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REVIEW

Biological effects of obestatin

Jiang-Bo Li · Akihiro Asakawa · KaiChun Cheng · Yingxiao Li · Huhe Chaolu · Minglun Tsai · Akio Inui

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Abstract Obestatin, a 23-amino-acid peptide, is derived from the preproghrelin precursor. Obestatin was identified in 2005 as a hormone regulating food intake and energy, and having opposite effects to those of ghrelin. However, as studies have progressed, many disputes on the physiological function of obestatin have emerged. The food intake suppressive effects of obestatin have not been replicated in many studies. Nonetheless, many biological roles of obestatin have been revealed, and obestatin is thought to be associated with a variety of biological functions such as feeding, drinking, incretion, memory, and sleep, and with neuropsychiatric manifestations. The biological effects of obestatin will be reviewed in this article.

Keywords Obestatin · GPR39 · Acylated ghrelin · Des-acyl ghrelin · Biological effects

Introduction

Over the past few decades, there has been a dramatic increase in the number of people who are overweight or obese. The obesity epidemic has become a major public health problem in many countries. Obesity increases the risk of diabetes, hypertension, cardiovascular diseases, hyperlipidemia, osteoarthritis, and several cancers. Effective control of obesity will be of great significance in preventing and treating these diseases. Many research groups have been focusing on obesity-related peptides. In 1994, Zhang et al. cloned the *ob* gene of both mice and humans, and identified its product as leptin [1]. Leptin, which is secreted from adipose tissue, circulates in the blood and acts on the central neural networks that regulate ingestive behavior and energy balance [1, 2]. In 1996, a heterotrimeric GTP-binding protein (G protein)-coupled receptor found in the pituitary and acute ventro-medial and infundibular hypothalamus of swine and humans was cloned and shown to be the target of growth hormone (GH) secretagogues [3]. In 1999, ghrelin, a 28-amino-acid peptide, was discovered in rat gastric extracts as a natural ligand of the orphan growth hormone secretagogue receptor type 1a (GHS-R1a) [4]. Ghrelin expression occurs mainly in the stomach. In addition, the ghrelin gene is ubiquitously distributed in various organs, including the stomach, intestines, kidney, pancreas, placenta, testicles, pituitary, and hypothalamus. On the other hand, GHS-R is also ubiquitously distributed in various organs and tissues, including the hypothalamus, pituitary, heart, lungs, intestines, pancreas, accessory adrenal glands, ovaries, adipose tissue, and blood vessels [3, 5]. Therefore, ghrelin plays various roles in physiological processes: GH release; food intake; energy and glucose homeostasis; gastrointestinal, cardiovascular, pulmonary, and immune function; cell proliferation and differentiation; and bone physiology [4, 6–10]. Two major molecular forms of ghrelin, acylated ghrelin and des-acyl ghrelin, exist in the stomach and serum. The former comprises <10% of the total circulating ghrelin but performs most of the ghrelin function. Initially, des-acyl ghrelin was thought to have no endocrine function, but was subsequently found to play an endocrine role. Some actions of des-acyl ghrelin and acylated ghrelin are completely different, while others are identical [11–13].

J.-B. Li · A. Asakawa (✉) · K. Cheng · Y. Li · H. Chaolu · M. Tsai · A. Inui
Division of Psychosomatic Internal Medicine, Department of Social and Behavioral Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
e-mail: asakawa@m2.kufm.kagoshima-u.ac.jp

In 2005, Zhang et al. first isolated a novel peptide produced by the ghrelin gene from rat stomach and named it obestatin. Contrary to the appetite-stimulating effects of ghrelin, obestatin suppressed food intake, inhibited jejunal contraction, and decreased body-weight gain. In addition, obestatin was found to bind to the orphan G protein-coupled receptor39 (GPR39) [14]. Perhaps as a result of the special connection between obestatin and ghrelin, obestatin has received considerable attention, and researchers have found that it has many biological effects (Fig. 1). In this article, the current knowledge on obestatin is reviewed and discussed.

