Disordered food intake and obesity in rats lacking cholecystokinin A receptors

TIMOTHY H. MORAN,1 LAURA F. KATZ,1 CARLOS R. PLATA-SALAMAN,2 AND GARY J. SCHWARTZ1
1Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and 2School of Life and Health Sciences, University of Delaware, Newark, Delaware 19720-2590

Moran, Timothy H., Laura F. Katz, Carlos R. Plata-Salaman, and Gary J. Schwartz. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R618–R625, 1998.—Otsuka Long-Evans Tokushima Fatty (OLETF) rats develop obesity, hyperglycemia, and non-insulin-dependent diabetes mellitus and do not express cholecystokinin A (CCK-A) receptors, the receptor subtype mediating the satiety actions of CCK. In short-term feeding tests, male OLETF rats were completely resistant to exogenous CCK, and their response to bombesin was attenuated. Comparisons of liquid meal consumption in OLETF and control Long-Evans Tokushima (LETO) rats demonstrated that 1) OLETF rats had greater intakes during 30-min scheduled daytime meals and significantly larger and fewer spontaneous nighttime meals and 2) although the initial rates of licking were the same, OLETF rats maintained the initial rate longer and the rate at which their licking declined was slower. In 24-h solid food access tests, OLETF rats consumed significantly more pellets than LETO controls, and this increase was attributable to significant increases in meal size. Together, these data are consistent with the interpretation that the lack of CCK-A receptors in OLETF rats results in a satiety deficit leading to increases in meal size, overall hyperphagia, and obesity.

satiety; peptides; hyperphagia; bombesin

A ROLE FOR THE BRAIN GUT peptide cholecystokinin (CCK) in the control of food intake has been demonstrated. After exogenous peripheral administration, CCK reduces food intake in a dose-related manner across a range of experimental situations and in a variety of species (1, 11, 15). The actions of CCK in food intake are specific to reductions in meal size (32) and the earlier appearance of a behavioral satiety sequence (2). The feeding-inhibitory effects of the exogenously administered peptide appear to be mimicking a physiological role for endogenous CCK. Administration of CCK antagonists results in increases in food intake (9, 24, 28, 30, 31), specifically increases in meal size and meal duration (23, 32). The feeding-inhibitory actions of both exogenously administered and endogenously released CCK are mediated through their interaction with CCK-A receptors (22).

Otsuka Long-Evans Tokushima Fatty (OLETF), an outbred strain of Long-Evans rats that had been established as an animal model of non-insulin-dependent diabetes mellitus (NIDDM) and obesity, has recently been demonstrated to have a congenital defect in the expression of the CCK-A receptor gene (10). The characteristic features of OLETF rats include 1) accelerated rates of weight gain beginning at 5 wk of age, resulting in an obesity of ~40% higher weight than the control Long-Evans Tokushima (LETO) strain; 2) development of hyperglycemia and NIDDM at ~18 wk of age; and 3) eventual insulin deficiency after 65 wk of age (14). In experiments aimed at characterizing the overall pancreatic function in the OLETF rats, it was discovered that pancreatic acini isolated from the OLETF rats did not release amylase in response to CCK, whereas sensitivity to carbachol/choline, bombesin, and secretin was unaltered or even slightly increased (26). Subsequent receptor binding studies failed to demonstrate any 125I-CCK-8 binding to pancreatic acini prepared from OLETF rats (26). It has now been established that there is no CCK-A receptor gene expression in pancreas or brain from OLETF rats and that there appears to be a difference in the structure of the CCK-A receptor gene in these animals (10).

The present experiments were aimed at characterizing the feeding behavior of OLETF rats to begin to determine whether the obesity in OLETF animals may be a result of a satiety deficit secondary to the absence of the CCK-A receptors. We have examined the feeding responses of OLETF and the LETO rats to exogenously administered CCK and bombesin. We have also characterized the patterns of food intake of OLETF and LETO rats in three testing situations. In the first, patterns of licking in a daily scheduled access to 0.5 kcal/ml glucose was assessed. In the second, balanced liquid diet meal patterns during the first 6 h of the dark cycle were compared between the two strains. Finally, the patterns of solid food intake over the 24-h light-dark cycle in OLETF and LETO animals were compared. Overall, the results demonstrate that OLETF rats are resistant to the feeding-inhibitory actions of exogenous CCK and, independent of feeding test, exhibit hyperphagia and patterns of food intake that are consistent with the absence of one of the physiological controllers of meal size.

