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Gastric mammalian target of rapamycin signaling, hormone production and energy metabolism

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Abstract

The obesity epidemic imposes a significant health burden on human beings. Current understanding of the mechanisms underlying the development of obesity is incomplete and contemporary treatment is often ineffective. Gastrointestinal hormones are important regulators of food intake and energy metabolism. Previous studies indicate that the mammalian target of rapamycin signaling pathway in the gastric mucosa is crucially involved in fuel sensing in the gastrointestinal tract and plays a critical role in the coordination of nutrient availability and ingestive behavior *via* the production of gastric hormones. As an important component of the brain-gut axis regulating food intake and energy homeostasis, energy sensing in the gastrointestinal tract may provide a novel insight into our understanding of the precise coordination between the organism and cellular energy state.

Key words: Gastric mammalian target of rapamycin; Hormones; Energy metabolism

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INTRODUCTION

Food intake and energy metabolism are regulated by the reciprocal actions of a group of anorexigenic peptides, which include leptin, insulin, cholecystokinin, peptide YY and glucagon-like peptide, and by the actions of a group of orexigenic peptides, including ghrelin. The majority of these hormones are secreted by endocrine cells scattered throughout the gastrointestinal tract[1]. All these hormones are proposed to modulate the activity of the energy metabolism center within the hypothalamus, ultimately leading to a change in feeding behavior and the control of metabolic homeostasis[2]. While many studies reveal that nutrient sensing molecules within the hypothalamic neurons are critical in the control of energy homeostasis[3] and defects in fuel sensing at the hypothalamic cellular level may lead to energy imbalance at the organism level and to the development of obesity[4], a recent study suggests that there also exists a fuel sensing mechanism in the gastric mucosa[5]. This finding suggests that the

interaction between peripheral and central fuel sensing mechanisms is a crucial feature of feeding behavior and energy homeostasis[5]. The peripheral fuel sensing mechanism in the gastric mucosa may function to regulate the production of gastric hormones and therefore contribute to the modulation of energy metabolism.

FUEL SENSING MECHANISM

Obesity is defined as the condition in which energy intake consistently outpaces energy expenditure leading to the accumulation of excess fat to an extent that health is negatively affected. The development of obesity is linked to small but cumulative discrepancies between caloric intake and energy expenditure[6]. Under normal conditions, balance in energy metabolism is maintained by a precise regulation of cellular activity in multiple organs that matches nutrient supply at the organism level[7]. The link between the energy status of individual cells and the overall energy balance of the entire organism is complex and remains largely unknown. Many studies have identified the hypothalamus as a critical organ for integrating intracellular metabolic processes with energy homeostasis at the organism level[7], adjusting food intake to match the level of overall cellular activity. Recent investigations have identified 5' AMP-activated protein kinase (AMPK)[8] and mammalian target of rapamycin (mTOR)[9] as key fuel sensors in hypothalamic neurons. AMPK is a serine-threonine protein kinase which serves as a cellular fuel sensor to protect cell viability in response to ATP depletion[10]. AMPK is tightly regulated, monitoring changes in the cellular ratio of adenosine monophosphate (AMP) and adenosine triphosphate (ATP). Recent studies have suggested that AMPK in the hypothalamus regulates energy metabolism by integrating inputs from multiple peptide hormones, neurotransmitters and nutrients. Alteration of hypothalamic AMPK activity leads to change in food intake and body weight[11-13]. mTOR, a highly conserved serine-threonine kinase, has been reported to serve as an intracellular ATP sensor. *In vitro* studies have demonstrated that cellular levels of ATP regulate mTOR signaling[14]. Aberrant mTOR activity is linked to the development of cancer, diabetes and obesity[15]. Significant elevation of mTOR signaling has been observed in liver and skeletal muscle of insulin-resistant obese rats maintained on a high fat diet[16]. In contrast, absence of the mTOR downstream target, S6 kinase 1, protects against diet-induced obesity and improves insulin sensitivity in mice[17]. mTOR signaling in hypothalamic neurons is involved in neuronal sensing of nutrient availability and regulates food intake and energy balance[9]. These observations suggest that mTOR plays an important role in central neuronal control of nutrient intake and energy balance. Further studies indicate that mTOR signaling is a potential downstream pathway for food intake regulation in response to hypothalamic AMPK[18], likely through the mediation of tuberous sclerosis complex 2, a known inhibitor of mTOR signaling[19]. Thus, food intake and nutrient metabolism may be coordinately regulated by linking AMPK and mTOR signaling pathways in the hypothalamus. These observations have motivated extensive studies of hypothalamic fuel sensing mechanisms and hypothalamic regulation of energy metabolism[7]. In contrast, virtually no attention has been focused on fuel sensing by the gastrointestinal tract, despite its critical role in the regulation of food intake.

