

Preproghrelin-derived Peptide, Obestatin, Fails to Influence Food Intake in Lean or Obese Rodents

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Abstract

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Objectives: Obestatin has been initially characterized as a new peptide derived from the ghrelin precursor, which suppresses food intake and inhibits the orexigenic and prokinetic actions of ghrelin when injected peripherally or centrally in lean mice. However, reproducing these data remains controversial. Reasons for the disparity may be the use of different doses, routes, and animal models. We aimed to investigate the effects of peripheral and intracisternal (IC) injection of obestatin on feeding, gastric motility, and blood glucose in rats as well as in diet-induced obese (DIO) mice.

Research Methods and Procedures: Food intake and gastric emptying of a semi-liquid caloric meal were measured after intraperitoneal (IP) injection of obestatin in rats and DIO mice. Gastric phasic motility and blood glucose were monitored in urethane-anesthetized rats after IC or intravenous (IV) injection of obestatin.

Results: Obestatin injected intraperitoneally at doses ranging from 0.1 to 3 mg/kg influenced neither acute food intake nor gastric emptying in rats. Obestatin injected intravenously at 0.3 or 3 mg/kg and IC at 7.5 or 30 μ g/rat modified neither fasted gastric phasic motility nor blood glucose levels, while ghrelin (30 μ g/kg, IV) increased and vagotomy suppressed gastric motility, and an oligosomatostatin analog (3 μ g/rat, IC) decreased blood glucose. Obestatin, injected intraperitoneally (0.3 mg/kg) in DIO mice, did not alter feeding response to a fast, while urocortin 1 (10 μ g/kg, IP) induced a 73.3% inhibition at 2 hours.

Discussion: Our data demonstrate that peripheral administration of obestatin did not modify food intake in rats or obese mice or gastric motor function in rats.

Key words: ghrelin, gastric motility, high-fat diet, glucose, gut-brain axis

Introduction

The prevalence of obesity is increasing dramatically (1) and so is the discovery of molecular mechanisms through which food intake and body weight are regulated (2,3). In this context, ghrelin is an acylated 28-amino acid peptide produced by the endocrine cells of the oxyntic mucosa of the stomach that is derived from the post-translational processing of preproghrelin (4). Its ability to increase food intake, and to exert an important role in the regulation of weight gain in rodents and humans has been extensively characterized over the past years, along with its prokinetic action on gastric motor function (5–7). Recently, Zhang et al. characterized a 23-amino acid peptide derived from preproghrelin processing (proghrelin 53–75) that was named obestatin to denote its food-intake- and body-weight-reducing actions through binding to GRP39 receptors (8). The food intake effect was observed when obestatin was injected intraperitoneally or intracerebroventricularly in doses ranging from 0.02 to 2.5 mg/kg in lean mice (8).

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Obestatin was also initially reported to delay gastric emptying under these conditions of administration and to counteract ghrelin's orexigenic and prokinetic effects in mice (8). However, the majority of subsequent studies (9–15) did not reproduce the initial report (8). Recent evidence indicates that obestatin is rapidly degraded after peripheral injection in mice as shown by the lack of intact obestatin at 20 minutes after intravenous (IV)¹ injection and the 85% disappearance of the intact peptide within 10 minutes (16). As some of the negative studies (9,11,14) were performed using the lowest effective dose of obestatin (0.3 mg/kg), this may explain the lack of anorexic response as was initially reported (8). Therefore, in the present study, we investigated whether high doses of obestatin (i.e., superior to the highest dose tested in mice) injected peripherally or intracisternally influence food intake, gastric emptying, gastric motility, and blood glucose in rats. In addition, we tested the ability of intraperitoneal (IP) obestatin to influence food intake in diet-induced obese (DIO) mice, which, to our knowledge, has not been investigated. These studies were performed in parallel with control substances known to influence food intake (urocortin 1; Ucn 1) (17), to inhibit (vagotomy) or enhance (peripheral injection of ghrelin) gastric motility (18,19), and to decrease blood glucose [intracisternal (IC) injection of Des-AA^{1,2,4,5,12,13}-[D-Trp⁸] somatostatin (ODT8-SST)] (20).

Research Methods and Procedures

Animals

Male Long-Evans (Harlan, Indianapolis, IN) and Sprague Dawley (SD) rats (Harlan, San Diego, CA) or adult male DIO mice (C57BL/6; Harlan, San Diego, CA) were housed individually and maintained under controlled conditions of temperature (20 °C to 24 °C) and illumination (12 hours light:12 hours dark). Rats were fed ad libitum with standard rodent chow (Purina 5001 chow; Ralston-Purina, St. Louis, MO) and tap water. Diet-induced obesity was achieved by feeding lean mice for 3 months with high fat chow containing 20% protein, 20% carbohydrate, and 60% fat (D12492; Research Diet, Inc., New Brunswick, NJ). All animal experiments were approved by the Animal Care Committees (Eli Lilly and Co. and Veterans Administration, Animal Component of Research Protocols 99-127-07 and 03-008-05) and conducted in accordance with principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

¹ Nonstandard abbreviations: IV, intravenous; IP, intraperitoneal; DIO, diet-induced obese; Ucn 1, urocortin 1; IC, intracisternal; ODT8-SST, Des-AA^{1,2,3,4,12,13}-[D-Trp⁸] somatostatin; SD, Sprague-Dawley; IGP, intra-gastric pressure; AUC, area under the curve; SE, standard error; ICV, intracerebroventricular.

