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Review

Lack of obestatin effects on food intake: Should obestatin be renamed ghrelin-associated peptide (GAP)?

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Abstract

Obestatin is a newly identified ghrelin-associated peptide (GAP) that is derived from post-translational processing of the prepro-ghrelin gene. Obestatin has been reported initially to be the endogenous ligand for the orphan receptor G protein-coupled receptor 39 (GPR39), and to reduce refeeding- and ghrelin-stimulated food intake and gastric transit in fasted mice, and body weight gain upon chronic peripheral injection. However, recent reports indicate that obestatin is unlikely to be the endogenous ligand for GPR39 based on the lack of specific binding on GRP39 receptor expressing cells and the absence of signal transduction pathway activation. In addition, a number of studies provided convergent evidence that ghrelin injected intracerebroventricularly or peripherally did not influence food intake, body weight gain, gastric transit, gastrointestinal motility, and gastric vagal afferent activity, as well as pituitary hormone secretions, in rats or mice. Similarly, obestatin did not alter ghrelin-induced stimulation of food intake or gastric transit. Therefore, the present state-of-knowledge on obestatin and GPR39 is leaving many unanswered questions that deserve further consideration. Those relate not only to redefining the biological action of obestatin that should be renamed GAP, but also the identification of the native ligand for GPR39.

Keywords: Ghrelin; Obestatin; Food intake; Gastric emptying; GPR39

Contents

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1. Prepro-ghrelin generates ghrelin and ghrelin-associated peptide, originally named obestatin

During the past decade, numerous gastrointestinal (GI)-derived peptides have been associated with significant effects on food intake and GI motility [1,2]. Among those peptides, ghrelin, a 28-amino acid acylated peptide identified by Kojima et al. in 1999, is to date the only gut peptide that is orexigenic [3,4]. Ghrelin is mainly synthesized in the endocrine cells of the oxyntic mucosa of the stomach and is also found at lower levels in the intestine, pituitary and hypothalamus [4,5]. Ghrelin binds the growth hormone secretagogue receptor subtype 1a (GHS-R1a), which is a G protein-coupled receptor located in the pituitary, hypothalamus and several peripheral tissues [3,4]. Consistent reports established that ghrelin injected centrally or peripherally increases food intake in several animal models and that the orexigenic activity of the peptide is conferred by the acylated group on Ser3 [3–5]. Interestingly, while des-acyl ghrelin injected peripherally loses its ability to increase feeding, central injection can mimic the orexigenic effects of acylated ghrelin through a GHS-R1a independent pathway [3,5,6]. Among other actions, ghrelin is a potent prokinetic that accelerates gastric propulsive motor activity under basal and inhibited conditions [4]. Ghrelin contributes to the regulation of many other functions and has been one of the most extensively investigated gut peptides in the last decade with more than 1500 publications in 7 years listed on PubMed.

Recent prominent discovery in the ghrelin field came from the identification of a peptide derived from the ghrelin precursor. Recently, Zhang et al. compared prepro-ghrelin sequences from 11 mammalian species and identified a conserved region flanked by a potential cleavage site from which originated a novel 23-amino acid aminated peptide that was named obestatin based on the initial reported biological actions [7]. Most noticeable were the surprising findings that two antithetical peptides are generated from the processing of prepro-ghrelin. Indeed, the initial report in mice indicated that human obestatin, when administered both peripherally or into the lateral brain ventricle, could decrease food intake, body weight gain, gastric transit and the stimulatory effects of ghrelin on food intake and gastric motor function [7]. These "Yin and Yang personalities of ghrelin and obestatin" [8] triggered a flurry of investigations that, however, dampened the impact of this first discovery and will be reviewed.

2. Obestatin does not hold its promise to regulate satiety in rodents

Zhang et al.'s initial findings indicated that amidated human obestatin suppressed food intake during the first 5 h following a single injection into the peritoneal cavity (intraperitoneal, ip) in doses ranging from 320 μg/kg to 2.5 mg/kg (125–1000 nmol/kg) in fasted mice and reduced the spontaneous body weight gain upon repeated daily ip administrations at large doses in lean mice [7]. In addition, the peptide injected into the mouse lateral brain ventricle at 20 μg/kg inhibited food intake almost completely for the 1st hour and significantly reduced cumulative food intake for the following 5-h experimental period.

