a separate group of age-matched male OT KO and WT mice (N = 8 per genotype) given two-bottle access to water and 10% Intralipid available ad libitum for 3 days. Volumes of water and Intralipid consumption were recorded daily at 1000. Intralipid preference scores were calculated each day by dividing Intralipid intake by total fluid intake. On the 3rd day of exposure, hourly intakes of each fluid were recorded from 1700 to 2300 (i.e., from 2 h before lights out until 4 h after lights out). This time period was selected based on our previous experience that this time corresponds to the maximal rate of palatable fluid intake.

RESULTS

Experiment 1. Daily intake of 10% Intralipid emulsion in male mice during first-time exposure. Male OT KO mice manifested an immediate and sustained preference for Intralipid over a range of descending concentrations (i.e., 98%; Fig. 1). In contrast, male WT mice displayed an incremental increase in preference (i.e., 56% on day 1, 70% on day 2, and 86% on day 3). Preference for Intralipid was significantly different between genotypes (ANOVA, F(1,14) = 4.922, P = 0.044) only on exposure day 1 (Bonferroni t-tests, P < 0.05) (Fig. 1A).

There was a significant interaction between genotype and exposure day on Intralipid intake across the 3 days of exposure (ANOVA, F(2,28) = 4.568, P = 0.019). On the 1st day, OT KO mice consumed almost twice as much Intralipid emulsion as WT mice (post hoc Bonferroni t-test, P < 0.05) (Fig. 1A). This difference was not sustained on subsequent days of exposure. In WT mice, Intralipid intake on day 1 was significantly lower than on days 2 and 3. In contrast, daily intake of Intralipid by OT KO mice did not differ among days. Water intake on each of the 3 days was <3 ml for WT mice and <0.5 ml for OT KO mice. There was a significant difference in water consumption between genotypes (ANOVA, F(1,14) = 5.69, P = 0.032, post hoc Bonferroni t-test, P < 0.05) on day 1, but not on days 2 or 3 (Fig. 1B). Total daily fluid intake (i.e., Intralipid plus water) was not different between genotypes on any of the 3 days.

At the end of the 3rd exposure day, hourly monitoring of 10% Intralipid and water intake for 6 h during the evening (1700 to 2300) confirmed that intake was not different between genotypes (Fig. 2A). In a parallel experiment, hourly intakes of 10% sucrose solution and water were monitored during the same evening period in a separate group of OT KO and WT male mice at the end of 3 days of exposure to 10% sucrose...
available ad libitum, along with water and pelleted chow. OT KO mice consumed significantly more 10% sucrose compared with intake by WT mice at each time point after lights out [ANOVA, F(1,14) = 8.35, P = 0.012; Bonferroni t-test, P = 0.05] (Fig. 2).

There was no genotypic difference in body weight at the beginning or end of the 3-day Intralipid experiment. Pre- and postexposure body weights for OT KO mice were 28.8 ± 1.0 and 28.7 ± 1.1 g and for WT mice were 30.3 ± 1.2 and 31.1 ± 1.4 g, respectively. There was also no genotypic difference in body weight at the beginning or end of the 3-day sucrose experiment. Pre- and postexposure body weights for OT KO mice were 26.5 ± 0.4 and 27.5 ± 0.6 g and for WT mice were 26.4 ± 0.9 and 26.6 ± 1.2 g, respectively.

Experiment 2: Daily Intralipid intake over a range of descending concentrations in nonnaive animals. Male OT KO and WT mice again demonstrated a marked preference for Intralipid over water when reexposed to 10% emulsion during a 3-day period that began 1 wk after the conclusion of experiment 1. Mice drank 12–15 ml of 10% Intralipid emulsion daily with no significant differences between genotypes (Fig. 3A) and no significant differences across exposure days. Intralipid preference was >98% on all 3 days in both genotypes. Water intake was minimal, averaging <1.0 ml/day and did not differ between genotypes (data not shown).

As with the 10% concentration, there were no significant differences between genotypes in their intake of 5% (Fig. 3B) or 2.5% (Fig. 3C) Intralipid emulsion over 3 days of exposure. OT KO and WT mice previously exposed to 10% Intralipid subsequently consumed significantly larger volumes of 5% [ANOVA, F(1,30) = 4.22, P = 0.049; Bonferroni t-test, P < 0.05] on day 2 and 2.5% Intralipid [ANOVA, F(1,30) = 6.31, P = 0.018; Bonferroni t-test, P < 0.05] on days 1 and 2 compared with intake of 10% Intralipid emulsion (Fig. 3).

Hourly monitoring of fluid intake (Intralipid and water) for 6 h during the evening (1700 to 2300) at the end of the 3rd
exposure day during access to 5 and 2.5% Intralipid confirmed that intake was not different between genotypes at either concentration (data not shown). Mice of both genotypes consumed little or no water during the 6-h monitoring period.