Peptides and Treatments

The human and rat/mice obestatin were synthesized as the C-terminal amide using standard automated 9-fluorenylmethoxycarbonyl/t-butyl (Fmoc/tBu)-based chemistry on Rink amide support. The peptide resin was cleaved and de-protected in trifluoroacetic acid in the presence of thiol scavengers and precipitated with diethyl ether. The crude peptides were purified by reverse-phase high-performance liquid chromatography using a C-18 preparative column. The purified peptides were analyzed by analytical high-performance liquid chromatography and mass spectrometry. ODT8-SST (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA), an octapeptide analog of somatostatin (20,21), rat Ucn 1 (Clayton Foundation Laboratories) in powder form, and human octanoylated ghrelin (University of Montreal, Montreal, Canada) aliquots (1 µg/µL aliquots) were stored at -80 °C. Immediately before use, obestatin and ODT8-SST were dissolved in saline (0.9% NaCl, sterile, pH 5; Sigma Chemical Co., St. Louis, MO) and Ucn 1 was dissolved in distilled water. Ghrelin was diluted in saline (0.9% NaCl).

IV injections were made at 0.1 mL/rat followed by a 0.1 mL flush through an IV catheter (Intramedics PE-90 polyethylene tubing, 0.86 mm ID, 1.27 mm OD, and 8 cm length) implanted into the right external jugular vein of rats anesthetized with 25% urethane (1.5 g/kg IP). IC injections were performed through an IC cannula positioned as previously described (22). Briefly, rats were anesthetized with 25% urethane (1.5 g/kg, IP) and placed in a stereotaxic instrument (Model 1404, David Kopf Instruments, Tujunga, CA) with the head oriented at a 45° angle (nose down) to the horizontal plane. A catheter (Intramedics PE-10 polyethylene tubing, 0.28 mm ID, 0.61 mm OD, 4 cm length) was introduced into the cisterna magna and secured to the occipital brain membrane with instant Crazy glue. Successful cannulation was verified by the appearance of clear cerebrospinal fluid. The end of the catheter was connected to a Hamilton microliter syringe and IC injections were made in a volume of 10 µL/rat followed by 10 µL of flush with saline (0.9% NaCl). The IP injections were performed in conscious animals in 0.3 or 0.5 mL/rat and 0.1 mL/mouse.

Measure of Food Intake

The food intake was monitored as in our previous studies in rats or mice (9). Single housed animals were given ad libitum, pre-weighed rodent chow from 9:00 AM to 10:00 AM. At various time-points, the remaining food and spillage collected on papers placed at the bottom of the animal cages were weighed (± 0.01 g). Food intake was calculated as the difference between the food weight before and after the feeding period at each time-point, and cumulative food intake by adding the values at the different time-points.

Measure of Semi-Liquid Gastric Emptying

The gastric emptying rate was measured according to the modified method of Francis et al. (23). Briefly, the semi-liquid diet consisted of the following ingredients mixed in a blender: 240 mL distilled water, 20 g methyl cellulose (Sigma, M7140), 16 g casein (Sigma, C707), 8 g cornstarch (Sigma, S4126), 8 g sucrose (Sigma, S7903), and 10 mL intralipid 20% (Sigma, IM1). Conscious rats were given 3 mL of semi-liquid diet by orogastric gavage and, 30 minutes later, animals were killed by CO₂ inhalation followed by decapitation. Stomachs were clamped at pylorus and cardiac sphincters and were removed and weighed before and after removal of stomach contents. Gastric emptying rate was measured as: % in 30 minutes = $100 \times (1 - [\text{Gastric Content}/\text{Food Given (3 mL)}])$.

Measure of Gastric Motility

The intra-gastric pressure (IGP) was monitored in rats anesthetized with 25% urethane (1.5 g/kg, IP), followed by additional IP injection of 25% urethane (≤ 0.2 mL) to maintain surgical anesthesia. Animals were tracheally cannulated for breathing, and a cannula was inserted into the jugular vein to allow continuous IV infusion of 0.4 mL/h sterile saline to maintain hydration and for IV peptide injection. In some experiments, an IC catheter was implanted as described above. Then, a catheter pressure transducer (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX) was inserted orally into the stomach through a stainless steel gavage tube and was used to record IGP. The transducer was pushed 1 cm distal to the lower esophageal sphincter, which was localized using the real time pressure monitoring. The intra-gastric placement of the transducer was verified at the end of the experiment by opening the stomach. The pressure transducer was connected to a preamplifier (model 600; Millar Instruments), the signal was then amplified using a transducer amplifier (TBM4; World Precision Instruments, Boca Raton, FL) and was acquired via a Micro1401 A/D interface (Cambridge Electronic Design Ltd., Cambridge, UK) and recorded using Spike 2 version 5 data acquisition software. The phasic component of the signals was obtained by removing the DC component with a time constant of 10 seconds from the 2-second smoothed original trace as previously described (24). Gastric motility was calculated as the area under the curve (AUC) of the phasic component during the 5-minute baseline and then at each 5 minutes for 30 minutes after each injection.

Measure of Blood Glucose

In urethane-anesthetized rat, the tail tip (1 mm) was snipped to allow blood sampling. Glucose levels were measured using a commercially available glucose monitor (One-Touch Ultra; LifeScan, Milpitas, CA).

Experimental Protocols

Except if otherwise stated, all of the experiments were performed in overnight fasted animals.