METHODS

Twelve male OLETF and 12 male LETO rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. The animals were 6 wk old at the time they arrived in our laboratory. At that point, there were no differences in the body weight between the two groups. Animals 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, were maintained with ad libitum access to pelleted Purina rat chow and water.
Responses to peripheral exogenous CCK and bombesin. After the initial 2-wk period, the rats were adapted to a feeding schedule in which the food was removed from the cages at 0900. At 1400, the rats had 30-min access to 0.125 g/ml of glucose. After the glucose access period, Chow pellets were returned to the cages. Water was always available. Once glucose intakes had stabilized, animals’ responses to dose ranges of exogenous CCK and bombesin were assessed. Five minutes before glucose access, rats received intraperitoneal injections of peptide or saline vehicle (1 ml/kg). Doses of CCK (sulfated CCK-8) and bombesin were 1, 2, 4, 8, and 16 µg/kg (Bachem). The order of testing of CCK doses was 8, 16, 0, 4, 2, and 1 µg/kg. The order of testing of the bombesin doses was 4, 2, 0, 8, 16, and 1 µg/kg. Animals’ responses to the full CCK dose range were ascertained before testing with bombesin was initiated. A vehicle day was paired with each CCK and bombesin dose day, and intakes following peptide administrations were directly compared with the intakes on the corresponding vehicle days using a mixed-model analysis of variance (ANOVA). Differences in intake between drug and corresponding control days were assessed by analyses of simple effects and planned t comparisons using the pooled error term from the ANOVA.

Microstructural analysis of glucose intake. To ascertain the microstructural pattern of glucose ingestion in OLETF and LETO rats, animals were tested in lickometry cages. Lickometer devices consisted of stainless steel drinking tubes inserted in graduated bottles. The lickometer was connected to an interface (DIG LOG Instruments and Systems; Tallahassee, FL) that passed less than a 60-nA current through the rat each time tongue contact with the tube was made. The current was amplified, and a signal was fed to an IBM AT computer that recorded the time of each tongue contact to the nearest millisecond. At the end of the test session, data were transferred to diskette for later analyses using the Tongue Twister program (13). Animals were adapted to the lickometry cages for 1 wk, and data were collected for three consecutive days at the end of the adaptation period. Data from the third day were used to compare the patterns of 30-min glucose intake between the two groups of animals. The data were analyzed to obtain the total number of licks, licks per milliliter of consumption, burst size and number of bursts, and cluster size and number of clusters. Criterion for the end of a burst was an interlick interval longer than 0.23 s but less than 0.5 s. The criterion for ending a cluster was an interlick interval of 0.5 s or longer. To quantify the changes in the rate of licking during the test, the number of licks during successive minutes of the test were calculated for each of the animals in 1-min bins. Lick rate data for each individual animal were fit to a Weibull function, \( y = A \exp\left[-\left(Bt\right)^C\right] \) by the least-squares method to quantify changes in the rate of licking during the test. This function has been used by Davis and colleagues (7, 8) and has been shown to have both theoretical significance and to fit these types of curves well. The A parameter is the initial rate of licking, the B parameter is the slope of the decline, and the C parameter provides a shape parameter that indicates how the function deviates from an exponential. When \( C = 1 \), the Weibull function degenerates to a simple exponential curve. A value of \( C > 1 \) indicates that the initial rate of decline is less rapid than it would be for an exponential. Data for these variables from the OLETF and LETO rats were compared by ANOVA.

