Obese OLETF rats exhibit increased operant performance for palatable sucrose solutions and differential sensitivity to D2 receptor antagonism

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Hajnal A, Acharya NK, Grigson PS, Covasa M, Twining RC. Obese OLETF rats exhibit increased operant performance for palatable sucrose solutions and differential sensitivity to D2 receptor antagonism. Am J Physiol Regul Integr Comp Physiol 293: R1846–R1854, 2007. First published September 5, 2007; doi:10.1152/ajpregu.00461.2007.—CCK-1-receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rats are hyperphagic and exhibit a greater preference for sucrose compared with lean controls [Long-Evans Tokushima Otsuka (LETO)]. To directly assess motivation to work for sucrose reward in this model of obesity and type 2 diabetes, we examined the operant performance of OLETF rats at nondiabetic and prediabetic stages (14 and 24 wk of age, respectively) on fixed-ratio (FR) and progressive-ratio (PR) schedules of reinforcement. To evaluate the involvement of dopamine systems, the effects of the D1 receptor antagonist SCH23390 (100 and 200 nmol/kg ip) and the D2 receptor antagonist raclopride (200 and 400 nmol/kg ip), were also tested on PR responding for sucrose. Compared with age-matched LETO rats, 14-wk-old OLETF rats emitted more licks on the “active” empty spout operant on the FR-10 schedule of reinforcement to obtain 0.01 M and 0.3 M sucrose and completed higher ratio requirements on the PR schedule to gain access to 0.3 M and 1.0 M sucrose. At 24 wk, this effect was limited to 1.0 M sucrose. Both antagonists were potent in reducing operant responding to 0.3 M sucrose in both strains at both ages, and there was no strain effect to SCH23390 at either age. OLETF rats, on the other hand, showed an increased sensitivity to the higher dose of raclopride, resulting in reduced responding to sucrose reinforcement at 24 wk. Taken together, these findings provide the first direct evidence for an increased motivation for sucrose reward in the OLETF rats and suggest altered D2 receptor regulation with the progression of obesity and prediabetes.

THE EPIDEMIC OF OBESITY AND its associated health consequences represent a major cause of preventable morbidity and mortality in the United States and worldwide (31). Although the etiology of obesity is complex, the motivation to respond to palatable food correlates with obesity (3, 52, 60). Despite this relationship, little is known about food reward functions in the obese. The common view is that obese individuals are presumed to show enhanced liking for palatable foods. Several human studies investigating the relationship between preference and obesity support this notion, whereas others debate it (3, 19, 20, 38).

Due to the complexity of human eating behavior there has been growing interest in using animal models to decipher basic neural processes underlying the development of obesity. Our laboratory has been investigating the Otsuka Long-Evans Tokushima fatty (OLETF) rats that lack the CCK-1 receptor, are hyperphagic, obese, and gradually develop non-insulin-dependent diabetes mellitus (36). Unlike in other genetically obese rodent models, in OLETF rats an increased food intake is necessary for the development of obesity. The underlying cause of chronic hyperphagia in this strain is unknown and is not explained entirely by their peripheral satiety deficits resulting from the missing CCK-1 receptors (39). Rather, a dysfunction in central pathways critical to the control of meal size is the most likely contributor. Indeed, accumulating evidence suggests that this strain suffers from both altered hypothalamic control of satiety (9, 40), as well as impairments in dopamine regulation (1, 15, 18, 21).

Although its exact role in reward has been debated (8, 12), it is generally accepted that dopamine is critical for natural and drug reinforcement and for operant behaviors (59). Furthermore, anatomical and functional evidence demonstrates interactions at multiple levels between dopamine and CCK systems (30, 32, 33, 46), and dopamine interacts with CCK to control food intake. For instance, treatment with a D1 or D2 antagonist augments the inhibitory effect of CCK on intrarostral infused sucrose (4). Thus, it is feasible that in OLETF rats the absence of CCK-1 receptors may result in altered dopamine receptor regulation of sucrose intake. In support of this notion, we have recently shown that compared with age-matched lean control Long-Evans Tokushima Otsuka (LETO) rats, OLETF rats have an increased dopamine release in the nucleus accumbens (1) and an accentuated preference for higher concentrations of sucrose (26). More direct evidence for a role of dopamine in the increased avidity for sucrose in this strain comes from findings demonstrating an increased sensitivity to dopamine antagonism in reducing two-bottle sucrose preference in both real and sham feeding conditions in prediabetic OLETF rats. These observations together with the proposed role of insulin in regulating dopamine and reward functions in general and sucrose reward in particular (22, 23) provide the rationale for our hypothesis that altered dopamine regulation affects the OLETF rats’ motivation to work for sucrose reward and that this effect will differ in nondiabetic and prediabetic stages.

