Increased oral and decreased intestinal sensitivity to sucrose in obese, prediabetic CCK-A receptor-deficient OLETF rats

Bart C. De Jonghe, Andras Hajnal, and Mihai Covasa. Increased oral and decreased intestinal sensitivity to sucrose in obese, prediabetic CCK-A receptor-deficient OLETF rats. Am J Physiol Regul Integr Comp Physiol 288: R292–R300, 2005. First published September 9, 2004; doi:10.1152/ajpregu.00481.2004.—CCK-A receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rats are hyperphagic and develop obesity and Type 2 diabetes. In this strain, taste preference functions have not been investigated. Therefore, a series of short-access, two-bottle tests were performed in age-matched prediabetic OLETF and nonmutant Long-Evans Tokushima Otsuka (LETO) rats to investigate preference for sucrose (0.03, 0.1, 0.3, or 1.0 M) presented with a choice of water. To discern orosensory from postgastric factors that may contribute to this preference, in a separate experiment, rats were allowed to sham feed sucrose in the absence or presence of duodenal sucrose infusion (0.3, 0.6, or 1.0 M). In the two-bottle real-feeding tests, OLETF rats exhibited a greater preference for 0.3 M sucrose (91.2 ± 1.7 and 78.5 ± 3.4% for OLETF and LETO, respectively; P < 0.01) than LETO rats. OLETF rats also sham fed less of the lowest (0.03 M; 33.8 ± 4.8 and 58.3 ± 7.3 ml for OLETF and LETO, respectively; P < 0.05) than LETO rats. OLETF rats also sham fed the most (1.0 M; 109.9 ± 6.5 and 81.0 ± 3.9 ml for OLETF and LETO, respectively; P < 0.01) concentration of sucrose relative to LETO rats. Finally, intraduodenal sucrose infusions (0.6 and 1.0 M) produced a smaller reduction of 0.3 M sham sucrose intake [14.1 ± 8.1 vs. 52.5 ± 3.3 ml and 49.4 ± 8.0 vs. 82.4 ± 3.2 ml for 0.6 M (P < 0.01) and 1.0 M (P < 0.05) infusions in OLETF and LETO, respectively]. These findings demonstrate that OLETF rats display an increased preference for sucrose, an effect that is at least partially influenced by the orosensory stimulating effect of sucrose. This enhanced responsiveness to oral stimulation, coupled with the deficit in responding to the postingestive feedback of intestinal sucrose, may contribute additively to the development of hyperphagia and weight gain in OLETF rats.

The Otsuka Long-Evans Tokushima fatty (OLETF) rat, an outbred strain of the Long-Evans Tokushima Otsuka (LETO) rat, lacks functional CCK-A receptor expression (68). These animals provide an excellent model to study obesity and related metabolic abnormalities, such as non-insulin-dependent diabetes mellitus (NIDDM), because of their spontaneous manifestation of hyperglycemia and hyperinsulinemia relative to age-matched control, nonmutant LETO rats (39). Also, OLETF rats exhibit accelerated rates of weight gain, beginning at 5 wk of age, which eventually culminate in body weights that are ~40% higher than the control LETO strain (48, 59). Relevant to the present work is the demonstration that OLETF rats ingest larger meals and are less sensitive to inhibition of food intake by intraintestinal nutrients than LETO control rats (13, 48). These animals have also been shown to consume normally preferred foods in excess amounts compared with LETO rats (59, 69).

Several recent data provide evidence that deficits in peripheral signaling mechanisms may account for the excessive meal size and food intake observed in OLETF rats (3, 13, 48, 59). However, our studies with CCK receptor antagonists in OLETF and LETO rats suggest that not all satiation deficits and hyperphagic behaviors in OLETF rats are directly related to the lack of CCK-A receptors (13). Furthermore, Bi et al. (2–6) recently showed that altered central neuropeptide Y (NPY) signaling within the dorsomedial hypothalamus is another contributor to hyperphagia in these animals in a variety of real feeding paradigms. In contrast, there are scant data examining altered orosensory functioning in OLETF rats as a possible cause for hyperphagia and subsequent weight gain even though it is well established that orosensory components of food, independent of postdigestive effects, can have a significant impact on the amount of food consumed. In fact, rats will sham feed sucrose in a concentration-dependent manner, when no postabsorptive effects are elicited (23, 49, 71).

