Chronic administration of OB protein decreases food intake by selectively reducing meal size in female rats

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Chronic administration of OB protein decreases food intake by selectively reducing meal size in female rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R186–R193, 1998.—The mechanisms by which OB protein controls food intake and energy balance are unknown. Therefore, we investigated the effects of a novel modified human recombinant OB protein (Mod-OB) on spontaneous feeding patterns, body weight, running wheel activity, and ovarian cycling in female rats. Mod-OB or vehicle was injected (4 mg·kg−1·day−1·sc) for 2 ovarian cycles (8 days) using a within-subjects design. Observations were continued for five ovarian cycles after injections; treatments were then reversed. Mod-OB reduced food intake 20% from injection day 1 to postinjection day 2. Body weight was reduced from injection day 3 to postinjection day 15 (maximum decrease, 25 ± 4 g, postinjection days 3 and 4). Food intake was reduced due to decreases in nocturnal meal size, which appeared to be superimposed on the normal pattern of spontaneous feeding (i.e., reductions in meal size at estrus). Mod-OB did not significantly affect diurnal food intake or meal patterns, failed to alter wheel running, and did not disrupt the rats’ ovarian cycles. We conclude that chronically administered Mod-OB reduces food intake in female rats by selectively affecting the mechanisms controlling meal size.

leptin; spontaneous meal patterns; satiety; feeding; obesity; estrus cycle; activity

UNDER MANY,ALTHOUGH NOT ALL, conditions, adults maintain remarkably stable levels of body weight and fat mass in the face of large fluctuations in energy intake and energy expenditure (6, 35). Kennedy (24) hypothesized that a “lipostatic” regulation of body fat mass results from a negative feedback control of feeding exerted by a circulating signal secreted in proportion to the degree of adiposity. Recently, OB protein, the product of the ob gene (42), has become the leading candidate signal for this lipostatic control of feeding. OB protein is expressed by adipocytes (44), and plasma OB concentration is an increasing linear function of fat mass (15). In rodents, several genetic models of obesity have been linked to a disruption in the OB signaling system. A non-sense mutation in the ob gene that results in secretion of no OB protein or a biologically inactive isoform produces the obesity phenotype of the ob/ob mouse (42), and mutations that result in defective OB protein receptors produce the nearly identical obesity phenotypes of the db/db mouse and the fa/fa Zucker rat (12, 13). The dependence of the ob/ob phenotype on insufficient OB protein was confirmed when chronic administration of recombinant OB protein normalized food intake and body weight in the ob/ob mouse (9, 22, 30). OB protein treatment also reversed the other components of the ob/ob phenotype, including decreased metabolic rate, hypothermia, decreased locomotor activity, and hypothalamic infertility (2, 10, 30). Reductions in food intake and body weight by exogenous OB protein have also been reported in lean mice (9, 25) and rats (36).

The mechanism by which OB protein controls food intake is not known. Because meal size and meal frequency are differentially affected by various test procedures (8, 33, 37), a necessary initial step in the analysis of OB protein’s feeding effect is to determine its influence on these parameters. In the accompanying paper (23), we reported that chronic administration of OB protein reduced meal size without affecting meal number in male Sprague-Dawley rats. In the present study, we examined the effects of chronic OB protein administration on spontaneous meal patterns in female Long-Evans rats. We chose the female rat model because of the dramatic effects on feeding produced by fluctuations in circulating estradiol, the prominent sex differences in human eating disorders and obesity, and the emerging sex differences in the physiology of OB protein (20, 21, 32, 40). For example, the function relating adiposity and plasma concentrations of OB protein is steeper in women than men (32). Furthermore, plasma OB concentrations are decreased in postmenopausal women who do not receive estrogen replacement (32). Thus, in the present study, we investigated the hypothesis that the OB signaling system may interact with the control of feeding exerted by estradiol, including the cyclic changes in food intake that are associated with the ovarian cycle in women and animals (4, 7, 20, 21).

