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Ghrelin and the Endocrine Pancreas

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Ghrelin is a 28-amino-acid peptide predominantly produced by the stomach, while substantially lower amounts derive from other tissues including the pancreas. It is a natural ligand of the GH secretagogue (GHS) receptor (GHS-R1a) and strongly stimulates GH secretion, but acylation in serine 3 is needed for its activity. Ghrelin also possesses other endocrine and nonendocrine actions reflecting central and peripheral GHS-R distribution including the pancreas. The wide spectrum of ghrelin activities includes orexigenic effect, control of energy expenditure, and peripheral gastroenteropancreatic actions. Circulating ghrelin levels mostly reflect gastric secretion as indicated by evidence that they are reduced by 80% after gastrectomy and even after gastric by-pass surgery. Ghrelin secretion is increased in anorexia and cachexia but reduced in obesity, a notable exception being Prader–Willi syndrome. The negative association between ghrelin secretion and body weight is emphasized by evidence that weight increase and decrease reduces and augments circulating ghrelin levels in anorexia and obesity, respectively, and agrees with the clear negative association between ghrelin and insulin levels. In fact, ghrelin secretion is increased by fasting whereas it is decreased by glucose load as well as during euglycemic clamp but not after arginine or free fatty acid load in normal subjects; in physiological conditions, however, the most remarkable inhibitory input on ghrelin secretion is represented by somatostatin as well as by its natural analog cortistatin that concomitantly reduce β-cell secretion. This evidence indicates that the endocrine pancreas plays a role in directly or indirectly modulating ghrelin secretion. As anticipated, ghrelin, in turn, is expressed within the endocrine pancreas, although it is still matter of debate if it is expressed by β-, α-, or non-α/non-β cells. Moreover, GHS-R1a expression in the pancreas has been demonstrated by many authors. Some impact of synthetic GHS on insulin secretion and glucose metabolism had been reported in both animal and human studies. Depending on dose and experimental conditions ghrelin has been shown able to inhibit or stimulate insulin secretion in animals. In humans, ghrelin administration is followed by transient inhibition of insulin levels that surprisingly follows persistent increase in plasma glucose levels suggesting that ghrelin would also directly or indirectly activate glycogenolisis. Current studies indicate that ghrelin also blunts the insulin response to arginine but not that to oral glucose load in humans. These acute effects of ghrelin are independent of any cholinergic mediation and are not shared by synthetic, peptidyl GHS indicating they are likely mediated by a non–GHS-R1a receptor. These acute effects of ghrelin on insulin secretion would be short-lasting, and it has to be remembered that long-term treatment with synthetic non peptidyl GHS in healthy elderly subjects was followed by insulin resistance. In all, it is already clear that ghrelin has remarkable impact in modulating insulin secretion and glucose metabolism. Insulin and ghrelin secretions seem linked by a negative functional relationship that strengthens the hypothesized role of ghrelin in participating in the management of the neuroendocrine and metabolic response to variations in energy balance.

Key Words: Ghrelin; GH secretagogues; insulin; glucose metabolism; energy balance.

Introduction

Ghrelin is a 28-amino-acid peptide predominantly produced by the stomach, while substantially lower amounts derive from bowel, pancreas, kidneys, gonads, placenta, pituitary, and hypothalamus *(1–4)*. Ghrelin has been discovered as a natural ligand of the orphan growth hormone

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(GH) secretagogues (GHS) receptor (GHS-R) type 1a that, in turn, had been shown specific for synthetic GHS *(1–5)*. Like synthetic GHS, ghrelin possesses strong GH-releasing effect in humans as well as in animal though acylation in Serine3 is needed for its activity *(6–8)*.

GHS-R are particularly concentrated in the hypothalamus–pituitary unit, but are also present in other areas of the central nervous system and in peripheral, endocrine, and nonendocrine animal and human tissues including the pancreas *(2–5,9,10)*. This GHS-R distribution explains ghrelin's GH-releasing effect and also other endocrine and nonendocrine activities. These activities include: (a) stimulation of lactotroph and corticotroph secretion and inhibition of gonadotroph secretion; (b) modulation of mechanisms involved in the control of sleep and behavior; (c) orexant activity coupled with control of energy expenditure; (d) modulation of gastric motility and acid secretion as well as of the endocrine pancreatic function; (e) modulation of the endocrine pancreatic function and glucose metabolism; (f) cardiovascular actions; and (g) modulation of neoplastic cell proliferation *(3,11,12)*.