On this basis, the goal of the present study was twofold: 1) to determine the preference of prediabetic OLETF rats for sucrose solutions of various concentrations compared with age-matched (6–8 wk) control LETO rats and 2) to assess whether increased preference for sucrose in the OLETF rat is due to pre- or postdigestive feedback mechanisms. Therefore, in a series of experiments, we employed short-access, two-bottle preference tests to examine potential strain differences in sucrose preference functions between OLETF and LETO rats. To assess participation of orosensory as well as postgastric effects of sucrose solutions, in a separate study, rats were
allowed to sham feed in the absence or presence of intraduodenal sucrose infusion.

METHODS

Subjects

Five-week-old male OLETF and LETO rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. All animals were individually housed in mesh-floored, stainless-steel hanging cages in a temperature-controlled vivarium and were maintained in a constant 12:12-h light-dark cycle (lights on at 0600). Rats were handled daily for a minimum of 1 wk before experimental procedures began. Tap water and pelleted rat chow (Purina 5001) were available ad libitum during the acclimation period. All protocols used were approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Surgical Procedures: Gastric Fistula and Duodenal Catheter Implantation

All rats were fasted overnight and anesthetized before surgery via intramuscular injection with 1 ml/kg mixture of xylazine (20.0 mg/ml), ketamine HCl (100.0 mg/ml), and acepromazine maleate (10.0 mg/ml), obtained from Burns Veterinary Supply (Rockville Centre, NY). OLETF and LETO rats used for sham and intraduodenal infusion experiments were surgically implanted with chronic gastric fistulae and duodenal catheters according to Yox and Ritter (75). Briefly, the inner flange of a gastric fistula (stainless steel; 13 mm length, 6 mm ID, 8 mm OD) was introduced through the ventral wall of the nonglandular portion of the stomach near the greater curvature and subsequently secured with a purse-string suture. A piece of Marlex mesh was centered to adhere flush with the outer flange of the fistula. The nonflanged end of the fistula was then externalized through a left paramedian abdominal incision. A stainless steel screw served to seal off the fistula lumen and maintain isolation of gastric contents in feeding conditions outside of experimental paradigms. For duodenal catheter implantation, one end of the catheter (0.025 in. ID, 0.047 in. OD; Dow Corning, Midland, MI) was inserted via the gastric fistula through the pylorus and advanced beyond the pyloric muscle. A small silicone nub, 6 cm from the intraduodenal end of the catheter, was passed through the pylorus and sutured on either side to one area of the basolateral surface of the duodenum. The peritoneum and abdominal muscles were simultaneously closed with absorbable sutures postimplantation. Wound clips were employed to close the abdominal skin incision, which were removed 7 days after surgery. The free end of the catheter was occluded with a stainless steel wire, which was removed immediately before each experiment when the catheter was coupled to a syringe pump (Harvard, PHD 2000) to begin intestinal infusions. Between infusion sessions, the plugged free end of the catheter was tucked inside the outer fistula lumen before the steel screw was fastened. Rats were allowed a minimum of 1 wk to recover from the fistula and catheter implantation surgery.

Procedures and Experiments

Experiment 1: sucrose preference of OLETF and LETO rats using short-access, two-bottle choice test. Twenty rats (10 OLETF and 10 LETO) with average body weights of 244.1 ± 4.4 and 197.3 ± 3.6 g, respectively, were divided into two groups (n = 5/strain) matched for body weight within each strain. Rats received two of the following sucrose concentrations during the training and testing period: 0.1 and 1.0 M sucrose (group 1) and 0.03 and 0.3 M sucrose (group 2). Rats were then placed on a water regimen consisting of overnight deprivation before the first training day and exclusive water access for 1 h in the evenings (1800–1900) thereafter, throughout the whole experiment. Pelleted chow was available ad libitum except during the two-bottle tests. Rats were familiarized with the drinking schedule by giving them concurrent access to two bottles both filled with distilled water in the mornings between 1000 and 1100 for 1 h and in the afternoon between 1500 and 1600 for 2 days. Rats were then familiarized with the sucrose solution by presenting the average concentration of the two sucrose solutions (0.55 or 0.165 M, for group 1 and group 2, respectively) in one bottle, with distilled water in the other bottle for 2 consecutive days to reduce neophobia. To control for side preference, the position of each cylinder was changed between days and counterbalanced within groups on the same day. For the next 8 days, the two-bottle tests were performed with the first concentration of sucrose vs. water in the morning and the second concentration within the pairing vs. water in the afternoon, alternating every other day. Rats that received 0.1 or 1.0 M sucrose never received other concentrations (i.e., 0.03 or 0.3 M for sucrose). Conversely, the other half of the rats received only 0.03 or 0.3 M sucrose solutions and never 0.1 or 1.0 M sucrose solutions. Sucrose and water intakes were measured to the nearest 0.5 ml.