Effects of Obestatin Injected Intraperitoneally at Various Doses on Food Intake in Non-fasted Rats. Long-Evans rats (6.5 weeks old, 161.1 ± 1.4 g), housed individually for 2 weeks and freely fed, were injected intraperitoneally with vehicle (saline, $n = 6$) or obestatin at 0.1, 1, and 3 mg/kg ($n = 6/\text{group}$) at the onset of the dark cycle. Food intake was monitored at 60, 120, and 180 minutes after the IP injection.

Effect of Obestatin Injected Intraperitoneally at Various Doses on Gastric Emptying in Conscious Fasted Rats. Long-Evans rats (400–450 g) were fasted overnight and then injected intraperitoneally with vehicle (saline, $n = 5$) or obestatin at 0.1, 0.3, 1, or 3 mg/kg ($n = 5/\text{group}$), and 30 minutes later, they received 3 mL of the semi-liquid nutrient meal intra-gastrically. Gastric emptying was monitored 30 minutes later.

Effect of Obestatin Injected Intravenously or Intracisternally and IV Ghrelin on Gastric Phasic Motility in Fasted Anesthetized Rats. Urethane-anesthetized SD rats (300 to 325 g) with a catheter pressure transducer positioned in the stomach were left undisturbed for at least 30 minutes. The occurrence of spontaneous gastric motility with a fasting pattern [i.e., high amplitude contractions (>20 mm Hg at a rhythm of 1 to 2 per minute)] was required to perform injections of IV ($n = 8$) or IC saline ($n = 5$), IV obestatin (0.3 and 3 mg/kg; $n = 4$ for each dose), IV ghrelin (30 $\mu\text{g}/\text{kg}$; $n = 4$), or IC obestatin (7.5 $\mu\text{g}/\text{rat}$, $n = 4$ or 30 $\mu\text{g}/\text{rat}$, $n = 5$). Gastric motility was assessed by measuring the AUC in 5-minute bins for 30 minutes after the IV or IC injection, and by calculating the mean AUC for the 30-minute response. In one experiment, gastric motility was monitored before and after acute bilateral cervical vagotomy performed through a cervical midline incision ($n = 7$).

Effect of Obestatin Injected Intravenously or Intracisternally on Blood Glucose Levels in Fasted Anesthetized Rats. In urethane-anesthetized SD rats (300 to 325 g), blood glucose was monitored at 1 minute before and at 5, 10, 20, and 30 minutes after the injection of saline (IV, $n = 8$; IC, $n = 4$), obestatin (3 mg/kg, IV, $n = 4$; 7.5 $\mu\text{g}/\text{rat}$, IC, $n = 4$; 30 $\mu\text{g}/\text{rat}$, IC, $n = 5$), or ODT8-SST (3 $\mu\text{g}/\text{rat}$, IC, $n = 3$).

Effect of Obestatin Injected Intraperitoneally on Food Intake in Fasted DIO Mice. DIO mice (40 to 55 g) were randomized in 3 groups ($n = 7$ in each group) and were injected intraperitoneally with saline, obestatin (300 $\mu\text{g}/\text{kg}$), or Ucn 1 (10 $\mu\text{g}/\text{kg}$) and given a high-fat diet ad libitum. Food intake was monitored at 30, 60, and 120 minutes after injection.

Data Analysis

Values are mean \pm standard error (SE). Statistical comparisons for food intake and gastric emptying experiments

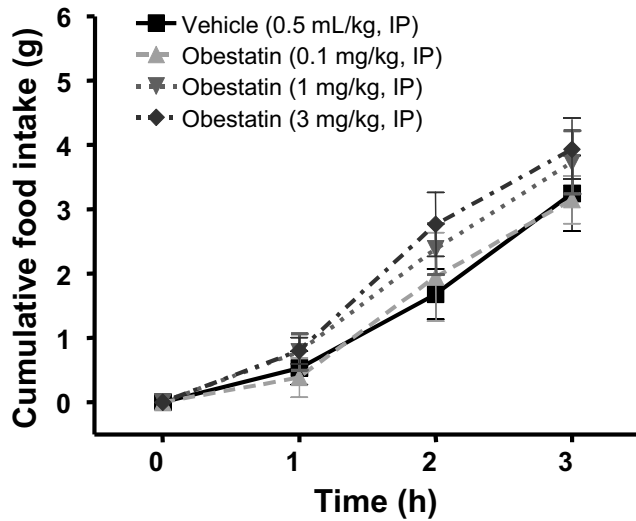


Figure 1: Obestatin injected intraperitoneally did not influence cumulative food intake in young male Long-Evans non-fasted rats. Vehicle or obestatin (0.1, 0.3, 1, or 3 mg/kg) was administered intraperitoneally at the onset of the dark period and food intake was monitored hourly. The data are presented as mean \pm SE for 6 rats per each dose group.

were made by ANOVA followed by a Newman-Student-Keuls post hoc test. The dose-response was assessed using the non-parametric Spearman correlation test. Influence of treatments on gastric phasic motility and blood glucose were analyzed using ANOVA for repeated measures followed by the Wilcoxon test. A p value <0.05 was considered as significant.

Results

Obestatin Intraperitoneally Does Not Decrease Food Intake in Non-fasted Rats

Non-food-deprived Long-Evans rats injected intraperitoneally with vehicle at the onset of the dark cycle ate 0.53 ± 0.26 , 1.68 ± 0.39 , and 3.25 ± 0.58 g of normal chow as monitored at 60, 120, and 180 minutes post-injection, respectively. The IP administration of obestatin at 0.1, 1, or 3 mg/kg did not modify the 60- to 180-minute post-injection cumulative food intake compared with the saline group ($p > 0.05$ at each time-point; Figure 1). There were no dose-response changes of cumulative food intake at any time-point after IP obestatin injection at doses ranging from 0 to 3 mg/kg (Figure 1).

