Obestatin acts in brain to inhibit thirst

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Obestatin acts in brain to inhibit thirst. Am J Physiol Regul Integr Comp Physiol 292: R637–R643, 2007. First published August 24, 2006; doi:10.1152/ajpregu.00395.2006.—Derived from the same prohormone, obestatin has been reported to exert effects on food intake that oppose those of ghrelin. The obestatin receptor GPR39 is present in brain and pituitary gland. Since the gene encoding those two peptides is expressed also in those tissues, we examined further the possible actions of obestatin in vivo and in vitro. Intracerebroventricular administration of obestatin inhibited water drinking in ad libitum-fed and -watered rats, and in food- and water-deprived animals. The effects on water drinking preceded and were more pronounced than any effect on food intake, and did not appear to be the result of altered locomotor/behavioral activity. In addition, obestatin inhibited ANG II–induced water drinking in animals provided free access to water and food. Current-clamp recordings from cultured, subfornical organ neurons revealed significant effects of the peptide on membrane potential, suggesting this as a potential site of action. In pituitary cell cultures, log molar concentrations of obestatin ranging from 1.0 pM to 100 nM failed to alter basal growth hormone (GH) secretion. In addition, 100 nM obestatin failed to interfere with the stimulation of GH secretion by GH-releasing hormone or ghrelin and did not alter the inhibition by somatostatin in vitro. We conclude that obestatin does not act in GH-releasing hormone or ghrelin and did not alter the inhibition by somatostatin in vitro, and we tested a wide range of concentrations of obestatin in rat anterior pituitary cell cultures for these potential actions. To verify the biologic activity of obestatin in rats, as had been described in mice (14), we examined the effects of centrally administered peptide on food intake and water drinking in fed and fasted male rats.

MATERIALS AND METHODS

Animals. All procedures have been approved by the Animal Care Committee of Saint Louis University or Queen’s University. Adult male rats (Harlan Sprague Dawley, Indianapolis, IN or Charles River, Quebec, Canada) were employed as tissue donors and for in vivo protocols. They were maintained (12:12-h light-dark cycle, lights on 0600, 23–25°C) with free access to food and water, unless otherwise indicated. In vitro studies. Dispersed anterior pituitary cell cultures. Rats (250–300 g) were killed by decapitation (9). Anterior pituitary glands were collected into minimum essential medium containing HEPES (20 mM), 1% penicillin-streptomycin (all obtained from Invitrogen, Carlsbad, CA), 0.1% BSA (Sigma, St. Louis, MO), and 0.1% trypsin (1:250, Difco, Detroit, MI) and mechanically dispersed until a single-cell suspension was obtained (37°C). Single-cell suspensions were aliquoted into 12 × 75-mm test tubes (~200,000 cells/tube) and incubated for 72 h at 37°C (room air) in Medium 199 (Sigma) containing 20 mM HEPES and 10% horse serum and 1% penicillin-streptomycin (both obtained from Invitrogen). On the day of experimentation, cells were pelleted by centrifugation (600 g, 10 min, room temperature). The medium was removed and replaced with test medium [Medium 199, 0.1% BSA, 20 mM HEPES, 1% penicillin-streptomycin, and 2.5 mM bacitracin (Sigma)] alone or medium containing log molar concentrations (1.0 pM to 100 nM) of obestatin or obestatin in combination with 1.0, 10, or 100 nM GH releasing hormone (GHRH) or ghrelin. In additional cultures, the effects of obestatin on the GH-release inhibiting effects of 0.01, 0.1, or 1.0 nM somatostatin was examined. All peptides were obtained from Phoenix Pharmaceuticals (Belmont, CA). Incubations lasted 60 min and were terminated by centrifugation and collection of medium for subsequent determination of GH content by radioimmunoassay, as previously described (10). The effect of obestatin alone on basal GH secretion was examined in six cell harvests/experiments. Experiments examining the interactive effects of obestatin with GHRH, ghrelin, or somatostatin were repeated in two separate cell harvests. Data are presented from one representative harvest/experiment. Data were analyzed by ANOVA with Scheffé’s multiple comparison testing.