Finally, despite recent indirect evidence gleaned from stronger taste preference conditioning in OLETF rats (16), the rewarding value of sapid sucrose has not been directly assessed in this strain. Various operant procedures are used in animals to measure the reinforcement value of foods, fluids, and drugs, with the most common being lever pressing on progressive ratio (PR) reinforcement schedules. With a PR schedule, the...
response requirement to obtain successive reinforcement increases according to some predetermined rule throughout the test session until the subject stops responding. The highest ratio completed in the session is referred to as the “break point” and is interpreted as the point at which the effort is no longer worth the reward. It has been shown that PR performance provides a measure of the reward value of taste stimuli (43). More recently, Sclafani and Ackroff (45) demonstrated that a modification of this method in which the rats engage directly in foraging by licking on spouts is as effective as the operant lever-pressing procedure in measuring the reward value of sucrose solutions in rats. Postigestation satiation is also minimized with the PR-operant licking schedule because it greatly limits sucrose consumption during the test sessions. Consequently, PR licking, like sham drinking and brief access licking, reflects the reward value of sweet taste as perceived by the rat. An additional advantage of the operant lick task is that it does not require extensive operant pretraining because licking is a natural response of rodents. Performance on a PR reinforcement schedule is also particularly sensitive to changes in dopamine system (for a review, see Ref. 44).

Based on the above rationale, the present study used a PR-operant licking task as a measure of motivation for sweet reward in the OLETF rat. In addition, to evaluate the involvement of different classes of dopamine receptors with respect to progression of obesity and type 2 diabetes, we repeated the PR tests following peripheral administration of the D1 receptor antagonist SCH23390 and the D2 receptor antagonist raclopride at two ages representing nondiabetic and prediabetic conditions.

METHODS

Subjects. Six OLETF and six LETO male rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. All rats were individually housed in mesh-floored, stainless-steel, hanging cages in a temperature-controlled vivarium while being constantly maintained on a 12:12-h light-dark cycle (lights on at 0700 h). At the beginning of the experiments, rats were 10 wk old with an initial body weight (mean ± SE) of 350 ± 7.0 g vs. 314.5 ± 7.7 g for OLETF and LETO rats, respectively. Rats were handled daily for a minimum of 1 wk prior to the onset of experimental procedures. Tap water and pelleted rat chow (Harlan Teklad 2018 rodent diet) were available ad libitum throughout the onset of experimental procedures. Tap water and pelleted rat chow (Harlan Teklad 2018 rodent diet) were available ad libitum throughout the onset of experimental procedures. The procedure for the PR schedule was identical to that described for the FR except that the requirement for spout 1 sucrose access increased by 10 licks with every reinforcement (PR-10, i.e., -10, -20, -30, -40, etc.) instead of remaining constant (FR-10, i.e., -10, -10, -10, etc.). In addition, the session was terminated when 10 min elapsed without having earned a reinforcement. PR responding was evaluated for each of four concentrations of sucrose (0.03 M, 0.1 M, 0.3 M, 1.0 M) and spouts 2 (middle) and 3 (right) were empty. Upon initiation of the program, the rat was given access to spouts 2 and 3. Empty spout 3 served as an inactive spout with no programmed consequences. Empty spout 3 served as the active spout and completion of the FR-10 lick contingency on this spout resulted in retraction of spouts 2 and 3 and the presentation of spout 1 (the sucrose spout). Sucrose was available for 10 s, beginning with the first recorded lick on that spout. At the termination of the 10-s interval, the sucrose spout retracted and the procedure was repeated. Each session was limited to 30 min. Again, the stimuli were presented in ascending order with each concentration being presented for a minimum of two consecutive days.