METHODS

Subjects and Housing

Nine adult female Long-Evans hooded rats (Taconic Farms, Germantown, NY; weighing 270–335 g at study onset) were housed individually in Plexiglas cages (floor area 205–475 cm2; height 40–50 cm) with grated stainless steel floors and perforated lids. Five of the nine cages were connected to stainless steel running wheels (35 cm diameter) by a 5-cm Plexiglas tube (7 cm diameter). A Plexiglas feeding niche (8 × 9 × 13 cm) protruded from each cage ~4 cm above the floor. A circular opening (4.5 cm diameter) in the floor of each niche allowed access to a spill-resistant food bowl that was mounted on an electronic balance (EW 300, A&D, Tokyo, Japan; ± 0.1 g). Water bottles with sipper tubes containing ball bearings to
minimize spillage were clipped to the cages. Throughout the experiment, ground rat chow (no. 5001, Ralston Purina, St. Louis, MO), with 4.5% fat and a metabolizable energy content of 12.7 kJ/g, and tap water were available ad libitum. The room was maintained at 20 ± 2°C with a 12:12-h light-dark cycle (lights on 1300-0100). Six 34-W fluorescent ceiling lamps were lit during the light period and two red 40-W incandescent bulbs provided dim illumination during the dark period. A white noise generator (Lafayette Instruments, Lafayette, IN) masked extraneous noise, except from 0800 to 0930. Daily maintenance and all experimental procedures were done during this period, and rats remained undisturbed otherwise. Rats were adapted to the housing conditions and daily maintenance for at least 8 days before tests began.

Meal Patterns

Outputs from the balances were fed via an interface (Plus 8, Stargate Technologies, Solon, OH) into a computer (Dell 325D, Austin, TX) located in another room. A custom-designed program (VZM, Software Entwicklung Krügel, Munich, Germany) recorded the weight of each balance at 30-s intervals. A meal was defined as any feeding bout of at least 0.2 g that was separated from other feeding bouts by at least 15 min. Recorded meals accounted for 96% of total daily food intake.

Ovarian Cycles

Vaginal smears were taken daily by inserting a cotton swab moistened with warm 0.15 M saline ~1.5 cm into the vagina and making gentle contact with the vaginal walls. The sample was then transferred to an untreated microscope slide, fixed with alcohol (Surgipath Cytology Spray, Richmond, IL), and stained with hematoxylin and eosin (HHS-32). Microscopic examination of the sample (Olympus Provis AX, Tokyo, Japan; ×10–40 magnification) was used to label the phases of the ovarian cycle using standard criteria (26) before inspection of the feeding data. Day 1 of diestrus (D1) was characterized by leukocytes interspersed with small clusters of non-nucleated cornified cells and/or nucleated epithelial cells. Day 2 of diestrus (D2) was characterized by leukocytes and nucleated epithelial cells. Proestrus (P) was characterized by large clumps of round, nucleated epithelial cells, and occasional small clusters of cornified cells. Estrus (E) was characterized by large clumps of nonnucleated squamous cornified cells. The ovarian phases of the smears were assigned to the 24-h period ending at the time of sampling. As a result, E included the luteinizing hormone surge, ovulation, and the entire nocturnal period during which female rats are sexually receptive. All rats had regular 4-day ovarian cycles before study onset.

Procedure

Maintenance. Each day at 0800, the VZM system was halted and activity (number of revolutions in the running wheel), water intake (±0.1 ml), and food intake and any spillage (±0.1 g) were recorded for the preceding 22.5-h period. Food bowls were refilled daily: water bottles and tray liners spread under the cages were changed two or three times weekly. This was completed by 0900. Vaginal smears were then taken and body weights were measured, and, on test days, injections were administered. At 0930, the VZM system was restarted and the rats were left undisturbed until the following day.

Injections. Rats were adapted to single intracardicular subcutaneous injections of isotonic saline between 0915 and 0930 for one complete ovarian cycle beginning on D1 (baseline cycle). A modified recombinant human OB protein preparation (Mod-OB; Hoffman-La Roche, Nutley, NJ) and Mod-OB vehicle were then tested using a within-subjects design. Mod-OB was produced by the covalent linking of authentic recombinant human OB protein and polyethylene glycol. The result of polyethylene glycolation of several other proteins has been a prolonged half-life (18,28,29). In pharmacokinetic experiments, Mod-OB had a prolonged half-life in the serum of rats (>48 h). Mod-OB binds to and activates the long form of the OB protein receptor (OB-Rb or OB-Rb, in transfected cell lines and exhibits the same spectrum of biological activities as recombinant human OB protein in rats. Mod-OB was formulated into a stock solution in a 10 mM sodium acetate buffer, pH 4.5, containing 80 mM sodium chloride. The vehicle was identical except for the absence of Mod-OB.