Liquid diet meal patterns. After the completion of testing with scheduled glucose consumption, cohorts of 4 OLETF and 4 LETO rats were adapted to consumption of a liquid diet (Ensure) as their sole source of food. Immediately before the beginning of the 12-h dark cycle, animals were transferred to the lickometry cages and presented with a fresh supply of Ensure. Animals had access to the Ensure (Miles Laboratories) for a total of 15 h per day. Water was also available during this period. Ensure was presented in the same bottles, using the same licking spouts that had been used in the glucose consumption tests. At the end of the 15-h Ensure access period, rats were returned to their home cages where chow was available for 3 h and water was available ad libitum. Animals were maintained on this schedule for 2 wk before data acquisition. On experimental days, the licking behavior of the animals during the first 6 h of the dark cycle was monitored. After testing it was discovered that one of the lickometers was not reliably recording contacts. Data from animals that were in that lickometer cage were not included in the analyses. Thus data from 11 OLETF and 9 LETO rats were successfully obtained. The time of each individual lick was recorded, and data were summed over 1-min intervals throughout the 6-h period. Licks were divided into meals using the criteria of three licks with interlick intervals of less than 0.25 s to initiate a meal. This criterion was adopted to identify licks that were occurring within a burst of licking (6). The intermeal interval was defined as 5 min without licking, an intermeal interval criterion that has recently been validated for spontaneous liquid diet meals by Rushing et al. (29). Meal data were analyzed for meal size (by number of licks), meal frequency, intermeal intervals, and satiety ratios (the intermeal interval in minutes divided by the size of the previous meal in number of licks) using ANOVAs. Withinmeal lick data were analyzed with the Tongue Twister program as in the 30-min glucose access test above. Analyses included assessments of differences in microstructural variables as well as Weibull analysis of lick rate functions. Microstructural and Weibull data for OLETF and LETO rats were compared by ANOVA.

Pelleted chow meal patterns. While some cohorts were being tested with Ensure, cohorts of two OLETF and two LETO rats were adapted to test cages containing computerized feeding devices (Coulbourn Instruments), which delivered 45-mg chow pellets. Animals had ad libitum access to pellets and water. Electromechanical pellet dispensers were controlled by infrared pellet-sensing photo beams. Individual pellets were delivered in response to the removal of the previous pellet. Animals were adapted to the testing apparatus and feeding paradigm for 10 days before experimental data collection. Data were summed and recorded in 10-min intervals 24 h per day. Data for the dark cycle, light cycle, and total 24 h for the variables of total intake, meal frequency, meal size, intermeal interval, and satiety ratio were recorded and calculated. A meal was defined as the acquisition of at least five pellets preceded and followed by at least 20 min of no feeding. Meal size was defined as the number of pellets delivered during a meal. Postprandial intermeal interval was defined as the time from the delivery of the last pellet of one meal to the delivery of the first pellet of the subsequent meal. Satiety ratio was defined as the intermeal interval in minutes divided by the size of the previous meal in pellets. Data were analyzed by Student’s t-test when data passed the normality (Kolmogorov-Smirnov) and equal variance (Levene median) tests. Otherwise, data were analyzed using the Mann-Whitney test. Eight LETO and eight OLETF rats were tested in these chambers. Two of the OLETF rats did not adapt to the chambers and lost weight. Data from these animals were not included in the analyses.

RESULTS

Figure 1 plots body weights for the OLETF and LETO rats throughout the period of testing. The OLETF
rats became significantly heavier than the LETO rats at 9 wk of age and continued to be significantly different for the duration of the testing paradigms.

Responses to peripheral exogenous CCK and bombesin. As presented in Fig. 2, peripheral exogenous administration of CCK caused a dose-related suppression of intake in LETO rats but had no effect on glucose intake in OLETF animals. Analysis of variance demonstrated significant effects of both strain \( F(1,22) = 61.369, P < 0.00001 \) and dose \( F(5,110) = 9.216, P < 0.00001 \) and a significant strain-by-dose interaction \( F(5,110) = 6.104, P < 0.00001 \). Analyses of simple effects indicated that baseline intakes were also significantly higher in OLETF rats \( F(1,54) = 8.882, P < 0.004 \).

In contrast to results with CCK, peripheral exogenous bombesin reduced glucose intake in both OLETF and LETO rats (Fig. 3). Analysis of variance demonstrated significant effects of strain \( F(1,22) = 31.793, P < 0.00001 \) and dose \( F(5,110) = 21.857, P < 0.0001 \), but no significant strain-by-dose interaction \( F(5,110) = 1.132, P > 0.3 \). Planned \( t \) comparisons indicated that bombesin significantly suppressed intake in both groups beginning at a dose of 2 µg/kg. The maximal bombesin-induced intake suppression in LETO rats was significantly greater than that found in OLETF rats. At a dose of 16 µg/kg, bombesin resulted in a maximal suppression 57.1 ± 9.0% in LETO rats, whereas that dose only reduced intake in OLETF rats by 26.6 ± 8.0% in OLETF rats \( F(1,22) = 5.913, P < 0.05 \). This was due to both higher baseline in OLETF rats and the relative, but nonsignificant, decrease in the volumes consumed [4.9 vs. 7.4 ml; \( F(1,22) = 1.851, P = 0.187 \)].