The initial promise of a new regulator of appetite and body weight gain was curtailed by convergent studies negating these findings. A number of reports failed to reproduce obestatin's anorexigenic effect upon acute administration and the reduction of body weight gain after chronic treatment (Table 1). Such a discrepancy between the initial and subsequent reports is hardly explained by the different experimental protocols, species or origin of the peptide used. Site and modalities of obestatin injection, either ip [9,10], subcutaneous (sc) [11] or intracerebroventricular (icv) [8,11,12], were equally inefficient to influence food intake. This was observed under conditions in which test anorexigenic control substances such as cholecystokinin-8 (CCK-8), melanocortin-receptor agonist, MT-II, exendin, sibutramine or dexfenfluramine resulted in the expected satiety effect or body weight reduction, or test control orexigenic peptide, such as ghrelin, increased food intake and body weight gain [9–11,13]. In addition, doses of obestatin injected centrally or peripherally were in a similar range with those reported by Zhang et al. [7] (including highest doses tested of 2.5 mg/kg, ip and 200 μg/kg, icv) [10,12] (Table 1). The duration of the experimental period (within the first 1 to 5 h) was comparable to the initial report along with the chronic administration of the peptide up to one week [11]. It is also unlikely to reflect species differences since obestatin was injected centrally and peripherally both in the same strains of adult rats and mice [8–12,14] (Table 1). Lastly, peripheral human and rat/mouse obestatin that shared 87% homology have been investigated under a variety of conditions, all resulting in the same negative outcome: nocturnal fasting, 50% food deprived and freely fed conditions followed by exposure to standard pellet rodent chow either dry or wet, in the dark and light phase, with or without prior habituation to a daily presentation of food and with or without adaptation to the injection and handling procedure [8–11,14]. In addition, negative findings were also obtained under testing conditions that mimicked those used in the initial report [8,10,11]. Molecular approaches confirmed the absence of obestatin effects on the expression of key hypothalamic peptides regulating food intake as observed in the different functional studies. The icv injection of obestatin (20 μg/kg/day for 7 days) did not influence the expression of neuropeptide Y, proopiomelanocortin, agouti-related peptide, or cocaine- and amphetamine-regulated transcript [11]. Of note is a recent study showing that obestatin injected ip at 320 μg/kg reduced food intake significantly by 34% at 1 h postinjection and no longer thereafter in fasted rats [15]. In this study, obestatin was dissolved in dimethyl sulphoxide (DMSO)/saline immediately prior to being injected ip, while the control group received saline injection without DMSO [15]. The use of such a vehicle raises some concerns about the influence of DMSO *per se* on the results obtained since studies have shown that DMSO is not recommended as a vehicle for peripheral administration in feeding studies, while it may be suitable for solubilization of drugs injected into the brain [16]. None of the other negative studies used DMSO to dissolve obestatin (Table 1). However,

Table 1
Effects of obestatin on food intake both in lean mice (C57BL6) or rats (Sprague Dawley) after either intraperitoneal (ip), subcutaneous (sc) or intracerebroventricular (icv) injections