Fixed ratio schedule. Once lick responses for continuous sucrose reinforcement stabilized for an individual rat across concentrations (2–3 days), training for operant sucrose procurement began using a fixed ratio (FR) schedule. In the morning (0800 h), rats were weighed and then placed into the operant chambers. Spout 1 (left) contained one of four concentrations of sucrose (0.03 M, 0.1 M, 0.3 M, 1.0 M) and spouts 2 (middle) and 3 (right) were empty. Upon initiation of the program, the rat was given access to spouts 2 and 3. Empty spout 3 served as an inactive spout with no programmed consequences. Empty spout 3 served as the active spout and completion of the FR-10 lick contingency on this spout resulted in retraction of spouts 2 and 3 and the presentation of spout 1 (the sucrose spout). Sucrose was available for 10 s, beginning with the first recorded lick on that spout. At the termination of the 10-s interval, the sucrose spout retracted and the procedure was repeated. Each session was limited to 30 min. Again, the stimuli were presented in ascending order with each concentration being presented for a minimum of two consecutive days.

Drugs and treatment schedule. Intraperitoneal injection of either SCH23390 (SCH23390 hydrochloride; Sigma-Aldrich, St. Louis, MO) or raclopride [(--)-raclopride (+)-tartarate; Sigma-Aldrich] was employed to selectively antagonize the D1 or D2 family of dopamine receptors, respectively. Doses administered were 100 and 200 nmol/kg for SCH23390 and 200 and 400 nmol/kg for raclopride. We selected these doses based on our previous studies investigating the effects of dopamine antagonists on two-bottle preference in OLETF rats (18). Briefly, rats were injected with either 0.9% saline or their respective dopamine antagonist doses 20 min prior to the PR sessions. In all tests, 0.3 M sucrose was used as the rewarding stimulus. This concentration was chosen based on prior work in this strain (15–17, 27) and results of the initial FR and PR experiments. Different doses of the drugs were tested randomly with only one dose tested each day.
Half of the rats were given the D1 antagonist first, followed by the D2 antagonist, whereas the other half received the drugs in the opposite order. Each drug day was bracketed with saline days to control for residual drug effects or altered sensitivity.

**Oral glucose tolerance test.** OGTTs were performed once before each series of tests, at 14 and 24 wk of age and at least 2 days before the operant conditioning sessions. After an overnight fast (minimum 16 h), glucose (2 g/kg, 500 g/l) was administered orally using latex intragastric gavage, and tail blood was taken by tail nips without anesthesia at 0, 30, 60, and 120 min. Blood glucose was determined with a glucometer (Elite Glucometer, Bayer, Elkhart, IN). Animals were classified as diabetic if the peak level of plasma glucose at any time point was 16.8 mmol/l (300 mg/dl) or glucose level at 120 min > 11.2 mmol/l (200 mg/dl) (36).

**Statistical analysis.** Body weight data were analyzed using unpaired Student’s t-tests. Data from OGTT tests were analyzed using two-way ANOVA (strain and age as main factors), and the area under the curve (AUC) was calculated for each group and compared between strains at each time point using unpaired Student’s t-tests.

The lick data during the operant tests were averaged over the last two sessions at each sucrose concentration. For the continuous reinforcement schedule, the total number of licks made on the sucrose spout served as the dependent measure, whereas for the FR-10 schedule, the number of reinforcements earned and the total number of licks made on both the active and the inactive spout served as dependent measures. These data were analyzed using mixed factorial two-way ANOVAs with strain and sucrose concentration as factors. For PR sessions, break point (last ratio completed) served as a dependent measure. A three-way mixed factorial ANOVA was used varying strain, age, and concentration to assess differences in break points. In addition, the number of licks emitted on the inactive and sucrose spouts, as well as the latency to initiate the ratio requirement for the next session, also were analyzed using mixed factorial two-way ANOVAs with strain, age, and sucrose concentration as factors. Separate two-way mixed factorial ANOVAs for each age, as well as strain, were also performed to further assess concentration response functions.

For the drug tests, three-way mixed factorial ANOVAs were conducted varying strain, age, and drug dosage to assess the impact of the dopamine antagonists on break point. Separate analyses were performed for each drug administered. For all experiments, post hoc Tukey’s (honestly significant difference) tests were conducted where appropriate. All data were expressed as means ± SE. Differences were considered statistically significant if P < 0.05. Statistical analyses were computed with Statistica software for PC (Version 6.1; StatSoft, Tulsa, OK).

