Endogenous Ghrelin Regulates Episodic Growth Hormone (GH) Secretion by Amplifying GH Pulse Amplitude: Evidence from Antagonism of the GH Secretagogue-R1a Receptor

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Ghrelin was purified from rat stomach as an endogenous ligand for the GH secretagogue (GHS) receptor. As a GHS, ghrelin stimulates GH release, but it also has additional activities, including stimulation of appetite and weight gain. Plasma GH and ghrelin secretory patterns appear unrelated, whereas many studies have correlated ghrelin variations with food intake episodes. To evaluate the role of endogenous ghrelin, GH secretion and food intake were monitored in male rats infused sc (6 μg/h during 10 h) or intracerebroventricularly (5 μg/h during 48 h) with BIM-28163, a full competitive antagonist of the GHS-R1a receptor. Subcutaneous BIM-28163 infusion significantly decreased GH area under the curve during a 6-h sampling period by 54% and peak amplitude by 46%. Twelve hours after the end of treatment these parameters returned to normal. Central treatment was similarly effective (−37 and −42% for area under the curve and −44 and −49% for peak amplitude on the first and second days of infusion, respectively). Neither peripheral nor central BIM-28163 injection modified GH peak number, GH nadir, or IGF-I levels. In this protocol, food intake is not strongly modified and water intake is unchanged. Subcutaneous infusion of BIM-28163 did not change plasma leptin and insulin levels evaluated at 1200 and 1600 h. On the contrary, central BIM-28163 infusion slightly increased leptin and significantly increased insulin concentrations. Thus, endogenous ghrelin, through GHS-R1a, acts as a strong endogenous amplifier of spontaneous GH peak amplitude. The mechanisms by which ghrelin modifies food intake remain to be defined and may involve a novel GHS receptor. (Endocrinology 146: 3836–3842, 2005)

The secretory pattern of GH is mainly driven by two counterregulatory neurohormones, GHRH and somatostatin. However, the existence of a third factor regulating GH rhythmicity was suggested 20 yr ago when met-enkephalin analogs without opiate-like activity were found to exert potent GH-releasing activity (1). Since then, numerous synthetic secretagogues (GHS) have been developed and shown to act through a specific G protein-coupled receptor, GHS-R1a (2). GHS-R1a is distinct from the GHRH receptor and is mainly expressed in the hypothalamus and pituitary gland. In 1999, a 28-amino-acid acylated peptide was identified as an endogenous ligand for the GHS-R1a and termed ghrelin (3). It has been demonstrated that the acylation is essential for GHS-R1a binding. Ghrelin is produced mainly by A-like cells in oxyntic glands of the stomach, and it is secreted into the bloodstream (4). In addition to the gastrointestinal tract, ghrelin expression, at either the mRNA or protein level or both, has been identified in a number of tissues, including the hypothalamus (5). In this brain structure, low levels of ghrelin have been measured in the arcuate nucleus (3, 6), which is involved in the control of GH secretion and food intake. As expected, ghrelin stimulates GH release (3, 4, 7–9) but also exerts multiple activities, including increased feeding (9) and weight gain (10). The circulating levels and patterns of ghrelin and GH appear weakly related (11), whereas many studies have established strong correlations between ghrelin variations and food intake episodes. Fasting increases circulating ghrelin levels, which decrease after food intake (11–13). Ghrelin gene deletion in mice impair growth neither growth nor appetite (14, 15), although deleting the GHS-R gene abolishes both ghrelin and synthetic GHS effects on these two functions (16). Thus, the role of endogenous ghrelin in the regulation of pulsatile GH secretion and food intake is not yet clear. The aim of the present study was to define this role by examining the effects of treating rats with a novel analog of ghrelin, BIM-28163, recently developed as a full competitive antagonist of the GHS-R1a (17).