GASTRIC mTOR IS A FUEL SENSOR INTEGRATING FUEL SUPPLY WITH HORMONE PRODUCTION

A series of studies have identified mTOR as a potential candidate of fuel sensor in the gastric mucosa because of its expression in a distinct group of the gastric endocrine cells, its reciprocal relationship with energy status and its role in the regulation of gastric hormone production[1,5,20].

Co-localization of mTOR signaling molecules in gastric neuroendocrine cells

Chromogranin A is a widely recognized marker of neuroendocrine cells, including those of the stomach, large and small intestine, adrenal medulla and pancreatic islets[21]. It is also an excellent marker for neuroendocrine tumors[22]. In the gastric fundus, the active forms of mTOR signaling molecules express in cells located in the basal one third of the gastric mucosa. One third of chromogranin A-immunoreactive cells express phospho-S6K1, the downstream target of mTOR. The majority of the mTOR positive endocrine cells are ghrelin positive with a small fraction of cells stained positive for gastrin immunoreactivity. No mTOR signaling molecule is located within somatostatin immunoreactive cells[20]. These studies suggest that mTOR signaling may selectively influence the function of a subpopulation of gastric endocrine cells.

A reciprocal relationship between gastric mTOR signaling and energy status at the organism level

Gastric mTOR signaling also senses the body energy status. Gastric mTOR activity decreases in 48 h fasted mice relative to fed animals. In contrast, there is a significant increase in gastric phospho-mTOR (Ser2448) and phospho-S6 (Ser235/236) expression in obese mice relative to lean animals[5]. Gastric mTOR signaling is therefore reciprocally related with the short- and long-term changes in nutritional status at the organism level.

Gastric mTOR and hormone production

Numerous peptides are synthesized and released from distinct populations of secretory neuroendocrine cells throughout the gastrointestinal tract[23]. Their roles in the regulation of gastrointestinal function have been well characterized for many years and it is now becoming evident that they also modulate feeding behavior and energy metabolism *via* distinct mechanisms. Major neuroendocrine products have been identified as gastrin in G

cells, histamine and uroguanylin in enterochromaffin-like (ECL) cells, somatostatin in D cells, serotonin in EC cells and ghrelin in X/A-like cells[23,24]. Hormones secreted from gastric endocrine cells bind to receptors located in the hypothalamus to regulate food intake and energy metabolism[1]. The fuel sensing mechanism is critical for the regulation of gastrointestinal hormone synthesis and secretion and therefore provides a fine tuning for the peripheral and central control of feeding behavior and energy homeostasis.