**RESULTS**

**Body weight.** At the time of the first iteration of the PR tests, i.e., 14 wk of age, OLETF and LETO rats weighed 401.7 ± 12.8 g and 352.3 ± 8.5 g, respectively. By the time of the second phase, i.e., over a period of 10 additional weeks, OLETF rats gained up to 613.29 ± 34.38 g, whereas LETO rats gained up to 509.2 ± 19.91 g. The differences between strains were statistically significant at both ages (P < 0.02; P < 0.01, for 14 and 24 wk, respectively).

**OGTTs.** Fasting blood glucose levels and responses to intragastric glucose load were tested twice, once at 14 and once at 24 wk of age, at the start of each series of PR testing (Fig. 1). The AUC above fasting plasma glucose concentrations was calculated for each group and is depicted in Fig. 1B. ANOVA revealed a significant age × strain interaction for blood glucose concentration [F(1,12) = 5.96, P < 0.05]. At 14 wk despite no strain difference in fasting blood glucose levels, OLETF rats had a nonsignificant trend of reduced glucose tolerance relative to LETO rats (at 30 min: 183.67 ± 26.4 mg/dl vs. 152.0 ± 10.87 mg/dl, P = 0.12) and a 23% increase in AUC, P = 0.09]. By 24 wk, prediabetes progressed dramatically in OLETF rats as indicated by significantly elevated fasting blood glucose levels (112.75 ± 7.86 mg/dl vs. 90.25 ± 6.39 mg/dl, P < 0.05) and a twofold rise in blood glucose at 30 min in the OGTTs compared with LETO (275.25 ± 29.16 mg/dl vs. 133.4 ± 13.07 mg/dl, P < 0.01). Blood glucose levels remained significantly elevated through the 90-min sample (P < 0.01). This response profile resulted in a 216% higher AUC in the OLETF group (P < 0.002). Although one rat of the five rats tested was already diabetic at this point, the 24-wk cohort overall did not meet the criteria for overt diabetes.

**Continuous reinforcement sucrose licking.** As part of the habituation training, rats received daily 30-min continuous access to different concentration of sucrose in the operant test...
cages at the age of ~12 wk. At this point, all rats had ad libitum access to food and fluid except when in the test chambers. Sucrose intake stabilized within 2 or 3 days of access to each of the three concentrations. Both OLETF and LETO rats demonstrated an inverted U-shaped concentration response function (data not shown). Whereas responses to the weakest solution were statistically identical between strains (1156.84 ± 241.24 vs. 1302.34 ± 548.87, P = 0.81 for the LETO and OLETF, respectively), OLETF rats emitted about twice as many licks of sucrose at the 0.3 and 1.0 M concentrations compared with LETO rats (2190.5 ± 457.25 vs. 1137.84 ± 137.22, P < 0.05, and 1661.34 ± 277.71 vs. 764.67 ± 162.97, P < 0.02, respectively).

**FR-10 performance for sucrose.** Similar to the continuous reinforcement schedule, OLETF rats at 14 wk exhibited an overall increased FR-10 performance for sucrose (Fig. 2). Specifically, two-way ANOVAs revealed a significant main effect of strain [F(1,10) = 24.43, P < 0.001] and concentration [F(3,30) = 7.33, P < 0.001] on the number of reinforcements earned. Subsequent post hoc analyses with Tukey’s (honestly significant difference) tests indicated that the OLETF rats earned more access periods to 0.1 M and 0.3 M sucrose than the LETO controls (P < 0.01 for both comparisons; Fig. 2A). Furthermore, separate one-way repeated-measures ANOVAs on the concentration response function revealed that only the OLETF rats increased FR-10 responding as a function of concentration [OLETF: F(3,18) = 5.9, P < 0.01, LETO: F(3,12) = 4.45, P = 0.02] with post hoc tests showing a higher number of reinforcement gained for the 0.1 M concentration relative to the lowest 0.03 M concentration. Once having gained access to the sucrose reward, however, the OLETF rats made more licks/10-s access period than age-matched LETO controls for 0.03, 0.1, and 0.3 M sucrose (0.03 M, P < 0.05; 0.1 M, P < 0.01; 0.3 M, P < 0.01; Fig. 2B).