Microstructural analysis of glucose intake. Analysis of microstructural variables during scheduled 30-min glucose access revealed a number of differences between the OLETF and LETO animals (Table 1). The increased volume intake noted above was also represented in an increased number of licks in the OLETF compared with the LETO rats \( F(1,18) = 12.055, P < 0.003 \). The increased number of licks was not exclusively the outcome of increases in either the number of bouts of licking or the number of licks per bout. As shown in Table 1, although burst and cluster size and burst number and cluster number were all elevated in...
the OLETF rats compared with the LETO, none of
these differences was significant. Comparisons of the
mean variables from the Weibull analysis of lick rates
(Table 2) revealed that variable A, the initial lick rate,
was not statistically different between the two groups.
However, variable B, the slope variable, was signifi-
cantly higher in LETO compared with obese animals
$[F(1,18) = 13.33, P < 0.002]$. The C variable or shape
function was significantly higher in OLETF compared
with LETO animals $[F(1,18) = 8.158, P < 0.01]$. Figure
4 provides plots of the lick rates for the two groups of
animals generated from the mean Weibull functions.
Although the initial rates of licking are comparable
between the two groups, the OLETF animals continue
to maintain this higher initial rate of licking for a
longer period of time, and the rate of licking declines
more slowly than occurs in the lean LETO animals.

Liquid diet meal patterns. Analysis of the licking
behavior through the first 6 h of the dark cycle on
access to Ensure liquid diet revealed a number of
differences in the patterns of licking between the
OLETF and LETO rats. Data for lick rate throughout
the 6-h period for representative OLETF and LETO
rats are plotted in Fig. 5. Consistent with the mean
data presented in Table 3, OLETF rats recorded nearly
twice the number of licks per meal as the LETO
animals $[F(1,19) = 9.67, P < 0.006]$. In contrast, the
mean number of meals in the OLETF animals was not
greater than in the lean animals (Table 3). In fact, there
was a trend for the mean number of meals to be reduced
in the OLETF rats $[F(1,19) = 3.79, P < 0.07]$. Mean
intermeal intervals were not different between OLETF
and LETO rats $[F(1,19) = 2.22, P > 0.15]$. Consistent
with the greater meal size and the lack of difference in
intermeal interval, satiety ratios in OLETF rats were
significantly lower than in LETO rats $[F(1,19) = 12.60,$
P < 0.005]. Analysis of microstructural variables re-
vealed that although burst size, cluster size, and clus-
ter number were not significantly different between the
two groups, mean burst number was significantly
greater in the OLETF compared with the LETO rats
$[F(1,19) = 5.48, P < 0.05]$. Weibull analysis of the mean
lick rates (Table 2) demonstrated that while there were
no differences in the initial rate of licking, there were
again significant differences in both the B slope func-
tion and C shape functions. The slope function was
significantly higher in lean LETO compared with obese
OLETF rats $[F(1,19) = 9.49, P < 0.01]$. The C or shape
function was significantly greater in the obese OLETF
compared with the lean LETO rats $[F(1,19) = 6.81, P <
0.02]$. The obese OLETF animals maintained a high
rate of licking for a longer period than the LETO rats.

Comparison of Weibull parameters from the sched-
uled 30-min glucose access test and the spontaneous
meals taken during Ensure liquid diet nighttime access
revealed that patterns of food intake within the meals
taken in the two situations differed. ANOVA demon-
strated that there was a significant effect of test
protocol on the initial lick rate $[F(1,16) = 7.004, P <
0.05]$, with higher initial rates in the spontaneous
meals during the 6-h dark cycle liquid diet test in the
OLETF rats. There was also a significant difference in
the B slope variable between the testing paradigms
$[F(1,16) = 17.132, P < 0.001]$, with higher slopes in the
spontaneous than in the scheduled meals. Finally, the
shape functions were significantly higher in the sponta-
eous nighttime compared with the scheduled daytime
glucose access meals $[F(1,16) = 27.365, P < 0.001]$. Thus
initial rates of licking were maintained for longer periods in the spontaneous meals.

