Decreased responsiveness to dietary fat in Otsuka Long-Evans Tokushima fatty rats lacking CCK-A receptors

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Materials and Methods

The present experiments were designed to further evaluate the relationships among the OLETF rats' deficient CCK signaling pathway, OLETF hyperphagia, and obesity. Specifically, we examined the ability of dietary fat, a macronutrient CCK secretagogue, to modulate food intake and energy balance in OLETF rats in two paradigms. In the first, daily food intake and body weight were compared throughout a 3-wk period in which OLETF and Long-Evans Tokushima (LETO) rats were maintained on powdered chow or a high-fat semisolid diet. In the second, we compared the ability of gastric and duodenal preloads of equal caloric fat, carbohydrate, and protein solutions to suppress subsequent liquid glucose intake in LETO and OLETF rats.

Methods

Male OLETF and LETO rats, obtained as a generous gift from the Tokushima Research Institute, Otsuka Pharmaceutical Tokushima, Japan, served as subjects in all studies. Rats were 4–5 wk old when they arrived in our laboratory. On arrival, there were no differences in the body weight between the two groups. Rats were individually housed in hanging wire mesh cages maintained on a 12:12-h light-dark cycle (lights on at 7:00 AM), and for the initial 2 wk in the laboratory, rats were maintained with ad libitum access to pelleted Purina chow and water.

Responses to high-fat feeding. Twenty male OLETF and twenty-one male LETO rats were used in these experiments. Rats were maintained on daily ad libitum pelleted Purina chow until their body weight curves began to reach asymptotic levels (25 wk). At this time, tail vein blood (2 × 300 µl)
was drawn twice on a single day, at 0700 and 1900, and spun at 10,000 rpm × 7 min, and plasma was extracted and frozen at −20°C for subsequent determinations of plasma leptin, glucose, and insulin levels from 11 OLETF and 9 LETO rats. Plasma glucose was measured with a YSI glucose analyzer (YSI Instruments, Yellow Springs, OH), and both plasma insulin and leptin were determined with rat-specific radioimmunoassay kits (Linco). Rats were then maintained for 1 wk on powdered Purina chow to familiarize them with this form of the diet. Ten OLETF and ten LETO rats continued to be maintained ad libitum on standard powdered Purina Rat Chow diet (3.8 kcal/g), whereas 10 OLETF and 11 LETO rats were switched to a high-fat diet (powdered Purina chow mixed with Mazola corn oil, 30% by weight, 5.4 kcal/g). Daily food intake and body weight were measured for 3 wk. Rats were then euthanized, all epididymal white fat and interscapular brown fat were harvested and weighed, and tail blood was obtained for plasma glucose determinations as above. Determination of body fat in the remaining carcass was performed in accordance with the ether extraction method of Bell and Stern (1). Briefly, carcasses were freeze-dried and ground in a Wiley mill, triplicate 1-g samples were taken, and fat was extracted with ether until asymptotic weight was achieved (~4 h). The ether was evaporated, and the weight of the remaining extract was expressed as a percentage of the initial 1-g dried sample.

Data analysis. Plasma leptin, glucose, and insulin values obtained before 3 wk high-fat feeding were analyzed by two-way mixed-model ANOVA with strain and time of day (0700 vs. 1900) as factors. Significant differences (P < 0.05) among individual strain × time pairs were assessed with planned t comparisons, employing the pooled error term from the ANOVA. Carcass fat content and plasma glucose determinations after high-fat feeding were analyzed by two-way ANOVA with strain (OLETF vs. LETO) and maintenance diet (low vs. high fat) as factors. Significant differences (P < 0.05) among individual strain × diet pairs were assessed with planned t comparisons, employing the pooled error term from the ANOVA. Daily food intake (in grams) and daily caloric consumption throughout the 3-wk diet regimen were analyzed with three-way mixed-model ANOVA with strain, macronutrient, and caloric concentration as factors. Significant differences (P < 0.05) among individual strain × caloric concentration and strain × macronutrient × caloric concentration pairs were assessed with planned t comparisons, employing the pooled error terms from the ANOVAs.