**PR performance for sucrose.** All rats received multiple (4–6) sessions to each sucrose concentrations in continuous blocks with the block randomized with respect to concentration. In this arrangement, all rats learned the operant task readily, and their performance stabilized by the 3rd day. Neither the main effect of day [F(1,10) = 0.73, P = 0.79] nor the day × concentration interaction [F(3,30) = 0.39, P = 0.76] was significant. Therefore, data from the last 2 days for each concentration were pooled for analysis. In addition, there was no statistical difference in the number of licks emitted on the inactive spout between strains or across the three higher concentrations (0.1 M: 33.65 ± 13.65 vs. 34.14 ± 8.11, P = 0.95; 0.3 M: 28.8 ± 4.18 vs. 39.71 ± 6.12, P = 0.21; 1.0 M: 24.80 ± 11.40 vs. 18.57 ± 7.39, P = 0.64, in LETO and OLETF, respectively). There was, however, large individual variability in inactive licks emitted during testing the 0.03 M sucrose solution with the LETO rats tending to generate more inactive licks than the OLETF rats (52.6 ± 8.96 vs. 27.6 ± 8.99, P = 0.054). Furthermore, lick rate on the sucrose spout was rather inflexible across concentrations with a nonsignificant trend for higher lick rates for the higher concentrations in both the OLETF and LETO rats (ranging from 6.98 ± 2.57 licks/s for 0.03 M to 7.23 ± 1.77 licks/s for 1.0 M in OLETF, and from 6.69 ± 8.41 licks/s for 0.03 M to 7.66 ± 1.28 licks/s for 1.0 M in LETO).

A three-way ANOVA varying strain, age, and sucrose concentration for break point revealed a significant main effect of strain [F(1,10) = 4.96, P < 0.05], age [F(1,10) = 5.21, P < 0.05], and concentration [F(3,30) = 5.89, P < 0.001]. In addition, there was a significant concentration × strain interaction [F(3,30) = 3.73, P < 0.03], but not age × strain interaction [F(1,10) = 0.82, P = 0.39] or three-way age × concentration × strain interaction [F(3,30) = 0.96, P = 0.43]. OLETF rats, overall, worked harder to gain access to 0.3 M and 1.0 M sucrose than did LETO rats (P < 0.02, for both comparisons).

To further scrutinize strain and concentration effects, we performed separate ANOVAs at 14 and 24 wk. At 14 wk, there was a significant main effect of strain [F(1,10) = 9.14, P <
The trend was not significantly significant (0.3 M: 15.8%, consumed more 0.3 M and 1.0 M sucrose at 24 wk, but this In addition, compared with the 14-wk intake, OLETF rats both 0.3 and 1.0 M sucrose than for LETO cohorts (Fig. 3A). At 24 wk, there was a significant main effect of strain [F(1,10) = 5.28, P < 0.05] but not concentration [F(3,30) = 0.93, P = 0.95] or strain × concentration interaction [F(3,30) = 1.86, P = 0.75] for break point. OLETF rats reached a higher break point at (i.e., worked harder for) 1.0 M sucrose (P < 0.05; Fig. 3B).

Once having earned access to sucrose, the consumption of sucrose mirrored the break point data (Fig. 3, C and D). However, in contrast to the effect for break point for 0.3 M sucrose at 24 wk, the actual number of licks emitted on the sucrose spout by the OLETF rats was significantly higher for both 0.3 and 1.0 M sucrose than for LETO cohorts (P < 0.05). In addition, compared with the 14-wk intake, OLETF rats consumed more 0.3 M and 1.0 M sucrose at 24 wk, but this trend was not significantly significant (0.3 M: 15.8%, P = 0.96, 1.0 M: 28.6%, P = 0.71).

Finally, once having completed what would become the final ratio (i.e., the break point), the OLETF rats were faster than lean controls to initiate completion of the next ratio requirement at both ages [main effect of strain: \(F(1,10) = 8.75, P < 0.01\)]. The latency to begin PR sessions was shorter in OLETF rats compared with LETO for the 0.1 and 1.0 M sucrose at 14 wk (17.53 ± 4.51 s vs. 27.68 ± 5.13 s, P < 0.02; 10.68 ± 2.16 vs. 28.79 ± 5.12, P < 0.01, respectively) and for 0.3 M and 1.0 M at 24 wk (13.07 ± 4.51 s vs. 35.57 ± 5.49 s, P < 0.01; 16.88 ± 4.75 vs. 35.59 ± 6.99, P < 0.01, respectively).