Injection solutions of Mod-OB were prepared daily by diluting an aliquot of the stock solution of 10 mg/ml Mod-OB to 8 mg/ml with sterile bacteriostatic saline (Abbott Laboratories, North Chicago, IL). Vehicle was diluted in the same manner. All test injections began on D1. Initially, one-half of the rats from each activity group (access to running wheels or no access to running wheels) received single daily subcutaneous injections of 4 mg/kg Mod-OB (0.5 ml/kg of 8 mg/ml Mod-OB) for two consecutive ovarian cycles (8 days). We selected this dose on the basis of preliminary results indicating that 2 mg Mod-OB/kg was about the threshold dose for an inhibition of feeding under similar conditions. The dose of 4 mg/kg Mod-OB is similar to the doses used in the original studies demonstrating feeding effects in ob/ob mice (22,30). The remaining rats received daily subcutaneous injections of 0.5 ml/kg of vehicle. After a postinjection period of five ovarian cycles during which no injections were given, treatments were reversed. The second (crossover) injection period was again followed by a five-cycle postinjection period. The two injection cycles were labeled C1 and C2, and the five postinjection cycles were labeled C3-C7.

Data Analysis

Because we were interested in the possibility that OB protein interacts with ovarian hormonal rhythms to control feeding, data from each consecutive ovarian cycle (C1–C7) were analyzed separately for each dependent variable. Data were first analyzed by three-factor split-plot ANOVAs in which activity (running wheel access or no access) was the result of subjects factor and phase of the ovarian cycle (D1, D2, P, E) and drug treatment (Mod-OB, vehicle) were the within-subjects factors. Because these analyses revealed no significant interaction effects of activity, data were collapsed across this factor and reanalyzed using two-factor repeated-measures ANOVAs (phase of ovarian cycle by drug treatment). When significant effects were detected, differences between individual means were tested with Tukey’s honestly significant difference test. Differences were considered significant when P < 0.05. The standard error of the difference (SED) is reported as an estimate of experiment-wide residual variability. Data were analyzed with the SAS (PC-SAS; SAS, Cary, NC) or the BMDP (SOLO V6.0; SPSS, Chicago, IL) statistical package.

RESULTS

Baseline Measures

Data collected during the baseline cycle are displayed in Table 1. During E, body weight decreased, activity increased in rats with access to running wheels,
Table 1. Baseline body weight, food intake, water intake, spontaneous meal patterns, and activity during one ovarian cycle before vehicle and Mod-OB treatment

<table>
<thead>
<tr>
<th>Phases of the Ovarian Cycle</th>
<th>D1</th>
<th>D2</th>
<th>P</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>296 ± 6</td>
<td>298 ± 6</td>
<td>295 ± 6</td>
<td>294 ± 6†</td>
</tr>
<tr>
<td>Daily food intake, g</td>
<td>25.5 ± 1.3</td>
<td>24.9 ± 1.1</td>
<td>23.7 ± 1.5</td>
<td>19.9 ± 0.7*</td>
</tr>
<tr>
<td>Daily water intake, ml</td>
<td>54.8 ± 8.3</td>
<td>56.7 ± 6.7</td>
<td>53.5 ± 7.8</td>
<td>48.9 ± 4.8</td>
</tr>
<tr>
<td>Diurnal food intake, g</td>
<td>2.3 ± 0.9</td>
<td>2.9 ± 1.1</td>
<td>1.6 ± 0.5</td>
<td>2.6 ± 1.0</td>
</tr>
<tr>
<td>Nocturnal food intake, g</td>
<td>24.0 ± 2.4</td>
<td>23.0 ± 2.7</td>
<td>21.8 ± 2.7</td>
<td>17.8 ± 1.6‡</td>
</tr>
<tr>
<td>Nocturnal meal size, g</td>
<td>2.9 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>2.5 ± 0.4§</td>
</tr>
<tr>
<td>Nocturnal meal frequency</td>
<td>8.2 ± 0.8</td>
<td>6.4 ± 0.5</td>
<td>6.6 ± 0.7</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>Nocturnal intermeal interval, min</td>
<td>82 ± 8</td>
<td>108 ± 10</td>
<td>103 ± 12</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Activity, revolutions/day</td>
<td>2,753 ± 904</td>
<td>3,234 ± 1,406</td>
<td>3,747 ± 1,392</td>
<td>6,831 ± 2,227*</td>
</tr>
</tbody>
</table>

