Products of the Ghrelin Gene, the Pancreatic β-Cell and the Adipocyte

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Abstract

The ghrelin system comprises acylated ghrelin (AG), unacylated ghrelin (UAG) and obestatin, besides the receptor for AG, the growth hormone (GH) secretagogue receptor type 1a (GHS-R1a), and the enzyme-promoting ghrelin acylation, ghrelin O-acyltransferase (GOAT). The ghrelin peptides exert a variety of biological actions, including regulation of energy homeostasis and glucose metabolism, as well as survival and proliferative effects in different cell types. Besides the stomach, its main site of production, ghrelin is expressed in pancreatic islets, where it represents an independent islet cell population. AG exerts insulinostatic actions, UAG has been shown to oppose the AG inhibitory effects on insulin secretion, and obestatin has demonstrated insulinotropic activities. Although differences exist in the regulation of glucose homeostasis, all peptides display survival and antiapoptotic actions in pancreatic β-cells, preserving β-cell mass and function both in vitro and in vivo. The ghrelin system is also expressed in adipose tissue, and ghrelin effects have been demonstrated in both white and brown adipocytes. Indeed, AG, UAG and obestatin promote adipogenesis and glucose uptake, and inhibit adipocyte lipolysis. Interestingly, despite similar effects at the cellular level, results from ghrelin, GHS-R and GOAT knockout mice have indicated that AG display diabetogenic effects in vivo. Conversely, UAG and obestatin positively regulate glucose metabolism, and a new role has been recently proposed for obestatin on adipocyte function and insulin sensitivity.

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Ghrelin, a 28-amino acid peptide produced by the stomach, was discovered as endogenous ligand for the growth hormone (GH) secretagogue receptor type 1a (GHS-R1a) [1]. Ghrelin potently stimulates GH release and food intake, induces adiposity and exerts a wide range of central and peripheral actions [2]. Ghrelin acylation on the third serine residue, promoted by ghrelin O-acyltransferase (GOAT), is essential for binding to GHS-R1a and for the central activities of the peptide [3–5]. Besides the stomach, acylated ghrelin (AG) and GHS-R1a are produced in different central and peripheral tissues, including the pancreas and adipose tissue [6–10].
Ghrelin peptides exist in two major forms, acylated ghrelin (AG) and unacylated ghrelin (UAG), of which UAG is the most abundant [1, 11]. UAG, like AG, is mainly produced in the stomach; however, it is devoid of binding to GHS-R1a and therefore unable to stimulate GH release and food intake. Nonetheless, UAG exerts many biological activities, some of which similar and other opposed to or independent of AG. Interestingly, AG and UAG display the same effects in different cell types and recognize common binding sites, suggesting the existence of a yet unknown receptor which is bound by both peptides [12–15].

Obestatin was recently discovered as a 23-amino acid amidated peptide encoded by the ghrelin gene [16]. Initially identified as the ligand of the orphan receptor GPR39, obestatin was claimed to oppose ghrelin effects on food intake [16, 17]; however, both findings have been questioned and to date obestatin physiological role has yet to be defined [18, 19]. Like ghrelin, obestatin is produced in the gastrointestinal tract, as well as in central and peripheral tissues [20]. Moreover, it exerts both central and peripheral actions and has been recently found to play a role in pancreatic β-cell survival and function [21], as well as in adipocyte function and glucose metabolism [22–24].

The role of the ghrelin gene-derived peptides in the pancreas and adipose tissue is becoming an important topic, particularly in light of the potential therapeutic applications in conditions such as obesity and insulin resistance. The present review highlights the most recent findings on AG, UAG and obestatin functions in pancreatic β-cells and adipocytes; indeed, these cells play a major role in glucose and lipid metabolism (fig. 1).

**Ghrelin System in the Endocrine Pancreas**

Ghrelin and GHS-R1a expression have been demonstrated in both human and rodent pancreatic islets [6, 7, 12, 20, 25, 26] and ghrelin in fetal life was found to be more abundant in the pancreas than in the stomach, suggesting a paracrine/autocrine role of the ghrelin system in hormonal secretion and/or islet growth and differentiation [27, 28]. Interestingly, ghrelin-producing ε-cells have been identified in pancreatic islets, and during fetal development ε-cells showed an ontogenetic and morphogenetic pattern distinct from that of glucagon-producing α-cells and insulin-producing β-cells [28–30].