Obestatin Intraperitoneally Does Not Inhibit Gastric Emptying in Fasted Conscious Rats

Vehicle IP-pretreated Long-Evans rats had $50.4 \pm 1.4\%$ of the semi-liquid caloric meal emptied at 30 minutes after the orogastric administration. Obestatin injected intraperi-

toneally at 0.1, 0.3, 1, or 3 mg/kg did not influence significantly the gastric emptying rate of a semi-liquid meal ($54.9 \pm 3.8\%$, $46.2 \pm 3.7\%$, $52.3 \pm 1.6\%$, and $54.6 \pm 2.5\%$, respectively; $p > 0.05$; Spearman test; Figure 2).

Obestatin Injected Intravenously or Intracisternally Has No Effect on Gastric Phasic Motility in Anesthetized Fasted SD Rats

Saline injected intravenously did not modify significantly the phasic component of IGP from baseline, as shown by the 1.62 ± 0.21 mm Hg \times sec mean AUC for the 30-minute period after IV saline compared with 1.96 ± 0.95 mm Hg \times sec for the 5-minute baseline (Figure 3). Obestatin injected intravenously at 0.3 and 3 mg/kg did not alter significantly the phasic component of gastric motility. The mean AUC monitored for 30 minutes was not different from the one observed in the saline-treated group (mm Hg \times sec: 1.65 ± 0.24 and 1.49 ± 0.27 for IV obestatin at 0.3 and 3 mg/kg, respectively; $p > 0.05$ vs. saline, Figure 3). By contrast, IV injection of ghrelin at $30 \mu\text{g}/\text{kg}$ induced a significant and sustained increase of phasic activity starting from the first 5 minutes (mm Hg \times sec: 6.68 ± 3.18 vs. saline: 1.71 ± 0.66 at 5 minutes; $p < 0.05$; Figure 3), with a mean AUC for the 30-minute period significantly higher than that observed in the saline group (5.23 ± 0.91 mm Hg \times sec; $p < 0.05$ vs. saline; Figure 3). Bilateral cervical vagotomy induced a profound inhibition of gastric phasic motility reaching over 95% at each time-point and a mean AUC for the 30-minute period of 0.09 ± 0.01 mm Hg \times sec ($p < 0.05$ vs. saline, Figure 3).

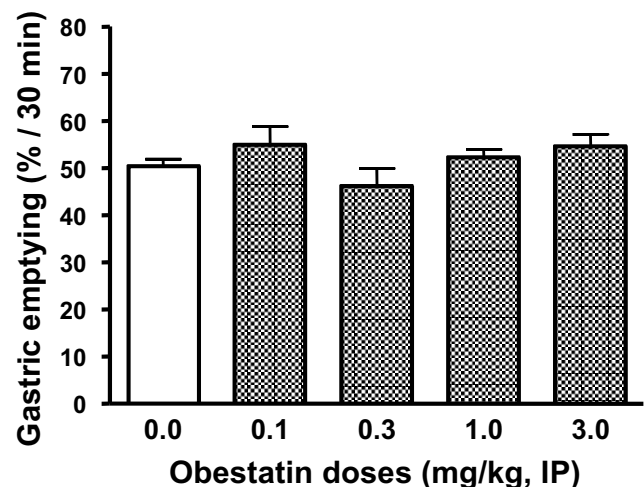


Figure 2: Obestatin injected intraperitoneally did not influence the gastric emptying rate of a semi-liquid caloric meal in fasted Long-Evans rats. Vehicle or obestatin (0.1, 0.3, 1, or 3 mg/kg) was administered intraperitoneally 30 minutes before orogastric gavage of the test meal (3 mL) and gastric emptying was measured 30 minutes later. Data are mean \pm SE for 6 rats per each dose group.