Experiment 2: sham feeding of sucrose solutions in OLETF and LETO rats in the absence of intestinal feedback. Three days before experiment, naive OLETF and LETO rats (n = 6/strain, weighing 284.3 ± 6.5 and 209.3 ± 5.5 g) were placed on a fluid deprivation schedule during which they were allowed 6 h of water access each day from 1400 to 2000. Pelleted food was continuously available in the home cages except during licking sessions. After habituation, rats were acclimated to the sham feeding procedure by presenting them with 0.03 M sucrose for 60 min over 3 consecutive days. During testing and after a brief 2-h fast, the stainless steel screws occluding the gastric fistulas were removed, and stomach contents were lavaged with warm tap water to ensure minimal gastric volume and distension upon start of sham feeding. Rats were placed into Plexiglas sham feeding boxes and presented with either 0.03 M (1.03% wt/vol), 0.3 M (10.2% wt/vol), or 1.0 M (34.2% wt/vol) sucrose three times each over 9 consecutive days in a randomized fashion. Sham intake was measured to the nearest 0.1 ml every 5 min over a 60-min feeding period (1030–1130). In all sham feeding tests, gastric drainage was collected in plastic graduated cylinders placed beneath the cages and the volume was recorded at experiment termination. If the volume of fluid ingested was greater than the volume of gastric drainage or if gastric drainage did not occur within 15 s of the start of sham feeding, the data from that subject were discarded on the basis that the gastric fistula was not properly placed or functioning (75).

Experiment 3: sham intake of sucrose in OLETF and LETO rats after intraduodenal sucrose infusion. OLETF and LETO rats (n = 6/strain, weighing 331 ± 5.4 and 268 ± 7.0 g, respectively) previously used in experiment 2 were used in intestinal infusion experiments. Scheduled access to chow and water and sham feeding protocols were identical to those described in experiment 2, except that the sham-feeding session was extended to 90 min. All duodenal catheters were flushed with 0.5 ml of 0.15 M saline 30 min before infusion to ensure patency. Rats were presented with a single concentration (0.3 M) of sucrose solution throughout the experiment so that the effects of infusions with various sucrose concentrations could be compared. During testing and after the stomachs had been cleared of gastric contents, rats were placed in sham feeding cages as previously described. The free end of the intraduodenal catheter was connected to a motorized digital syringe pump (Harvard, PHD 2000) to deliver at a rate of 0.4 ml/min. Rats were presented with burettes filled with 0.3 M sucrose solution, and intestinal infusion began 10 min after sucrose presentation and continued for 20 min, according to the procedure employed by Greenberg et al. (27). Each rat received a total volume of 8.0 ml of infusate. After infusion ceased, rats were allowed to sham feed sucrose for an additional 60 min to detect changes in sham intakes after sucrose infusion. Thus rats had access to 0.3 M sucrose for a total of 90 min. Before sucrose infusion sessions began, 2 days of 0.15 M saline infusions were performed to establish a stable baseline of 90-min sham intake. Rats then received each of three concentrations of sucrose infusions: 0.3 M (0.83 g/8 ml), 0.6 M
Oral Glucose Tolerance Test

Two days after experimental testing, an oral glucose tolerance test was performed in a subset of eight rats (n = 4/strain, 12 wk of age, weighing 342 ± 6.41 and 274 ± 6.8 g for OLETF and LETO, respectively) within each of the two experimental groups to determine whether OLETF rats had developed NIDDM. After a 16-h fast, an oral glucose load (2 g/kg) was delivered to each rat via oral gavage. Blood glucose was measured before gavage and 30, 60, 90, and 120 min postglucose administration via a glucometer (LifeScan, One-Touch Basic). Animals were classified as diabetic if the peak level of plasma glucose was measured before gavage and 30, 60, 90, and 120 min postglucose administration via a glucometer (LifeScan, One-Touch Basic). Animals were classified as diabetic if the peak level of plasma glucose was ≥16.8 mM and a peak glucose level at 120 min was >11.2 mM (39).