Approximately 3 yr after the discovery of ghrelin, literature reports an impressive number of major contributions that are now mostly focusing on the central orexant activity and the endocrine and nonendocrine, gastroenteropancreatic and metabolic actions *(3,11,12)*. Indeed, there is already clear evidence that ghrelin is an hormone signaling the metabolic balance and managing the neuroendocrine and metabolic response to starvation *(12,13)*. Ghrelin might be complementary to leptin informing the central nervous system (CNS) about the status of energy balance *(14)*. Concurrently, both also would be exerting their critical role at the peripheral level adapting the endocrine pancreatic function (mostly including insulin secretion and sensitivity) to variations in the nutritional status and glucose metabolism. To support this hypothesis there is already evidence suggesting that ghrelin secretion would be, in turn, modulated by the endocrine pancreas and metabolic fuels *(15,16)*.

The potential clinical implications for ghrelin in the metabolic field are even obvious. Obesity, diabetes type 2, and related disorders as well as anorexia and cachexia are among the leading causes of illness and mortality in the developed world *(17)*. The ghrelin system promises to be of critical importance to understand the central and peripheral pathophysiological mechanisms underlying metabolic disorders. The orexigenic action of ghrelin, however, also engenders the dream that orally active ghrelin analogs acting as agonists and antagonists would be useful for treatment of some eating and/or metabolic disorders.

The role of ghrelin in the control of appetite, food intake, and energy balance will be reviewed elsewhere in this issue. The aim of this article is to update the present knowledge about the functional link between ghrelin and the endocrine pancreas and glucose metabolism.

Endocrine and Metabolic Actions of Ghrelin: from the Concept of a Natural "GH Secretagogue" to the Concept of a Driver of the Neuroendocrine and Metabolic Response to Variations in Energy Balance

Ghrelin, although only in its octanoylated form, as well as synthetic GHS possess a strong and dose-related GH-releasing effect that is more marked in humans than in animals. This effect is mostly mediated by hypothalamic actions including enhancement of GHRH activity and functional antagonism of somatostatin activity *(3,18)*. This latter property likely explains why ghrelin and GHS stimulatory action on GH secretion is synergistic with that of GHRH and is also partially refractory to the inhibitory effect exogenous somatostatin *(19)*. Note that it is also partially refractory to the inhibitory effect of cholinergic antagonists, beta-adrenergic agonists, glucose, free fatty acids, rhGH, and rhIGF-I as well as totally refractory to the potentiating effect of cholinergic agonists and arginine; all these factors affect somatotroph function, at least partially, modulating hypothalamic somatostatin release *(3,20)*.

The endocrine activity of both natural and synthetic GHS is not fully specific for GH; in fact, they stimulate both lactotroph and corticotroph secretion, likely via direct pituitary and full central actions, respectively *(21,22)*. The ACTH-releasing activity of ghrelin and GHS is remarkable in physiological conditions and, particularly, much more in patients with pituitary ACTH-dependent Cushing's syndrome, despite their hypercortisolism *(21)*.

It has to be taken into account that ghrelin's discovery is the result of a story born more than 20 yr ago with synthetic, orally active GHS. This evidence suggested the dream they would pharmacologically replace rhGH for treatment of GH deficiency and as anabolic drug intervention in aging and catabolic states *(5,21)*. The research showed this is unlikely to be the case, and recent studies even questioned the hypothesis that the stimulatory action on somatotroph function is the most remarkable biological activity of ghrelin. In fact, pulsatile ghrelin secretion is not strictly correlated to GH pulsatility whereas it is associated with food intake episodes and even sleep–wake patterns *(23–25)*. Moreover, (a) although circulating ghrelin levels are 70% generated by gastric secretion in humans as well as in humans, gastrectomy is not associated to GH insufficiency; (b) a clear feedback mechanism between GH and ghrelin levels has not been demonstrated in humans or animals; (c) age-related changes in GH secretion are not coupled to changes in ghrelin secretion *(3,11)*. Taking this evidence into account, on the other hand, it has to be emphasized that changes in GH secretion associated with variations in the nutritional status would reflect changes in ghrelin secretion. In fact, it has been shown that GHS-R1a disruption within the arcuate nucleus in the rat is followed by GH insufficiency and retarded