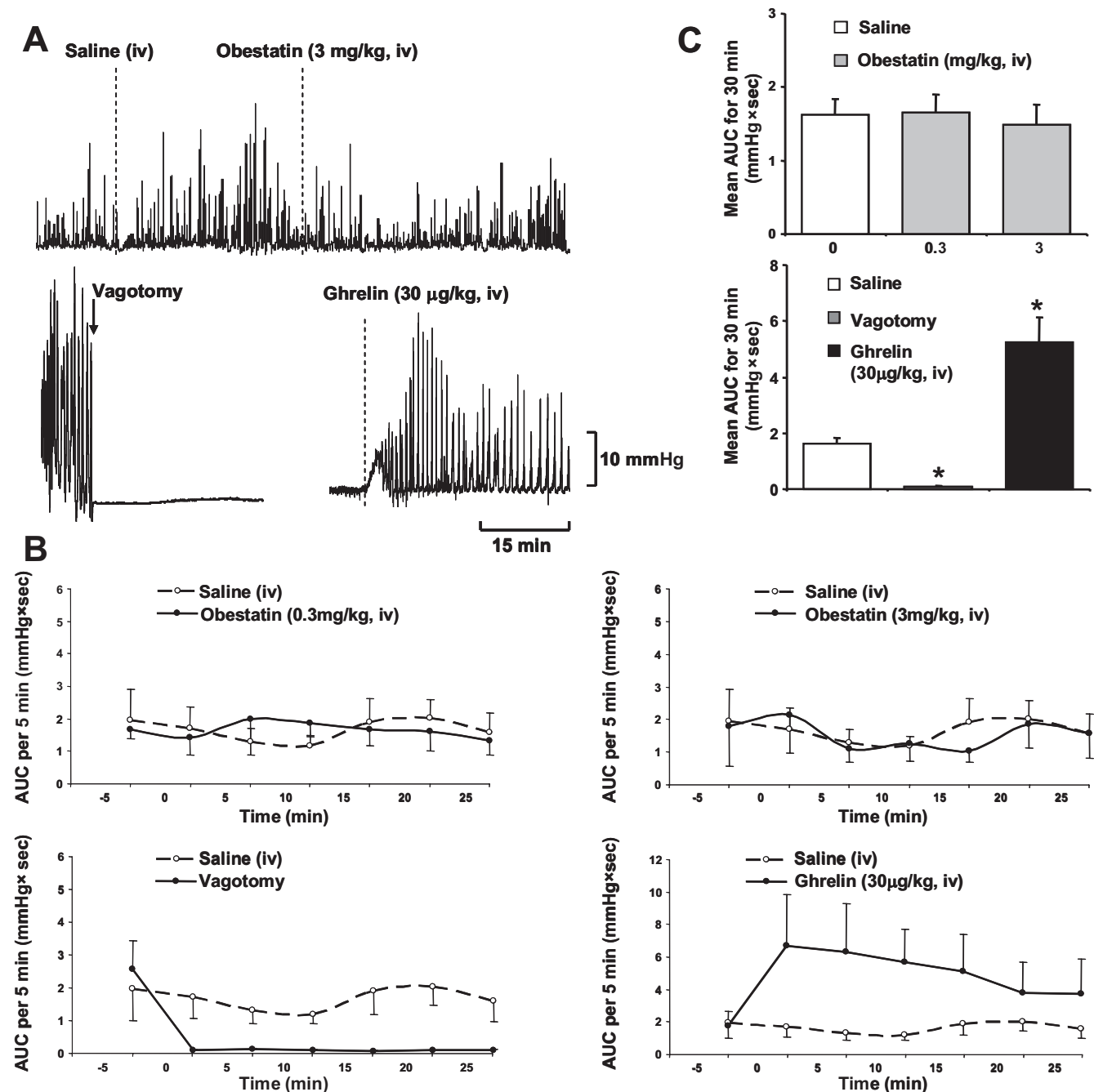


Figure 3: Obestatin injected intravenously did not inhibit gastric phasic contraction in anesthetized SD fasted rats. (A) Representative traces of the time course of the phasic component of IGP after IV injection of saline, obestatin, and ghrelin, and bilateral cervical vagotomy in urethane-anesthetized rats (IV injections are represented as dotted lines, vagotomy as a black arrow). (B) Time course of IGP 5 minutes before and 30 minutes after bilateral cervical vagotomy and IV injection of either saline, obestatin, and ghrelin (IV injection and vagotomy were performed at time 0). The phasic component of IGP is expressed as mean \pm SE of the AUC (mm Hg \times sec) for each 5-minute period (4 to 8 rats/group). The upper graphs represent the influence of IV obestatin at 0.3 and 3 mg/kg on AUC, while the two lower graphs show the response observed after vagotomy and IV ghrelin injection. (C) Total AUC measured for 30 minutes after each IV injection and bilateral cervical vagotomy. Results are expressed as mean \pm SE. * $p < 0.05$ compared with saline (ANOVA).

Saline injected intracisternally did not produce significant changes in the phasic component of IGP throughout the

experiment with a mean AUC of 2.62 ± 0.85 mm Hg \times sec at baseline and 2.42 ± 0.38 mm Hg \times sec for the 30-minute