Data are means ± SE for 1 ovarian cycle before onset of daily injections of vehicle or recombinant modified OB protein (Mod-OB). Phases of the ovarian cycle are D1, diestrus day 1; D2, diestrus day 2; P, proestrus; and E, estrus. Daily food intake, meal pattern, and body weight data are for all 9 rats; other data are for 5 rats with access to the running wheels. *P < 0.05 vs. D1, D2, and P; †P < 0.01 vs. D2; ‡P < 0.05 vs. D1, and D2; §P < 0.05 vs. D1 and P; by Tukey’s honestly significant difference test after significant 1-way ANOVA.

and total daily and nocturnal food intake decreased due to a decrease in nocturnal meal size (all P < 0.05), as observed previously in Long-Evans rats (21). Throughout the experiment, daily injections of vehicle failed to disrupt these normal variations in behavior that are associated with the ovarian cycle.

Body Weight

Mod-OB treatment produced a long-lasting reduction in body weight (Fig. 1A). There were interactive effects of Mod-OB and phase of the ovarian cycle on body weight during C1 [F(3,24) = 5.46, P < 0.0001, SED = 0.5 g] and C2 [F(3,24) = 8.85, P < 0.001, SED = 0.5 g]. That is, the difference in body weight between Mod-OB- and vehicle-treated rats was more on P and E than on D1 and D2 during C1 and C2 (all P < 0.01). Body weight remained significantly reduced after the end of Mod-OB injections for four ovarian cycles, C3-C6 [F(1,8) = 7.32–18.09, all P < 0.01, SED = 0.6–1.0 g], with a significant difference between groups last detected on postinjection day 15 (i.e., P of C6). The maximum body weight reduction was 25 ± 4 g on postinjection days 3 and 4 (i.e., P and E of C3).

Food and Water Intakes

Chronic administration of Mod-OB protein significantly reduced daily food intake during C1 [F(1,8) = 33.26, P < 0.001, SED = 0.3 g] and C2 [F(1,8) = 23.58, P < 0.0001, SED = 0.4 g] (Fig. 1B). During this 8-day period, Mod-OB reduced food intake ~20% (range = 11–29%). Daily food intake was also reduced during C3 in rats treated previously with Mod-OB [F(1,8) = 9.39, P < 0.01, SED = 0.4 g]. The decreases between treatment groups were first significant on injection day 1 (i.e., D1 of C1) and last on postinjection day 2 (i.e., D2 of C1). During the period of reduced food intake (C1-C3), no interactions between Mod-OB and phase of the ovarian cycle were detected [F(3,24) = 1.07–1.49, NS], indicating that Mod-OB did not disrupt the usual rhythmicity of food intake associated with the ovarian cycle. That is, food intake was significantly reduced on E of C1-C3 compared with the other phases of the cycle.

Fig. 1. Chronic administration of recombinant modified OB protein (Mod-OB) reduced body weight and daily food intake in female rats. Data are mean body weight (A) and food intake (B) across 7 ovarian cycles (C1-C7). During C1 and C2, rats received single daily subcutaneous injections of 4 mg/kg Mod-OB. No injections were given during C3-C7. Chronic administration of Mod-OB produced a decline in body weight that reached a maximum at the end of C3 and then gradually recovered during C4-C6. Standard errors of the difference (SEDs) for the means displayed ranged from 0.5 to 1.0 g. Mod-OB significantly reduced 24-h food intake during C1-C3 and increased 24-h food intake during C4. Mod-OB’s inhibitory effects on food intake appeared to be superimposed on the normal cyclic changes in food intake that are associated with the ovarian cycle. SEDs for the means displayed ranged from 0.3 to 0.4 g. D1 and D2, days 1 and 2 of diestrus, respectively; P, proestrus; E, estrus. *Less than vehicle, P < 0.05. + Greater than vehicle, P < 0.05.
in both Mod-OB- and vehicle-treated rats (all P < 0.01). An interaction effect of Mod-OB treatment and ovarian phase was detected, however, during the second postinjection cycle, C4 [F(3,24) = 9.26, P < 0.001, SED = 0.4 g], when food intake was significantly increased on E in rats treated previously with Mod-OB (P < 0.05). No differences in daily food intake were observed during C5-C7.