Biological characteristics, structure, and distribution

Obestatin, a 23-amino-acid peptide with a molecular mass of 2516.3, was derived from the preproghrelin precursor and was originally purified from rat stomach mucosa. Its amino-acid sequence is FNAPFDVGIKLSGAQYQQH-GRAL [14]. Obestatin is also present in the gastric mucosa, duodenum, jejunum, colon, pancreas, spleen, mammary glands, breast milk, Leydig cells of the testis, saliva, and plasma [14–19]. While 24-h fasting resulted in elevated ghrelin levels in mice, obestatin levels were significantly reduced. This suggested that secretion of these two peptides is regulated by nutritional status in an opposite manner [20]. Fasting plasma concentrations of obestatin in humans have been reported to be 268 and 5 ng/ml [21, 22]; this 20-fold difference may indicate the non-reliability of the obestatin assay. In lactating mothers, obestatin levels in colostrum (539 pg/ml) and mature milk (529 pg/ml) were more than twice the corresponding blood levels (270 pg/ml) [17]. In normal-weight subjects, postprandial obestatin levels showed a significant decrease between 60 and 90 min, rising to basal levels thereafter. Postprandial obestatin levels correlated inversely and significantly with body mass index (BMI) and basal insulin levels in normal-weight female and male subjects. Basal obestatin levels in females were higher compared to males [23]. The half-life of obestatin was only 2 min [24]. However, Morash et al. recently reported that they did not detect fasting-induced changes in tissue obestatin expression or plasma obestatin level [25].

Receptor for obestatin

Zhang et al. reported that GPR39 is the specific receptor for obestatin. I^{125}-obestatin binding to GPR39 competed with obestatin binding [14]. GPR39 was initially identified as a GHS-R-related orphan receptor containing seven transmembrane domains and a G protein interaction domain [26, 27]. Many rat tissues express GPR39 mRNA at relatively higher levels in digestive tissues such as the jejunum, duodenum, stomach, and ileum, and moderate levels in brain regions including the hypothalamus. The identified GPR39 expression sites were consistent with the results of obestatin binding studies [14]. Recently, the same research group obtained mono-iodo-obestatin following high-performance liquid chromatography purification and demonstrated its binding to the jejunum, stomach, ileum, pituitary, and to adipose tissue, suggesting that obestatin is a metabolic hormone capable of binding to GPR39 to regulate the functions of diverse gastrointestinal and adipose tissues [28]. Other research demonstrated that GPR39 is expressed in adipose tissue and the amygdala, hippocampus, auditory cortex, gastrointestinal tract, liver, pancreas, and kidney; at intermediate levels in the lung and heart; and at low levels in the spleen. Surprisingly, no expression of GPR39 was found in the hypothalamus [29–31]. Moechars et al. reported that gastric half-emptying

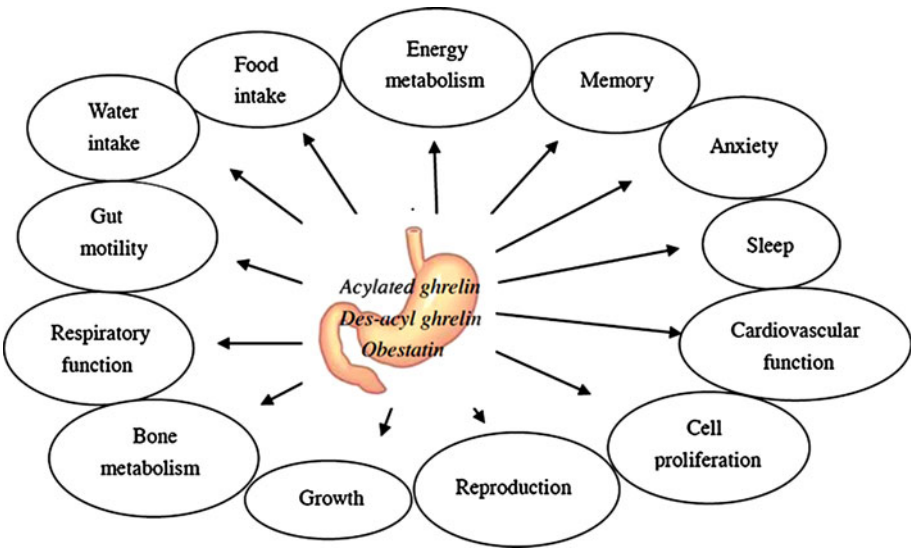

Fig. 1 Various roles of ghrelin associated family

time of a solid meal was significantly accelerated in GPR39$^{-/-}$ mice versus in GPR39$^{+/+}$ mice and that the volume of gastric secretion was significantly increased; however, gastric acid secretion was unchanged 4 h after pylorus ligation in GPR39$^{-/-}$ mice. The mature body weight and body fat composition of GPR39$^{-/-}$ mice was significantly higher compared with that of GPR39$^{+/+}$ mice. These findings suggested that obestatin plays a functional role in the regulation of gastrointestinal and metabolic function through interaction with the GPR39 receptor [31]. However, Holst et al. reported that no specific binding of obestatin could be detected in two different types of GPR39-expressing cells using three different radioiodinated forms of obestatin. They then suggested that GPR39 is probably not the obestatin receptor [32]. Some other studies also clearly showed that obestatin could not bind to GPR39 or activate this receptor in the hypothalamus. Therefore, the role of the obestatin/GPR39 system in the regulation of energy balance remains unclear [33–35].