Pelleted chow meal patterns. Analysis of the data on
spontaneous food intake of chow pellets also revealed a
number of significant differences between the OLETF
and LETO animals (Table 4). Total daily intake ($t =
5.08, P < 0.001$) as well as intake in both the dark cycle

---

**Table 1. Thirty-minute liquid glucose consumption: microstructural variables**

<table>
<thead>
<tr>
<th></th>
<th>OLETF</th>
<th>LETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal Size</td>
<td>3.398±0.281*</td>
<td>2.272±0.162</td>
</tr>
<tr>
<td>Burst Size</td>
<td>35.6±8.4</td>
<td>25.7±13.9</td>
</tr>
<tr>
<td>Burst Number</td>
<td>138.8±25.1</td>
<td>103.0±12.7</td>
</tr>
<tr>
<td>Cluster Size</td>
<td>55.9±14.0</td>
<td>42.0±5.4</td>
</tr>
<tr>
<td>Cluster Number</td>
<td>94.7±17.5</td>
<td>64.0±7.1</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. OLETF, Otsuka Long-Evans Tokushima Fatty rats; LETO, Long-Evans Tokushima rats.

*Significant difference from corresponding LETO value; †significant difference from corresponding 30-min glucose consumption test values.

---

**Table 2. Weibull analysis**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-min Glucose consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLETF</td>
<td>223±13</td>
<td>0.0001±0.0002*</td>
<td>4.75±1.38*</td>
</tr>
<tr>
<td>LETO</td>
<td>262±17</td>
<td>0.0022±0.0003</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td>6-h Dark cycle liquid diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLETF</td>
<td>285±7.3</td>
<td>0.0023±0.004*</td>
<td>59.2±11.9†</td>
</tr>
<tr>
<td>LETO</td>
<td>270±12</td>
<td>0.0051±0.0008#</td>
<td>26.2±4.51#</td>
</tr>
</tbody>
</table>

Values are means ± SE. A, initial rate; B, slope; C, shape.
*Significant difference from corresponding LETO value; †significant difference from corresponding 30-min glucose consumption test values.

---

**Table 3. Analysis of microstructural variables**

<table>
<thead>
<tr>
<th></th>
<th>OLETF</th>
<th>LETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst Size</td>
<td>138.8±25.1</td>
<td>103.0±12.7</td>
</tr>
<tr>
<td>Cluster Size</td>
<td>55.9±14.0</td>
<td>42.0±5.4</td>
</tr>
<tr>
<td>Cluster Number</td>
<td>94.7±17.5</td>
<td>64.0±7.1</td>
</tr>
</tbody>
</table>

**Table 4. Analysis of spontaneous food intake**

<table>
<thead>
<tr>
<th></th>
<th>OLETF</th>
<th>LETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal Size</td>
<td>3.398±0.281*</td>
<td>2.272±0.162</td>
</tr>
<tr>
<td>Burst Size</td>
<td>35.6±8.4</td>
<td>25.7±13.9</td>
</tr>
<tr>
<td>Burst Number</td>
<td>138.8±25.1</td>
<td>103.0±12.7</td>
</tr>
<tr>
<td>Cluster Size</td>
<td>55.9±14.0</td>
<td>42.0±5.4</td>
</tr>
<tr>
<td>Cluster Number</td>
<td>94.7±17.5</td>
<td>64.0±7.1</td>
</tr>
</tbody>
</table>
the 24-h observation period (t = 2.72, P < 0.05) and light cycle (t = 2.43, P < 0.05) was significantly greater in the OLETF than in the LETO rats (Fig. 6). As plotted in Fig. 7, the increases in total food intake were a result of significant overall increases in meal size in the OLETF rats (t = 6.41, P < 0.001) as well as increases in both the dark (t = 8.09, P < 0.001) and the light cycle (t = 3.91, P < 0.01). In contrast, meal frequency was not elevated in the OLETF animals. In fact, the number of meals taken during the dark cycle was significantly less in the OLETF than the LETO rats (t = 6.16, P < 0.001), whereas the number of meals taken in the light did not differ between the two groups. Overall, as shown in Fig. 8, this resulted in the obese animals taking significantly fewer meals throughout the 24-h observation period (t = 4.11, P < 0.01). Intermeal intervals were significantly longer in the OLETF in comparison to the LETO rats (t = 2.74, P < 0.05). This difference was due to the relative duration of the intermeal intervals during the dark cycle (t = 4.36, P < 0.001). Intermeal intervals during the light cycle were not significantly different. Satiety ratios defined as the ratio of the intermeal interval divided by the preceding meal size were lower overall in the OLETF rats (t = 3.88, P < 0.01). Analysis of the satiety ratios indicated that there were no differences between the groups during the dark, whereas satiety ratios were significantly lower in the OLETF during the light cycle (t = 2.27, P < 0.05). Table 4 also presents the mean body weights for the LETO and OLETF rats at the time of testing of their spontaneous solid food meal patterns. The OLETF rats were significantly heavier than the LETO rats during these experiments (t = 9.50, P < 0.001).