growth together with reduced body mass *(26)*. Moreover, in humans: (a) ghrelin hypersecretion is associated with increased GH secretion triggered by fasting and caloric restriction as well as with GH hypersecretion in anorectic and cachectic patients; (b) ghrelin hyposecretion is associated to GH decrease after feeding as well as to GH hyposecretion in obese patients *(3,27–29)*. This negative association between ghrelin secretion and body mass predicts negative association between insulin and ghrelin secretion that has clearly been demonstrated in humans as well as in animals *(15,25,27, 30–33)*. In humans, ghrelin peaks anticipate food episodes and are coupled with the lowest insulin levels *(25)*. On the other hand, food intake is followed by a prompt decrease in ghrelin coupled with an increase in insulin levels. The same strict negative association between insulin and ghrelin levels is present at night *(25)*. Strict inverse association between ghrelin and leptin levels has not been demonstrated in humans, but fasting synchronizes negative association between ghrelin and leptin pulses in rats *(34)*. Moreover, it has been widely demonstrated that ghrelin and leptin act within the arcuate nucleus to modulate the NPY/AGRP system and exert their influence on food intake and energy balance; at this level the orexigenic effect of ghrelin seems to override the anorexant action of leptin *(35,36)*. This evidence suggested the hypothesis that ghrelin and leptin play a role in driving the neuroendocrine and metabolic response to starvation; in other words, ghrelin and leptin might really be complementary players of one regulatory system that has developed to inform the CNS about the status of energy balance and, concurrently, activates a series of neuroendocrine adaptations *(12,13,36)*. As discussed more extensively in the following paragraphs, further evidence now emphasizes this role of ghrelin.

Regulation of Ghrelin Secretion: Metabolic Factors Play the Major Role

Ghrelin secretion, mostly represented in its acylated form, occurs in a pulsatile manner, and it is noteworthy that there is no strict correlation between ghrelin and GH levels in rats; interestingly, ghrelin pulses are correlated with food intake episodes and sleep cycles. Particularly, in humans it has been shown that peaks in ghrelin levels anticipate food intake, suggesting the latter is triggered by ghrelin discharge *(25)*.

In agreement with the major influence of nutrition on ghrelin secretion, circulating ghrelin levels are increased in anorexia and cachexia but reduced in obesity and overfeeding *(27–29,37)*. In every condition ghrelin secretion is normalized by recovery of ideal body weight *(38,39)*. These changes are opposite to those of leptin, suggesting that both ghrelin and leptin are hormones signaling the metabolic balance and managing the neuroendocrine and metabolic response to starvation *(12,13)*. Circulating ghrelin levels mostly reflect gastric secretion; in fact, they are reduced by 70% after gastrectomy *(28)*.

In humans, ghrelin levels are increased by fasting and energy restriction but decreased by food intake and overfeeding; note that ghrelin increase anticipates fasting-induced GH hypersecretion, suggesting the latter is triggered by enhanced ghrelin release *(40)*. It had been reported that ghrelin secretion is not inhibited by simple gastric distension in animals, but, more recently, it has been demonstrated that gastric by-pass strongly inhibits it *(28,29,37,41)*. Either oral and intravenous glucose loads inhibit ghrelin secretion in humans as well as in animals; on the other hand, free fatty acid load as well as arginine load do not affect circulating ghrelin levels *(16,31,42)*.