Mod-OB affected water intake similarly. That is, daily water intake was reduced, 11% (range = 5–15%) during C1-C3 [F(1,8) = 5.94–9.61, all P < 0.05, SED = 0.7–1.0 ml] (data not shown).

Diurnal and Nocturnal Food Intakes

Rats ate 83% (range = 78–89%) of their food during the nocturnal period, and Mod-OB reduced nocturnal food intake (Fig. 2) similarly to total daily food intake (Fig. 1B). Nocturnal food intake was reduced during C1-C3 [F(1,7) = 8.39–17.91, all P < 0.01, SED = 0.4 g], and the typical suppression of food intake at E was similar in Mod-OB- and vehicle-treated rats (all P < 0.05). Nocturnal food intake was increased on P and E of C4 in Mod-OB-treated rats (all P < 0.05). No differences in nocturnal food intake were detected during C5-C7. Mod-OB appeared to affect diurnal food intake similarly, but these effects were not significantly different (data not shown).

Meal Patterns

The inhibitory effect of Mod-OB on nocturnal food intake was due entirely to a reduction in meal size (Fig. 3) with no change in meal frequency (Fig. 4). Mod-OB significantly reduced nocturnal meal size on 10 of the 13 days from the first day of treatment through postinjection day 5 (i.e., D1 of C4) [F(1,8) = 7.87–8.82, all P < 0.05, SED = 0.1–0.3 g] (Fig. 3). Mod-OB did not alter the cyclic reduction in meal size associated with E. Nocturnal meal size was increased on P of C4 and D1 of C5 after Mod-OB injections (all P < 0.01), but this may have been due to anomalously small meal sizes in the control group on these days. No changes in meal size were detected during C6 and C7. Mod-OB tended to reduce nocturnal meal duration but these differences were not significant (data not shown). No alterations in nocturnal meal frequency [F(1,8) = 0.08–2.57, NS, SED = 0.2–0.4 g] (Fig. 4) or intermeal interval (data not shown) were detected.

Activity

The cyclic pattern of daily activity associated with the ovarian cycle was not altered by chronic Mod-OB (Fig. 5). No significant differences were detected during
or after Mod-OB treatment [F(1,4) = 2.65–5.98, NS, SED = 246–265 revolutions/day].

Ovarian Cycles

Chronic administration of Mod-OB did not alter the appearance of the vaginal smears or change the length of the ovarian cycles. Three of the nine rats switched from 4-day to 5-day cycles during the postinjection period of the second half of the crossover design. We do not attribute this to Mod-OB treatment for three reasons. First, the ovarian cycle can lengthen spontaneously over time or with repeated handling (26). In our experiment rats were handled daily, and the changes in cycle length occurred near the end of the study. Second, a prolonged diestrus characterizes 5-day cycles, and this is what occurred in these three rats. Third, only one of these rats received Mod-OB injections during the part of the crossover design when the cycle lengthened. Therefore, we conclude that these three rats switched spontaneously to 5-day ovarian cycles independently of Mod-OB treatment. To allow statistical comparison between rats with 4- and 5-day cycles, the 3rd day of diestrus during the 5-day cycle (i.e., D3) was omitted from data analysis.