DISCUSSION

OLETF rats were developed from a spontaneously diabetic rat with polyuria, polydipsia, and obesity that

### Table 3. Six-hour dark cycle liquid diet: meal patterns and microstructural variables

<table>
<thead>
<tr>
<th>Meal Parameter</th>
<th>OLETF</th>
<th>LETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal size, no. of 45-mg pellets</td>
<td>2,862 ± 318*</td>
<td>1,485 ± 129</td>
</tr>
<tr>
<td>Meal frequency, no. of meals</td>
<td>4.45 ± 0.25</td>
<td>5.44 ± 0.37</td>
</tr>
<tr>
<td>Intermeal interval, min</td>
<td>96.9 ± 8.8</td>
<td>80.8 ± 6.1</td>
</tr>
<tr>
<td>Satiety ratio</td>
<td>4.27 ± 0.45*</td>
<td>6.81 ± 0.51</td>
</tr>
<tr>
<td>Burst size, no. of licks</td>
<td>38.2 ± 6.5</td>
<td>43.6 ± 9.8</td>
</tr>
<tr>
<td>Burst number</td>
<td>97.4 ± 15.7*</td>
<td>51.9 ± 8.5</td>
</tr>
<tr>
<td>Cluster size, no. of licks</td>
<td>151.1 ± 39.5</td>
<td>119.9 ± 45.1</td>
</tr>
<tr>
<td>Cluster number</td>
<td>34.4 ± 6.8</td>
<td>26.6 ± 4.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Satiety ratio, intermeal interval/meal size.

*Significant difference from corresponding LETO value.

### Table 4. Meal parameters in OLETF and LETO rats

<table>
<thead>
<tr>
<th>Meal Parameter</th>
<th>n</th>
<th>Dark Period</th>
<th>Light Period</th>
<th>Total Daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total intake, no. of 45-mg pellets</td>
<td>6</td>
<td>426 ± 31*</td>
<td>244 ± 43*</td>
<td>670 ± 36t</td>
</tr>
<tr>
<td>OLETF</td>
<td>8</td>
<td>351 ± 12</td>
<td>148 ± 18</td>
<td>499 ± 16</td>
</tr>
<tr>
<td>LETO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meal frequency, no. of meals</td>
<td>6</td>
<td>4.83 ± 0.2†</td>
<td>3.50 ± 0.5</td>
<td>8.33 ± 0.4†</td>
</tr>
<tr>
<td>OLETF</td>
<td>8</td>
<td>7.25 ± 0.3</td>
<td>3.75 ± 0.4</td>
<td>11.00 ± 0.5</td>
</tr>
<tr>
<td>LETO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meal size, no. of 45-mg pellets</td>
<td>6</td>
<td>88.0 ± 5†</td>
<td>72.5 ± 10†</td>
<td>81.5 ± 6.5†</td>
</tr>
<tr>
<td>OLETF</td>
<td>8</td>
<td>49.0 ± 2.5</td>
<td>39.6 ± 2.9</td>
<td>45.8 ± 2.0</td>
</tr>
<tr>
<td>LETO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermeal intervals, min</td>
<td>6</td>
<td>132 ± 8†</td>
<td>181 ± 24</td>
<td>146 ± 10t</td>
</tr>
<tr>
<td>OLETF</td>
<td>8</td>
<td>89 ± 7</td>
<td>194 ± 26</td>
<td>116 ± 7</td>
</tr>
<tr>
<td>LETO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satiety ratio</td>
<td>6</td>
<td>1.51 ± 0.1</td>
<td>2.67 ± 0.4*</td>
<td>1.84 ± 0.2t</td>
</tr>
<tr>
<td>OLETF</td>
<td>8</td>
<td>1.82 ± 0.1</td>
<td>3.11 ± 0.9</td>
<td>2.54 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Satiety ratio, intermeal interval/meal size. Body weights at time of testing: LETO = 499.8 ± 9.8, OLETF = 666.7 ± 17†. Significant differences from LETO values: †P < 0.05; ‡P < 0.01; * Significant differences from LETO values: †P < 0.05; ‡P < 0.01 [2-sample t-test when data passed the normality (Kolmogorov-Smirnov) and equal variance (Levene median) tests; otherwise the data were analyzed with the Mann-Whitney test].