In agreement with the negative association between ghrelin secretion and body mass, clear negative association between ghrelin and insulin secretion has been found in humans as well as in animals *(25,27,30)*, suggesting inhibitory influence of insulin on ghrelin secretion *(15,44)*. Indeed, during euglycemic clamp, steady-state increase in insulin levels is associated with a clear reduction in circulating ghrelin levels *(31)*. This evidence suggests that ghrelin has no role in mediating the neuroendocrine response to hypoglycemia and that, on the other hand, the lack of any change in ghrelin secretion during insulin-induced hypoglycemia would reflect balance between inhibitory influence exerted by insulin and stimulatory influence exerted by hypoglycemia. The exact mechanisms by which insulin and glucose variations regulate ghrelin secretion is still unknown, although it has been demonstrated that insulin directly modulates ghrelin expression at the gastric level *(45)*.

The most remarkable inhibitory input on ghrelin secretion is represented by somatostatin as well as by its natural analog cortistatin that reduce beta-cell secretion *(46)*. Somatostatin receptor subtypes are present in the gastric mucosa *(47,48)*. Moreover, gastric carcinoids, previously classified as silent, nonfunctioning tumors but indeed able to synthesize and secrete ghrelin, mostly express sst2 and sst5 receptors, suggesting that these somatostatin receptor subtypes would mediate the inhibitory influence of SRIF on ghrelin secretion (unpublished data).

Overall, evidence that insulin and somatostatin exert a critical inhibitory action on ghrelin secretion indicates that the latter is under major influence, mostly inhibitory, from the endocrine pancreas. A notable exception to the negative association between insulin and ghrelin secretion is represented by the Prader–Willi syndrome, which is generally connoted by obesity but, nevertheless, is associated with clear-cut ghrelin hypersecretion *(49)*. This peculiar exception to the rule that ghrelin secretion is negatively associated to BMI as well as to insulin has suggested that overweight in this syndrome would be the result of exaggerated food intake triggered by the orexigenic effect of ghrelin hypersecretion *(49)*.

Influence of Ghrelin on Endocrine Pancreas and Glucose Metabolism

Ghrelin is a new gastric hormone showing so high an homology to motilin that, a few months after its discovery by Kojima et al. *(1)*, it was also isolated by Tomassetto et al. and named motilin-related peptide *(50)*. It is therefore not surprising that this new gastric hormone exerts gastroenteropancreatic actions. In fact, it has been demonstrated that ghrelin modulates gastric motility and acid secretion *(3,11,51)*, is able to stimulate ileal peristalsis *(51)*, and also inhibits CCK-induced pancreatic protein secretion *(52)*.

Remarkably, GHS-R1a and 1b have been found expressed in either animal or human endocrine pancreas by the majority of authors *(4,10)*. Interestingly, however, significant specific binding for peptidyl GHS had not been demonstrated *(9)*. On the other hand, ghrelin mRNA and protein have been demonstrated to be expressed in the endocrine pancreas; this expression either in animals or in humans has been variably localized in α- *(53)*, in β- *(10)*, and in non-β/non-α cells *(54)*. Moreover, it has been shown that ghrelin expression within the endocrine pancreas anticipates that in the stomach occurring very early in fetal life and decreasing after birth *(54)*. Thus, this evidence suggests that the ghrelin system would be operative upon the endocrine pancreas; this influence would occur in an endocrine manner but also in a paracrine/autocrine manner.

Old studies with synthetic GHS suggested their potential impact on the endocrine pancreatic function. In fact, it had been demonstrated that chronic treatment with non-peptidyl GHS: (a) was followed by hyperglycemia and insulin resistance in obese rats—these findings were explained hypothesizing they were probably reflecting GHS-induced HPA hyperactivation *(55)*; (b) induced hyperglycemia and insulin resistance in normal elderly subjects, but this result was explained as consequence of GHS-induced enhancement in GH secretion *(56)*; (c) induced increase in fat mass and worsened insulin resistance in obese patients *(57)*. These findings were particularly surprising because GHS-induced increase in GH secretion had been expected to exhibit lypolitic action possibly favoring fat mass decrease *(57)*.