Dispensed subfornical organ (SFO) neuron cultures. Rats (100–250 g body wt) were decapitated, the tissue containing the SFO was quickly isolated, and the SFO was microdissected free. SFO tissue

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was incubated for 30 min at 30°C in 2 mg/ml papain (Worthington, Lakewood, NJ), washed twice with Hibernate/B27 (Invitrogen), and then underwent three rounds of trituration with the supernatant removed following each round of trituration. The collected supernatant was then centrifuged for 4 min at 200 g, the resulting supernatant discarded, and the pellet was resuspended in 2 ml of Neurobasal media (Invitrogen) supplemented with B27 and glutamine. The cell suspension was then plated onto glass-covered 35-mm culture dishes and allowed to settle for 1 h in a 5% CO2 incubator at 37°C after which 2 ml of media was added to each dish. SFO neurons prepared in this way have been demonstrated to express ion channels and peptide receptors similar to those described for SFO neurons in vivo (2).

After 4–7 days in culture, SFO neurons were used for electrophysiological experiments. Culture plates were transferred to the stage of an upright microscope (Nikon) equipped with infrared diffusion interference contrasting optics, and viable cells were viewed on a video monitor with aid of a video camera (Dage, Michigan City, IN). Neurons were continuously perfused with artificial cerebrospinal fluid (ACSF) comprising (in mM): 140 NaCl, 5 KCl, 1 MgCl2, 10 glucose, 10 HEPES, and 2 CaCl2. An inline heater was used to heat the ACSF to between 30 and 32°C. Whole cell recordings in the current clamp mode were made by using a Multiclamp 700B (Molecular Devices, Sunnyvale, CA), and data acquired using a Micro 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge UK). Digitization rate was 5 kHz, and signals were filtered at 10 kHz. The microelectrodes used, when filled with (in mM): 130 K-gluconate, 2 MgCl2, 1.1 EGTA, 10 KCl, 2 NaATP, and 10 HEPES (pH 7.2) had resistances of between 3 and 7 MΩ. Obestatin (Phoenix Pharmaceuticals, Belmont, CA) was dissolved in distilled water and stored at −20°C until just prior to use when it was diluted into ACSF. Application was through the bath perfusion and a lag of ~50 s existed between the start of obestatin perfusion and the first sign of its action. Neurons were classified as responsive to the peptide if the membrane potential deviated from baseline by more than two SDs of the mean baseline noise measured over a 50-s interval prior to peptide application. Statistical analysis of the effects of obestatin on membrane potential were via one-way ANOVA with Newman-Keuls multiple comparison post hoc test done using GraphPad Prism (San Diego, CA).

In vivo experiments. Under ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA)/xylazine (TranquiVed, Vedco St. Joseph, MO) anesthesia (60 mg · 8 mg mixture −1 · ml −1, 0.1 ml/100 g body wt, ip injection) rats were placed in a stereotaxic device and a 23-gauge, stainless steel cannula (17 mm) implanted into the right lateral cerebroventricle, as previously described (12). Rats were allowed to recover to presurgery weights, a minimum of 5 days prior to experimentation. Placement and patency of the lateral ventricular cannula were verified by the dyespecific response to intracerebroventricular administration of ANG II (50 pM ANG II) at the end of all experiments.

To examine the effect of obestatin on food intake, rats (250–300 g body wt) were habituated to metabolic cages (Nalgene; Harvard Apparatus, Holliston, MA) for a minimum of 4 days with free access to pelleted lab chow (Rodent A/I diet; Research Diets, New Brunswick, NJ) and tap water (11). Daily food and water intakes and body weights were recorded. Two protocols were conducted. In the first, animals were denied food and water for 18 h prior to receiving an injection at 1000 via the indwelling catheter of saline vehicle (2 μl, sterile 0.9% NaCl) or vehicle containing 1.0 or 3.0 nM obestatin. Twenty minutes later, food cups and water bottles were replaced to the cages and intakes were monitored at 30-min intervals until 2100 and again the next day beginning at 0400 and continuing until 0700.

Twenty-four-hour food and water intakes were also recorded, as were body weights.

The effect of obestatin on pharmacologically driven thirst was examined in ad libitum-fed and -watered rats (0900–1000). Ten minutes prior to the intracerebroventricular administration of a dispogenic dose of ANG II (50 pM in 2 μl, sterile 0.9% NaCl) animals were administered 2 μl saline vehicle or vehicle containing 0.3, 1.0, or 3.0 nM obestatin. Water bottles were returned to the cages immediately following ANG II administration. Food was present at all times. Water intakes were recorded 5, 15, 30, and 60 min following ANG II administration.