Materials and Methods

In vitro experiments

Binding assay. The cDNA of the human GHS-R1a was cloned by PCR using human brain RNA (Clontech, Palo Alto, CA) as the template. The PCR product was cloned into the pCR2.1 vector using the TA Cloning Kit (Invitrogen, Carlsbad, CA) and subcloned into the mammalian expression vector pcDNA 3.1 (Invitrogen). The plasmid was transfected into the Chinese hamster ovary cell line, CHO-K1 (American Type Culture Collection, Manassas, VA), by the calcium phosphate method. Single-cell clones stably expressing the human GHS-R1a were obtained by selecting transfected cells using 0.8 mg/ml G418-containing selection media (Life Technologies, Carlsbad, CA).

Membranes were prepared for radioligand binding studies by homogenization of CHO-K1 cells expressing the human recombinant GHS-
R1a in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkmann Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 × g for 10 min), and the final pellets were resuspended in 50 mM Tris-HCl, containing 2.5 mM MgCl₂ and 0.1% BSA. For assay, aliquots (0.4 ml) were incubated with 0.05 nM [³²P]h-grehelin (~2000 Ci/mmol), and with and without 0.05 ml of unlabeled competing test peptide. After a 60-min incubation (4 C), the bound [³²P]h-grehelin was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine/0.1% BSA. The filters were then washed three times with 5-ml aliquots of ice-cold 50 mM Tris-HCl and 0.1% BSA, and the bound radioactivity trapped on the filters was counted in the presence of 1000 nM unlabeled h-grehelin (Rachem, Torrance, CA).

**Intracellular calcium mobilization assay.** CHO-K1 cells, expressing the GHS-R1a, were harvested by incubating in a 0.3% EDTA/PBS solution (25 C) and washed twice by centrifugation. The washed cells were resuspended in Hanks' buffered saline solution for loading of the fluorescent calcium indicator fura-2 AM. Cell suspensions of approximately 10⁶ cells/ml were incubated with 0.05 ml [³°Ca²⁺] (Westbury, NY). The suspensions were transferred to a spectrofluorometer (Hitachi F-2000) equipped with a magnetic stirring mechanism and a temperature-regulated cuvette holder. After equilibration to 37 C, the h-grehelin analogs were added for measurement of intracellular calcium mobilization. The excitation and emission wavelengths were 340 and 510 nm, respectively.

**In vitro experiments**

**Acute in vitro GH stimulation.** Male Sprague-Dawley rats (250 g) were anaesthetized and fit with a jugular-right atrial cannula 18 h before the experiment. To determine basal hormone levels, blood samples were withdrawn into heparinized syringes at time −10 and 0 min. To demonstrate antagonist activity, immediately after the time 0 blood sample, the rats were injected with either BIM-28163 (1000 µg/kg) or vehicle via the indwelling cannula. Ten minutes later, a blood sample was withdrawn, and the rats were immediately injected with either h-grehelin (10 µg/kg) or vehicle via the cannula. Subsequent blood samples were withdrawn 10, 20, and 40 min after injection of h-grehelin or vehicle. Plasma was separated and stored until assay for GH.

**Chronic in vivo treatments.** Four weeks before sampling experiments, adult male Sprague-Dawley rats weighing 100–125 g (Charles River Laboratories, Inc., L’arbresle, France) were housed individually in transparent plastic containers placed in a soundproof room with controlled temperature (22–24 C) and illumination (12 h light, 12 h dark schedule with lights off at 1900 h). They had free access to food and water and were regularly handled and weighed to minimize stress effects. Guidelines of the National Institute of Health for the Care and Use of Laboratory Animals were followed.

**Subcutaneous treatment.** Six days before the experiment, an osmotic minipump (Alzet model 2002, flow rate 0.5 µl/h for 14 d) (Charles River) was connected to a polyethylene catheter and implanted sc in the back of the animals under ether anesthesia. The catheter had previously been filled with a combination of saline and BIM or saline. This resulted in delivery of saline during 144 h followed by BIM-28163 (6 µg/h) or saline during 10 h. On the day of the experiment (d 0), sc treatment started at 0900 h, and rats received ip injection of 300 µg/0.2 ml BIM-28163 or 0.2 ml NaCl at 1000 h.