Effects of duodenal nutrient preloads on subsequent food intake. In Male LETO and OLETF rats (n = 6/strain), we evaluated the feeding inhibitory effects of duodenal macronutrient preloads. Before surgery all rats were trained for 1 wk to consume liquid Ensure (Ross Laboratories, 1 kcal/ml) as their maintenance diet, available from 1200 until 1700 daily, with tap water available ad libitum. Both LETO and OLETF rats maintained their pretesting body weight throughout this feeding regimen. Pretesting body weight averages ± SE for each group were: LETO, 401 ± 14.5 g; OLETF, 518 ± 11.2 g. Rats were handled daily and adapted to the morning test protocol as follows. They were placed individually in a wire cage next to an infusion pump, and the pump was turned on for 10 min before each rat was returned to its home cage and given 30-min access to 12.5% glucose solution. All rats adapted well to this procedure and, by the end of the 1-wk training period, consumed at least 10 ml of glucose during the 30-min morning access period.

Duodenal cannulation surgery. After training, rats were deprived of food but not water overnight before surgery. Rats were anesthetized with a mixture of ketamine (Vetalar, 100 mg/ml) and xylazine (Rompun, 20 mg/ml) (4:3, 0.1 ml/100 g body wt) to maintain a surgical level of anesthesia. A laparotomy incision was made on the ventral flank, and the proximal 1.5 cm of the duodenum was exposed and kept warm and moist with saline-soaked sterile cotton gauze. A 25-gauge needle was used to make a small puncture wound on the ventral aspect of the duodenum in a region where the vascular arcade was as sparse as possible to minimize bleeding. Small-diameter Silastic tubing (0.012 in. ID, 0.025 in. OD) with a blunt-tipped tip was inserted 2 cm into the puncture wound in an anadirectional, such that the tip rested ~3.5 cm anad to the pyloric sphincter. The cannula was anchored to the outer serosal surface of the proximal duodenum around the puncture wound with a drop of cyanoacrylic cement (Vetbond) and a 0.5-cm² piece of Marlex mesh sewn to the serosal surface with 6-0 silk suture. The proximal portion of the cannula exited to the abdominal cavity through another small puncture wound in the right lateral abdominal wall, and this proximal portion of the duodenal cannula was then drawn subcutaneously to exit from a 2-cm midline incision on the skin of the back of the neck, just rostral to the interscapular area. The abdominal wall incision was closed with 4-0 silk, and the skin incision was closed with stainless steel wound clips. The exteriorized portion of the duodenal cannula was anchored to the ventral neck musculature with Marlex mesh sutured in place with 4-0 silk suture. The mesh was bound to the Silastic tubing with silicone adhesive, and the duodenal cannula tubing was pressure fitted to a 2.5-cm length of
larger diameter (0.025 in. ID, 0.05 in. OD) exteriorized Silastic tubing. The neck skin incision was closed with cyanoacrylic cement (Vetbond). A 2-cm length of blunted 23-gauge stainless steel tubing, pressure fitted into the larger Silastic tubing, served as an obturator to block the duodenal cannula when it was not in use. All rats received 10,000 U ampicillin intramuscularly on the day of and 2 days after surgery. All rats recovered their presurgical body weights within 4–6 days of surgery. Intake tests were begun 10 days after the completion of duodenal cannula surgeries.

Duodenal preload intake tests. On weekdays, rats received one intake test per day. Tests began at 1000 and were completed by 1200. A test consisted of a 0.44 ml/min 10 min duodenal preload delivered by a syringe pump followed immediately by 30-min access to 12.5% glucose. Diet intake (in milliliters) was measured at 30 min. Duodenal preloads of glucose, peptone (Sigma; type II, from meat) and Intralipid (10%, 1.1 kcal/ml; Kabi-Vitrum) were delivered at 1 kcal/ml randomly across days. All duodenal infusates were warmed to 37°C before infusion. Distilled water was the solvent for glucose and peptone solutions, and physiological saline was used to dilute 10% Intralipid to 1.0 kcal/ml. The infusion rate and caloric concentration were chosen based on values of Walls et al. (27) used to suppress food intake after duodenal nutrient infusions. All rats received the same type of preload on a given day, and a physiological saline preload day always intervened between two successive nutrient preload days.

Duodenal cannula verification. At the end of experiments, rats were reanesthetized as above, 0.3 ml of a 1% fast green dye solution was slowly infused into the duodenal cannula, and the laparotomy incision was reexposed to reveal the gastrointestinal spread of duodenal cannula infusates. In all cases, the dye was localized to points including and distal to the proximal 2 cm of the duodenum and did not appear in the stomach at all.

Data analysis. Because intake data after 4.4 ml/10 min physiological saline duodenal preloads failed to differ significantly from no preload condition [F(2,23) = 0.83, P > 0.5, data not shown], glucose intake data were expressed as percentage suppression below the average baseline saline preload condition for the trials preceding and after each macronutrient preload trial. Data were analyzed by two-way repeated-measures ANOVA with strain and macronutrient as factors. Significant differences (P < 0.05) among individual strain × preload pairs were assessed with planned t comparisons, employing the pooled error term from the ANOVAs.