To determine whether centrally administered obestatin caused changes in locomotor activity that might contribute to any effects of food or water intake, a separate group of animals was studied in the Opto-Max Activity Monitor (Columbus Instruments, Columbus, OH). Animals were moved to the recording room and allowed to rest for a minimum of 1 h in their home cages. Individual animals were then placed in the recording chamber and left undisturbed for 1 h during which time behavior was recorded. Following this habituation period, either saline vehicle (2 μl, sterile 0.9% NaCl) or saline vehicle containing 3.0 nM obestatin was injected intracerebroventricularly, and recording continued for 2 h. Data were collected in intervals of 1 min and grouped into 15-min averages.

Differences between groups or within groups across time (in vivo experiments) were determined by ANOVA with Scheffé’s multiple comparison testing. In experiments with only two experimental groups, the independent Student’s t-test was employed. An outcome with a probability of <5% was considered significant. All data are presented as means ± SE.

RESULTS

Log molar concentrations of obestatin ranging from 1.0 pM to 100 nM failed to significantly alter basal GH release from dispersed pituitary cells (Table 1). In addition, the stimulatory actions of GHRH and ghrelin and the inhibitory action of somatostatin were not altered by coincubation with 100 nM obestatin (Table 2).

Animals deprived of food and water for 18 h displayed a robust food appetite and thirst following central administration of saline vehicle (Fig. 1, A and B). Animals administered 1.0 nM obestatin icv 10 min prior to return of the water bottles and food cups drank significantly less water during the initial 15 (P < 0.001)- and 30 (P < 0.05)-min interval compared with saline vehicle-injected controls (Fig. 1A). The inhibitory effect of obestatin on water consumption was longer lasting in animals administered 3.0 nM icv peptide. These animals consumed significantly less water, compared with controls, at 15 (P < 0.001); 30 and 45 (P < 0.01); and 60, 90, and 120 min

Table 1. Failure of obestatin to significantly alter growth hormone release from dispersed anterior pituitary cells harvested from male rat donors

<table>
<thead>
<tr>
<th>Obestatin Treatment, nM</th>
<th>GH Released, ng/ml</th>
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<tbody>
<tr>
<td>0</td>
<td>28.7 ± 2.4</td>
</tr>
<tr>
<td>0.001</td>
<td>32.1 ± 5.0</td>
</tr>
<tr>
<td>0.01</td>
<td>29.2 ± 1.6</td>
</tr>
<tr>
<td>0.1</td>
<td>31.6 ± 2.6</td>
</tr>
<tr>
<td>1.0</td>
<td>30.8 ± 3.3</td>
</tr>
<tr>
<td>10</td>
<td>28.2 ± 3.0</td>
</tr>
<tr>
<td>100</td>
<td>28.9 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. GH, growth hormone.
(P < 0.05) following water bottle return to the cages (Fig. 1A). At no time point following return of the food cups to the cages (P < 0.05) were returned to the cages. *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline vehicle-injected controls. 

In rats provided tap water and chow at all times, drinking behavior normally observed prior to and immediately following lights out was significantly inhibited by intracerebroventricular administration of obestatin 2 h before lights out (Fig. 2A). Cumulative water intakes 120, 150, and 180 min after water bottle reinstatement, which occurred 10 min after obestatin or vehicle injection intracerebroventricularly, were significantly less than controls in animals administered 3.0 nM obestatin (P < 0.01). Thereafter, significant differences in cumulative water intakes were observed in both the 1.0 and 3.0 nM obestatin treatment groups. Cumulative water intakes remained significantly depressed compared with controls for the next four sampling intervals (210, 240, and 270 min: 1.0 nM obestatin or 3.0 nM obestatin treatment groups). Cumulative water intakes in the 1.0 and 3.0 nM obestatin treatment groups remained depressed thereafter (Fig. 3, F1,5,52 = 6.82, P < 0.001) and were significantly different (P < 0.05) than control at 24 h. When actual intakes during each 30-min sampling interval were analyzed between groups during the final sampling periods before and 1 h following lights on (0400–0700, day 2), no significant differences among groups were detected. Thus the inhibitory effect of obestatin on water drinking was expressed only during the first 4–5 h after intracerebroventricular administration. These same animals consumed less food during the same time intervals; however, at no time point did these differences attain significance. (Figs. 2B and 3). There were no significant differences in body weights between groups prior to the testing interval; however, while animals in the saline vehicle treatment group gained weight, those in the 1.0- and 3.0-nM obestatin treatment groups lost weight over the 24-h testing period (Fig. 4). This loss of body weight attained significance (F3,52 = 4.88, P < 0.01) in the 3.0 nM obestatin treatment group (P < 0.01, vs. saline vehicle-injected controls).
The observation that obestatin inhibits ANG II-induced thirst, which has been demonstrated to occur as a result of ANG II actions at the SFO led us to specifically examine whether obestatin exerted direct effects on SFO neurons. Current-clamp recordings from cultured SFO neurons \( (n=46) \) demonstrated that nanomolar concentrations of obestatin \( (10^{-10} - 100 \text{ nM}) \) resulted in the depolarization of 38% of the cells tested by \( 6.6 \pm 1.4 \text{ mV} \) (Fig. 6A). Depolarization was seen to be reversible in many of the cells on washing, especially at the lower concentration. Furthermore, in 42% of the cells, a hyperpolarization was observed, with a mean amplitude of \(-7.5 \pm 2.1 \text{ mV} \) (Fig. 6B). The remaining 20% of cells did not show any clear changes in membrane potential in response to obestatin (Fig. 6C).