**Intracerebroventricular (icv) treatment.** Six days before the experiment, a chronic icv cannula (Alzet Brain Infusion Kit II; Charles River) was inserted into the lateral ventricle of the brain under pentobarbital anesthesia (60 mg/kg of body weight ip; Centravet, Plancoet, France). The following coordinates were used: anterior −0.8 mm to the bregma; lateral −1.5 mm to the midline; height −3.8 mm to the bregma, with the incisor bar set at −3.3 mm below the interaural line. The cannula was secured to the skull of the animal with stainless steel and dental cement and connected to an osmotic minipump (Alzet model 2002; flow rate, 0.5 µl/h for 14 d) (Charles River) implanted under the dorsal skin of the animal. The flow moderator of the pump was connected to the polyethylene catheter containing 72 µl saline, which was administered during the first 6 d in all animals. A small air bubble was inserted to separate the saline solution from an additional 24 µl BIM-28163 (5 µg/h) or saline to be delivered for an additional 48 h. Treatment started at 0800 h on d 0 until 0800 h on d 2.

**Blood sampling schedule**

Experiments were performed on freely moving rats. Two days before data collection, an indwelling cannula was inserted into the right atrium under ether anesthesia as previously described (18). Two hours before the sampling and recording period, the distal extremity of the cannula was connected to a polyethylene catheter filled with 25 IU/ml heparinized saline. Blood samples were withdrawn every 20 min and centrifuged immediately. Red blood cells were resuspended in saline and reinfected every hour to attenuate hemodynamic modifications. Plasma was stored at −20 C until assayed for GH.

**Feeding behavior**

Experiments on food and water intake were performed on a separate group of animals that underwent the same treatments. Food and water intake were measured continuously with a PC-controlled monitoring system (feeding/drinking monitor; Technical and Scientific Equipment, Bad Homburg, Germany). Animals were acclimated to this new environment for 1 wk before actual monitoring.

**Hormonal determinations**

Plasma GH concentrations were measured by enzyme immunoassay as previously described (19). Values are reported in terms of rGH-RP2, with sensitivity of 5 ng/ml and intra- and interassay coefficients of variation less than 7%.

Plasma insulin, total leptin, and IGF-I levels were determined with RIA kits following manufacturer instructions (Linco, Saint Charles, MO, and DSL, Webster, TX, respectively). The sensitivity was 0.2, 0.5, and 160 ng/ml, respectively. For all assays, the intra- and interassay coefficients of variation were less than 10%.

**Statistical analysis**

GH pulse analysis was performed using the Cluster program (20) with the t value set to 2 to maintain false positive rates under 1%. Cluster size was set to two prepeak and two postpeak nadir values. False positive error for peak detection was 7%.

Areas under the GH response curves (AUC) were calculated by mean of trapezoidal analysis.

Values are given as means ± SEM, and statistical analysis was performed by ANOVA and paired t test using the JMP IN 5.1 software (SAS Institute Inc., Cary, NC).

**Results**

**In vitro experiments**

BIM-28163 was found to displace labeled h-grehelin binding to the human GHS-R1a with an IC₅₀ (Ki) of 8.1 ± 1.3 nM. Human ghrelin, under the same conditions, had an IC₅₀ of 1.6 ± 0.3 nM.

In the calcium mobilization assay, h-grehelin induced concentration-dependent calcium mobilization in cells stably transfected with the human GHS-R1a between the concentrations of 0.1 and 100 nM, with an EC₅₀ of 5.4 ± 1.4 nM. In contrast, BIM-28163 had no effect on calcium mobilization, even when tested at concentrations as high as 10⁻⁶ M (data not shown). When added in combination, BIM-28168 attenuated the ability of h-grehelin to induce calcium mobilization in a dose-dependent manner, with complete inhibition achieved with a ratio of 100:1 BIM-28163:h-grehelin (Fig. 1),
indicating that BIM-28163 acts as a full, competitive antagonist of ghrelin at the GHS-R1a.