Fig. 5. Representative examples of patterns of licking of Ensure liquid diet in OLETF (A) and LETO (B) rats over the first 6 h of the dark cycle. Data are plotted as minute-by-minute lick rate over the 6-h period. OLETF rats take larger meals than LETO rats.
had been identified in an outbred colony of Long-Evans rats in 1984 (14). Through selective matings, both OLETF and control LETO lines have been established. No evidence for diabetes or obesity in the over 20 generations of the LETO line have been found (14). OLETF rats begin to gain weight more rapidly than controls from week 5 and demonstrate persistent glucose intolerance and hyperinsulinemic by 18 wk of age. Eventually, OLETF rats become hypoinsulinemic and develop insulin-dependent diabetes corresponding to deterioration of pancreatic islets. In examining pancreatic exocrine function in these animals, Otsuki et al. (26) demonstrated a selective and total loss of sensitivity to CCK in acini prepared from OLETF rats. Experiments addressing whether this lack of sensitivity was due to an abnormality in the receptor or in postreceptor transduction, they determined that 125I-CCK binding was totally absent in acini from OLETF rats (26). Subsequent experiments demonstrated a lack of expression of the CCK-A receptor gene in the pancreas of OLETF rats, and Southern blot hybridization analysis of genomic DNA from LETO and OLETF rats indicated differences in the restriction fragments in the two strains, suggesting a change in the structure of the CCK-A receptor gene in OLETF rats (10). These data have established the OLETF rat as a model system for examining the various physiological roles of CCK.

The lack of a satiety response to peripheral exogenously administered CCK in OLETF rats is consistent with their defect in CCK-A receptor gene expression. The satiety actions of exogenously administered CCK have been demonstrated to depend on CCK’s interactions with CCK-A rather than CCK-B receptors (22). Prior work has demonstrated that OLETF rats are also insensitive to the feeding inhibitory actions of centrally administered CCK (21). Thus, although the relationship between the satiety actions of peripheral and central CCK remains unclear, both feeding inhibitory actions occur through an interaction with CCK-A receptors.

The OLETF rat’s attenuated response to exogenously administered bombesin was unanticipated. One interpretation of this finding is that the feeding-inhibitory actions of bombesin depend in part on actions of CCK at CCK-A receptors. Evidence for a role for CCK in some actions of bombesin has been found. For example, the CCK-A receptor antagonist devazepide has been demonstrated to attenuate the gastric inhibitory actions of bombesin-like peptides (16). However, a similar action...
of devazepide against the feeding-inhibitory actions of bombesin has not been reported. Furthermore, although some data have suggested synergistic actions between CCK and bombesin in inhibiting food intake (12), this has not always been found to be the case (25, 33). At this point, it is not clear whether OLETF rats have reduced sensitivity to many satiety-provoking stimuli or whether this attenuated response is specific to bombesin.