In a final experiment, the effect of obestatin on locomotor activity was examined in ad libitum-fed and -watered rats. Testing was conducted during the light phase between 0900 and 1400. No significant effects of central administration of 3.0 nM obestatin were observed (compared to saline vehicle-administered controls) at any time point. Variables analyzed included total behavioral activity, ambulatory activity, ambulatory time, stereotypy bursts, stereotypy time, margin time, margin distance, rearing counts, rearing time, plane breaks, and total distance traveled. The data for total behavioral activity, ambulatory activity, and stereotypy bursts are presented in Table 3.

DISCUSSION

Although the receptor for obestatin GPR39 is expressed in rodent pituitary gland (14), we, like Hsueh and colleagues (14), did not observe any significant effect of obestatin on basal GH release from cultured anterior pituitary cells. In addition, we report here for the first time the failure of obestatin to interact with two known stimulators (GHRH and ghrelin) and an inhibitor (somatostatin) of GH release from the anterior pituitary gland. Thus, unlike ghrelin, a product of the same prohormone, for which there are unique receptors in the pituitary

![Figure 2](http://ajpregu.physiology.org/)

**Fig. 2.** Effect of intracerebroventricular administration of saline vehicle or vehicle containing obestatin on water consumption (A) and food intake (B) in animals provided ad libitum access to food and water. Water bottles and food cups were removed just prior to the intracerebroventricular injections and then returned to the cages 10 min later. (ANOVA between groups, \(*P < 0.05, **P < 0.01, ***P < 0.001;\) see text for results of Scheffé’s multiple comparisons).

![Figure 3](http://ajpregu.physiology.org/)

**Fig. 3.** Cumulative food and water consumptions over the 24-h-period following intracerebroventricular administration of saline vehicle or vehicle containing obestatin under ad libitum drinking and feeding conditions (same animals as displayed in Fig. 2). N.S., nonsignificant; \(*P < 0.05, \) vs. saline vehicle-injected controls (ANOVA with Scheffé’s multiple comparisons).

![Figure 4](http://ajpregu.physiology.org/)

**Fig. 4.** Body weight changes over the 24-h-period following intracerebroventricular injection of saline vehicle or vehicle containing obestatin under ad libitum drinking and feeding conditions (same animals as displayed in Figs. 2 and 3). **P < 0.01, vs. saline vehicle-injected controls (ANOVA, with Scheffé’s multiple comparisons).
gland (4), obestatin does not appear to exert significant effects on the secretory activity of somatotrophs, at least in the in vitro setting.

To be sure that our failure to observe significant effects of obestatin in pituitary cell cultures was not due to inactivity of the commercially obtained peptide, we attempted to repeat the anorexigenic results obtained in mice by Zhang et al. (14) in our rat feeding model using similar peptide doses. Thus, initially, we restricted from food and water our rats overnight and tested two doses of obestatin administered intracerebroventricularly on food and water intake. To our surprise, obestatin, in doses that have been employed in rats for other orexigenic and anorexigenic peptides (7), significantly altered water but not food consumption in these animals. In rats, an 18-h period of food and water deprivation is a strong stimulus for subsequent drinking and eating. It is possible that the intensity of the stimulus may have obviated any pharmacologic effects of the administered peptide on food intake. Indeed, animals in all three treatment groups displayed a robust appetite for food in the first 15 min of testing (saline controls, 2.23 ± 0.10 g food; 1.0 nM obestatin, 2.58 ± 0.23 g; 3.0 nM obestatin, 1.98 ± 0.23 g). Thus we employed a second protocol to examine the potential effect of obestatin on ad libitum drinking and eating.