Statistical Analyses

For experiment 1, two-way repeated-measures analysis of variance (rmANOVA) was performed with strain and sucrose concentration as main effects. Total intakes of both solutions within the pairing (sucrose + water) were used to calculate preference percentages according to the following formula: preference percentage = [volume of sucrose solution (ml) × 100/total volume of sucrose and water (ml)].

Sixty-minute sham intake in experiment 2 was analyzed by appropriate two-way rmANOVA with strain and sucrose concentration as main factors. One-way rmANOVA was performed to test concentration effects of sucrose within OLETF or LETO rats. ANOVA results were subsequently analyzed by Tukey’s honestly significant difference post hoc tests when applicable for both experiments 1 and 2.

For experiment 3, one-way rmANOVA was used to calculate all individual 5-min intake bins as well as cumulative 5-min intakes in both OLETF and LETO rats within each infusion group. Percent suppression of 0.3 M sucrose sham intake over the 90-min session was calculated with a two-way rmANOVA with strain and infusion concentration as main effects. Percent suppression was calculated according to the following formula: percent suppression = 1 – (experimental/baseline) × 100. One-way rmANOVA was calculated to test concentration effects of sucrose infusion on sham intake within OLETF or LETO rats. These results were again analyzed post hoc by Tukey’s honestly significant difference when necessary.

Planned t-tests were calculated to compare blood glucose levels in OLETF and LETO rats following an oral glucose tolerance test. All data are expressed as means ± SE. Differences were considered statistically significant if P < 0.05. All statistical analyses were carried out with PC-SAS (version 8.02, SAS Institute, Carey, NC).

RESULTS

Experiment 1: Sucrose Intake Following Short-Access, Two-Bottle Tests in Age-Matched OLETF and LETO Rats

There were no overall within-strain differences in sucrose intake in the morning vs. afternoon sessions. Therefore, data were pooled for the subsequent analyses. Prediabetic OLETF rats exhibited a greater preference for 0.3 M [91.2 ± 1.7% and 78.5 ± 3.4% for OLETF and LETO, respectively; F(1,8) = 11.4; P < 0.01] and 1.0 M sucrose [65.3 ± 1.2% and 57.5 ± 2.7% for OLETF and LETO, respectively; F(1,8) = 6.66; P < 0.05] than LETO controls in a 60-min two-bottle choice test (Fig. 1). OLETF rats consumed a greater absolute volume of 0.3 M sucrose [34.6 ± 2.9 and 21.5 ± 1.5 ml for OLETF and LETO, respectively; F(1,8) = 16.5; P < 0.01] and 1.0 M sucrose [22.1 ± 1.6 and 12.9 ± 0.6 ml for OLETF and LETO, respectively; F(1,8) = 29.8; P < 0.01], whereas paired water intakes during these sessions did not differ significantly (P > 0.05). No strain differences in intake or preference were noted for 0.03 and 0.1 M sucrose consumption as well as for the respective paired water intakes (P > 0.05).

Average daily caloric intake (chow + sucrose) was significantly higher [F(1,16)= 22.30; P < 0.01] in OLETF compared with LETO rats (89.2 ± 4.6 and 70.0 ± 3.1 kcal/day, respectively).