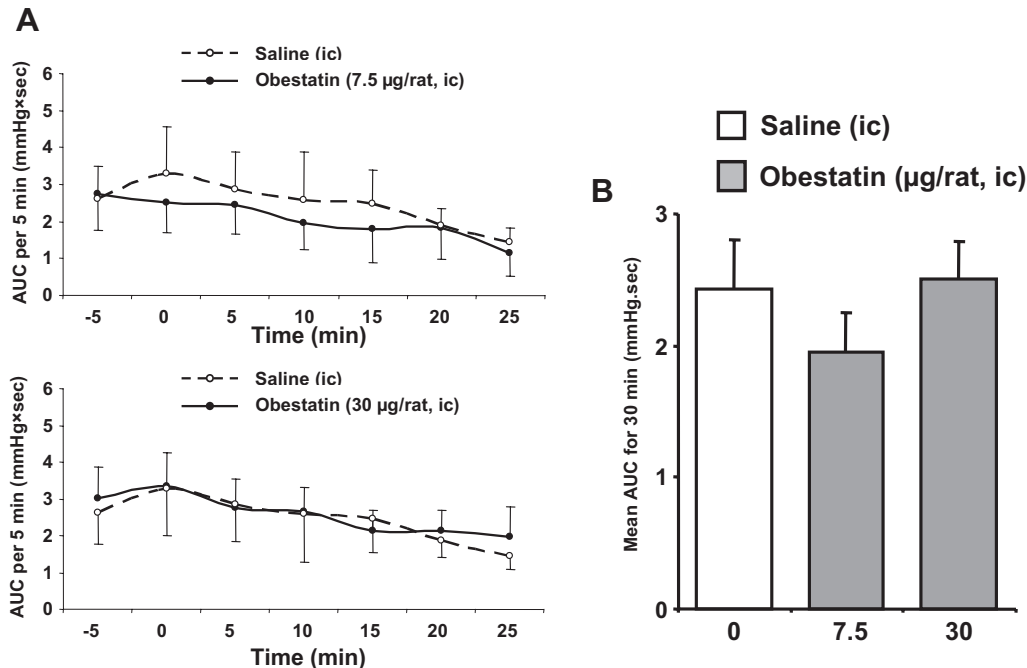


Figure 4: IC injection of obestatin did not inhibit gastric phasic contraction in anesthetized fasted rats. (A) Time course of IGP 5 minutes before and 30 minutes after IC injection of either saline or obestatin (IC injection was made at time 0). The phasic component of IGP is expressed as mean \pm SE of the AUC (mm Hg \times sec) for each 5-minute period (4 rats/group for each dose). (B) Total AUC measured for 30 minutes after IC injection of saline or obestatin. Data are mean \pm SE.

period after the IC injection ($p > 0.05$; Figure 4). When injected intracisternally, obestatin at a dose of 7.5 or 30 $\mu\text{g}/\text{rat}$ did not significantly modify the gastric phasic activity at any time-point compared with baseline with a mean AUC for 30 minutes of 1.95 ± 0.30 and 2.50 ± 0.29 mm Hg \times sec, respectively ($p > 0.05$ vs. saline; Figure 4).

Obestatin Injected Intravenously or Intracisternally Does Not Alter Blood Glucose in Anesthetized Fasted SD Rats

Saline injected intravenously produced a slight but non-significant trend to increase blood glucose compared with baseline (mg/dL: 212 ± 25 at baseline vs. 259 ± 24 at 30 minutes; $p > 0.05$, Figure 5). Obestatin injected intravenously (3 mg/kg) also resulted in a non-significant trend to elevate blood glucose as monitored at 5, 10, 20, or 30 minutes compared with baseline (mg/dL: 277 ± 13 at 30 minutes vs. 232 ± 27 at baseline; $p > 0.05$), and there was no significant difference with the saline-treated group at the same time period post-injection (Figure 5). Similarly, obestatin injected intracisternally at 7.5 or 30 $\mu\text{g}/\text{rat}$ did not produce any change in blood glucose at 5, 10, 20, or 30 minutes post-injection compared with baseline (mg/dL: 263 ± 30 and 234 ± 20 , respectively, at 30 minutes vs. 251 ± 37 and 237 ± 26 at baseline; $p > 0.05$, Figure 5) or saline IC group at the 5- to 30-minute period (mg/dL: 209 ± 44 at baseline vs. 254 ± 32 at 30 minutes; $p > 0.05$; Figure

5). By contrast, a significant decrease in blood glucose value was achieved 30 minutes after ODT8-SST injected intracisternally at 3 $\mu\text{g}/\text{rat}$ (mg/DL: 152 ± 22 at 30 minutes vs. baseline: 220 ± 18 ; $p < 0.05$; Figure 5).

Ucn 1 Injected Intraperitoneally Reduces Food Intake in Fasted DIO Mice While Obestatin Has No Effect

Cumulative food intake response to a fast in intraperitoneally saline-injected DIO mice was 0.07 ± 0.02 , 0.16 ± 0.04 , and 0.28 ± 0.08 g as monitored at 30, 60, and 120 minutes, respectively, after exposure to fatty food. Obestatin, injected intraperitoneally (0.3 mg/kg) in fasted DIO mice, did not significantly modify the cumulative food intake from 30 to 120 minutes post-injection compared with the saline group (-9% at 30 minutes, -10.7% at 60 minutes, and -14.2% at 120 minutes; $p > 0.05$ vs. saline-treated group at each time-point, Figure 6). Ucn 1 (10 $\mu\text{g}/\text{kg}$, IP) inhibited feeding compared with both vehicle- and obestatin-treated groups (-40% , -63.5% , and -73.3% at 30, 60, and 120 minutes, respectively, compared with saline; $p < 0.05$ at 60 and 120 minutes, Figure 6).

Discussion

In the present study, we showed that obestatin administered peripherally, even at doses up to 3 mg/kg, did not