In our colony, we have observed and characterized a period of eating and drinking that precedes the onset of darkness (lights out). Therefore, we administered obestatin just prior to this endogenous rhythm of drinking and eating and monitored water drinking and food consumption into a point in the dark phase when the initial behaviors of feeding and drinking plateau. In this model of ad libitum feeding and drinking, obestatin exerted significant, inhibitory effects on water drinking. Food intakes were also diminished, but these changes did not attain significance. Thus it appeared that obestatin did possess biologic activity but that the effect on food intake might be secondary to an initial action to inhibit thirst, a
phenomenon referred to by some authors as dehydration anorexia (13). Future studies can test this directly by monitoring plasma osmolalities during the testing period to determine whether the decreases in water intake in these animals are reflected by physiological manifestations that may then explain the decreases in total food consumed, and perhaps even in the ad libitum-fed and -watered animals, the decreased body weight at 24 h.

Ad libitum-fed and -watered animals consume little food and water during the initial hour of the light phase, and the drinking response to ANG II is a well characterized pharmacologic approach to the study of thirst mechanisms (1). We observed a rapid and significant drinking response to ANG II in saline vehicle pretreated rats. A dose-related inhibition of ANG II-induced drinking was observed in rats pretreated with increasing doses of obestatin. Since the peptide was administered intracerebroventricularly, we could only speculate on the potential sites of action of obestatin to inhibit ANG II-induced water drinking; however, peptides administered into the lateral cerebroventricle have ready access to the SFO located at the confluence of the lateral ventricles and the third cerebroventricle adjacent to the foramina of Monroe. The SFO is an important site for the expression of the dipsogetic action of ANG II (1), and we hypothesized that this circumventricular organ may also be the site of obestatin’s action to inhibit ANG II-induced thirst. Indeed, the message for the obestatin receptor GPR39 has recently been detected in isolated SFO tissue (D. Murphy and A. V. Ferguson, unpublished observation).

Our electrophysiological experiments demonstrated significant membrane effects of obestatin in cultured SFO neurons. Both hyperpolarizing and depolarizing effects were observed, effects that suggest differential action on separate, functionally distinct subpopulations of SFO neurons. We did not expose these neurons to other peptides during the cultures reported here; however, their responses to obestatin are quite similar to those we have reported previously for cultured SFO neurons exposed to ANG II (2) or ghrelin (8). Indeed, we have reported that similarly cultured SFO neurons respond to ANG II exposure (10 to 1,000 nM) with a mean depolarization of 18.3 ± 2.0 mV (41 out of 65 cells tested). In addition, cultured SFO neurons responded to ghrelin (1.0 nM) with a mean depolarization of 7.4 ± 0.69 mV (9 of 31 cells tested).

It is premature to speculate on the phenotypic identity (chemical or anatomical) of each population of responsive neurons. Future studies will be necessary to address this issue, as well as to examine the membrane events underlying such actions of obestatin on SFO neurons. At this time, our hypothesis that obestatin inhibits thirst when administered into the lateral cerebroventricle of conscious rats by an action in the SFO appears to have support from our in vitro studies. Additional sites of action are possible, and only site-specific microinjections in concert with the mapping of immediate early gene expression profiles in response to obestatin administration will allow us to further identify the exact GPR39 expressing neuronal population in which the peptide may act to inhibit water drinking.

Pan et al. (6) reported that obestatin does not readily cross the blood-brain barrier in appreciable amounts, and thus, if the peptide produced in the gut (5) were to have significant effects on food intake and water drinking, two potential routes of action could be hypothesized. One would be via actions on neural afferents to brain stem centers important in conveying peripheral information to appetite and thirst centers in brain, e.g., vagal afferents to the nucleus tractus solitarius and adjacent brain stem structures. Certainly, anorexigenic effects observed following intraperitoneal administration of obestatin by Zhang et al. (14) may be the result of peripheral actions of the peptide. We have not administered obestatin peripherally in our protocols, and it remains possible that more profound inhibitions of food and/or water intake would result from such routes of peptide administration. The alternative mode of access to brain centers important in appetite and thirst regulation would be through a circumventricular organ where access to the peptide’s receptors could be gained. We hypothesize that circulating obestatin, or obestatin of central origin, may exert a physiologically relevant action on thirst mechanisms via such a route.