Experiment 2: Sucrose Sham Feeding in OLETF and LETO Rats

For both OLETF and LETO rats, sham intake increased as a function of concentration [F(2,13) = 44.9; P < 0.0001 and F(2,15) = 7.48; P < 0.01 for OLETF and LETO rats, respectively]. However, OLETF rats drank significantly less 0.03 M sucrose [33.8 ± 4.8 ml and 58.3 ± 7.3 ml for OLETF and LETO, respectively; F(1,9) = 7.48; P > 0.05] than LETO controls during a 60-min sham-feeding session (Fig. 2).
contrast, OLETF rats sham fed significantly greater amounts of 1.0 M sucrose \([109.9 \pm 6.5 \text{ ml} \text{ and } 81.0 \pm 3.9 \text{ ml} \text{ for OLETF and LETO, respectively; } F(1,9) = 15.9; P < 0.01]\) than LETO animals. Both strains consumed a similar amount of 0.3 M sucrose during 60-min sham feeding \((P = 0.113)\). Post hoc testing revealed that, although OLETF rats increased consumption across all three concentrations administered \((P < 0.01 \text{ and } P < 0.001 \text{ for } 0.3 \text{ to } 0.3 \text{ M sucrose and from } 0.3 \text{ to } 1.0 \text{ M sucrose, respectively}), \) LETO rats only increased intake of 0.03 and 0.3 M sucrose \((P < 0.05)\) and did not increase intake of 0.3 and 1.0 M sucrose \((P = 0.439)\).

**Experiment 3: Sham Feeding in OLETF and LETO Rats After Intestinal Infusion of Sucrose**

Intraduodenal infusion of 0.15 M saline did not lead to significantly different 90-min sham intake of 0.3 M sucrose in OLETF and LETO rats \((P = 0.138)\). Figure 3B shows that no overall strain difference in sham intake was noted in response to 0.3 M sucrose infusion \((P = 0.21)\). OLETF rats, however, exhibited an initially higher \((P < 0.05)\) sham intake of 0.3 M sucrose than LETO controls, which was attenuated during infusion of 0.3 M sucrose (Fig. 3A). Cumulative sham intake was significantly enhanced \((P < 0.05)\) until 50 min postinfusion (Fig. 3B).

Infusion of 0.6 M sucrose resulted in significantly higher sham intakes in OLETF vs. LETO rats \((P < 0.05)\) (Fig. 4B). Intake volumes before infusion of 0.6 and 1.0 M sucrose were again significantly higher \((P < 0.05 \text{ and } P < 0.01 \text{ for } 0.6 \text{ and } 1.0 \text{ M sucrose infusion, respectively})\) for OLETF rats. In contrast to results from saline and 0.3 M sucrose infusions, however, intraduodenal infusion of 0.6 and 1.0 M sucrose in OLETF rats showed marked differences in sham intake compared with LETO controls after infusion termination. Figure 4A shows that, although both strains decreased their sham intake in response to infusion of 0.6 M sucrose, only OLETF rats showed an immediate increase in 5-min sucrose intake after the end of 0.6 M sucrose infusion, in contrast to LETO controls, which maintain their lower level of sham intake. This increased intake remained elevated relative to LETO controls for 30 min postinfusion.

**Fig. 3.** Ninety-minute sham intake of 0.3 M sucrose in response to 20-min duodenal infusion of 0.3 M sucrose. A: OLETF rats had an initially higher sham intake of 0.3 M sucrose than LETO controls before the start of the infusion. B: cumulative sham intake data show that OLETF rats had significantly higher sham intakes compared with LETO throughout the 70-min sham feeding. However, no overall strain difference in sham intake was noted at 90 min. *\(P < 0.05\) and **\(P < 0.01\) and ***\(P < 0.001\) between strains.

**Fig. 4.** Ninety-minute sham intake of 0.3 M sucrose in response to 20-min duodenal infusion of 0.6 M sucrose. A: OLETF rats show an immediate increase in 5-min sucrose intake after the end of 0.6 M sucrose infusion, in contrast to LETO controls, which maintain their lower level of sham intake. This increased intake remained elevated relative to LETO controls for 30 min postinfusion. B: cumulative sham intakes postinfusion of 0.6 M sucrose were significantly higher in OLETF compared with LETO rats. *\(P < 0.05\) and **\(P < 0.01\) between strains.