The latest research reported that obestatin showed specific binding to glucagon-like peptide-1 receptor (GLP-1R) on HIT-T15 and INS-1 β-cells, and that the GLP-1R antagonist exendin-(9-39) reduced obestatin effects on β-cell survival [36]. However, Unniappan et al. reported that obestatin did not bind to INS-1 β-cells or human embryonic kidney cells overexpressing GLP-1 receptors [37]. Further studies are required to clarify the issue of these contradictory investigations of the obestatin receptor.

Biological activities

Influences on the endocrine system

Obestatin concentrations progressively decreased from postnatal day 1 to 21, and there were significant positive correlations between postnatal obestatin and insulin concentrations in the pancreas [15]. Obestatin promotes survival and prevents apoptosis in both β-cells and human islets. Moreover, obestatin induces expression of genes that regulate β-cell fate, insulin biosynthesis, and glucose sensing [36]. In streptozotocin-treated animals, obestatin improved glucose metabolism and preserved islet cell mass, displaying therapeutic potential for medical conditions associated with impaired β-cell function [38]. Intravenous (i.v.) administration of obestatin was found to stimulate pancreatic protein output in anaesthetized rats via a cholecystokinin- and vagal-dependent mechanism [39, 40]. However, other reports state that obestatin more effectively inhibited insulin secretion [41–43]. Very recently, Egido et al. showed a dual effect of obestatin on insulin secretion in the rat pancreas. At 10 nM, obestatin inhibited glucose-induced insulin secretion, while at 1 nM, it potentiated the insulin response to glucose, arginine, and tolbutamide, indicating that its stimulation of insulin output is not mediated by an α- or δ-cell paracrine effect [44]. On the other hand, other reports suggested that obestatin did not alter glucose or insulin responses [37, 45].

Intravenous administration of rat/mouse ghrelin increased plasma GH levels as soon as 5 min after administration. The effect was maximal at 10 min and returned to basal levels after 45–60 min. Under the same conditions, obestatin did not change basal GH levels but markedly inhibited ghrelin-induced GH secretion [20]. Furthermore, not only did i.v. and intracerebroventricular (i.c.v.) administration of obestatin not influence plasma GH levels, it also did not influence plasma prolactin, adrenocorticotropic hormone, and thyroid stimulating hormone levels [46]. Recently, Qader et al. reported that acylated ghrelin and obestatin had almost identical effects in that each stimulated glucagon secretion and inhibited pancreatic polypeptide (PP) and somatostatin secretion from both mouse and rat islets [41].

Fasting obestatin and ghrelin levels were significantly lower in obese than in normal-weight individuals [21, 47–49]. Obestatin was negatively correlated with BMI, glucose, insulin, leptin, and the homeostasis model assessment of insulin resistance, and it was positively correlated with insulin sensitive index. These might suggest a certain role of obestatin in the regulation of energy homeostasis. A significant relationship between plasma obestatin and ghrelin levels, independent of anthropometric parameters, supports simultaneous secretion of both hormones from the common precursor [21, 47–51]. However, Vicennati et al. showed that obese women had higher obestatin levels, lower ghrelin blood levels, and a lower ghrelin/obestatin ratio compared with controls. In contrast, the ghrelin/obestatin ratio was significantly and negatively correlated with BMI, waist circumference, waist-to-hip ratio, fasting insulin levels, and homeostasis model assessment for insulin resistance, and it was positively correlated with insulin sensitivity index [52]. Obestatin imbalance might play a role in the pathophysiology of obesity. It is worth researching whether obestatin resistance exists.

In addition, plasma obestatin levels were lower in patients with impaired glucose regulation and type 2 diabetes mellitus, insulin resistance, chronic atrophic gastritis, ulcerative colitis, fatty liver, hemodialysis, and oral squamous cell carcinoma tissue. Plasma obestatin levels were high in anorexia nervosa and epileptic patients compared to normal subjects [19, 21, 22, 53–60]. Obestatin seem to play a significant role in the pathophysiology of these diseases.

