Ghrelin Function in Insulin Release and Glucose Metabolism

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Abstract
Given its wide spectrum of biological activities such as growth hormone (GH) release, feeding stimulation, adiposity and cardiovascular actions, the discovery of ghrelin opened many new perspectives within neuroendocrine, metabolic and cardiovascular research, thus suggesting its possible clinical application. Circulating ghrelin is produced predominantly in the stomach, and its receptor GH secretagogue receptor (GHS-R) is expressed in a variety of central and peripheral tissues. Ghrelin, GHS-R and ghrelin O-acyltransferase (GOAT), the enzyme that promotes the acylation of the third serine residue of ghrelin, are all expressed in pancreatic islets, and this peptide is released into pancreatic microcirculations. Ghrelin inhibits insulin release in mice, rats and humans. The signal transduction mechanisms of ghrelin receptor in islet β-cells are very unique, being distinct from those utilized for GH release. Pharmacological and genetic blockade of islet-derived ghrelin markedly augments glucose-induced insulin release in vitro. Ablation of ghrelin, GHS-R or GOAT enhances insulin release and prevents impaired glucose tolerance in high-fat, diet-induced and leptin-deficient obese models. Thus, manipulation of the insulinostatic function of the ghrelin–GHS-R system, particularly that in islets, could optimize the amount of insulin release to meet the systemic demand. Ghrelin antagonism provides a novel strategy to treat type 2 diabetes with dysregulated insulin release.

Ghrelin, a stomach-derived 28-amino acid hormone discovered as the endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R), potently stimulates GH release [1] and feeding [2] as well as exhibits adiposity [3] and positive cardiovascular effects [4]. Given this wide spectrum of biological activities, the discovery of ghrelin opened many new perspectives within neuroendocrine, metabolic and cardiovascular research, thus suggesting its possible clinical application. Recent evidence highlights an important role of ghrelin in glucose homeostasis. Ghrelin and GHS-R are also located in pancreatic islets [5, 6]. Ghrelin O-acyltransferase (GOAT), which has been identified as the enzyme that promotes the acylation of the third serine residue of ghrelin, is highly expressed in the pancreatic islets [7]. The concentrations of
plasma ghrelin in the pancreatic vein are significantly higher than those in the pancreatic artery in rats, indicating that ghrelin is released from pancreas [8]. Ghrelin inhibits insulin release in mice, rats and humans [9–11]. Here, we review the physiological role of ghrelin in the regulation of insulin release and glucose metabolism, and present a potential therapeutic avenue to manipulate ghrelin signaling and thereby counteract the progression of type 2 diabetes.

**Systemic Effects of Ghrelin on Insulin Release and Glucose Metabolism**

Systemic action of exogenous ghrelin to elevate blood glucose levels has been well documented in humans and rodents. When ghrelin was simultaneously injected into mice with glucose in glucose tolerance test (GTT), the insulin responses were markedly attenuated and the glucose responses were larger in comparison to the control without ghrelin [11]. It has recently been shown that in healthy humans ghrelin suppresses insulin secretion and elevates blood glucose in intravenous GTT [12]. Conversely, GTT performed in mice showed that insulin responses were markedly enhanced and increases in plasma glucose were markedly attenuated by simultaneous injection of GHS-R antagonist [D-Lys³]-GHRP-6 [11]. In insulin tolerance tests, hypoglycemic effect of insulin was indistinguishable between the ghrelin-injected, GHS-R antagonist-injected and control mice [11]. Esler et al. [13] confirmed these results by reporting that oral administration of a small molecule GHS-R antagonist improved glucose tolerance in rats by stimulating insulin secretion, while eliciting no apparent effect on insulin sensitivity. Pretreatment of mice with the GOAT inhibitor also showed increased insulin response and a reduced blood glucose in GTT [14]. Knockout studies support the pivotal role of ghrelin in glucose homeostasis. Mice lacking ghrelin [8, 15] have improved glucose tolerance mainly due to increased glucose-induced insulin secretion. Possible additional effects of ghrelin on glucose disposal or insulin sensitivity [15–18] cannot be disregarded.