**Effect of D1 receptor antagonists on PR-10 sucrose licking.** Since the behavioral tests showed a significant strain and age effect on PR-10 performance for 0.3 M sucrose and a similar strain effect was also apparent following saline injection in the pharmacological tests (break points, OLETF: 15.43 ± 0.81, LETO: 11.0 ± 0.77, P < 0.01), statistical analysis was conducted on normalized data calculating percent suppression by each dose of either drug relative to predrug saline baseline. Results from this comparison are depicted in Fig. 4.

The D1R antagonist SCH23390 reduced break points for 0.3 M sucrose uniformly in OLETF and LETO rats (Fig. 4A). A three-way ANOVA on the dose effects revealed no significant interaction between strain and age [F(3,30) = 1.73, P = 0.18]. However, post hoc tests of the significant main effect of drug [F(3,30) = 15.63, P < 0.00001] revealed that whereas both the 100 and 200 nmol/kg doses potently reduced break points on the PR-10 schedule in both strains at 14 wk (OLETF: from 15.43 ± 0.81 to 7.14 ± 1.61, P < 0.001, and to 5.14 ± 1.83, P < 0.001; LETO: from 11.0 ± 0.77, to 7.40 ± 1.75, P < 0.01).

**Fig. 3.** Break points (the highest ratio completed) on PR-10 schedule by OLETF (n = 6) and LETO (n = 6) rats at 14 wk of age (A) and 24 wk (B) representing nondiabetic and prediabetic stages, respectively. Sucrose licks on PR-10 by OLETF and LETO rats at 14 wk (C), and 24 wk (D). Values are expressed as means ± SE. Statistical symbols represent post hoc comparisons between strains (#P < 0.05, *P < 0.01).
0.05, and to 5.44 ± 1.60, \( P < 0.02 \), at 100 and 200 nmol/kg, respectively) at the older age, only the higher dose had a significant effect in lean LETO rats (reducing break point from 11.2 ± 1.28 to 6.20 ± 1.69, \( P < 0.02 \)). OLETF rats exhibited statistically significant suppression of break points to both doses (from 13.43 ± 0.81 to 8.14 ± 1.06, \( P < 0.01 \), and to 7.29 ± 1.54, \( P < 0.01 \), at 100 and 200 nmol/kg respectively). Analysis of the raw data, however, revealed that this abolished sensitivity of LETO to 100 nmol/kg SCH23390 (\( P = 0.6 \)) was actually due to a single rat that increased rather than suppressed its performance.

**Effect of D2 receptor antagonists on PR-10 sucrose licking.** As with the D1 antagonist, the D2 antagonist raclopride, also reduced PR performance for 0.3 M sucrose (Fig. 4B). Post hoc tests of the highly significant separate repeated-measures ANOVAs [OLETF: \( F(8,48) = 8.05, P < 0.00001 \); LETO: \( F(8,32) = 6.00, P < 0.0001 \)] indicated that both strains at either age responded less for sucrose on the PR-10 schedule following pretreatment with either the 200 and 400 nmol/kg dose of raclopride. However, post hoc tests of a significant two-way strain \( \times \) age interaction [\( F(1,10) = 5.6317, P < 0.0391 \)] revealed that 400 nmol/kg D2R antagonist dose had a greater effect on break points in the OLETF rats when they were prediabetic (at 24 wk) relative to when they were nondiabetic or to the age-matched lean controls (\( P < 0.05 \) for both comparisons).

**DISCUSSION**

In the present study, we sought to investigate whether OLETF rats, an increasingly popular model of dietary obesity and type 2 diabetes, were willing to expend more effort on operant tasks to obtain palatable sucrose solutions compared with their lean controls. The results showed that obese rats displayed increased operant responding for sucrose on an FR-10 or a PR-10 schedule by emitting more licks and completing higher ratio requirements than age-matched LETO controls. Specifically, compared with LETO, 14-wk-old OLETF rats expended more effort (i.e., more licks on the empty spout) on an FR-10 schedule and reached higher break points on the PR-10 schedule to gain access to 0.3 M and 1.0 M sucrose. Despite the fact that rats drank less sucrose during the PR sessions than the FR sessions overall, compared with lean rats, OLETF rats always consumed more sucrose. The most preferred concentrations by the OLETF rats, however, varied with respect to whether FR or PR regimen was used. When lighter effort was required to obtain the reward, i.e., on the FR-10 schedule, OLETF rats worked the hardest to gain access to 0.1 M sucrose. In contrast, on the PR schedule, OLETF rats expended significantly more effort to obtain both the 0.3 M and 1.0 M concentrations than they did for the weaker solutions including 0.1 M sucrose. This difference in the shape of the concentration-response function between the operant schedules is similar to the findings of Sclafani and Ackroff (45) and it is in concert with PR lever-pressing tasks for sucrose (43).