Neither \[^{125}\text{I}\]h-ghrelin binding nor ghrelin-induced mobilization of intracellular calcium was observed in the parent CHO-K1 cell line.

**In vivo experiments**

**Acute in vivo GH stimulation.** Ghrelin-induced GH stimulation was maximal 10 min after injection (data not shown). As illustrated on Fig. 2, injection of h-ghrelin (10 μg/kg) alone into freely moving, unanesthetized rats induced a significant increase in plasma GH, whereas injection of BIM-28163 (1000 μg/kg) was without effect. When BIM-28163 was injected 10 min before h-ghrelin, the h-ghrelin-induced increase in GH secretion was completely abolished.

**Chronic in vivo treatments.** BIM-28163 was infused either sc (at the rate of 6 μg/h during 10 h after an ip bolus of 300 μg) or icv (at the rate of 5 μg/h during 48 h) to unanesthetized, freely moving rats, and blood samples were collected every 20 min for 6 h the first day, 1 h after the beginning of infusion, and the following day during the same period. Individual profiles are illustrated on Fig. 3. BIM-28163 administration always resulted in a rapid decrease in spontaneous GH secretion. Throughout BIM-28163 treatment, total GH, calculated as AUC, was significantly lower in treated animals compared with their respective controls (Table 1). The decrease in total GH corresponded to a similar decrease in GH peak amplitudes. Basal values and GH pulse number were not modified (Table 1). The icv infusion was similarly effective on the first and second day of treatment. Fifteen hours after the end of sc treatment, pulsatile GH secretion was similar to that of controls.

Neither sc nor icv treatment modified plasma IGF-I levels, sampled at 1500 h [sc treatment: BIM-28163, 777 ± 84 ng/ml (n = 5), vs. saline, 855 ± 78 ng/ml; icv treatment: BIM-28163, 977 ± 94 ng/ml (n = 7), vs. saline, 1105 ± 133 ng/ml (n = 7), on the first day, and BIM-28163, 1199 ± 96 ng/ml (n = 7), vs. saline, 1162 ± 113 ng/ml (n = 7), on the second day]. The sc infusion of BIM-28163 changed neither plasma leptin nor insulin levels evaluated at 1200 and 1600 h. In contrast, icv BIM-28163 infusion slightly increased leptin and significantly increased insulin concentrations (Table 2).

Food intake was modestly increased only during the lights-on period on the second day after icv infusion of BIM-28163 (Fig. 4), and water consumption remained unchanged after either peripheral or central BIM-28163 infusion.

**Discussion**

In this study, either sc or icv infusion of a GHS-R1a antagonist, BIM-28163, was demonstrated to decrease spontaneous GH secretion without causing major changes in food intake. This h-ghrelin analog is a full competitive antagonist at the GHS-R1a. Indeed, BIM-28163 binds to the h-GHS-R1a receptor and can displace h-ghrelin binding, but BIM-28163 does not activate GHS-R1a and completely and dose-dependently inhibits the ability of ghrelin to stimulate calcium mobilization in h-GHS-R1a-transfected cells and GH secretion in vivo. Moreover, BIM-28163 blunts the GH-releasing effect of ghrelin but not the GHRH-induced GH rise (17). Thus, our results indicate that ghrelin, acting through the GHS-R1a, appears to be an endogenous regulator of spontaneous GH secretion but not necessarily of food intake.