**Ghrelin as a Potential Therapeutic Target for Type 2 Diabetes**

Circulating plasma ghrelin levels decrease immediately after a meal [19]. The meal-induced decrease of ghrelin levels is impaired in type 2 diabetic subjects [20], suggesting that the impaired suppression of circulating ghrelin during the meal intake may partly account for the glucose intolerance as well as ongoing weight gain in type 2 diabetes. When wild-type and ghrelin-KO mice were fed a high-fat diet (HFD), both mouse lines developed moderate increases in body weight to a similar extent [8]. In an apparent controversy, it was reported that another line of ghrelin-KO mice were protected from a rapid weight gain during post-weaning exposure to HFD, which was associated with decreased adiposity, increased energy expenditure and increased lo-
comotor activity as compared to wild-type mice [21]. HFD treatment significantly increased blood glucose levels in wild-type mice but not in ghrelin-KO mice [8]. HFD treatment increased plasma insulin levels in wild-type mice, and this increment was much greater in ghrelin-KO. This phenotype was even more prominent in GTT. Ghrelin-deficiency promotes insulin release and prevents glucose intolerance in an HFD-induced obese model [8]. Sun et al. [15] have reported that ablation of ghrelin in leptin-deficient ob/ob mice augmented insulin release and thereby markedly reduced hyperglycemia. Thus, the ghrelin blockade counteracts the obesity-associated glucose intolerance in both the life style-related and genetic obese models. As the underlying mechanism, we propose that lack of ghrelin and its insulinostatic activity increase the maximal capacity of glucose-induced insulin release and enable islets to secrete more insulin to meet an increased demand associated with obesity, thereby achieving normoglycemia.

**Insulinostatic Function of Ghrelin**

To examine the physiological roles of the pancreatic islet-produced ghrelin, insulin release from the perfused rat pancreas, an in vitro system that retains the intact circulation in pancreatic islets while excluding the influence of other organs, was employed. The glucose-induced insulin release was significantly enhanced by blockade of GHS-R with a GHS-R antagonist and by immunoneutralization of endogenous ghrelin with antighrelin antiserum [8]. Conversely, administration of exogenous ghrelin suppressed it [8, 22]. In isolated rat islets, GHS-R antagonists and antiserum against ghrelin markedly increased glucose-stimulated insulin release [11]. The glucose-induced insulin release from isolated islets of ghrelin-KO mice was significantly greater than that of wild-type mice, while basal insulin release was not altered. No difference was observed between KO and wild-type mice in insulin content per islet and mRNA expressions of insulin 1 and insulin 2 [8]. These findings indicate that the endogenous ghrelin suppresses glucose-induced insulin secretion within islets. Barnett et al. [14] reported that pretreatment of human islet cells with a peptide-based GOAT inhibitor promoted significant increase in insulin response to a glucose challenge. These results indicate that GOAT may catalyze acylation of ghrelin in islets and confirm the insulinostatic function of islet-derived acyl-ghrelin suppresses insulin response in human.

**Ghrelin Signaling Mechanisms in Islet β-Cells**

Insulinostatic effects of ghrelin were blunted in rats pretreated with pertussis toxin (PTX), a specific inhibitor of Gi and Go subtypes of trimeric GTP-binding proteins [23]. In perfused pancreas of PTX-treated rats, glucose-induced insulin release was...
markedly enhanced, and ghrelin failed to affect it (fig. 1a, b). A membrane-permeable cAMP analogue, dibutyryl-cAMP (db-cAMP), markedly enhanced glucose-induced insulin release, and in the presence of db-cAMP, ghrelin failed to suppress the insulin release (fig. 1c). In the pancreas preincubated with an irreversible adenylate cyclase inhibitor, MDL-12330A, the glucose-induced insulin release was attenuated, and was not further altered by administration of ghrelin (fig. 1d). These results suggest that
Ghrelin attenuates glucose-induced insulin release via the PTX-sensitive G-protein that is coupled to modulation of cAMP signaling. The glucose (8.3 and 22 mM)-induced cAMP production in isolated rat islets were significantly inhibited by exogenous ghrelin and augmented by GHS-R antagonist and antighrelin antiserum (fig. 2). Administration of ghrelin also suppressed the glucose-induced oscillatory rise in cytosolic cAMP concentrations in MIN6 β-cells transfected with a fluorescent-translocation biosensor using evanescent-wave microscopy, indicating that ghrelin directly inhibits glucose-induced cAMP signaling in β-cells [23].

At substimulatory glucose concentrations, β-cells maintain the resting membrane potential at a hyperpolarized level of around −70 mV. Elevation of the blood glucose concentration increases glucose uptake and metabolism by β-cells, resulting in closure of the ATP-sensitive K+ (KATP) channels. When K+ efflux is reduced, inward currents more effectively contribute to the membrane potential and depolarize the membrane, inducing bursting spike-like short action potentials at membrane potentials positive from −50 to −40 mV. These action potentials are produced by orchestrated openings of voltage-dependent Ca2+ channels and voltage-gated K+ channels. The electrical firings were characterized by spike-like and repetitively occurring action potentials on top of the plateau phase of slow waves, and these firings were attenuated...