When we compared the effect of age, associated with increasing obesity and insulin resistance in the OLETF rats (as inferred from reduced tolerance to oral glucose loads in the 24-wk cohorts) on operant performance for sucrose, we found only minor differences. Although there was only a nonsignificant trend (\( P = 0.062 \)) for an increased operant responsiveness to 1.0 M sucrose in the OLETF rats at 24 wk compared with 14 wk, it resulted in a higher number of actual sucrose licks (~20%). This finding is in agreement with our previous observation revealing an accentuated lick response to higher concentrations of sweet tastants with the progression of prediabetes in OLETF rats. Further analysis of the present data, however, showed that the observed age by strain interaction was actually due to an overall increased performance by the LETO rats at the older age rather than to changes in the behavior of the OLETF rats. Although the exact reason for this effect remains unknown, it is possible that experience with the PR regimen at the younger age played a role in improved performance of the LETO rats in the second phase of testing at
the older age more so than in OLETF rats. An alternative explanation is that body size may influence operant performance. Although there has been no study directly investigating this relationship, a recent report by la Fleur and colleagues (37) showed that, while fat mass correlated positively with PR performance for sucrose consumption, this was only the case in high-fat, high-sugar, diet-induced obese rats and not in lean rats fed chow. Furthermore, the present study used a more natural operant licking task which requires little effort compared with lever pressing. Thus, taken together, it is unlikely that body size, strength or adiposity directly influenced operant responsiveness in our study.

An additional observation was that, although the LETO rats readily learned the operant tasks and performed reliably on the PR sessions, they produced a flat concentration-response function with a tendency for reduced responsiveness for the higher concentrations. One interpretation of these data is that LETO rats are more sensitive than are OLETF rats to the development of conditioned satiety as a function of experience with postabsorptive consequences (11). In support, we recently reported that OLETF rats are less sensitive than LETO rats in forming a conditioned preference for a neutral stimulus paired with sucrose based on postigestive consequences in favor of orosensory effects (16). Thus, such an effect might contribute to strain differences seen in operant responsiveness tested here and cannot be excluded entirely. Nevertheless, to limit the possible confounding effects of such a scenario, the FR tests were performed only at 14 wk as part of the training preceding the PR tests and were omitted from the 24-wk tests.

Altered motivation to consume sugars may be presumed to result from altered glucose control and cellular metabolism associated with type 2 diabetes. However, there has been only sporadic and contradictory evidence suggesting altered preference for sugars in diabetes (e.g., increased, see Refs. 51, 55; and reduced, see Refs. 6, 35, 54). Plausible systems that may mediate diabetes’s effect (e.g., elevated blood glucose, high circulating insulin levels, or insufficient insulin effect) on sweet preference include the taste receptors and central relays (25, 41, 47), brain areas that are involved in the regulation of satiety with respect to metabolic states (48), or directly through the reward system (23). Of particular interest is the accumulating evidence showing that insulin can regulate dopamine function by increasing dopamine uptake (24). Thus, with progression of insulin resistance, the accompanying increase in circulating insulin levels may alter dopamine functions in the OLETF rats. Our observations that OLETF rats expended more effort for 1.0 M sucrose in their prediabetic stage may be related to increased circulating insulin levels and can be explained by any of the above discussed mechanisms. On the other hand, the overall lack of major changes in operant behavior for sucrose, despite the dramatic change of glucose control in the OLETF rats, however, mitigates the possibility that the initial preference for sweet stimuli with bias for higher concentrations were critically dependent on insulin or other metabolic factors. Indeed, we observed increased sucrose preference in the OLETF rats as early as 8 wk of age, which is enhanced for higher concentrations, well before signs of impairments in glucose control can be detected (26). In this context, it is plausible that an increased motivation for palatable meals may, in part, contribute to overconsumption, and this increase in reward sensitivity may further progress with increasing metabolic derangement.