Interestingly, antagonism of the GHS-R1a in freely moving male rats does not impair the pulsatile pattern of GH secretion; however, it significantly lowers pulse amplitude. These results suggest that endogenous ghrelin acts to amplify the basic pulsatile pattern of GH established by the interplay of hypothalamic GHRH and somatostatin (21). Indeed, it has been established that concomitant administration of ghrelin and GHRH produces an increase in GH secretion significantly greater than that induced by either factor alone (22). The observation that antagonism of the GHS-R1a receptor with BIM-28163 lowers spontaneous GH pulse amplitude and total GH secretion without altering the basic pulsatile pattern agrees well with the observation of Chapman et al. (23) that treatment of elderly men with MK677, a nonpeptide ligand and agonist of the GHS-R1a, results in a significant increase in GH secretion without changing the underlying pulsatile pattern. Previously, repetitive injections of \([\text{d-Lys}_3]\)-GHRP6, a compound reported to be a GHS-R1a antagonist, did not significantly reduce plasma GH levels (24); however, a very low dose of \([\text{d-Lys}_3]\)-GHRP6 was used (750 ng, less...
than 1 nmol) considering the in vitro IC$_{50}$ ($3.2 \times 10^{-6}$ M) of this particular analog (25) and its minimum effective dose on in vivo food intake ($200 \text{ nm}$) (26). On the other hand, GH secretion was reduced in female (but not in male) transgenic rats expressing an antisense GHS-R mRNA under the control of the tyrosine hydroxylase promoter, thus selectively attenuating GHS-R protein expression in the arcuate nucleus (27). Moreover, in ghsr-null mice, serum IGF-I levels and body weight are reduced compared with wild-type littermates, suggesting decreased GH secretion (16). Taken together, these data suggest that endogenous ghrelin (or another, yet unidentified, ligand of GHS-R1a) acts as an amplifier of GH pulsatility. At the present time, a strict correlation has not been established between GH and ghrelin secretory episodes (11); however, only total ghrelin was monitored, and it cannot be excluded that a stronger correlation occurs between GH and acylated ghrelin, which represents only a minor proportion of total plasma ghrelin and has a very short half-life (28).

In the subchronic experimental paradigm used herein, total plasma IGF-I levels were not modified by BIM-28163 infusion. This lack of effect is in keeping with the data obtained on male transgenic rats expressing an antisense GHS-R mRNA in the arcuate nucleus (27) as well as another study in which chronic icv administration of ghrelin for 7 d did not affect IGF-I levels (29). Moreover, 95% of IGFs circulate bound to IGF binding proteins and have a half-life of 12–15 h (30, 31); thus, it cannot be excluded that a longer treatment might affect IGF-I levels.

Interestingly, plasma leptin slightly and insulin concentrations were significantly elevated by icv BIM-28163 treatment but not by peripheral administration. The change in plasma leptin concentrations may represent triggering of counterregulatory mechanisms because leptin has been reported to suppress feeding, although the overt effect of ghrelin on feeding in the present study was minimal. Surprisingly, the effect on insulin secretion was observed only after icv administration of BIM-28163. Expression of the GHS-R1a

### TABLE 1. Cluster analysis of GH pulsatility parameters in BIM-28163-treated rats

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Sampling day</th>
<th>Treatment</th>
<th>AUC (ng/ml)</th>
<th>Number of peaks</th>
<th>Peak amplitude (ng/ml)</th>
<th>Nadir (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sc</td>
<td>0</td>
<td>Saline (n = 7)</td>
<td>12863 ± 1467</td>
<td>1.7 ± 0.2</td>
<td>69 ± 11</td>
<td>23 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BIM-28163 (n = 7)</td>
<td>5948 ± 1320$^a$</td>
<td>1.7 ± 0.7</td>
<td>37 ± 6$^b$</td>
<td>24 ± 7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Saline (n = 7)</td>
<td>16617 ± 1174</td>
<td>2.5 ± 0.5</td>
<td>101 ± 13</td>
<td>26 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BIM-28163 (n = 7)</td>
<td>13958 ± 2114</td>
<td>2.6 ± 0.5</td>
<td>81 ± 9</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>icv</td>
<td>0</td>
<td>Saline (n = 7)</td>
<td>13085 ± 1435</td>
<td>2.0 ± 0.4</td>
<td>70 ± 10</td>
<td>23 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BIM-28163 (n = 7)</td>
<td>8235 ± 1163$^b$</td>
<td>1.6 ± 0.5</td>
<td>38 ± 5$^b$</td>
<td>21 ± 2</td>
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<tr>
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<td>Saline (n = 7)</td>
<td>18681 ± 1497</td>
<td>3.0 ± 0.5</td>
<td>114 ± 11</td>
<td>31 ± 5</td>
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<td>BIM-28163 (n = 5)</td>
<td>10926 ± 489$^a$</td>
<td>2.2 ± 0.2</td>
<td>59 ± 3$^a$</td>
<td>19 ± 1</td>
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</table>