DISCUSSION

Chronic subcutaneous administration of Mod-OB, a novel modified form of recombinant human OB protein with a half-life >48 h, significantly decreased daily food intake and body weight by decreasing meal size without changing meal frequency in adult, cycling female Long-Evans hooded rats. There was no sign of tolerance to repeated administration of Mod-OB in these variables. Mod-OB treatment did not disrupt the normal cyclic variations in daily food intake, meal size, spontaneous activity, or vaginal cytology associated with the rat’s ovarian cycle. The present findings extend our demonstration that Mod-OB decreases food intake and body weight gain by selectively decreasing meal size in male Sprague-Dawley rats (23). These data are the first characterizations of the effects of chronic OB protein treatment on the organization of spontaneous feeding. Together, they suggest the hypothesis that a primary physiological function of endogenous OB protein secreted by the adult rat’s adipose tissue is to contribute a control of meal size that adjusts food intake so as to maintain a constant level of body adiposity. OB protein may be one of the long-sought lipostatic controls of feeding (24, 33).

It is unlikely that the reductions in food intake and body weight observed in the present study were due to a nonspecific action of Mod-OB. First, the reductions in meal size were selective and Mod-OB failed to reduce activity levels or the frequency of meals throughout the experiment. Second, the cyclic variations in food intake, meal size, activity, and vaginal cytology normally observed in female Long-Evans hooded rats were not affected by chronic Mod-OB treatment. Rather, we conclude that the inhibitory actions of Mod-OB on feeding are behaviorally and physiologically specific in female rats, as they appear to be in male rats (23).

Mod-OB’s inhibitory effect on meal size was clearer during the nocturnal period than the diurnal period, presumably because nocturnal food intake accounted for 80–90% of total daily food intake. Nocturnal meal size was decreased on the first day of Mod-OB treatment and, with three exceptions, on every day thereafter until 5 days after treatment ended. Nocturnal meal frequency did not change during this period. The sustained decrease in nocturnal meal size led to a significant decrease in total daily food intake and body weight by the 3rd day of treatment. The sustained decrease in nocturnal meal size after Mod-OB treatment ended may reflect the slow clearance of Mod-OB. A sex or strain difference in sensitivity to OB protein may also have contributed to Mod-OB’s inhibitory effect on nocturnal meal size. This is because the same regimen of Mod-OB decreased nocturnal meal size sooner (1st vs. 3rd day of treatment) and more persistently (5 vs. 3 days after treatment ended) in female Long-Evans rats than male Sprague-Dawley rats (23).

The selective inhibitory effects of chronic Mod-OB treatment on spontaneous meal size in male and female rats extend the accompanying report of Flynn et al. (17) that single intracerebroventricular injections of OB protein elicited dose-related decreases in nocturnal meal size with no change in nocturnal meal frequency. This suggests that both centrally and peripherally administered OB protein may decrease meal size by acting on the same central OB protein receptors (39). The site of such receptors is unclear. Blevins et al. (5) reported that acute intravenous injections of OB protein and acute injection of OB protein directly into the paraventricular nucleus of the hypothalamus each reduced meal frequency, but not meal size. It will require additional research to determine the relevance of these findings to the chronic effects of OB protein reported here.

The selective decreases in meal size elicited by chronic OB protein treatment correspond to the sponta-
neous meal patterns of rodents that are obese due to genetic lesions in the OB signaling system. That is, ob/ob mice, which lack biologically active OB protein, and fa/fa Zucker rats, which lack functional OB protein receptors, both display significantly increased meal size accompanied by partially compensatory decreases in meal frequency (38). We did not detect any compensatory change in meal frequency during chronic OB administration in normal male or female rats. It is not clear whether this represents a fundamental difference in the controls of decreased and increased meal size or is related to the magnitude or duration of the changes in the OB signaling system in genetically obese and normal rodents. The selective decreases in meal size produced by Mod-OB also correspond with the results of manipulation of adipose tissue mass on meal parameters. Changes in body adiposity often lead to compensatory over- or underfeeding, and these transient hyperphagic or hypophagic periods are typically caused by changes in meal size rather than meal frequency (16). The similarity of the changes in spontaneous feeding produced by the manipulation of adiposity, by genetic lesions of the OB signaling system, and by chronic OB protein administration is consistent with the hypothesis that OB protein is a lipostatic signal that relays information about the size of the adipose depot to brain mechanisms that are specific for the control of meal size. Whether the same is true for the other leading candidate lipostatic signal, basal insulin level (34), is less clear. This is because chronic intracerebroventricular infusion of insulin decreased meal frequency as well as meal size and significantly altered the circadian rhythm of feeding (27, 31).