Infusion of 0.6 M sucrose increased 5-min intakes in OLETF vs. LETO rats \((P < 0.05)\) (Fig. 4B). Intake volumes before infusion of 0.6 and 1.0 M sucrose were again significantly higher \((P < 0.05 \text{ and } P < 0.01 \text{ for } 0.6 \text{ and } 1.0 \text{ M sucrose infusion, respectively})\) for OLETF rats. In contrast to results from saline and 0.3 M sucrose infusions, however, intraduodenal infusion of 0.6 and 1.0 M sucrose in OLETF rats showed marked differences in sham intake compared with LETO controls after infusion termination. Figure 4A shows that, although both strains decreased their sham intake in response to infusion of 0.6 M sucrose, only OLETF rats showed an immediate increase in 5-min sucrose intake directly after infusion end. This increased intake remained significantly elevated \((P < 0.05)\) relative to LETO controls for each 5-min recording until 30 min postinfusion. Sham intake at each 5-min time point recorded after infusion onset was significantly higher \((P < 0.05)\) in OLETF rats compared with LETO rats (Fig. 4B).

Figure 5B illustrates that OLETF rats had significantly higher \((P < 0.05)\) overall intakes of sucrose compared with LETO rats when intraduodenally infused with 1.0 M sucrose. Infusion of 1.0 M sucrose again decreased 5-min intakes in both strains; however, LETO rats consumed
almost no sucrose after infusion ceased (Fig. 5A). Conversely, OLETF rats increased their sham intake after infusion ended compared with LETO controls. B: cumulative sham intake at all time points in the 90-min sham session were significantly higher in OLETF rats compared with LETO animals. *P < 0.05 and **P < 0.01 between strains.

For both strains, suppression of the 0.3 M sucrose sham intake increased as a function of infusate concentration [F(2,10) = 55.6; P < 0.0001 and F(2,11) = 6.33; P < 0.05 for OLETF and LETO rats, respectively]. Figure 6 shows that infusions of 0.6 and 1.0 M sucrose produced significantly enhanced suppression of the 0.3 M sucrose sham intake in LETO rats relative to OLETF rats [F(1,8) = 18.10; P < 0.01 and F(1,8) = 11.04; P < 0.05 for 0.6 and 1.0 M infusions, respectively]. OLETF and LETO rats showed no differential responsiveness to infusion of 0.3 M sucrose (P = 0.394). Post hoc tests revealed that LETO rats suppressed intake significantly across all three concentrations of infusate (P < 0.001 for both 0.3 to 0.6 M and 0.6 to 1.0 M sucrose). In contrast, suppression of sham intake in OLETF rats was enhanced only between 0.6 and 1.0 M sucrose infusion (P < 0.05), whereas no difference in suppression was noted between 0.3 and 0.6 M sucrose infusion (P = 0.873).

Oral Glucose Tolerance Test

Results of the oral glucose tolerance test performed at the end of the study on samples of rats from each experimental group showed that 12-wk-old OLETF rats were prediabetic because they did not show elevated blood glucose levels indicative of NIDDM (Fig. 7). However, OLETF rats showed significantly higher [F(1,6) = 42.2; P < 0.001] blood glucose peaks at 30 min relative to LETO rats. At all other time points, blood glucose in OLETF and LETO rats did not differ significantly (P > 0.05).

DISCUSSION

These results show that in short-access, two-bottle preference tests prediabetic OLETF rats exhibit a markedly enhanced preference for sucrose compared with nonmutant LETO rats. We also show that OLETF rats consumed more sucrose than LETO rats in the absence of gastric or intestinal feedback stimulation. Finally, OLETF rats were less responsive to the inhibitory effects of intraduodenal infusion of sucrose compared with LETO rats. To our knowledge, these are the first studies to demonstrate an altered preference for sucrose in the...
CCK-A receptor-deficient rat using two different paradigms: the short-access, two bottle test and sham feeding preparation.

Intake and preference for sucrose in rats have been shown to depend on solution concentration (17). In our short-access tests, OLETF as well as LETO rats displayed an equal preference for sucrose solutions at the lower concentration range (0.03 and 0.1 M). As sucrose concentration increased beyond 0.1 M, the overall preference decreased. However, OLETF rats had an increased preference for sucrose solutions of the two highest concentrations (0.03 and 1.0 M). In other words, OLETF rats display a right-shifted sucrose preference curve relative to LETO rats.