RESULTS

Table 1 summarizes the body weights that OLETF and LETO rats maintained on standard Purina chow before the high-fat feeding regimen began. At this time,

<table>
<thead>
<tr>
<th>Initial Weight</th>
<th>Chow diet</th>
<th>High-fat diet</th>
</tr>
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<tbody>
<tr>
<td>LETO 548 ± 13</td>
<td>15.7 ± 3.7</td>
<td>31.5 ± 3.9</td>
</tr>
<tr>
<td>OLETF 697 ± 32</td>
<td>6.3 ± 5.9</td>
<td>39.2 ± 7.8*</td>
</tr>
</tbody>
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Data are expressed as means ± SE in grams. LETO, Long-Evans Tokushima rats; OLETF, Otsuka Long-Evans Tokushima fatty rats. *Significantly greater than same strain under chow diet regimen (P < 0.05).

OLETF rats were significantly larger than LETO rats [F(1,18) = 32.5, P < 0.001]. As shown in Fig. 1, OLETF rats were hyperglycemic [F(1,18) = 10.0, P < 0.005] and hyperleptinemic [F(1,18) = 4.5, P < 0.04] compared with LETO controls (Fig 1). There was no significant effect of sample time [0700 vs. 1900; glucose: F(1,18) = 2.85, P > 0.1; leptin: F(1,18) = 2.0, P > 0.15] nor a significant strain × sample time interaction for either plasma glucose or plasma leptin [glucose: F(1,18) = 0.110, P > 0.3; leptin: F(1,18) = 0.967, P >
0.3], indicating that the hyperglycemia and hyperleptinemia in OLETF rats relative to LETO rats tended to be stable across these sampling times. OLETF rats also showed a trend toward hyperinsulinemia relative to LETO controls, but this was not significant ($P < 0.2$).

Table 1 also shows the body weight gain of OLETF and LETO rats after 3 wk diet maintenance with powdered Purina chow or high-fat diet (powdered chow contained 30% corn oil by weight). There was a significant overall effect of diet regimen on body weight gain over the 3-wk period [F(1,18) = 18.1, $P < 0.001$]. Specifically, OLETF but not LETO rats gained significantly greater amounts of weight under high-fat feeding compared with their respective chow diet-fed controls [OLETF: F(1,18) = 16.4, $P < 0.03$; LETO: F(1,18) = 3.8, $P < 0.064$].

Figure 2, top, shows the daily food intake (in grams) for both groups of rats under the two dietary conditions. There was an overall significant effect of diet on grams consumed [F(1,37) = 6.98, $P < 0.01$]. LETO rats consumed significantly fewer grams of high-fat chow than those maintained on chow throughout the 3-wk period [F(1,37) = 15.04, $P < 0.001$]. Thus LETO rats reduced their food intake in response to the high caloric content of the high-fat diet. In contrast, OLETF rats maintained on high-fat chow did not differ in their daily gram intake compared with OLETF maintained on chow diet [F(1,18) = 0.009, $P > 0.9$].

Figure 2, bottom, shows daily food intake in calories for both groups of rats under the two dietary conditions. There was an overall significant effect of diet on calories consumed [F(1,37) = 97, $P < 0.001$], where high-fat diet promoted more caloric intake than did chow diets. Both LETO and OLETF rats on high-fat chow consumed more calories than their counterparts maintained on chow diet [LETO: F(1,37) = 67.1, $P < 0.001$; OLETF: F(1,37) = 32.6, $P < 0.001$]. Planned t comparisons revealed that this effect was maintained throughout the 3-wk period in OLETF rats ($P < 0.05$), whereas it was absent after day 8 in LETO rats ($P > 0.1$). Thus LETO rats maintained on high-fat diets demonstrated complete caloric compensation for the more nutrient-dense high-fat diet within a week, whereas OLETF rats continued to overconsume high-fat diet across the 3-wk period compared with chow-fed OLETF rats.