Obestatin origin	μg/kg, route	Species (nb/group)	Status	Food	Duration of monitoring	Effect on food intake	References
Human	320–2500, ip	Mice, (NS)	Fasted	Standard	1–5 h	Inhibition	[7]
Rat/mouse	300, ip	Mice (5)	Fasted	Standard	30 min–5 h	None	[9]
Rat/mouse	314, ip	Mice (6)	Fasted	Standard	6 h	None	[29]
Rat/mouse	300, ip	DIO mice (7)	Fasted	Standard	2 h	None	[19]
Human	250–2500, ip	Mice (10–11)	Fasted/nonfasted	Wet mashed diet	24 h	None	[10]
Human	2500, ip	Mice (8)	Fasted/nonfasted	Standard	1–8 h, 7 days	None	[11]
Human and rat/mouse	2520, ip	Mice (5, 9)	Fasted	Standard	1–24 h	None	[13]
Rat/mouse	30–300, ip	Rats (5)	Fasted	Standard	30 min–2 h	None	[9]
Rat/mouse	320, ip	Rats (5–7)	Fasted	Standard	1–5 h	Decrease	[15]
Human	400–800, ip and sc	Rats (8,9)	Nonfasted	Standard	1–24 h, ; 7 days	None	[11]
Human	100–3000, ip	Rats (6)	Nonfasted	Standard	1–3 h	None	NP
Human	20, icv	Mice (NS)	Fasted	Standard	5 h	Decrease	[7]
Rat/Mouse	20, icv	Mice (4)	Fasted	Standard	1–5 h	None	[12]
NS	10–30, icv	Rats (6–9)	Fasted/non fasted	Standard	5h/24h	None	[8]
Rat/mouse	20, icv	Rats (8–10)	Fasted/nonfasted/food restricted	Standard	6 h	None	[14]
Human	100–400/day, icv	Rats (8–12)	Fasted/nonfasted	Standard	1–24 h/7–9 days	None	[11]

DIO: diet-induced obesity, NS: not specified; nb: number, NP: Gourcerol et al., unpublished observation.

all together these recent data should be put in the context of another anorexigenic peptide, such as peptide YY $(PYY)_{3-36}$ for which concerns about the robustness and reproducibility for the peptide to decrease food intake in rodents were subject of initial debate [17].

It has been underlined early on that the given name of obestatin was premature since the inhibitory effect of the peptide to reverse feeding in obese animal models was not originally tested [18]. Recent data seems to confirm this statement since acute ip injection of obestatin at 300 μg/kg was unable to decrease food intake in high fat diet-induced obese mice [19]. However, additional higher doses and other obesity models need to be tested before ruling out the absence of action of obestatin in experimental models of obesity. Further developments may shade some light as to whether obestatin will live up to its name as a peptide curtailing obesity.

3. Lack of reproducible interaction between obestatin and other gut peptides influencing satiety

Over the past years, it has become increasingly recognized that different signals governing meal initiation, meal-ending satiation and inter-meal satiety could modulate each other. Interaction between these signals results in a more potent satiety effect than each signal alone or alternatively, one signal can reduce the effects of that induced by another. For instance, we and others have previously established the synergistic interactions between CCK and leptin at the level of capsaicin-sensitive vagal afferents resulting in an enhanced satiety response [20–22]. Kobelt et al. also established recently that subthreshold doses of CCK or bombesin injected ip blocked the orexigenic effect of ip-administered ghrelin while amylin did not modulate ghrelin-induced food intake in rats [23,24]. In this context, obestatin and ghrelin, which both arise from post-translational cleavage of the prepro-ghrelin peptide, were well positioned to interact. This was highlighted in the original Zhang et al.'s report. Both ghrelin and obestatin where co-injected ip at the same molar ratio (1 μmol/kg) resulting in the normalization of food intake compared to the inhibitory effect of obestatin administered alone [7]. Conversely, repeated daily injections of obestatin prevented the body weight gain induced by ip ghrelin in lean mice over a 1-week period [7]. Subsequent reports, using central or peripheral injections of obestatin-ghrelin, however, failed to confirm such an interaction (Table 2). Acute icv injections of obestatin and ghrelin did not modify icv ghrelin-induced increase in food intake in three rat experimental models: fed *ad libitum*, 50% food restricted or nocturnally fasted [14]. Under conditions of chronic obestatin and ghrelin administration at the same molecular ratio (1 μmol/kg, ip), obestatin did not yield to a reduction of ghrelin-induced increased food intake and body weight gain over a 7-day treatment period in mice [11]. Considering that studies showing the lack of obestatin effect to influence food intake were performed in fasted rodents (Table 1), a condition associated with the highest endogenous circulating levels of ghrelin [25–27], it can be inferred that obestatin also did not alter the orexigenic action of endogenous ghrelin. Obestatin serum levels, unlike those of ghrelin, are not influenced by feeding or fasting [7]. Therefore, the uncoupled changes in obestatin and ghrelin circulating levels in response to feeding status do not favor an interaction between the peptides under these physiological conditions.