It is well established that both orosensory and postgingestive components of a food can modulate intake (18, 27, 36, 63). Given that sucrose is both a toothsome stimulus and also contains calories, data from the first experiment did not allow us to interpret the relative contribution of orosensory and postgastric effects of sucrose on preference. Prior work has focused primarily on gastric and postgastric mechanisms mediating regulatory deficits in CCK-A receptor rats (13, 48, 59). The sham feeding preparation allowed us to test participation of orosensory components of sucrose in the absence of any appreciable gastric and postgastric stimulation (71). In this paradigm, ingested sucrose empties from the stomach via a drainage tube, providing minimal contact of the solution with the gastric mucosa (65).

We observed that OLETF rats naive to the postgastric effects of sucrose showed an increased sham intake of the highest sucrose concentration presented (1.0 M) and a decreased intake of the lowest concentration (0.03 M) relative to LETO control animals. These data are in concert with results from the two-bottle preference testing in that strain differences occurred primarily at relatively higher concentrations of sucrose; that is, OLETF rats exhibit an unaltered concentration discrimination function with a right-shifted preference function. These observed changes in sucrose intake are suggestive of alterations in the motivational modulation of taste functioning in OLETF rats, rather than diminished responsiveness to the primary gustatory signal.

The mechanisms by which OLETF rats increase their intake and preference for sucrose are not known; however, the absence of CCK-A receptors is one likely candidate. Numerous studies have shown that exogenous CCK administration decreases sham intake (20, 22, 24, 43), whereas blockade of the CCK-A receptor with a specific antagonist potently inhibits or abolishes this diminution (8, 9). Administration of CCK-A receptor antagonists alone, however, has not been shown to alter sham intake, suggesting that activation of the CCK-A receptor by endogenous levels of CCK does not play a regulatory role in sham feeding (55). Furthermore, data examining the effects of CCK on the orosensory components of palatable foods have not provided conclusive evidence supporting a role for CCK-A receptors in the orosensory control of food intake (19, 26, 70). Lastly, administration of a CCK-A receptor antagonist does not alter conditioned flavor preferences in intact rats (51). Together, it appears that increased sham intake in the OLETF rat is not due to a primary deficit in CCK-A receptor activation.

Our data also show that sham-fed OLETF rats are less responsive to intestinal infusion of sucrose than LETO controls. In addition, the kinetics of sham intake in OLETF rats revealed an initially increased rate of sucrose consumption when postgastric effects of sucrose were not yet present, as well as increased consumption after infusion of the two highest concentrations of sucrose infusate. Such discrepancies in intake between strains both pre- and postinfusion appear to additively magnify increased sham intake in OLETF rats. These latter findings are in agreement with prior demonstrations in OLETF rats that illustrate reduced sensitivity to gastric and intestinal preload nutrient infusions in real feeding tests (13, 59).

There is ample evidence attesting to the role of CCK-A receptors in nutrient-induced suppression of food intake (56). For example, CCK-A receptor antagonists have been shown to attenuate suppression of both real and sham feeding after intraduodenal nutrient infusion (55, 74). Therefore, one mechanism by which OLETF rats are less responsive to the suppression of intake by sucrose infusion might be due to their lack of CCK-A receptors. However, in addition to the well-characterized CCK-A receptor deficits, OLETF rats may have alterations in other feeding-related signaling pathways, such as those involved in food reward processes.

The intake of preferred foods has been shown to affect opioid, serotonergic, and dopaminergic systems (35, 40, 67). When given intermittent access to sugar solutions, rats exhibit increased binding of D1 dopamine (DA) receptors and μ-1 opioid receptors in the nucleus accumbens (10). Conversely, opioid antagonists reduce intake of palatable foods in real (11, 12, 45) and sham (41, 42) feeding and decrease sucrose palatability in taste reactivity tests (30). Although it seems that CCK-A and opioid receptors do not interact to alter food intake (54), an enhanced sucrose preference in the OLETF rats may also be due to deficits in opioid signaling in addition to those attributed to lack of CCK-A receptor. However, this possibility has yet to be tested.

