Section A-Research paper

Obestatin; Overview and Physiological actions

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Abstract

Background: The word obestatin is a contraction of obese, and derives from Latin 'obedere', meaning 'to devour' and 'statin', denoting suppression. First discovered in 2005 using bioinformatics, obestatin is a 23-amino acid peptide that is derived from the same 117-residue prepropeptide as ghrelin. High levels of GPR39 mRNA were found abundantly in the amygdala, the hippocampus, and the auditory cortex but not in the hypothalamus. Obestatin was first reported to inhibit jejunal contraction, food intake and body weight gain in rats, in addition to antagonising ghrelin-induced contraction of isolated jejunum muscle. Furthermore, obestatin is incapable of preventing ghrelin-mediated acceleration of gastric emptying or intestinal motility. In addition to its proposed physiological actions, it appears that obestatin may also confer some benefits in GI disease. For example, in rats, obestatin protects against experimental ulcerative colitis via acute attenuation of lipid peroxidation and TH1-mediated inflammation, chronic suppression of polymorphonuclear leukocyte infiltration, induction of glutathione synthesis, improved mucosal blood flow and stimulation of cell proliferation in colonic mucosa, effects that may be mediated by activation of anti-inflammatory cytokines

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Introduction

The word obestatin is a contraction of obese, and derives from Latin 'obedere', meaning 'to devour' and 'statin', denoting suppression. First discovered in 2005 using bioinformatics, obestatin is a 23-amino acid peptide that is derived from the same 117-residue prepropeptide as ghrelin **(1).** It displays a post-translational amide modification of the C-terminal, which was initially suggested to be essential for binding of obestatin **(1).** and later demonstrated to be essential for stabilization of the peptide into its regular conformation **(2).** which has now been determined.

Obestatin is largely produced throughout the GI tract (e.g. stomach, pancreas and duodenum) with predominant expression in the gastric mucosa **(3),** although its distribution is somewhat species specific. For example, in the rat, obestatin is found in the GI tract, within the A-like cells and oxyntic glands of the gastric mucosa and cholinergic neurons of the myenteric plexus, and in the Leydig cells of the testis where it is co-localized with its precursor peptide, preproghrelin **(3).**

Obestatin is also expressed in the brain where it promotes calcium signalling via stimulation of intracellular calcium store release **(4),** which may mediate some of its proposed central actions. Similarly, in humans, the majority of obestatin production is localized to the GI tract, with predominance in the stomach versus the duodenum, jejunum and ileum (where it is specifically found in the crypts of Lieberkuhn and Brunner's glands), and absence from the colon, while obestatin is also expressed in both the periphery of the pancreatic islets and the exocrine pancreatic ducts **(5).**Furthermore, obestatin has been identified in epithelial ducts of the human mammary gland **(5).**

Once obestatin enters the circulation, it is rapidly degraded by a number of proteases, such as aminopeptidase and post-prolyl endopeptidase, which are largely located in the blood, liver and kidney **(6).** Its half-life in the

plasma is a critical determinant of whether obestatin is able to reach and act upon its target tissues, and published figures in rodents are highly variable. For example, the half-life of native mouse obestatin in mouse plasma is reported to be 42.2 min, compared with 12.6 min in liver and 138 min in kidney membranes **(6),** while the half-life of rodent obestatin in rat liver homogenate was found to be 21.7 min and increased over threefold by the addition of a polyethylene glycol (PEG) group to the N-terminal **(7),**

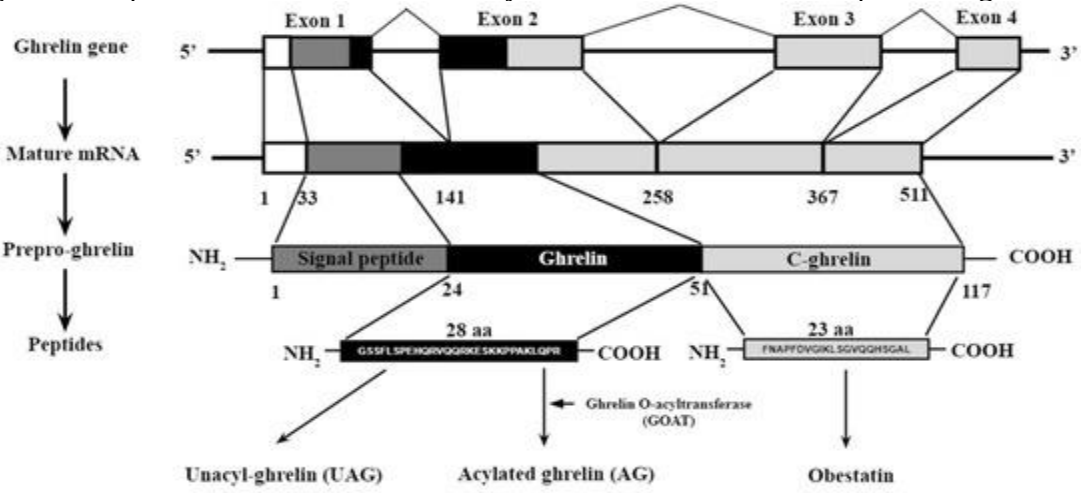
A large number of groups have investigated the circulating physiological levels of obestatin in both rodents and humans, with a wide range of values reported (rodents: from 1.34 to 2560; humans: from 8.4 to 22 057 $pg \cdot mL^{-1}$). The most likely explanation for these markedly different results is due to variations in the sensitivity of the employed detection methods and their specificity for obestatin versus proghrelin **(8).**