Figure 2 summarizes the plasma glucose and body fat analyses from the LETO and OLETF rats after the 3-wk high fat vs. chow dietary regimen. OLETF rats were hyperglycemic relative to LETO controls [F(1,18) = 27.9, $P < 0.001$], and there was no significant diet x strain interaction ($P > 0.3$). OLETF rats had significantly more carcass fat than did LETO rats [F(1,19) = 10.97, $P < 0.004$], and high-fat feeding caused significant increases in carcass fat in both strains [F(1,19) = 52.5, $P < 0.001$]. OLETF rats also had significantly more white epididymal fat than did
LETO controls \( F(1,19) = 13.3, P < 0.05 \), and high-fat feeding caused significant increases in carcass fat in both strains \( F(1,19) = 12.7, P < 0.002 \). OLETF rats fed high-fat diets had significantly higher interscapular brown fat than did chow-fed OLETF rats \( F(1,19) = 4.6, P < 0.04 \), who did not differ from LETO rats \( P > 0.1 \).

Effects of gastric nutrient preloads. The feeding inhibitory effects of gastric nutrient preloads are shown in Fig. 4. There was a significant overall effect of caloric content \( F(1,11) = 37.2, P < 0.01 \); overall, the 10-kcal loads suppressed intake to a greater degree than did the 5-kcal loads. In LETO rats, increasing caloric concentrations of glucose, peptone, and Intralipid gastric preloads dose-dependently suppressed subsequent 30-min glucose intake \( P < 0.05 \). Also, for LETO rats, there was no simple effect of nutrient on the degree of suppression \( F(2,22) = 0.344, P > 0.5 \); i.e., within a
caloric concentration across the three macronutrients, glucose, peptone, and Intralipid produced statistically similar levels of intake suppression in LETO rats.

In OLETF rats, the profile for feeding suppression after gastric glucose and peptone preloads was similar to that of LETO rats, where there was a dose-dependent increase in the degree of suppression with increasing preload concentration [F(1,6) = 8.54, P < 0.01]. However, there was a significant simple effect of nutrient in OLETF rats [F(2,22) = 6.74, P < 0.01]. Specifically, gastric Intralipid was significantly less effective than equicaloric glucose and peptone at the 10-kcal load (P < 0.05).

Within-concentration comparisons for a single macronutrient preload type revealed that glucose and peptone preloads suppressed intake to similar degrees in both OLETF and LETO rats. (P > 0.9). In contrast, Intralipid gastric preloads were much less effective in suppressing food intake in OLETF rats than in LETO controls at the high (10 kcal/5 ml; P < 0.01) but not at the low (5 kcal/5 ml) concentration (P > 0.5).

Effects of duodenal nutrient preloads. The feeding-inhibitory effects of 1 kcal/ml × 10 ml duodenal macronutrient preloads are shown in Fig. 5. Duodenal preloads produced significantly less intake suppression in OLETF rats than in LETO controls [F(1,10) = 9.71, P < 0.01]. There was also a significant overall effect of nutrient [F(2,20) = 6.04, P < 0.01]. Duodenal fat was significantly less effective in suppressing intake than was glucose or peptone in OLETF [F(2,20) = 3.48, P < 0.05] but not in LETO rats [F(2,20) = 2.63, P > 0.09].

DISCUSSION

Overall, OLETF rats maintained on a standard chow diet 1) weighed significantly more, 2) had more white adipose tissue and carcass fat, and 3) were hyperglycemic and hyperleptinemic and tended toward hyperinsulinemia relative to LETO controls. This profile is consistent with previous results characterizing the obese and diabetic features of the adult OLETF rat at comparable ages (27–35 wk) (29). We and others (12) have previously suggested that the obesity seen in the OLETF rat is at least in part caused by increased ingestive behavior; OLETF rats are hyperphagic, and this hyperphagia is manifested as an increase in the meal size rather than meal frequency (15). We suggest that the increased meal size is consistent with a lack of meal-elicited CCK satiety signals due to the absence of functional CCK-A receptors in OLETF rats (15). The hyperglycemia and hyperleptinemia seen in obese OLETF rats are likely secondary to the increase in body weight; OLETF rats given the opportunity to exercise on a running wheel do not become obese, and have plasma glucose and total body fat that do not significantly differ from LETO controls (23).

The current findings reveal that OLETF rats lacking CCK-A receptors have decreased responsiveness to dietary fat. OLETF rats persistently overconsumed a high-fat diet throughout a 3-wk access period and failed to compensate for the extra energy provided by this calorically dense diet. LETO rats maintained on a high-fat diet initially consumed greater numbers of calories of the diet compared with controls maintained on standard chow, but they began to compensate for the increased caloric density immediately, and by day 8 of the 3-wk trial their caloric intake on high-fat and chow diets did not differ. Although there was a trend for fat diet-fed LETO rats to gain more weight than chow-fed controls, this did not reach statistical significance. In contrast, OLETF rats consistently consumed more calories on the high-fat diet, resulting in significantly greater body weight gain and body fat accumulation. This persistent overconsumption of a high-fat diet is similar to the fa/fa Zucker fatty rat, which has a defect in the leptin receptor (18). When given access to a high-fat diet, Zucker rats fail to compensate for the increased caloric density, and their caloric intake remains elevated for up to 7 wk (26). Adipose mass also increases over this period, and it has been suggested that there is a causal relationship between increased fat deposition and the gradual decrease in overconsumption of high-fat diets in Zucker fa/fa rats (26). Osborne-Mendel rats, another obesity-prone rat strain, also overconsume high-fat diets, resulting in significant weight gain and fat deposition (30).