Other investigations showed that there is no interaction between obestatin and CCK known to induce satiety signaling through vagal pathways when both peptides were injected ip in rats [9]. This was further demonstrated *in vitro* in a gastric vagus-stomach preparation with cannulated gastric artery. Obestatin (30 μg) injected intra arterially into the stomach did

Table 2
Effects of obestatin in combination with other peptides on food intake in mice (C57BL6) or rats (Sprague Dawley) after either intraperitoneal (ip) or intracerebroventricular (icv) injections

Obestatin (origin, μg/kg, route)	Peptide co-injected (μg/kg, route)	Species (nb/group)	Effects on food intake	References
Human, 300–2500, ip	Ghrelin (3300, ip)	Mice (NS)	Obestatin-induced reduction of food intake for 3–5 h was prevented by ghrelin	[7]
Rat/mouse, 300, ip	CCK (1, ip)	Rats (5)	Obestatin had no effect on basal- or CCK-induced reduction of food intake	[9]
Human, 2500, ip	Ghrelin (3300, ip)	Mice (8)	Obestatin had no effect on basal or ip ghrelin-stimulated food intake/body weight	[11]
Rat/mouse, 5 μg/rat, icv	Ghrelin (6 μg/rat, icv)	Rats (8–10)	Obestatin had no effect on basal or icv ghrelin-stimulated food intake	[14]

NS: nonspecified; nb: number.

not significantly alter gastric vagal afferent discharge in the same fibers that responded with a marked spike activity to a low dose (10 ng) of CCK-8 [9].

4. Lack of reproducible inhibitory action of obestatin on upper gastrointestinal motor function

Most of the gut peptides that display significant effects on food intake also exert biological actions on digestive motor function, especially on the upper GI [28]. Such effects were also tested in the first report describing the anorexigenic properties of obestatin [7]. Obestatin diminished the contractile activity of jejunal muscular strips *in vitro* while ghrelin displayed the opposite effect, suggesting a role for obestatin to slow down upper GI transit [7]. This was expended by *in vivo* assay where obestatin injected ip in doses ranging from 320 μg/kg to 2.5 mg/kg delayed gastric emptying of a caloric meal in mice for the 1–2-h period postinjection [7]. However, these findings so far have also not been replicated (Table 3). Recent reports showed that obestatin injected ip at 320 μg/kg did not influence basal gastric emptying of an acaloric meal in rats while under similar conditions, 1 μg/kg of CCK-8 induced a significantly delayed gastric emptying [9]. The CCK-8 inhibitory effect was also not influenced by co-administration of obestatin [9]. Other studies showed that obestatin injected ip at doses ranging from 150 to 640 μg/kg influenced neither basal nor ghrelin accelerated gastric emptying of a chow meal as measured by C^{14} breath test in mice [29]. Lastly, a recent report indicated that obestatin infused intravenously at 75 μg/kg min^{-1} for 30 min did not influence gastric retention of either a non-nutrient or nutrient meal in rats [30].

Similar consistent lack of obestatin action on GI motility has been reported. Obestatin injected intravenously (iv) at 300 μg/kg in anesthetized rats did not influence intragastric pressure monitored continuously while the preparation responded to a subsequent injection of CCK-8 (0.3 μg/kg) by the well defined gastric relaxation [9]. Intravenous infusion of obestatin in conscious rats at 25 μg/kg min^{-1} for 1 h altered neither the fasted pattern of small intestinal motility in conscious rats nor ghrelin-induced decrease in migrating motor complex [30]. Confronted to the absence of biological action *in vivo*, recent reports explored obestatin action on gastric and intestinal contractility *in vitro*. However, obestatin (1 μM) was unable to induce any response on rat and mouse jejunal, duodenal and fundic strips in presence or absence of electrical field stimulation [29,30]. Again, it is unlikely that divergent results between the initial and subsequent reports may result from experimental procedures. Gastric emptying was tested using different techniques, with acaloric and caloric test meals in both rats and mice using established positive controls. Furthermore, doses were within the ranges to those previously tested initially (Table 3).