Interestingly, one group reported human plasma obestatin levels of 267 ± 10 $pg \cdot mL^{-1}$ **,** while another published values of 68.3 ± 14.8 $pg \cdot mL^{-1}$ **,** that is, fourfold lower, despite using the apparently same detection method. However, these differences may also be due to diurnal variations in obestatin production, which has been reported to follow a pulsatile pattern **(9).** Such observations highlight the importance of following rigorous sampling and analysis protocols in order to achieve reliable estimates of circulating obestatin levels, which to date have been both conflicting and largely uninformative.

Biosynthesis: Obestatin is one of the three ghrelin gene products. Ghrelin and obestatin are derived from the same ghrelin gene following post-translational cleavage of the 117 amino acid pre-proghrelin peptide**.** Mature pre-proghrelin is then post-translationally modified by signal peptidase, prohormone convertase 1/3 (PC 1/3) and carboxypeptidase-B like enzyme into 28 amino acid unacyl ghrelin (UAG) and 23 amino acid obestatin; the unacyl ghrelin peptide is further post-translationally modified into acyl ghrelin, also known as ghrelin**.** Acylation of ghrelin is potentiated by ghrelin O-acyltransferase (GOAT) **(10).**

Growth Hormone Secretagogue receptor (GHS-R), a G-protein coupled receptor (GPCR), is the biologically relevant receptor for ghrelin**.** The acylation of ghrelin is essential for its binding to GHS-R**.** Traditionally, acyl ghrelin has been considered the biologically active isoform, whereas UAG has been considered the biologically "inactive" isoform**.** Subsequent studies have revealed that UAG also has biological functions, although the receptor of UAG is still unknown **(11).**

Obestatin is produced by the post-translational modification of the same pre-proghrelin peptide following post-translational amination of the C-terminal **(1).** which is essential for the stable conformation of obestatin **(2).** Currently, the receptors of obestatin and the enzymes involved in obestatin processing are still unclear.



	Acylated ghrelin (AG)	Obestatin
Expression	GI tract (Stomach, Intestine), Brain, Pancreas, etc.	GI tract, Pancreas, etc.
Receptor	GHS-R1a	GPR39?, GLP-1R?, GHS-R?

Figure 1: Post-translational processing of preproghrelin to unacyl ghrelin, ghrelin, and obestatin. The figure was adapted from **(12)**

Obestatin in plasma and its degradation Similar to ghrelin and growth hormone, obestatin is secreted in a pulsatile manner. Obestatin and ghrelin amounts in human plasma were found to be 6.9±0.28 and 132.4±13.1 fmol/ml, respectively with ratio of obestatin to ghrelin 5.21% **(13).**

Biological effects of obestatin: It was originally projected that obestatin binds to an orphan G protein-coupled receptor, termed GPR39 **(1).** High levels of GPR39 mRNA were found abundantly in the amygdala, the hippocampus, and the auditory cortex but not in the hypothalamus**.** However, **Lauwers et al., (14)** indicated that obestatin is not the endogenous cognate ligand for GPR39, whereas a more recent study demonstrated that obestatin was a metabolic hormone capable of binding to GPR39 and, in turn, of regulating the diverse biological functions of gastrointestinal and adipose tissues **(1).** Furthermore, **Granata et al., (15)** reported that obestatin promotes beta cell and human islet survival by binding to glucagon like peptide-1 receptor (GLP-1R), the receptor through which incretins act. To date, the receptor for obestatin remains unknown and further studies are required to reveal the exact relationship between obestatin, GPR39 and GLP-1R.

