Modulation of Ingestive Behavior and Gastrointestinal Motility by Ghrelin in Diabetic Animals and Humans

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Acyl ghrelin, a 28-amino acid peptide hormone, is the endogenous cognate ligand for the growth hormone secretagogue receptor. Ghrelin is involved in stimulating growth hormone release, eliciting feeding behavior, inducing adiposity and stimulating gastrointestinal motility. Ghrelin is unique for its post-translational modification of *O-n*-octanoylation at serine 3 through ghrelin *O*-acyltransferase, and is the only peripheral signal to enhance food intake. Plasma ghrelin levels manifest "biphasic changes" in diabetes mellitus (DM). In the early stage of DM, the stomach significantly increases the secretion of ghrelin into the plasma, and elevated plasma ghrelin levels are correlated with diabetic hyperphagic feeding and accelerated gastrointestinal motility. In the late stage of DM, plasma ghrelin levels may be lower, which might be linked with anorexia/muscle wasting, delayed gastrointestinal transit, and even gastroparesis. Therefore, the unique ghrelin system may be the most important player compared to the other hindgut hormones participating in the "entero-insular axis". Further studies using either knockdown or knockout of ghrelin gene products and ghrelin *O*-acyltransferase may unravel the pathogenesis of DM, and show benefits in combating this disease and metabolic syndrome. [*J Chin Med Assoc* 2010;73(5):225–229]

Key Words: acyl ghrelin, diabetes mellitus, feeding, gastrointestinal motility, ghrelin *O*-acyltransferase

Introduction

Acyl ghrelin, a 28-amino acid peptide hormone, has been identified as the endogenous cognate ligand for the growth hormone secretagogue receptor (GHS-R).[1] It was discovered by "reverse pharmacology".[1,2] After acyl ghrelin binds to GHS-R, it induces the release of growth hormone.[3] Ghrelin is mainly synthesized in specific endocrine cells, designated X/A-like cells, in the gastric oxyntic glands.[1,4] Des-acyl ghrelin, the major form of ghrelin in plasma,[2] may be acylated into acyl ghrelin through ghrelin *O*-acyltransferase (GOAT) in the stomach.[5] In addition to inducing growth hormone release, acyl ghrelin enhances food intake, and it is the only peripheral signal to increase meal size.[6] Acyl ghrelin also stimulates adiposity, which is independent of its hyperphagic effects.[7] Therefore, ghrelin is an interesting molecule of high clinical relevance to human obesity and metabolic syndrome.[8] With regard to the gastrointestinal tract, acyl ghrelin accelerates gastric emptying[9] and elicits gastroduodenal phase III-like contractions[10] in rats.

Diabetes mellitus (DM) is a common clinical problem with increasing prevalence in the world. There are 2 main types of DM. Both types are caused by derangement of insulin's function and activity in the body.[11] Type 1 DM most often develops in childhood or adolescence and causes hyperglycemia due to insufficient production of insulin, while over 90% of all DM cases are type 2 DM. DM may manifest many

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gastrointestinal symptoms such as nausea, vomiting, diarrhea, constipation, abdominal pain,[12] and even hyperphagia.[13,14] Some symptoms can be attributed to gastrointestinal dysmotility. Acyl ghrelin dose-dependently inhibits insulin secretion in mice,[15] and the relationship between ghrelin and glucose metabolism has been previously discussed in our review.[8] A recent study revealed that MK-677, an acyl ghrelin mimetic, increases blood glucose and HbA1c levels in healthy aged volunteers after 1 year of administration.[16] Mice lacking acyl ghrelin demonstrate lower fasting blood glucose, a better insulin-induced blood-glucose-lowering effect, as well as higher plasma insulin and lower blood glucose levels after intraperitoneal glucose injection.[17] These *acyl ghrelin* knockout mice are protected against hyperinsulinemia and hyperglycemia induced by a high-fat diet.[18,19] *GHS-R* knockout mice exhibit lower blood glucose and serum insulin levels,[20,21] and greater "metabolic flexibility" under diet-induced metabolic stress.[22] Furthermore, *acyl ghrelin* and *GHS-R* double knockout mice show a greater blood glucose drop during 50% caloric restriction.[21] Inhibition of GOAT[8] and *GOAT* knockout[23] is proposed to have potential for anti-diabetic therapeutics. Since acyl ghrelin plays an important role in inducing food intake, eliciting gastroduodenal phase III-like contractions, accelerating gastric emptying, and glucose homeostasis, the present mini-review will focus on the influence of ghrelin on ingestive behavior and gut motility in diabetic animals and humans.