Depending on dose and experimental conditions, ghrelin has been shown able to inhibit or stimulate insulin secretion in animals *(32,33,43,53)*. Ghrelin has been reported to stimulate insulin secretion from isolated rat pancreatic islets *(32,53)* and also in rats in vivo *(43)*. Conversely, ghrelin has been reported able to blunt insulin secretion from isolated rat pancreas after stimulation with glucose, arginine, and carbachol *(33)*. Interestingly, exposure to ghrelin also blunted the somatostatin response to arginine *(33)*. In humans, the acute administration of ghrelin has been found followed by a significant increase in plasma glucose levels surprisingly followed by a reduction in insulin secretion *(58)*; these findings have been recently confirmed and are independent of age *(59)*.

The prompt increase in plasma glucose levels after ghrelin injection cannot be explained by gluconeogenetic action, although it has also been demonstrated that ghrelin affects glucose metabolism at this level. In fact, ghrelin has been found to up-regulate several insulin-induced activities, but, unlike insulin, to up-regulate gluconeogenesis of hepatoma cells *(60)*. The ghrelin-induced increase in glucose levels is unlikely to be reflecting stimulation of glucagon release that, in turn, would have stimulated insulin secretion. Theoretically, ghrelin would activate glycogenolisis either indirectly via stimulation of catecholamine release *(58,60)* or acting directly on hepatocytes.

At the hepatic level, the ghrelin-induced variations in either glucose or insulin levels are unlikely to be mediated by the classical GHS-R1a; in fact, these effects are not shared by peptidyl GHS that normally bind the GHS-R1a *(58)*. In agreement with this hypothesis is also evidence that within the liver GHS-R1a expression and binding studies never showed the significant presence of the classical ghrelin receptor *(4)*.

It has been shown that acetylcholine mediates the influence of ghrelin on gastric acid secretion and motility and its major role in the gastroenteropancreatic function could predict its involvement in the impact of ghrelin on insulin secretion *(3,11)*. However, it has recently been demonstrated that cholinergic agonists and antagonists do not modify the ghrelin-induced insulin and glucose variations in humans *(61)*. On the other hand, in humans, although unable to modify the insulin and glucose response to oral glucose load, ghrelin has been found able to blunt the arginine-induced insulin response while enhancing the arginine-induced increase in glucose levels (personal unpublished results).

More recently, ghrelin administration has been shown able to increase circulating SRIF and even PP levels in humans *(33)*. On one hand, these findings suggest the existence of a functional feedback mechanism linking somatostatin and ghrelin secretion in humans. On the other hand, ghrelin-induced SS increase would theoretically explain insulin decrease, but this hypothesis remains to be demonstrated *(33)*.

Independently on the mechanisms mediating the endocrine or paracrine/autocrine impact of ghrelin on insulin secretion, it is clear that ghrelin influences insulin secretion and glucose metabolism; as noted above, insulin and glucose levels, in turn, negatively influence ghrelin secretion *(15,31,44)* thus indicating the existence of a feedback mechanisms linking ghrelin with the endocrine pancreas and glucose metabolism.

As in normal subjects, in obese patients, i.e., a condition of ghrelin hyposecretion, acute ghrelin administration is followed by a rise in glucose levels as well as by a more marked, although transient, decrease in insulin levels. On the other hand, in anorectic patients, i.e., a condition of ghrelin hypersecretion, ghrelin administration does not further inhibit reduced insulin levels and is unable to modify glu-

cose levels, possibly reflecting exhaustion of glycogen stores (personal unpublished results).

It has to be emphasized, however, that, as anticipated by the discrepant results in animals *(32,33,53)*, ghrelin influence on insulin secretion cannot be defined as simply inhibitory. The acute effects of ghrelin on insulin secretion would be short-lasting and would be followed by different adaptations depending mostly on the metabolic environment and/or by the length of exposure to elevated ghrelin levels. In this context, as mentioned above, it has to be taken into account the evidence that long term treatment with synthetic, non-peptidyl GHS in healthy elderly subjects was followed by insulin resistance and, often, by hyperglycemia *(56)*.

Conclusions

Overall, it is already clear that ghrelin has a remarkable impact in modulating insulin secretion and glucose metabolism. Insulin and ghrelin secretions seem linked by a negative functional relationship that strengthens the hypothesized role of ghrelin in participating in the management of the neuroendocrine and metabolic responses to variations in energy balance.

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