Obestatin was first reported to inhibit jejunal contraction, food intake and body weight gain in rats, in addition to antagonising ghrelin-induced contraction of isolated jejunum muscle **(1).** actions that are clearly relevant to T2DM. These initial findings with regard to GI transit have since been confirmed by the same authors **(1).** and others, who have reported obestatin to reduce antral and duodenal motility in the fed state and to impede restoration of normal fasted-state duodenal activity **(16).**

Decreased duodenal and jejunal motility in adult rats have also been confirmed by a study, although increased GI contractility was demonstrated in suckling and adolescent rats in response to obestatin in this same investigation**.** A clinical investigation reported increased preprandial obestatin levels in children with unexplained delayed gastric emptying**.** However, a significant number of investigators have failed to reproduce such effects of obestatin on GI motility **(17).**

Furthermore, obestatin is incapable of preventing ghrelin-mediated acceleration of gastric emptying or intestinal motility **(16),** and obestatin levels and the ghrelin/obestatin ratio are unchanged in patients with gastroparesis, a condition associated with delayed gastric emptying challenging the proposed actions of obestatin on GI motility. Obestatin immune-reactivity in the stomach has also been questioned**.** Similarly, the originally reported beneficial effects of obestatin on food intake and body weight have also been questioned, with more studies disputing **(7; 9)** rather than confirming the initial findings **(18)** on feeding behaviour.

Within these negative studies, obestatin was found not to influence cholecystokinin (CCK)-mediated satiety signalling and to inhibit water more potently than food intake, leading the authors to suggest that previously reported effects of obestatin on food intake may occur secondary to those on water intake**,** although these data have not been reproduced by other groups **(7),** despite demonstrating significant effects of obestatin administration on food intake in rats in response to 24 h food and water deprivation, a study reported no effects on water intake **(19).**

Further to its apparent controversial effects on GI motility, food intake and body weight, obestatin has also been reported to modulate the actions of hormone, ghrelin. For example, obestatin was shown to inhibit the orexigenic actions of ghrelin in rodents and fish **(9)**, although some groups found no effect **(20).**

Both native obestatin and a natural obestatin variant (preproghrelin polymorphism Gln90Leu) decreased ghrelin-induced food intake in mice, together with growth hormone secretion and c-Fos activation in the brain**.** Conversely, obestatin-mediated decreases in GI motility were prevented by injection of corticotrophin-releasing factor (CRF) receptor antagonists, while c-Fos expression was induced by obestatin administration, indicating that potential actions on food intake and GI motility may occur, at least in part, via the vagal afferent pathway and central CRF receptors **(1).**

In addition to its proposed physiological actions, it appears that obestatin may also confer some benefits in GI disease. For example, in rats, obestatin protects against experimental ulcerative colitis via acute attenuation of lipid peroxidation and TH_1-mediated inflammation, chronic suppression of polymorphonuclear leukocyte infiltration, induction of glutathione synthesis, improved mucosal blood flow and stimulation of

cell proliferation in colonic mucosa, effects that may be mediated by activation of anti-inflammatory cytokines **(21).**

Obestatin administration has been shown to confer protective effects against ischaemia–reperfusion injury in rat ileum**,** while the ghrelin/obestatin ratio (but not obestatin levels) is reported to be elevated in patients with active inflammatory bowel diseases (Crohn's disease and colitis) compared with those in remission **(22),** suggesting that obestatin signalling may play a role in this setting.

❖ ***Physiological actions of obestatin:***

On Gastrointestinal system it decreases food intake, slow gastric emptying, reduce jejunal motility, reduce body weight, increase the secretion of pancreatic juice enzymes and inhibits glucose-induced insulin secretion. On Central nervous system it improves memory and explicates an anxiolytic action, Regulates sleep and Inhibitory effects on water drinking **(23).**

On Hormone secretion it doesn't modify GH and corticosterone secretion, No effects on plasma Prolactin, ACTH and TSH levels,it decreases plasma vasopressin levels but not oxytocin levels with no influence on serum leptin levels **(1).** It inducse cell proliferation in cultures of human retinal pigment epithelial cells also it induces ovarian cell proliferation, apoptosis and secretion **(24).**

❖ ***Obestatin and the pancreas***

Pancreatic beta cell loss, reduced beta cell function and inflammation are characteristic of both type 1 diabetes mellitus (T1DM) and T2DM and so are a major focus of research aimed at development of novel metabolic therapies. Obestatin and Ghrelin are co-expressed in both fetal and adult endocrine pancreas with co-localization at the islet periphery, thereby suggesting a synergistic relationship that may be connected with pancreatic beta cell function **(15).**

In 2008, obestatin was reported to be secreted by human pancreatic islets and pancreatic beta cell lines, to enhance their viability in response to both serum starvation and cytokines and to inhibit apoptosis **(15).** In addition, survival of these cells was compromised upon incubation with an anti-obestatin antibody, while genes associated with insulin production, beta cell survival, mass, growth and differentiation (insulin receptor substrate 2, cAMP response element binding protein, pancreatic and duodenal homeobox-1, and glucokinase) were up-regulated by obestatin, together with activation of phosphoinositide 3-kinase (PI3K)/Akt, ERK1/2 and cAMP **(15).** highlighting a potential autocrine/paracrine role.