Regulation of Ghrelin Secretion and the Influence of Ghrelin on Ingestive Behavior in Diabetes

Ghrelin-immunoreactive cell density was found to be reduced in type 1 non-obese and type 2 obese diabetic mice.[24] This observation could explain the slow gastric emptying and slow intestinal transit encountered in human diabetic gastroenteropathy, based on the fact that ghrelin has gastrointestinal prokinetic effects. However, the correlation between ghrelin levels and diabetic gastrointestinal dysmotility needs to be further investigated before drawing a definite conclusion. In streptozotocin-induced diabetic rats, the number of ghrelin-immunoreactive cells in the gastric fundus is consistently found to be decreased, whereas insulin treatment reversed this finding, implying that a decrease in ghrelin-immunoreactive cells reflects a decrease in ghrelin content in X/A-like cells but not a decrease of ghrelin-producing cells.[25] Body weight and serum insulin levels in the streptozotocin-induced rats was decreased, whereas plasma acyl ghrelin and total ghrelin levels and gastric preproghrelin mRNA expression levels were significantly increased.[25] When considered together, these results indicate that DM, a negative energy balance condition, may enhance preproghrelin mRNA expression in the stomach and ghrelin secretion into the bloodstream. Acyl ghrelin and des-acyl ghrelin have been demonstrated to inhibit apoptosis and stimulate proliferation of pancreatic β cell lines and human islets of Langerhans.[26] This finding indicates that acyl ghrelin, as well as des-acyl ghrelin, might protect β cells against apoptosis and increase β cell survival. A subsequent study revealed that acyl ghrelin treatment for 21 days increases pancreatic insulin, pancreatic and duodenal homeobox 1 gene (*Pdx1*) mRNA and the number of replicating cells in streptozotocin-treated neonatal rats.[27] This finding showed that acyl ghrelin and des-acyl ghrelin promote regeneration of β cells in streptozotocin-treated animals. Collectively, in addition to the effects compensatory for the loss of body weight and serum insulin levels in streptozotocin-induced rats, the increases in plasma acyl ghrelin and total ghrelin levels and gastric preproghrelin mRNA expression levels could prevent further β cell damage and facilitate β cell regeneration. Therefore, early administration of acyl ghrelin might prevent or ameliorate the development of DM in disease-prone subjects after β cell destruction.[27]

Uncontrolled DM is characterized by marked behavioral perturbations, such as severe hyperphagia and increased circulating ghrelin levels could cause the development of diabetic hyperphagia.[12,13] In streptozotocin-induced rats, plasma total ghrelin levels are increased well before the onset of hyperphagic feeding, supporting the hypothesis that increased ghrelin signaling contributes to the stimulatory effect on food intake in the early stage of DM.[13] A subthreshold dose of intracerebroventricular administration of acyl ghrelin was found to increase food intake by 357% in diabetic rats compared with that in controls, indicating increased behavioral sensitivity to acyl ghrelin in the absence of the opposing effects of leptin and insulin in DM.[13] Similarly, plasma fasting acyl ghrelin levels are increased, whereas des-acyl ghrelin levels are decreased in patients with obesity-related type 2 DM compared with lean subjects.[28] Metformin therapy was found to prolong the postprandial fall in total plasma ghrelin levels, and thus had concomitant effects on appetite in type 2 DM, contributing to its actions in promoting weight loss and attenuating weight gain in these patients.[29] A recent study demonstrated that barley intake dose-dependently decreases plasma glucose and insulin levels, whereas postprandial reduction of plasma

des-acyl ghrelin is suppressed by barley intake in a dose-dependent manner, compared with glucose and white rice.[30] Since des-acyl ghrelin might have anorexigenic[31,32] and insulin-mimetic[33] effects, either through binding to an additional as-yet unidentified receptor[8] or buffering[33] of acyl ghrelin's actions, it has been advocated that a combination of white rice and barley may play a beneficial role in preventing and treating human type 2 DM.[30] However, total plasma ghrelin levels are negatively correlated with HbA1c in diabetic patients, suggesting that long-term poor glycemic control might impair ghrelin secretion,[34] and that plasma ghrelin levels could be lower in the late stage of DM. Consistently, fasting total plasma ghrelin levels are decreased in insulin-resistant obese adults compared with those in equally obese insulin-sensitive controls, implying that insulin resistance and compensatory hyperinsulinemia are independently associated with suppression of ghrelin.[35] In addition, salivary levels of acyl ghrelin and des-acyl ghrelin are similarly decreased in obese diabetic subjects in comparison with non-obese diabetic and healthy controls.[36] These alterations may have a causal role in the development and severity of disease.