There was no effect of genotype on body weight measured at the beginning or end of each 3-day exposure to Intralipid. There was also no effect of genotype on body weight measured before the reexposure to 10% Intralipid and the end of 2.5% Intralipid exposure. Pre- and postexposure body weights for OT KO mice were 28.8 ± 1.1 and 29.7 ± 1.2 g and for WT mice were 30.3 ± 1.2 and 31.0 ± 1.4 g, respectively.

**Experiment 3:** Daily intake of 4.1% Intralipid emulsion in female mice during first-time exposure.

The preference of female OT KO mice for Intralipid was 96% on day 1, 97% on day 2, and 87% on day 3. In contrast, the preference of female WT mice for Intralipid was only 58% on day 1 and then became similar to that of OT KO mice (i.e., 97 and 98% on days 2 and 3, respectively). There was a significant interaction between genotype and exposure day on preference for 4.1% Intralipid [ANOVA, F(2,28) = 6.850, P = 0.004] on day 1 (Bonferroni t-test, P < 0.05). Female OT KO mice consumed approximately twice as much 4.1% Intralipid emulsion as WT mice on the 1st day of exposure (Fig. 4A). The effect of genotype on 4.1% Intralipid intake on experimental day 1 in naive female OT KO mice was similar to that seen on the 1st day of 10% Intralipid exposure in naive male OT KO mice (experiment 1). There was a significant interaction between genotype and exposure day in daily intake of 4.1% Intralipid in naive female mice [ANOVA, F(2,28) = 4.622, P = 0.018] on day 1 (Bonferroni t-test, P < 0.05). There was also an overall significant interaction between genotype and exposure day in daily water intake [ANOVA, F(2,28) = 6.161, P = 0.006] (Fig. 4B). Water intake by WT mice on day 1 was significantly higher than water intake by WT mice on days 2 and 3 (Bonferroni t-test, P < 0.05). However, there was no overall effect of genotype on total daily fluid intake.

There was no effect of genotype on body weight measured at the beginning or end of the 3-day exposure to 4.1% Intralipid emulsion. Pre- and postexposure body weights for female OT KO mice were 28.8 ± 1.0 and 28.7 ± 1.2 g and for female WT mice were 30.3 ± 1.2 and 31.1 ± 1.4 g, respectively.

**Experiment 4:** Daily caloric intake over a range of ascending Intralipid concentrations.

A separate group of female OT KO and WT mice naive to Intralipid were exposed to an...
ascending concentration series of Intralipid emulsions (i.e., 0.5, 1.0, 2.1, 4.1, and 8.2%) for 3 days each during consecutive weeks. There was a significant effect of concentration on the volume of Intralipid consumed [ANOVA, $F(1,4) = 8.25, P < 0.001$], but there were no intake differences between the genotypes (Fig. 5A). Intralipid consumption increased as concentration increased up to 4.1%, but declined during exposure to the highest concentration tested (i.e., 8.2%). As Intralipid concentration increased, calories obtained from Intralipid also increased [ANOVA, $F(1,4) = 54.51, P < 0.001$] (Fig. 5C). However, total daily caloric intake (powdered chow plus Intralipid) did not differ among Intralipid concentrations or between genotypes (Fig. 5B). WT and OT KO animals decreased chow intake to compensate for the increased calories consumed from Intralipid (Fig. 5D). There was no difference between genotypes in calories obtained from powdered chow, but there was a significant effect of Intralipid concentration [ANOVA, $F(1,4) = 15.99, P < 0.001$]. Significantly fewer calories were obtained from chow during exposure to 8.2% Intralipid compared with chow calories consumed during exposure to each of the other Intralipid concentrations (Bonferroni t-test, $P < 0.05$). As Intralipid concentration and calories obtained from Intralipid increased, chow intake decreased similarly in both genotypes.

There was no effect of genotype on body weights measured at the beginning or end of the experiment or at any time point during the consecutive 3-day exposure periods. Pre- and post-exposure body weights for OT KO mice were 27.4 ± 1.0 and 26.9 ± 0.8 g and for WT mice were 27.3 ± 0.9 and 27.1 ± 0.9 g, respectively.

**DISCUSSION**

Previous work in our laboratory demonstrated that, compared with WT mice, male and female OT KO mice display a marked preference for and immediate ingestion of water sweetened with either sucrose or saccharin during their 1st day of exposure (2, 3). With continued exposure to either 10% sucrose or 0.2% saccharin solution, OT KO mice persist in consuming significantly greater daily volumes compared with WT cohorts (2, 3). Recent work has also shown that OT KO mice consume greater daily volumes of palatable but nonsweet carbohydrate-containing solutions (i.e., Polycose and cornstarch) (9). The present study sought to determine whether the enhanced intake of palatable carbohydrate-containing solutions manifested by OT KO mice extends to enhanced intake of palatable Intralipid emulsions.