Our results should facilitate analysis of the mechanism of OB protein’s feeding effects. The basic controls of meal size appear to be initiated by the action of food stimuli on preabsorptive receptors during meals. These have been called the direct controls of meal size (37). They include oropharyngeal flavor stimuli and gastrointestinal satiety signals mediated by gut peptides, such as cholecystokinin and glucagon. In contrast, controls of meal size exerted by stimuli that are not generated by ingested food during meals and do not act preabsorptively are indirect controls (37). As a tonic signal related to the mass of body fat, OB is an indirect signal and is likely to exert its action by modulating the potency of one or more direct controls. The identification of such interactions is a crucial issue for future investigation of the physiological mechanism of OB protein’s inhibitory effect on feeding.

In the present study, the maximal mean body weight loss by Mod-OB treatment was 25 g, which was reached on the 3rd day after injections ended. On the assumption that the energy content of adipose tissue is 30 kJ/g (19) and that the entire weight loss represented a decrease in adipose tissue mass, as appears to be the case when OB protein is administered to mice (22), then this weight loss would supply 750 kJ metabolizable energy (25 g × 30 kJ/g). The reduction in cumulative food intake to postinjection day 3 was 47 g of chow, or ~600 kJ metabolizable energy, which is not significantly different from 750 kJ (t(8) = 2.03, NS). Thus, although increased energy expenditure appears to contribute to OB protein’s effect on body weight in lean and obese mice (2, 22, 25) and also did so in our test of male rats (23), it did not appear to do so here. The cause of this will require further research.

OB protein may play a role in the control of reproductive function in rodents. For example, chronic administration of OB protein corrected the hypothalamic infertility of ob/ob mice (3, 10, 41); reduced the latency to vaginal opening, ovarian cyclicity, and sexual function in normal prepubertal mice (1, 11); and prevented the delay in vaginal estrus that is induced by starvation in adult mice (2). Chronic OB treatment in the present study, however, failed to induce any changes in the appearance of vaginal smears or the length of the ovarian cycle in normal ad libitum-fed rats.

Finally, chronic OB protein administration also failed to produce any dramatic effect on the behavioral rhythms associated with the ovarian cycle. That is, neither the decreases in meal size and total food intake, the increase in activity, nor the decrease in body weight that occur during E in most strains of rats (4, 7, 21) was disrupted during OB treatment. The normal decrease in daily food intake on E was significantly blunted, however, during C4, the second cycle after Mod-OB treatment (Fig. 1B). This may be an indication of some compensatory hyperphagia in the OB-treated rats. It would be interesting to determine whether more marked degrees of compensatory hyperphagia (16) interact more clearly with the ovarian cycle.

Perspectives

The mechanism by which OB protein controls food intake and energy balance is unknown. However, converging evidence suggests that OB protein secreted from adipose tissue exerts a negative feedback control of feeding that is proportional to the degree of adiposity. The present results demonstrated that chronic administration of OB protein reduced daily food intake and body weight by reducing meal size without affecting meal frequency in female rats, as it did in male rats (23). These studies provide the first evidence that chronic administration of OB protein reduces food intake and body weight in rats by selectively affecting the mechanisms controlling meal size. Because spontaneous meal size and meal timing are under differential controls (8, 37), our results should facilitate analysis of the mechanism by which OB protein controls food intake and energy balance.

Previous studies have reported sex-related differences in the physiology of OB protein (1, 2, 11, 32). In the present study, we found that the normal cyclic changes in activity, food intake, meal size, and vaginal cytology that are associated with the ovarian cycle in most rat strains were not affected by our regimen of OB protein treatment. These findings do not suggest that the potency of the OB signaling system varies across the ovarian cycle under the present experimental condi-
tions. However, the same regimen of OB protein treatment appeared to decrease nocturnal meal size sooner and more persistently here than under similar conditions in male rats (23). Furthermore, Mod-OB appears to stimulate metabolic energy expenditure in male rats (23) but not female rats. These differences indicate the importance of future analysis of the influences of sex differences on the physiological function of OB protein.

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