5. G protein-coupled receptor 39 (GPR39) still orphan?

Biological activities of peptides are mediated by receptors located at the site of action. Zhang et al. [7] reported that obestatin is the cognate ligand for the orphan GPR39 receptor. GPR39 was originally cloned in 1997 and characterized to belong to the class A of 7 transmembrane domain G protein-coupled receptors (R) as part of the ghrelin subfamily that also includes ghrelin R (GHS-R1a), motilin R (GPR38), neurotensin R1, neurotensin R2, neuromedin U-R1 (GPR66) and neuromedin U-R2 [31–33]. The GPR39 receptor was first reported by Northern blot analysis to be widely expressed in the human

Table 3
Effects of obestatin on other endpoints such as GI motility, hormone secretion or water intake in mice (C57BL6) or rats (Sprague Dawley) after either intra-arterial (ia), intraperitoneal (ip), intravenous (iv), subcutaneous (sc) or intracerebroventricular (icv) injections

Obestatin (origin, μg/kg, route)	End points	Species (nb/group)	Effects	References
Human, 300–2500, ip	Gastric emptying	Mice, (NS)	Inhibition	[7]
Rat/mouse, 150–630, ip	Gastric emptying	Mice (10,11)	None	[29]
Rat/mouse, 300, ip and iv, ia	Gastric: emptying, relaxation, vagal afferent activity	Rats (6–7)	None	[9]
Human, 100–3000, ip	Gastric emptying	Rat (6)	None	NP
NS, 25–750, iv	Gastric emptying,	Rats (4)	None	[30]
NS, 1500, iv	Small bowel motility	Rats (7)	None	[30]
Rat/mouse 300, iv	Gastric motility	Rats (4–8)	None	[19]
Human	GH	*In vitro*	None	[7]
Rat/mouse, 10, iv and icv	GH, ACTH, Prolactine, TSH	Rats (4)	None	[12]
Human, 300, iv	GH	Rats (8)	None	[11]
Rat/mouse, 320, sc	GH and corticosterone	Rat pups (5–7)	None	[15]
NS, 7.5, icv	Arterial pressure, heart rate	Rats (NS)	None	[8]
NS, 2.5–7.5, icv	Water intake	Rats (6,9)	Reduction	[8]

NS: not specified. NP: Gourcerol et al., unpublished observations.

brain, while its peripheral distribution was restricted to the stomach and small intestine [31]. In mice, Zhang et al. reported that the highest levels of GPR39 are expressed in the small intestine, stomach, liver and hypothalamus as detected by Northern blot and reverse transcriptase-polymerase chain reaction analyses [7]. In contrast, subsequent reports using *in situ* hybridization in mice and real time quantitative polymerase chain reaction analysis in rats failed to detect the expression of GPR39 mRNA in the hypothalamus [10,11,34], a region primarily involved in food intake regulation [35]. By contrast, the amygdala subnuclei, which underlie fear-associated learning behavior, and the ventral hippocampus were identified as the most prominent in expressing GPR39 mRNA in mice [34].