In the sc group, rats were infused at d 0 with saline or BIM-28163 (6 µg/h) from 0900–1900 h and received an ip injection of saline or BIM-28163 (300 µg) at 1000 h. In the icv group, rats were infused with saline or BIM-28163 (5 µg/h) for 48 h from 0800 h at d 0. Data are mean ± SEM.

$^a$P < 0.01 vs. saline.

$^b$P < 0.05 vs. saline.
has been described in the pancreas, yet direct interaction with BIM-28163 via peripheral administration produced no significant effect on insulin secretion. Results from both GHS-R (16) and ghrelin null (14) mice also suggest that ghrelin exerts only a minimal role in regulating insulin or leptin levels, because these parameters remain largely unchanged in both animal models. Indeed, the relationship between ghrelin and insulin secretion is far from clear because, depending on the dose, model, and species, ghrelin has been reported to both stimulate and suppress insulin secretion (32). The present results may suggest a central mechanism for stimulation of insulin secretion as well as provide evidence that this analog does not cross the blood-brain barrier; however, additional knowledge is clearly needed to fully interpret the present results on leptin and insulin secretion.

The fact that BIM-28163 treatment, either icv or sc, does not change feeding behavior is surprising because both GHS and exogenous ghrelin do stimulate food intake via GHS-R1a (16). Moreover, suppression of feeding has been reported in mice after icv injections, using two other antagonists: [d-Lys3]-GHRP6, an analog of one of the synthetic GHSs or [d-Arg,d-Phe,d-Trp,Leu]-substance P, an analog of sub-

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Sampling day</th>
<th>Treatment</th>
<th>Insulin (ng/ml)</th>
<th>Leptin (ng/ml)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1200 h</td>
<td>1600 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saline (n = 5)</td>
<td>1.53 ± 0.09</td>
<td>1.77 ± 0.08</td>
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<td>BIM-28163 (n = 5)</td>
<td>1.78 ± 0.23</td>
<td>1.90 ± 0.24</td>
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<td>sc</td>
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<td>1.83 ± 0.13</td>
<td>1.30 ± 0.10</td>
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<td></td>
<td>BIM-28163 (n = 5)</td>
<td>1.65 ± 0.12</td>
<td>1.81 ± 0.08</td>
</tr>
<tr>
<td>icv</td>
<td>0</td>
<td>Saline (n = 7)</td>
<td>1.76 ± 0.22</td>
<td>2.02 ± 0.23</td>
</tr>
<tr>
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<td>BIM-28163 (n = 7)</td>
<td>2.70 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.07 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Saline (n = 7)</td>
<td>1.92 ± 0.36</td>
<td>2.27 ± 0.54</td>
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<tr>
<td></td>
<td></td>
<td>BIM-28163 (n = 5)</td>
<td>2.86 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.17 ± 0.70</td>
</tr>
</tbody>
</table>

For sc administration, hormones were measured in freely moving male rats at 1200 and 1600 h on d 0 (3 and 7 h after the start of the treatment) and at the same time on d 1 (17 and 21 h after the end of the treatment). For icv administration, hormones were measured in freely moving male rats at 1200 and 1600 h on d 0 (4 and 8 h after the start of the treatment) and at the same time on d 1 (28 and 32 h after the start of the treatment). Data are mean ± SEM.