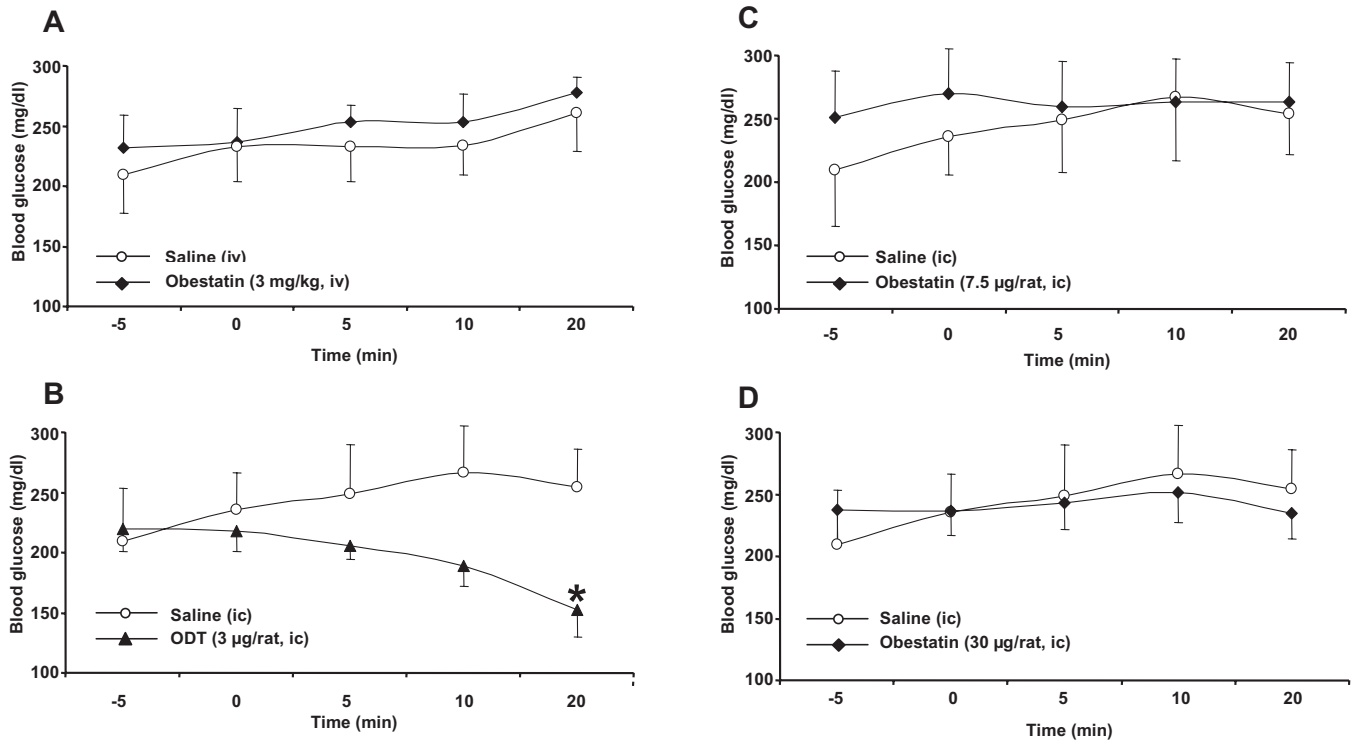


Figure 5: Time course of blood glucose before and 30 minutes after IV injection of saline and obestatin (A), IC injection of saline or ODT8-SST, a somatostatin receptor agonist (B), obestatin at 7.5 µg/rat (C), or obestatin at 30 µg/rat (D). The injections were made at time 0, and blood glucose was measured 1 minute before and 5, 10, 20, and 30 minutes after each injection. Data are mean ± SE. * $p < 0.05$ compared with saline (ANOVA).

modify food intake and gastric emptying of an acaloric meal in conscious rats. Moreover, in DIO mice, IP obestatin at 300 µg/kg did not decrease food intake of a high fat diet as

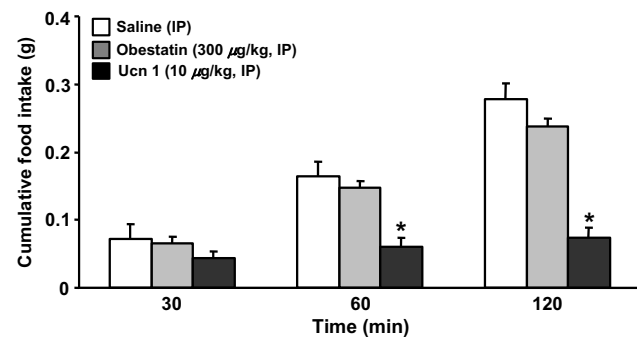


Figure 6: Urocortin 1 (Ucn 1), but not obestatin, injected intraperitoneally inhibited food intake in DIO mice. DIO mice were overnight-fasted and then injected intraperitoneally with either obestatin (300 µg/kg) or Ucn 1 (10 µg/kg). Cumulative food intake of the high fat diet was monitored at 30, 60, and 120 minutes. Each column is the mean ± SE of 7 mice per group. * $p < 0.05$ compared with both vehicle and obestatin groups (ANOVA).

monitored for 120 minutes post-injection, while Ucn 1 given at 10 µg/kg resulted in a significant 73.3% decrease in feeding. In addition, we did not observe any short-term effects of peripherally or intracisternally administered obestatin, even at high doses, to modify gastric phasic motility, while ghrelin and vagotomy increased and decreased, respectively, the phasic component of IGP in urethane-anesthetized rats. Lastly, obestatin given intravenously (0.3 or 3 mg/kg) and intracisternally (7.5 or 30 µg/rat) did not produce a significant change in blood glucose, while ODT8-SST given intracisternally at 3 µg/rat lowered blood glucose levels at 30 minutes. These results are at variance with the initial report of Zhang et al. (8), which characterized the novel peptide obestatin as an inhibitor of both feeding and upper gastrointestinal motility in mice. Indeed, they originally showed that obestatin injected at doses ranging from 0.2 to 2.5 mg/kg IP or at 20 µg/kg intracerebroventricularly significantly decreases food intake in fasted lean mice at 1, 3, and 5 hours post-injection (8). However, our findings on food intake extend recent reports that failed to demonstrate an inhibitory effect of peripherally administered obestatin on food intake in lean mice or rats (9–12,15,25–28). For example, we provided the first evidence that obestatin injected intraperitoneally in doses