Obestatin enhances generation of pancreatic islet-like clusters together with increased insulin gene expression during endocrine pancreatic precursor cell selection and differentiation, which appears to occur via pathways involving fibroblast growth factor receptors, notch receptors and neurogenin 3, suggesting a role in pancreatic development and regeneration**.** The reported anti-apoptotic actions of obestatin in the pancreas appear to extend to its microvascular endothelial cells, indicating that such protection may be mediated indirectly via support of islet vascularization **(25).**

Obestatin has been shown to protect against acute pancreatitis in rats, induced by either cerulein or ischaemia/reperfusion, via increasing pancreatic blood supply in parallel with reduced inflammation and digestive enzyme activity, and also to promote pancreatic repair and regeneration in these animals**.** Also circulating obestatin levels are increased in patients with acute pancreatitis **(26),** supporting a protective function in this setting.

Although obestatin appears to activate pancreatic insulin gene expression, at least *in vitro*, its effects on insulin secretion are unclear due to highly variable reports **(15).** Several studies have shown obestatin to have no effect on circulating glucose or insulin in normoglycaemic mice and rats **(7),** although glucose-induced insulin secretion in rats *in vivo* and in mouse and rat isolated islets was inhibited by obestatin**,** which is consistent with reports of an inverse relationship between obestatin and insulin levels in humans. In contrast, other studies have shown obestatin to stimulate insulin secretion in human islets in both the presence and absence of glucose **(15).**

Obestatin was found to be capable of regulating secretion of other pancreatic hormones (glucagon, pancreatic polypeptide and somatostatin) in isolated rodent islets and increases pancreatic protein output in rats via vagal activation **(27).** Although the precise pancreatic actions of obestatin remain unclear, the evidence of effects on beta cell metabolism and survival coupled with its ability to modulate insulin levels and inflammation clearly supports further investigation of this peptide as a potential therapeutic target in diabetes.

Obestatin and adipose tissue

Adipose tissue is considered to be endocrine in nature, further to adipokine-mediated regulation of glucose, lipid and energy homeostasis, as well as inflammation. Notably, obesity and deregulation of these processes, which appear to be modulated by obestatin, are frequently associated with insulin resistance and diabetes **(28).**

Similar to the GI and pancreatic actions of obestatin, its effects on adipose tissue function, production and survival are subjected to some debate. Several groups have demonstrated obestatin secretion from rat white adipose tissues and adipocytes from both mice and humans **(15).** Expression of the obestatin precursor, preproghrelin, has been reported in mouse epididymal and subcutaneous adipose tissue, while both neutralization of preproghrelin protein products (including obestatin) and inhibition of preproghrelin gene expression decrease adipocyte differentiation **(15).** In addition to its secretion, obestatin may mediate important actions on adipose tissue, pointing towards a potential autocrine/paracrine role **(29).**

Obestatin has been demonstrated to promote pre-adipocyte differentiation, lipid accumulation and leptin secretion, whilst decreasing and increasing lipolysis during differentiation and adipogenesis, respectively**,** indicating that the actions of obestatin in these settings may be complex.

Effects of obestatin on both tissue and circulating lipid levels have also been widely investigated. For example, acute obestatin treatment in 3T3-L1 differentiating mouse adipocytes increased triglyceride levels **(30)**, although circulating concentrations were reduced in rats or mice subjected to chronic treatment with native or modified obestatin, with activation of glycerolipid metabolism and PPAR signalling proposed as a potential mechanism **(7).** Although circulating cholesterol levels remained unaltered in obestatin-injected rats, decreased expression of cholesterol transporter ABCA1 was demonstrated in bovine WAT further to obestatin treatment **(7).**

Consistent with beneficial actions of obestatin on lipid metabolism, phosphorylation of AMP activated protein kinase (AMPK) is reported to be increased by obestatin in 3T3-L1 adipocytes and human adipose tissue, whilst in human subcutaneous adipocytes, this effect occurs in parallel with modulation of adiponectin and leptin expression **(15).**

With regard to glucose metabolism, obestatin has been shown to inhibit glucose transport in isolated rat adipocytes and to down-regulate glucose transporter type 4 (GLUT-4) in adipose tissue In contrast, glucose uptake is reported to be enhanced by obestatin in both 3T3-L1 and human subcutaneous adipocytes, together with increased translocation of GLUT-4 to the plasma membrane **(15).** Similar data have been generated by other groups upon investigation of WAT from obestatin-treated animals **(29),** suggesting that obestatin is likely to activate rather than inhibit glucose metabolism in adipose tissue.

Conflicts of Interest: The authors declare no conflict of interest.

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