<sup>a</sup> P < 0.01 vs. saline.
<sup>b</sup> P < 0.05 vs. saline.

FIG. 4. Effect of subchronic sc (left) or icv (right) administration of BIM-28163 (open symbols) or saline (closed symbols) on food intake. Top, Cumulative food intake during the 48 h after infusion; bottom, food intake measured during both the diurnal (0700–1900 h) and nocturnal (1900–0700 h) period before, during, and after treatment.
stance P (26). Results with these compounds may be difficult to interpret because the first also binds to four of the melanocortin receptors (33) and the second antagonizes many peptide receptors (34, 35). A recent report indicated that the GHS-R1a displays a high constitutive activity and that [D-Argd-Phe-d-TrpLeu]-substance P is a full inverse agonist, rather than a true antagonist (36). One might therefore hypothesize that the GHS-R1a involved in GH regulation and in food intake may have different levels of constitutive activity and, subsequently, different responses to inverse agonists and antagonists. Alternatively, it cannot be excluded that another, as yet unknown, ghrelin receptor may exist. Structural variants of GHS-R have been found in the human, swine, chicken, and fish genome (37–39). A truncated version of the GHS receptor, derived from alternative gene splicing, GHS-R1b, and not yet observed in the rat, cannot be the novel ghrelin receptor because it binds neither ghrelin nor any of the various GHS molecules. However, GHS-R1b, or another receptor, may interact with GHS-R1a, perhaps through receptor heterodimerization as shown in fish (39), thereby playing a counterregulatory role in controlling either GH secretion or food intake.

Another potential explanation of the discrepant results using GHS-R1a antagonists may rely on the central or peripheral location of the ghrelin systems involved. Central ghrelin probably stimulates food intake and GH secretion through neural mechanisms involving neuronal circuitry (40). An important site for GHS-R1a mediation of GH secretion is the hypothalamic arcuate nucleus (41), where GHS-R mRNA is expressed on both GHRH and neuropeptide Y-expressing neurons (21). However, peripheral ghrelin can also modulate food intake and GH secretion via vagal afferent fibers (42). Blockade of the vagal afferent, either through vagotomy or through perivagal capsaicin application, totally blocks peripheral ghrelin effects on feeding, whereas it only attenuates the stimulation of GH secretion.

In summary, this study shows that pharmacological dissociation of the effects of endogenous ghrelin on GH secretion and feeding is possible. It will remain to be determined whether other effects of this interesting peptide, such as those on adiposity or cardiac function, can also be separately affected. At any rate, the present data support the feasibility of creating ghrelin analogs with selective actions on either GH or feeding behavior.

Acknowledgments

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thalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141:4325–4328


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mone: validation and application to the determination of plasma levels and in vitro release. J Immunassay 18:335–356


Erratum

After publication of the articles titled “Glucocorticoid Receptor Blockade Disinhibits Pituitary-Adrenal Activity during the Stress Hyporesponsive Period of the Mouse” (Endocrinology 146:1458–1464, 2005) by M. Schmidt, S. Levine, M. S. Oitzl, M. van der Mark, M. B. Muller, F. Holsboer, and E. R. de Kloet and “Differential Expression of c-fos and Tyrosine Hydroxylase mRNA in the Adrenal Gland of the Infant Rat: Evidence for an Adrenal Hyporesponsiveness Period” (Endocrinology 143:1717–1725, 2002) by Darren K. Okimoto, Alison Blaus, Mathias Schmidt, M. Kathleen Gordon, Gersham W. Dent, and Seymour Levine, one of the authors, Mathias Schmidt, added a second initial to his name; the complete name is now Mathias V. Schmidt (or M. V. Schmidt).

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