ranging from 30 to 300 $\mu\text{g}/\text{kg}$ did not alter feeding response to an overnight fast in rats (9). Likewise, in the present study, obestatin injected intraperitoneally at the highest dose tested so far (i.e., up to 3 mg/kg) did not modify significantly the 3-hour dark-phase feeding in non-fasted young rats. Obesity could modify the gut sensitivity to several peptides released postprandially (29). As all of the previous studies on obestatin effects on food intake were carried out on lean rodents (15), we tested the ability of obestatin injected intraperitoneally at 300 $\mu\text{g}/\text{kg}$ to modify refeeding after a fast in DIO mice. However, IP obestatin did not influence food intake of a fatty meal in DIO mice as reported in lean mice (9–11,26–28). By contrast, Ucn 1 injected intraperitoneally at 10 $\mu\text{g}/\text{kg}$ induced a sustained inhibition of feeding of a fat-rich diet in DIO mice. These findings extend to DIO mice, the anorexic action of IP Ucn 1 previously reported in lean and *ob/ob* mice (17,30). Taken together, the present and previous functional data do not support a role for circulating obestatin in the neurohumoral regulation of appetite (15). In addition, a recent report has asserted that obestatin (proghrelin 53–75) is detected neither in rat stomach tissue extracts nor in rat and human plasma as determined by high performance liquid chromatography and radioimmunoassay (31). By contrast, ghrelin was readily measured in gastric tissues, and human plasma levels were inversely correlated with glucose and negatively correlated with mass index (31).

Peripheral injection of obestatin (0.3 to 2.5 mg/kg) was originally found to inhibit gastric emptying in mice (8). Subsequently, we reported that the peptide injected intraperitoneally at a dose of 0.3 mg/kg did not influence gastric emptying of a liquid a caloric meal in conscious rats (9). In the present study, obestatin injected intraperitoneally at doses ranging from 0.1 to 3 mg/kg did not modify gastric emptying of a nutrient semi-liquid meal in conscious rats. Moreover, real-time monitoring of spontaneous fasting gastric motility did not reveal any significant changes after IV injection of obestatin at 0.3 or 3 mg/kg. By contrast, ghrelin enhanced gastric phasic motility while bilateral cervical vagotomy inhibited such activity. These data corroborate previous comparable observations on ghrelin (19) and bilateral vagotomy (18) effects on gastric motility in rats and establish the responsiveness of the gastric preparation. While our manuscript was under review, two additional reports also showed that obestatin injected intraperitoneally or intravenously modified neither gastric emptying in rats and mice nor small bowel motility in vivo in rats and in vitro in rats and mice (14,28).

With regards to the central actions of obestatin, results on food intake are inconsistent. The intracerebroventricular injection of obestatin at doses ranging from 0.1 to 25 $\mu\text{g}/\text{rat}$ or mouse was reported to decrease food intake (8,32,33), while a number of studies found no change under similar conditions (11–13,25). There are also reports that obestatin

injected intracerebroventricularly inhibits thirst and alters sleep in rats (12,34), although those reports still need to be confirmed. In the present study, obestatin injected into the brain at the level of the cisterna magna at 7.5 or 30 $\mu\text{g}/\text{rat}$ did not modify basal gastric phasic motility in fasted urethane-anesthetized rats. This route of administration was previously shown to be responsive to several peptides regulating gastric motor function through modulation of dorsal vagal complex neurons innervating the stomach (35–37). These data along with recent reports provide consistent evidence that obestatin injected either centrally (present study) or peripherally (9,14,28) did not influence gastrointestinal motor function, casting doubt as to the peptide's role in the regulation of upper gastrointestinal digestive function (15).

The influence of obestatin in the regulation of energy metabolism also remains controversial. Indeed, Zhang et al. (8) observed in their first report a decrease in body weight after peripheral obestatin administration. Further studies failed to reproduce such findings (11). Another report described weight loss after intracerebroventricular obestatin administration, which was explained by a decrease in thirst but not by the inhibition of feeding (12), while other data showed no change in weight gain after intracerebroventricular infusion of obestatin in rats (32). In the present work, such a parameter has not been investigated; however, we examined the ability of peripheral and mainly central obestatin to alter blood glucose in rats. Obestatin was first proposed to oppose ghrelin's effects on feeding and gastric emptying (8), and ghrelin has been shown to play a key role in blood glucose regulation (5,38). In addition, a recent report indicates that obestatin is expressed in the pancreas, although at a lower level, consistent with the presence of proghrelin in this tissue (39). However, we failed to demonstrate that acute administration of central and peripheral obestatin influences blood glucose in rats. By contrast, under similar conditions, IC-injected ODT8-SST, an oligosomatostatin receptor agonist, reduced by 30.8% the elevated blood glucose. Previous studies established the central action of the oligosomatostatin peptide to curtail hyperglycemia induced by various stressors (20,40). It is of note that we conducted an acute experiment under anesthesia, which is known to induce hyperglycemia through sympathetic response and appears to be a reliable condition to observe a decrease in blood glucose (41). Nevertheless, experiments conducted in conscious animals with long-term monitoring of blood glucose are warranted to conclude the absence of effect of obestatin on blood glucose. However, our preliminary data, along with the fact that obestatin, unlike ghrelin, is not correlated with plasma levels of insulin in obese subjects (42), do not favor a modulation of glucose by obestatin.

Taken together, our present results along with recent studies using different paradigms (9–11,15,26–28) failed

to expand a role for peripheral administration of obestatin in the regulation of satiety signaling in lean and obese rodents. Moreover, the peptide does not act in the brain or periphery to regulate gastric motility. Additional studies are warranted to establish the biological actions of obestatin.

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