Impacts of Ghrelin on Gastrointestinal Motility in Diabetes

Circulating acyl ghrelin levels fluctuate and the peaks are associated with the gastric migrating motor complex cycle,[37] indicating the indispensable role of endogenous acyl ghrelin in modulating gastrointestinal motility. Experiments with a streptozotocin-induced DM rat model showed elevated plasma acyl ghrelin levels in diabetic rats, and the elevated levels were accompanied with accelerated solid gastric emptying and enhanced postprandial antro-pyloric coordination.[38] Treatment with anti-acyl ghrelin antibodies suppressed the accelerated gastric emptying and stimulated antro-pyloric coordination. An elevated plasma acyl ghrelin level-induced accelerated gastric emptying could predispose to overeating, which would, in turn, exacerbate DM in the diabetic early stage. In contrast, gastric emptying becomes slow in the late stage of DM, and severe gastroparesis sometimes occurs. These findings have clinical implications in the prevention for the development of complications in DM, such as diabetic gastroparesis, as in the late stage of DM. Sham feeding is characterized by an increase in pancreatic polypeptide and ghrelin in normal healthy humans, whereas changes in pancreatic polypeptide and ghrelin levels in diabetic gastroparesis are significantly less than those in normal subjects.[39] Ghrelin subsequent to lunch significantly decreases in patients without gastroparesis, but not in gastroparetic patients.[40] Taken together, these findings suggest that decreased plasma ghrelin levels are linked with a slow gastrointestinal transit in the late stage of DM. Loss of rhythmicity in ghrelin levels of diabetic gastroparesis highlights the importance of integrity of the neurohumoral-intestinal axis.[32] Patients with diabetic gastroparesis show no decrease of plasma acyl ghrelin after glucose loading, unlike patients without gastroparesis or healthy controls,[41] indicating that diabetic gastroparesis might be related to ghrelin-associated neurohormonal abnormalities. Conceivably, intravenous infusion of acyl ghrelin improves impaired gastric emptying in patients with diabetic gastroparesis, and this effect is independent of vagal tone.[42] Therefore, we propose that analogs of acyl ghrelin may represent a new class of prokinetic agents in future treatment for patients with diabetic gastroparesis.

Conclusions and Future Perspectives

Obesity has replaced cigarette smoking as a severe new burden on public health.[43] Obesity-related metabolic syndrome, and DM, which negatively affects quality of life and life expectancy, also cannot be overlooked. Ghrelin is an exceptionally intriguing gastric hormone, and actively participates in the modulation of ingestive behavior and gastrointestinal motility. Plasma ghrelin levels are elevated in the early stage of DM, which correlates with hyperphagic feeding and accelerated gastrointestinal motility. In contrast, plasma ghrelin levels can be decreased in the late stages of DM, which may be linked with poor appetite, body weight loss and gastroparesis. The "entero-insular axis" has clinical implications for the treatment of human DM.[44] Hindgut hormones, such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1, hold great promise. However, a recent study indicated that selective bypass of the proximal intestine by an endoluminal sleeve, mimicking human Roux-en-Y gastric bypass (the only way to resolve DM), reduces body weight and food intake, and improves fasting hyperglycemia and glucose tolerance in rats with diet-induced obesity.[45] These results suggest that the "foregut theory" may be preferable to the "hindgut theory". Therefore, ghrelin deserves more attention in the pathogenesis of DM. Two recent studies showed that measurement of total ghrelin did not adequately reflect acyl ghrelin and des-acyl ghrelin levels.[46,47] Therefore, in contrast to the original concept, levels of total ghrelin are not an ideal surrogate for those of

acyl ghrelin.[8] Further studies, particularly using state-of-the-art techniques to separately measure acyl ghrelin, des-acyl ghrelin, and obestatin, are necessary to clarify the differential roles of ghrelin gene products in the pathogenesis of DM. Ghrelin manifests "biphasically" in DM. GOAT enhancers, acyl ghrelin and/or des-acyl ghrelin, and GHS-R agonists, may rescue damaged β cells and even endothelial progenitor cell function in individuals with type 2 DM,[48] while GOAT inhibitors, immunization against acyl ghrelin/acyl ghrelin antibodies, des-acyl ghrelin, and GHS-R antagonists, may be useful in the treatment of hyperphagic feeding and accelerated gastrointestinal motility in the early stage of DM. Conversely, GOAT enhancers, acyl ghrelin, as well as its mimetics and GHS-R agonists, may provide therapeutic targets in the treatment of diabetic anorexia-cachexia and gastroparesis in the late stage of DM. In conclusion, manipulating the unique GOAT/ghrelin/GHS-R system may provide relevant approaches to prevent, ameliorate and treat disturbance of ingestive behavior and gastrointestinal motility in human DM.

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