In the developing mouse and rat pancreas, ghrelin immunoreactivity can be detected as early as embryonic day E9.5 and E15, respectively, and remains positive throughout gestation, peaking just before birth [30, 31]. Notably, ε-cells replaced β-cells and most of α-cells in two mouse models of pancreas development, suggesting a relationship between ghrelin-, insulin- and glucagon-producing cells in the developing pancreas [31, 32]. In normal adult rats, ghrelin immunoreactivity has been observed in the peripheral region, whereas in diabetic rats, it was found in the central region of the islets [33].
In humans, ε-cells have been detected from midgestation to early postnatal period, accounting for approximately 10% of all islet cells [28, 29]. The developmental pattern of the ghrelin cells in human pancreas, although clearly distinct, was found to be quite similar to that of glucagon-expressing cells. Indeed, in fetal pancreas, ε- and α-cells reside at the periphery of the islets, whereas β-cells are mostly located in the islet core [26, 34–36]. Interestingly, colocalization of ghrelin and glucagon has been shown in both human and rat pancreas, which further supports the possible developmental link between these two cell types [6, 30]. Recently, ghrelin immunostaining was found to be either dependent or independent of glucagon, suggesting the existence of two different pancreatic ghrelin cell populations [37].

With regard to GHS-R1a, its immunoreactivity in human pancreatic islets partially overlaps with that of insulin in β-cells, in agreement with the many findings on β-cell responsiveness to ghrelin stimulation [12, 38]. Moreover, like ghrelin, GHS-R1a is expressed in human and rat developing pancreatic islets, from early gestation to adult [12, 27, 39].

Obestatin colocalizes with ghrelin at the periphery of the islets in rat, as well as in fetal and adult human pancreas [20, 21, 37, 40, 41]. Furthermore, both human pancreatic islets and INS-1E β-cells were found able to release obestatin, suggesting autocrine/paracrine effects of the peptide [21]. Besides the different ghrelin gene products,
high levels of GOAT mRNA have been found in both stomach and pancreas [3], indicating regulatory mechanisms controlling AG versus UAG production and the consequent effects of these peptides on glucose metabolism and/or cell proliferation and survival.

**Ghrelin Gene-Derived Peptides and the Pancreatic β-Cell**

Different studies have shown that AG, UAG and obestatin play key roles in pancreatic β-cells and human pancreatic islets [38, 42]. We will not describe here their effects on insulin secretion and glucose metabolism, as they will be thoroughly covered in the chapter by Dezaki et al. [this vol.]. We will focus instead on β-cell proliferation and survival, and the underlying signaling pathways triggered by the peptides.

The survival and antiapoptotic effects of the ghrelin gene products have been described in a variety of cell types [43]. Interestingly, AG and UAG, when investigated together, showed similar ability to increase cell proliferation and survival and to inhibit apoptosis. Due to UAG inability to bind to GHSR-1a, it was proposed the existence of an alternative receptor involved in these effects [12, 13, 15, 44].

In HIT-T15 and INS-1E β-cells, as well as in human pancreatic islets, AG and UAG promote proliferation and inhibit apoptosis induced by serum starvation and cytokine synergism, which is a main cause of β-cell destruction in diabetes [12, 45, 46]. HIT-T15 cells do not express GHS-R1a, and possess common binding sites for AG and UAG, in agreement with previous studies which postulated the existence of a common receptor recognized by both peptides [15, 44, 47]. AG and UAG survival effects in β-cells involved signaling through the Go, protein-coupled receptor and adenylyl cyclase (AC)/3’,5’-cyclic AMP (cAMP)/protein kinase A (PKA) pathway, and activation of survival and proliferative cascades such as phosphatidyl inositol-3 kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK)1/2. Autocrine or paracrine effects of AG and UAG were also suggested, due to their release in β-cell conditioned medium and to increased β-cell apoptosis after incubation with anti-ghrelin antibodies [12]. Both peptides even reduced cytokine-induced nitric oxide production, which is also a cause of β-cell dysfunction and death [48]. Other studies have later shown antiapoptotic effects of AG in HIT-T15 β-cells [49] and inhibitory effects against lipotoxicity-induced apoptosis in MIN6 β-cells, where AG activated protein kinase B (PKB), inhibited JNK signaling and nuclear translocation of forkhead box protein O1 (Foxo1) [50, 51].

On the basis of the greater positive effects on insulin sensitivity and glucose homeostasis, UAG was suggested to be a more appealing candidate for the treatment of metabolic dysfunctions, such as insulin resistance and type 2 diabetes. Therefore, studies were performed to assess whether UAG fragments would mimic the actions of full length peptide on survival of β-cells and human pancreatic islets. These fragments either included or excluded serine-3, the site for ghrelin acylation.