An abundant expression of GPR39 receptor in the upper GI tract was, however, consistently reported in humans, mice and rats [7,11,13,31], supporting that the cognate ligand would exert an action within the GI tract. Zhang et al. observed in a crude plasma membrane preparation of rat jejunum that obestatin binds with high affinity to GPR39 while other members of the ghrelin subfamily did not [7]. Furthermore, obestatin displayed high affinity to GPR39 receptors overexpressed in CHO or HEK293T cells and also stimulated cAMP [7]. However, these findings have been recently challenged by several independent groups [10,13,36]. Holst et al., who previously established and characterized the constitutive activity of GPR39 [33], were unable to confirm that obestatin has agonist properties on the GPR39 receptor [10]. This was supported by the absence of obestatin signaling in GPR39 expressing cells as monitored by inositol phosphate turn over, cAMP, arrestin mobilization, as well as cAMP response element-dependent and serum response element-dependent transcriptional activity [10,13]. In addition, radiolabelled obestatin did not bind GPR39 in different types of GPR39 expressing cells [10]. Similarly, Lauwers et al. [36] were unable to show that obestatin elicits GPR39 signaling in transfected cells as monitored by cAMP under similar conditions of incubation as reported previously [7] and using another fluorometric Ca^{2+} flux method [36]. This contrasted with the stimulation of signaling induced by Zn^{++} that displays features of a potential agonist or modulator of GPR39 receptors [10,33,36]. These *in vitro* studies suggest that obestatin is not the cognate receptor for GPR39, as initially claimed. This is also supported by *in vivo* studies showing that the expression of GPR39, for instance in the pituitary or the stomach and intestine, does not translate into biological actions of obestatin at these sites at least for the end parameters monitored so far (Table 3). For instance, GPR39 is highly expressed in the pituitary [7], but obestatin injected iv influences neither basal growth hormone (GH) nor ghrelin-stimulated GH release as monitored 10 min after injection in rats or in dispersed rat pituitary cells [8,12,15]. Similarly, basal secretion of several other pituitary and corticomedullary hormones was not altered by the iv injection of obestatin [11,12,15] (Table 3). These findings do not rule out the involvement of GPR39 in other biological actions when activated by its yet to be discovered cognate ligand. Indeed, GPR39-deficient mice exhibit an accelerated gastric emptying rate and increased fecal pellet output compared to wild type suggesting a role of the receptor in the regulation of GI transit [13,37]. However, GPR39 knockout mice display similar body weight, adiposity and food intake compared to age matched littermate controls suggesting that GPR39 is not a major regulator of body weight at least in lean mice [13,37].

6. Obestatin: Toward new physiological role(s)?

Obestatin displays a very short half-life in blood circulation and does not have specific uptake by endothelia cells composing the blood-brain barrier [38], indicative that the peptide actions are most likely local. A recent study found obestatin immunoreactivity within the rat and guinea pig myenteric plexi, colocalizing with choline acetyltransferase [39]. Therefore, we cannot conclude so far that obestatin has no role to play within the GI tract, and its potential effects on sensory, secretion or immune functions still remain to be investigated.

New and unexpected actions for this peptide, especially within the central nervous system, were recently reported. Interestingly, Samson et al. found that obestatin administered icv inhibits water consumption associated with feeding or stimulated by the dipsinogen, angiotensin II while no change in locomotor activity, food intake or stereotypic behaviors were observed [8]. Another study suggests that obestatin injected centrally increased the number and shortened latency of non-rapid-eye-movement during sleep in rats [40]. These observations await further confirmation and physiological relevance in the context that gastric release of obestatin may not reach the brain due to its rapid metabolism and lack of uptake mechanisms [38]. The receptor involved in such effects also remains to be established.

7. Should obestatin be renamed ghrelin-associated peptide?

The discovery that the processing of ghrelin gene yields obestatin opens new insight to the post-translational processing of prepro-ghrelin [41]. Obestatin was initially reported to reduce food intake, body weight gain, gastric emptying and suppress intestinal motility through an interaction with the orphan receptor GPR39 [7]. In addition, obestatin was originally found to abrogate ghrelin stimulatory action on these end points [7]. Therefore, this new ghrelin-associated peptide was proposed to serve as a physiological opponent of ghrelin [7]. However, a number of subsequent studies undergone in different laboratories suggest that these initial results could be far less compelling. So far the assumptions that obestatin conveys a satiety signal and inhibits upper GI motility under basal- and ghrelin-stimulated conditions are largely unconfirmed (Tables 1, 2). Noteworthy, is also the fact that GPR39 that was deorphanized as the obestatin receptor [7] is again in search of its endogenous ligand since several groups were unable to confirm that obestatin binds with high affinity to GPR39 or activates signaling in transfected cells [10,36]. Therefore, the present state-of-knowledge on obestatin and GPR39 is leaving significant unsolved issues, most notably the basis for the lack of reproducible biological actions of obestatin on food intake and gastric motor function, and also the identification of the

native ligand for GPR39 along with that of the receptors on which obestatin exerts recently described central actions to influence drinking behavior and sleep [8,40]. In view of these findings, it is proposed that ghrelin associated peptide, GAP, will be a better-suited name for obestatin for which a gap in knowledge needs to be filled.

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