At death, high-fat fed OLETF rats had significantly greater percent dry carcass fat and interscapular brown adipose tissue compared with high-fat-diet fed LETO controls and chow-fed OLETF controls. High-fat feeding has been shown to increase brown adipose tissue (BAT) mass but also to increase sympathetic activity and BAT activity relative to chow-fed controls (21).
However, Osborne-Mendel rats fed a high-fat diet have lower BAT norepinephrine turnover, an index of reduced sympathetic activity, despite increased BAT mass (30). The increased brown fat mass after high-fat feeding in OLETF rats may also be a result of reduced sympathetic activation.

In short-term intake tests, OLETF rats also revealed a reduced responsiveness to dietary fats. The feeding-suppressive actions of gastric nutrient preloads were remarkably similar for LETO and OLETF rats, with one exception: the 2-kcal/ml gastric Intralipid preload was significantly less effective in suppressing food intake in OLETF rats compared with LETO controls. Duodenal lipid infusions were also particularly ineffective in suppressing food intake in OLETF rats compared with 1) equicaloric carbohydrate and protein in OLETF rats and 2) duodenal lipid in LETO controls. Duodenal fat is a potent secretagogue of CCK in rats (2, 9). Duodenal lipids have previously been shown to suppress feeding (8, 27), and CCK-A receptor antagonists reverse this suppression (28, 31). The relative inability of gastric and duodenal lipid to suppress feeding in OLETF rats is consistent with the inability of lipid-induced CCK release to provide CCK-A receptor-mediated negative feedback signals important in the control of food intake. The ability of duodenal carbohydrate and protein solutions to suppress intake was also significantly suppressed in OLETF rats relative to LETO controls, although to a lesser degree than that of fat. The ability of some duodenal protein and carbohydrate solutions to slow gastric emptying and suppress food intake has been demonstrated to depend on endogenous, nutrient-elicted CCK acting at CCK-A receptors. In the rat, the inhibition of gastric emptying produced by duodenal peptone and maltose solutions is reversed by the potent and specific CCK-A receptor antagonist devazepide (5, 19). In rhesus monkeys and humans, devazepide has been demonstrated to accelerate glucose gastric emptying (6, 14). In addition, Yox et al. (31) have shown that the suppression of feeding produced by intraintestinal maltose infusions is blocked by devazepide. The present attenuation of the feeding suppressive effects of duodenal carbohydrate and protein solutions is consistent with the lack of a functional CCK-A receptor-mediated negative feedback pathway in OLETF rats.

Perspectives

We have interpreted the hyperphagia and increased meal size in OLETF rats as reflecting a deficit in the ability to detect satiety signals provided by endogenous, meal-elicted CCK. The present data extend this interpretive framework by demonstrating that nutrient secretagogues of CCK, particularly lipid, are significantly less effective in suppressing food intake. From a mechanistic perspective, we view the present reduced responsiveness to dietary fat in OLETF as secondary to the deficit in the CCK signaling pathways in OLETF rats: the lack of functional CCK-A receptors. Both CCK and duodenal nutrient-induced satiety are mediated by a common neural substrate: the afferent subdiaphragmatic vagus nerve. CCK-A receptors have been localized to subdiaphragmatic vagal afferent fibers (3, 11, 16, 17), CCK stimulates gut vagal afferents (4, 22), and afferent subdiaphragmatic vagotomy blocks the satiety actions of low doses of peripherally administered CCK (24). Similarly, subdiaphragmatic vagal deafferentation of the duodenum attenuates or blocks the ability of duodenal nutrients to suppress food intake (27). Interpreted in the context of the CCK-A receptor mediation of CCK satiety, the current data underscore a critical role for endogenous CCK acting at vagal CCK-A receptors in the feeding suppressive actions of nutrients in the gut. These data also support the view that a chronic deficit in meal-elicitd CCKergic negative feedback signals can promote obesity.

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