Influences on the cardiovascular system

Ghrelin is of great importance in the cardiovascular system in that it can obviously decrease mean arterial pressure,

increase cardiac output and cardiac index, and so on [61, 62]. Ghrelin and des-acyl ghrelin inhibit apoptosis of primary adult and H9c2 cardiomyocytes and endothelial cells in vitro via activation of extracellular signal-regulated kinase-1/2 and Akt serine kinases [10]. Fasting plasma obestatin levels were negatively correlated with systolic blood pressure in humans [54]. However, obestatin levels and the ghrelin/obestatin ratio were significantly higher in spontaneously hypertensive rats than in Wistar-Kyoto rats [63]. Plasma obestatin concentrations were positively correlated with systemic blood pressure in normal pregnant women and patients with pregnancy-induced hypertension. Accordingly, obestatin may have a role in blood pressure regulation [64]. In an in vitro study, obestatin decreased vascular cell adhesion molecule-1 expression in endothelial cells when stimulated together with TNF-α. In addition, obestatin increased oxidized low-density lipoprotein binding to macrophages. The effects of obestatin on atherosclerosis using cell models seem to be similar to or less effective than ghrelin [65]. However, it was reported that obestatin did not modify the cell cycle or viability of HL-1 cardiomyocytes, and that it was not able to prevent cytosine arabinoside-induced apoptosis of these cells [66]. Recent findings demonstrated that bolus i.v. administration of obestatin from low to high dosage had no significant effects on mean arterial pressure, heart rate, or baroreflex sensitivity in spontaneously hypertensive rats [67].

Influences on sleep, feeling, and memory

Obestatin induced a significant increase in the time rats spent in non-rapid-eye-movement sleep (NREMS) in the first hour after i.c.v. administration at dark onset. In addition, obestatin treatment decreased NREMS latency and electroencephalographic slow-wave activity. These results suggested that the sleep-promoting effect of centrally administered obestatin may be part of the behavioral manifestation of satiety elicited by the peptide [68]. Carlini et al. reported that ghrelin clearly and dose dependently increased memory retention and, at different potencies, induced anxiogenesis. On the other hand, the effects of obestatin on anxiety were functionally opposite those of ghrelin, while both obestatin and ghrelin increased memory retention [69, 70].

Regulation of feeding and gastrointestinal motility

Influence on feeding

Zhang et al. reported that obestatin had the opposite effect of ghrelin, thereby reducing food intake and gastric transit time and decreasing body-weight gain [14]. Bresciani et al. reported that i.c.v. administration of obestatin resulted in a significant decrease in food intake [70, 71]. Intraperitoneal (i.p.) administration of obestatin inhibited feeding at doses of 10–100 nmol/kg in mice and 100–300 nmol/kg in lean and Zucker fatty rats [72]. Both glucose excursions and insulin responses were lowered in mice after i.p. administration of obestatin or 4 h prior to an allowed 15-min feeding period. However, these phenomena were accompanied by reductions in food intake [43]. When i.p. obestatin was administered to test rats or mice daily for 7–12 days in conjunction with a standard diet, daily caloric intake and body-weight gain decreased as compared to that in control (vehicle-treated) animals [72, 73]. The anorexic and weight reducing effects of obestatin treatment were evident as of treatment day 6 and day 8, respectively, and were consistent through the end of the treatment period [73]. In addition, exogenous obestatin inhibited the ghrelin orexigenic effect that was evident in fed mice [20]. On the other hand, many reports have stated that obestatin did not show any effect on food intake in mice or rats [24, 32, 34, 37, 74–76]. Obestatin treatment did not modify either daily calorie intake or body-weight gain in highly palatable cafeteria-style diet-fed rats [73]. As a result, great controversy surrounding the effects of obestatin on appetite regulation persists.

Influence on water drinking

Obestatin acts in the brain to alter the thirst mechanism. The i.c.v. administration of obestatin significantly inhibits water intake, attenuates vasopressin release induced by central angiotensin II administration, and leads to exaggerated basal vasopressin release in rats [77, 78]. However, Annemie et al. reported that central administration of obestatin failed to show inhibitory effects on water intake in mice [76].