Regarding the potential underlying mechanisms of increased appetite for, and intake of, sucrose in this strain, we have previously demonstrated an increased preference as well as an increased avidity for the orosensory stimulatory effects of palatable taste solutions in the OLETF rat (17). Moreover, the present study revealed an increased motivation driven by the reinforcing properties of sucrose. According to the proposed division of reward (7) to differentiate functions that facilitate instrumental behaviors and actions to achieve specific goals (termed “wanting”) from the assigned evaluative and affective values to the goal object (termed “liking”), it appears that both the liking and wanting aspects of reward are compromised in the OLETF rats. Our present findings that both D1 and D2 antagonists reduced operant performance on PR schedule further supports a proposed role for dopamine in reward in general and in sucrose reward in particular (28, 50, 59), and extends it to obesity. Specifically, systemic administration of dopamine antagonists suppressed both real and sham feeding of sucrose (50) as well as oils (58). Conversely, D1 receptor agonist SKF38393 enhances preference for high-palatability, energy-dense foods (14). Much less is known, however, about dopamine receptor functions in obese subjects. Thus, the novel finding of our study was that, whereas the D1 receptor antagonist had a uniform effect on PR performance in obese and lean rats, the obse OLETF rats were more sensitive to the inhibitory effect of the D2 receptor antagonist. It must be noted, however, that the use of two doses of antagonists in our experiment was neither intended, nor was it sufficient, to quantitate a sensitivity shift in this strain. Rather, our aim was to reveal a potential differential contribution from dopamine receptors to operant sucrose licking in this obese strain at nondiabetic and/or prediabetic stages.

The observation that a differential effect occurred only at 24 wk when OLETF rats exhibited increased obesity and impaired blood glucose control (discussed above) suggests that the differential D2 regulation of sucrose procurement may be secondary to the development of obesity and/or its correlates rather than being an antecedent of the hyperphagic behavior. In this respect, there is evidence in both humans and animal models of obesity for reduced D2 receptor binding (13, 42, 53, 57). Recently, we have found significantly lower [125I]iodosulpride binding in the nucleus accumbens of OLETF at 24 wk of age compared with age-matched LETO rats (29). Thus, in addition to, or as part of, the obesity-related D2 receptor alterations, elevated insulin levels also may further augment behavioral consequences of dopamine regulatory deficits. In support of this notion is the finding by Sipols et al. (49) showing that intracerebroventricular administration of insulin in a dose that is ineffective when administered alone, potentiated the suppressive effect of a subthreshold dose of raclopride on sucrose lick rate in brief access tests for concentrations higher than 0.2 M. Such an effect by insulin, coupled with reduced availability of D2 receptors and an altered regulation of basal and stimulated dopamine release, may explain why prediabetic OLETF rats were more sensitive to a higher dose of raclopride.

Finally, it must be noted that an altered food reward function in the OLETF rats may result from nondopaminergic mechanisms. Independent of obesity, CCK has been proposed to play a role in drug reward by fostering psychostimulant sensitization (5).
Whether this effect is direct or indirect, i.e., whether it is exclusively dependent on a CCK-dopamine interaction or whether it recruits alternative mechanisms is unknown. Nevertheless, extracellular CCK increases in the nucleus accumbens in response to psychostimulants (30, 33). Relevant to our model, systemic or intra-accumbens administration of the CCK-1 antagonist devazepide, but not the CCK-2 antagonist L-365,260, was shown to block acquisition of cocaine-conditioned activity on a subsequent drug-free test in normal rat (34). Based on this and other findings demonstrating cross-sensitization between sucrose and psychostimulants (2), consideration must be given to the hypothesis that the lack of CCK-1 receptors also may contribute to the increase in reward sensitivity and instrumental responsiveness to sucrose in the OLETF rats. A second candidate for a nondopaminergic mechanism is neuropeptide Y (NPY). In support, there is strong evidence demonstrating increased hypothalamic NPY expression in the OLETF rats (40), data link NPY expression with the hyperphagic trait (10), and evidence suggests that NPY is a potential contributor to increased motivation for sucrose intake (56).

In summary, the present results demonstrate increased reinforcing potency of palatable sucrose in prediabetic, obese OLETF rats that have been previously shown to be susceptible to palatability-driven overeating. This information supports the notion that a disregulation in the reward system, in addition to peripheral deficits controlling meal size, may contribute to overconsumption in this model of obesity. In addition, the present data, together with previous findings, suggest that altered D2 receptor function is involved in the heightened sensitivity to sweet reward in obese OLETF rats, and that this disregulation may progress with metabolic derangements associated with type 2 diabetes.

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