Serotonergic signaling may also play a role in sweet preference modulation in OLETF rats. Exogenous 5-HT administration has been shown to not only reduce intake but also diminish preference of preferred saccharin and sucrose solutions in intact animals (47). Furthermore, evidence for an interaction between the cholecystokininergic system on both central (33, 53) and peripheral (31, 32) serotonergic receptors in the control of food intake has been reported. The effects of serotonergic manipulations in the OLETF rat on food intake and preference, however, have not been addressed.

Finally, recent studies assessing DA functions in the OLETF rats provide indirect evidence that may shed some light on possible mechanisms of enhanced sucrose preference in the OLETF rats (21, 28, 66). Indeed, evidence supporting the role of central DA as a mediator of food reward in nonmutant animals has been postulated for some time (34, 52). Sham feeding sucrose in nonmutant (Sprague-Dawley) male rats has been shown to trigger DA release as a function of sucrose concentration (30). Furthermore, intraperitoneal administration of the DA D2 receptor antagonist raclopride reduced sham feeding of sucrose in a dose-dependent manner (37). Administration of D1 receptor blockers directly in the nucleus accumbens suppresses intake. Conversely, increasing DA levels provoke greater intakes and preference for sucrose solutions (29).

Learned preference for sucrose has also recently been shown to be decreased with administration of DA receptor blockers (76, 77). Furthermore, CCK has been shown to stimulate central

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DA release via activation of CCK-A receptors (14). Although there are only sporadic data to support the theory of altered DA signaling additional to CCK signaling abnormalities in OLETF rats (28), it is plausible that such deficits could interfere with reward mechanisms and, in turn, contribute to the manifestation of altered preferences for palatable food stimuli.

In summary, OLETF rats prefer sucrose to a greater extent than nonmutant LETO control rats in a short-access, two-bottle choice test. When postigestional caloric and chemosensory properties of sucrose are removed via gastric fistula preparation, OLETF rats sham feed more of a relatively high concentration of sucrose than LETO rats. In the presence of intestinal feedback stimulation, OLETF rats display diminished sensitivity to sucrose infusion, as evidenced by greater sham intakes of sucrose compared with nonmutant LETO animals. The specific mechanisms behind these phenomena are not well defined; however, it is likely that secondary anomalies beyond the lack of CCK-A receptors, such as those involving the dopaminergic system, may mediate the altered ingestion of sucrose solutions in OLETF rats.

Perspectives

Knockout and spontaneous mutant rodent models have been used in investigations of regulation of feeding in general and neural mechanisms underlying the development of dietary-induced obesity in particular. Among these is the CCK-A-deficient OLETF rat, a spontaneous CCK-A receptor knockout strain. This rat provides a unique model for the study of signals mediated by CCK-A receptors in control of food intake. The roles of CCK-A receptors, which are located predominantly in the periphery, in the control of food intake and gastrointestinal functions are well established (57). Therefore, until recently, this model has been used almost exclusively for studying a potential peripheral domain of food intake regulation. With the use of OLETF rats, several studies, including our own, have provided compelling evidence of CCK-A receptor participation in the control of food intake (3).

Previous work in intact animals has shown that CCK-A receptors do not participate in long-term control of food intake because overall food intake remains unchanged after exogenous administration of CCK (15, 72). However, CCK-A receptor-deficient rats are hyperphagic and become obese, suggesting two possibilities: 1) the absence of the CCK-A receptor leads to disordered energy balance or 2) other non-CCK-related defects may exist in the OLETF rat that regulate feeding and body weight. There is emerging support for both hypotheses. Recent work from Bi et al. (3, 6) showed that CCK-A receptors located in the dorsomedial hypothalamus and acting on NPY neurons may be responsible for the obese phenotype in the OLETF rat. In addition, dorsomedial hypothalamus NPY expression is elevated in the absence of CCK-A receptors, suggesting a central mechanism for CCK in control of food intake.

In the studies presented here, we have explored potential central mechanisms that may interfere with processing of critical information in the control of food intake. These results are the first to assess motivational and sensory processing of orosensory stimuli of palatable foods that may contribute to hyperphagia in the OLETF rat. The possible existence of food reward deficits proposed here, coupled with emerging data showing altered dopaminergic functioning, suggests that OLETF rats provide a largely untapped in vivo model to test the role of the CCK-A receptor in alterations of food reward mechanisms leading to disordered eating.

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GRANTS

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