Influence on gastrointestinal motility

Zhang et al. originally demonstrated that obestatin treatment decreased the contractile activity of jejunum muscle strips and antagonized the stimulatory effect of ghrelin on the jejunum muscle; this observed inhibition of jejunal contraction may trigger an afferent vagus signal to induce a central satiety response [14]. Ataka et al. reported that motor activity in the antrum and duodenum was inhibited when obestatin was given intravenously to freely moving conscious rats in the fed state but not when it was given to rats in the fasted state [79]. Between 30 and 90 min after i.v. administration, obestatin decreased the percentage motor index (%MI) in the antrum and prolonged the time required to return to fasted motility in the duodenum. However, obestatin failed to antagonize the ability of ghrelin either to stimulate the %MI in the antrum or to

accelerate the initiation of fasted motility in the duodenum when administered to rats in the fed state [79]. Corticotropin-releasing factor and urocortin 2-containing neurons in the brain might be activated by i.v. administration of obestatin. The vagal afferent pathways might be partially but not entirely involved in these actions of obestatin [79, 80]. The action of obestatin on gastrointestinal motility is different from that of acylated ghrelin but similar to that of des-acyl ghrelin in freely moving, conscious mice (Table 1). However, other reports suggested that obestatin does not influence gut motility, gastric emptying, or vagal afferent activity [74, 81–83].

Prospective view

Since the discovery of ghrelin, much attention has been paid to it, and it has been revealed that ghrelin is involved in food intake, water drinking, gastrointestinal motility, incretion, cardiovascular activity, reproduction, immunity, neuropsychiatry, energy metabolism, and so on. Studies on des-acyl ghrelin are also in progress and have made some advances. On the other hand, studies on obestatin are limited because of several of the abovementioned reasons. Further, these studies differ widely on whether or not obestatin has biological actions that are opposite to those of ghrelin. Some studies not only failed to detect opposite effects of these two peptides but contrarily discovered some similar actions, for example, on water intake, sleep, memory, glucagon, pancreatic polypeptide, somatostatin, and islet cells (Table 2). Most scholastic studies have focused on the effects of obestatin on food intake, but the results are controversial. The differences in the results of these studies may at least partially depend on the unusual dose effect of obestatin on feeding inhibition. Recently, Lagaud et al. reported that acute administration of obestatin

Table 1 Ghrelin associated family on gastroduodenal motility

Acylated ghrelin	↑	Phase III like contraction (duodenum)
		Motor index (antrum)
Des-acyl ghrelin	↓	Phase III like contraction (antrum)
Obestatin	↓	Phase III like contraction (duodenum)
		Motor index (antrum)

↑: increase; ↓: decrease

Table 2 Roles of ghrelin associated family in biological functions

Effect	Acylated ghrelin	Des-acyl ghrelin	Obestatin
Receptor	GHS-R	?	GPR39 ×
Vagal afferent firing	↓	×	?
GH	↑	↓ ×	↓ ×
ACTH, TSH	↑	×	×
Insulin secretion	↑ ↓	↓	↑ ↓ ×
Glucagon	↑	×	↑
Somatostatin	↓	×	↑
PP (pancreatic polypeptide)	↓	×	↓
Body weight	↑	↓	↓ ×
Water intake	↓	×	↓ ×
Food intake	↑	↑↓	↓ ×
Heart function	↑	↑	×
Memory	↑	×	↑
Anxiety	↑	×	↑
Sleep	↑	×	↓
Cell proliferation	↓ ↑	↓ ↑	↑
Gut motility	↑	↓	↓ ×
Osteoblast production	↑	↑	×
Islet cell	↑	↑	↑

↑: stimulation; ↓: inhibition; ×: no effect; ?: effect not determined

inhibited feeding and that the dose–response relationship was U-shaped such that both low and high doses were without effect in mice and in lean and Zucker fatty rats [72]. These findings may explain the difficulties in reproducing the effects of obestatin on feeding reported by some groups [24, 32, 34, 37, 72, 74–76]. In addition, the use of different test conditions, experimental animal species, and methods of administration may have greatly influenced the outcomes of the studies. It was recently suggested that the divergent conclusions about the activity of obestatin might be explained by the lack of consistency in the quality of the peptide used. Spiegeleer et al. analyzed obestatin provided by five different manufacturers and found that one of the examined products was an entirely different peptide in reality and that the quality of two-thirds of these examined products was low [84]. With the background of the existing controversy on obestatin, recent studies have proved that obestatin has an objective existence and plays many roles in organisms, such as promotes sleep [68], improves memory, resists anxiety [69, 70], inhibits water intake [77, 78], increases the secretion of pancreatic juice enzymes [39], promotes survival of pancreatic β-cells [36, 38], and regulates glucose-induced insulin secretion [41–44]. Thus, obestatin is a promising target in studies on the discovery of new drugs for the treatment of obesity, diabetes, and neuropsychiatric diseases. Future studies should thoroughly investigate the obestatin receptor and focus on identifying the biological functions of obestatin.

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