It is possible that an action of obestatin on food intake, unlike the effect on water drinking, is expressed at tissue sites distant from the cerebroventricles and that significant inhibitory effects would be observed only with higher doses given intracerebroventricularly or tissue-specific injections. Indeed, Zhang et al. (14) did report anorexigenic actions of the peptide when administered intracerebroventricularly in mice. The ex-

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Total Behavioral Activity (beam breaks/interval)</th>
<th>Behavior Monitored Ambulatory Activity (horizontal beam breaks/interval)</th>
<th>Stereotypy Bursts (No./interval)</th>
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<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Obestatin</td>
<td>Saline</td>
</tr>
<tr>
<td>0–15 min</td>
<td>231 ± 113</td>
<td>307 ± 153</td>
<td>150 ± 80</td>
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<tr>
<td>15–30 min</td>
<td>122 ± 72</td>
<td>50 ± 21</td>
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<tr>
<td>30–45 min</td>
<td>410 ± 138</td>
<td>683 ± 194</td>
<td>298 ± 109</td>
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<td>45–60 min</td>
<td>39 ± 13</td>
<td>126 ± 66</td>
<td>16 ± 7</td>
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<td>60–75 min</td>
<td>47 ± 16</td>
<td>58 ± 18</td>
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<td>135–150 min</td>
<td>34 ± 19</td>
<td>51 ± 16</td>
<td>15 ± 3</td>
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Data are means ± SE analyzed by independent t-test; n = 8 per group. Treatments occurred after the second observation interval at 30 min. No outcomes were observed at P < 0.05.
ant regional distribution of GPR39 expression in brain has not yet been extensively characterized; however, once those sites are identified, site-specific injections should be conducted in these behavioral models. In the end, the difference between our results and those of Zhang et al. (14) may be simply due to the species employed, such that in the rat inhibition of thirst takes precedence, whereas in mice, obestatin primarily affects appetite or metabolic state.

Obestatin may inhibit water drinking by three potentially indirect mechanisms. First, the intracerebroventricular injection of the peptide may reduce locomotor activity and, therefore, result in fewer approaches to the water bottle. However, since food consumptions did not differ among groups, particularly at the early time points following intracerebroventricular injections when the food cups were being accessed, this is unlikely. Second, since no behavioral changes in locomotor or stereotypy activity were observed in the behavioral testing arena, again a general effect to decrease motor activity is unlikely to be the cause of the decreased water drinking. Third, obestatin may act in brain to elevate sympathetic tone, as do other peptides that activate SFO neurons (e.g., ANG II), and the resulting increase in arterial pressure then may be the cause for the decreased water and food consumptions. However, in a preliminary trial, we failed to observe any significant action of 3.0 nM obestatin administered intracerebroventricularly in conscious, unrestrained male rats on mean arterial pressures or heart rates, even though these animals did respond to ANG II (data not shown).

Does this mean that obestatin is not, as hypothesized in the remarkable discovery report by Hsueh and colleagues (14), an important factor in the regulation of food intake? No. Our data add to the potential mechanisms by which this newly described peptide may regulate energy homeostasis. To be sure, our observation that ad libitum-fed and -watered animals administered obestatin intracerebroventricularly gained less weight, even lost weight, over the ensuing 24-h period compared with controls, argues in favor of the hypothesis advanced by Zhang et al. (14). The fact that cumulative, 24-h food intakes did not differ in these animals, while water intakes were significantly inhibited in rats treated with the two higher doses of peptide might suggest that the weight losses observed in those two groups were solely due to decreased fluid intake. If this were the case, then plasma osmolalities during the testing period should have been elevated in those two groups. If they were not, then an alternative mechanism should be examined as the underlying reason for the attendant weight loss, for instance, a potential catabolic effect of obestatin. The mechanisms for the antidiipsogenic actions of obestatin and the physiological relevance of those observations now deserve close attention.

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GRANT

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