Intralipid emulsions within the concentration range used in this paper are highly palatable to rats (4) and mice (5, 8). Our present results demonstrate that Intralipid concentrations ranging from 0.5 to 10% are preferred over water by both WT and OT KO mice. As observed during exposure to sucrose, there are no differences in intake pattern between male and female animals of the same genotype. Interestingly, the only time that OT KO mice showed greater preference for Intralipid compared with WT mice was on their 1st day of exposure to the novel emulsions at concentrations of 10 and 4.1%. There were no differences between genotypes in daily intake or preference for Intralipid at any of the eight concentrations tested, including no subsequent differences in hourly evening intakes. These new observations contrast with our previous findings that OT KO mice persistently consume significantly greater amounts of sucrose, saccharin, and nonsweetened carbohydrate-containing solutions than WT cohorts.

![Fig. 5. Volume of liquid and calories consumed from an ascending range of Intralipid concentrations in OT KO and WT mice given two-bottle access to water paired with Intralipid. Data shown represent intake on day 3.](http://ajpregu.physiology.org/)
Our cumulative findings support the view that the congenital absence of OT in OT KO mice is not associated with enhanced intake of a palatable fat-enriched emulsion (Intralipid), but is associated with enhanced intake of saccharin and carbohydrate-containing liquids. When male and female OT KO and WT mice were naive to Intralipid, OT KO mice consumed almost twice as much lipid emulsion and displayed nearly twice as much preference for Intralipid over water compared with WT mice on the 1st day of exposure to the novel emulsion. During initial exposure, OT KO mice demonstrate no delay in consuming novel palatable liquids, including both Intralipid (present study) and sucrose solutions (2). After mice gain experience with the palatable solutions, both genotypes consume equivalent amounts of Intralipid emulsion (present study), but OT KO mice continue to consume greater amounts of palatable sweetened (sucrose and saccharin) and nonsweetened carbohydrate-containing (Polycose and cornstarch) solutions (2, 3, 9).

Results shown here and in Ref. 2 demonstrate that OT KO animals have an increased acceptance of novel palatable ingesta. Briefly, we hypothesize that central OT signaling pathways inhibit ingestion in mice, and that these signaling pathways can be recruited in more than one way. Our data support the view that OT signaling pathways are recruited in WT mice as part of the mild stress response associated with initial exposure to all types of novel flavors (i.e., taste plus odor) and textures of ingesta. Habituation during repeated or continued exposure presumably is associated with reduced recruitment of OT signaling to thereby permit increased intake of the novel familiar ingesta. The absence of OT signaling in OT KO mice may account for the increased intake of novel palatable ingesta during initial exposure. In our view, neural systems that recruit hypophagic OT pathways during initial exposure may be separate from neural systems that recruit OT pathways during and after intake of familiar palatable sweet and nonsweet carbohydrate (2, 9) and saccharin (3) solutions. However, the present results suggest either that OT signaling pathways are not initially recruited when mice consume Intralipid, or that OT recruitment is overcome by other neural signaling pathways that increase Intralipid intake.

Our experiments included intake tests over a range of Intralipid concentrations, from 0.5 to 10%, including 4.1% Intralipid, which is isocaloric with 10% sucrose (0.41 kcal/ml). Thus it is unlikely that daily Intralipid intake by mice of either genotype was limited by a ceiling effect of caloric density. As shown in experiment 4, the absence of OT does not interfere with the ability of mice to regulate their total daily caloric intake. Mice of both genotypes maintained a relatively constant energy intake by reducing chow intake as calories derived from Intralipid increased. The compensatory reduction in solid food intake was also observed in our laboratory in OT KO and WT mice drinking 10% sucrose solution (2).

Several interesting behavioral and physiological traits distinguish OT KO mice from age- and sex-matched cohorts. After overnight water deprivation, male OT KO mice display blunted dehydration anorexia (7) and enhanced intake of hypertonic NaCl solution (1). However, we have shown that age- and sex-matched OT KO and WT mice have similar body weights and display similar hourly and cumulative daily food and water intake during basal conditions (6), similar water intake after overnight water deprivation (1), and similar food intake after an overnight fast with water available ad libitum (6). Because OT gene deletion does not disrupt food or water intake under basal conditions or after overnight deprivation, the immediate and sustained excessive intake of carbohydrate-containing solutions by male and female OT KO mice is striking (2, 3). Such observations supported our initial hypothesis that OT signaling pathways in mice play a special role in limiting excessive intake of highly palatable ingesta. Integration of past findings with the new data reported here suggest that this hypothesis should be refined, as OT signaling pathways do not appear to be involved in controlling intake of Intralipid.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance provided by Melissa BeHanna and Houming Cai.

GRANTS

Studies were supported by National Institute of Child Health and Human Development Grant HD 44898 (J. A. Amico).

REFERENCES