Analyses of liquid diet intake patterns within meals revealed clear differences between the OLETF and LETO rats. Fitting the lick rate data to Weibull functions produced estimates of 1) initial lick rates, 2) the rate of delay in licking across time, and 3) a shape parameter that expresses how the function differs from the exponential or the duration of maintaining a high initial rate of licking. The initial rate of licking has been shown to be sensitive to the oral stimulatory properties of a solution. For example, increasing the saccharin concentration in a glucose solution significantly increases the initial lick rate (4). OLETF and LETO rats did not differ on this parameter. In contrast to the initial rate parameter, both the slope and shape parameters have been suggested to be under the control of negative feedback signals arising from the postoral consequences of the ingested solutions (7, 8). Higher slope and lower shape parameters suggest increased negative feedback. In both scheduled and spontaneous meals, OLETF rats differed from LETO rats on both of these parameters. The direction of these differences (higher slope and lower shape parameters for the OLETF rats) is consistent with the interpretation of reduced negative feedback (satiety) in the OLETF rats. In all of the tests, the OLETF rats were hyperphagic relative to the LETO controls. Overall 24-h pellet intake was 34% higher in the OLETF rats, and the magnitude of increases in meal size either in the 30-min glucose access test, the nighttime liquid diet assessments, or on pelleted chow were even greater, 50, 96, and 79%, respectively. In the liquid diet and chow access tests, there were also decreases in meal frequency. Whether these decreases in meal number represent an unsuccessful attempt at compensation for the increased meal size is unclear. Because animals are more efficient at defending against a caloric deficit than compensating for a caloric surplus, incomplete compensation for large increases in meal size might be expected.

The effects of transient blockade of the actions of CCK at CCK-A receptors have been studied using potent and specific CCK antagonists. In a variety of experimental settings, administration of the CCK-A antagonist devazepide has resulted in increased food intake (9, 24, 28, 30, 31). Such data demonstrate a physiological action of endogenous CCK in the control of food intake. In testing situations where meal patterns have been studied following antagonist administration, devazepide has been demonstrated to produce increases in both meal size and duration (23) and, in lean Zucker rats (fa/?) devazepide both increased meal size and produced a reduction in meal number (34). Thus the meal patterns in the OLETF rat lacking CCK-A receptors resemble those resulting from antagonist-mediated transient blockade of CCK-A receptors in normal animals.

The patterning of food intake has been studied in a variety of genetic and surgical obesity models. In the ob/ob mouse, which lacks the Ob protein or leptin (38), food intake is increased and the hyperphagia is marked by increases in meal size, particularly during the dark phase of the light-dark cycle (35). Similar results have been reported in the fa/fa Zucker rat (3, 5), which has a deficit in the Ob (leptin) receptor (27). It is presently unclear how deficits in leptin signaling result in specific increases in meal size. In intact animals, leptin levels appear to be a function of the degree of adiposity (19). However, leptin or leptin-induced signals may modulate the efficacy of meal-related satiety signals. For example, leptin modulates the ability of CCK to activate vagal afferent fibers (36), and both the db/db mouse and the fa/fa Zucker rat have been reported to be less sensitive to exogenously administered CCK (20, 35). Animals with hyperphagia and obesity resulting from ventromedial hypothalamic lesions demonstrate a different pattern of food intake. As well as increasing meal size, these animals also increase meal frequency (17). Thus hyperphagia and obesity can be expressed in multiple food intake patterns.

Although OLETF rats may have additional genetic deficits other than in the gene for CCK-A receptors and may have developmental abnormalities arising from the absence of CCK-A receptors that may contribute to the disordered food intake and obesity, the patterns of food intake in the OLETF rats are consistent with the absence of an important physiological satiety signal. A straightforward hypothesis relating the lack of CCK-A receptors to the obesity in the OLETF rat is that the increased meal size, hyperphagia, and obesity in the OLETF rats are the direct result of the loss of a within-meal satiety signal.

Perspectives

The recent findings demonstrating roles for leptin and leptin signaling in body weight regulation in the ob/ob mouse (38) and db/db mouse (18) and Zucker rat (27) have focused attention on the importance of feedback signals arising from stored nutrients within adipose tissue in the long-term control of energy balance. The current data, showing that OLETF rats, lacking CCK-A receptors, develop hyperphagia and a degree of obesity comparable to that of the Zucker rat, expands the perspective of the controls of energy balance to include signals involved in the control of individual meals. Thus dysregulation within short-term control pathways, as well as within longer term, adiposity-related systems, can result in overeating and obesity.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-19302 and the generous gift of the OLETF and LETO rats from the Tokushima Research Institute, Otsuka Pharmaceutical.
REFERENCES