Ghrelin: In 1999, ghrelin was isolated from the human and rat stomach as the endogenous ligand for the growth hormone secretagogue-receptor (GHS-R)[25]; it is synthesized mainly by X/A-like cells in the gastric mucosa and secreted into the circulation[26]. Several molecular forms of ghrelin are found in the stomach and circulation: the 28 amino acid ghrelin with n-octanoylated serine in position 3; des-acyl ghrelin, an identical peptide in which the third amino acid serine is not acylated; and the 27 amino acid des-glutamine 14 ghrelin produced by alternative splicing of the ghrelin gene[24]. Another putative proghrelin peptide, termed "obestatin", has been proposed[27] but biochemical and functional evidence supporting its existence has not been forthcoming. Octanoylation is necessary for ghrelin to bind with its receptor, GHS-R. Ghrelin-O-acyltransferase, the enzyme responsible for ghrelin acylation, has been recently characterized as a member of the Membrane Bound O-Acyltransferases family[28,29]. Ghrelin has been reported to exercise a broad array of functions including control of food intake[30] and glucose metabolism[31]. Exogenous ghrelin induces adiposity in rodents by stimulating an acute increase in food intake, as well as a reduction in fat utilization[32]. Blocking the action of ghrelin by either its receptor antagonism[33] or interfering with its availability for its receptor by neutralizing antibodies[34] or Spiegelmer RNA[35] have been reported to show some effects on reduction of food intake and body weight, although the immunization against ghrelin fails to cause long-term body weight reduction. Ghrelin exerts its orexigenic effect *via* a mechanism involving the central nervous system; at least part of the orexigenic effect of ghrelin is mediated by up-regulating the genes encoding orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP)[36] in the hypothalamus. During fasting, ghrelin secretion increases[37]. Conversely, plasma ghrelin concentration decreases in most obese subjects[38] except in Prader-Willi syndrome[39]. Ghrelin and its receptor are expressed in human and rat pancreatic islets[40]. Ghrelin inhibits glucose stimulated insulin secretion in a dose-dependent manner *in vitro*[41]. Intravenous ghrelin injection decreases plasma insulin and increases plasma glucose levels, likely by inhibition of insulin secretion[41]. Absence of ghrelin in ob/ob mice lowers blood glucose substantially even though it does not decrease food intake or body weight[42].

The secretion of ghrelin is tightly coupled to the fasting or fed state[43]. While it is presumed that precise control in the production and secretion of ghrelin is critical for the maintenance of energy balance, the molecular mechanisms by which ghrelin producing cells modulate transcription and translation of ghrelin to match overall energy status remain largely unknown. A recent study has demonstrated that gastric mTOR is a critical molecule coordinating the ghrelin production with energy supply levels. In gastric mucosa, mTOR signaling molecules are located mainly in the ghrelin-positive cells. More than 90% of ghrelin-positive cells stain positively for mTOR signaling molecules. There exists a reciprocal relationship between gastric mTOR signaling and the expression and secretion of ghrelin during changes in energy status. Inhibition of gastric mTOR signaling increases expression of gastric ghrelin and circulating ghrelin. Conversely, activation of gastric mTOR signaling attenuates the expression and secretion of ghrelin. All these data support the concept that gastric mTOR activity is reciprocally linked to the production of ghrelin[5].

Gastrin: Gastrin is an acid secretagogue peptide discovered by Edkins[44] in 1906. Gastrin stimulation of ECL cells results in the increased synthesis and release of histamine, which then induces acid secretion by binding to the H receptors located on parietal cells[45]. Other major physiological functions of gastrin on the gastrointestinal tract includes functioning as a growth/differentiation factor[46]. Gastrin release is stimulated by vagal impulses during the cephalic phase and by intramural neural reflexes as well as by the presence of food constituent in the gastric lumen during the gastric phase of acid secretion[47]. Increased production of hydrochloric acid lowers intragastric pH and inhibits further secretion of gastrin[48]. Gastric mTOR signaling may be involved in the regulation of gastrin synthesis and secretion in a proportion of gastric G cells. Only 1/3 of gastrin cells contain mTOR signaling molecules, suggesting that regulation of gastrin synthesis and secretion may involve multiple mechanisms[20].

Somatostatin: Somatostatin was originally isolated as a hypothalamic somatotropin-release inhibiting factor[49] and was soon found to potently inhibit the secretion of multiple hormones, including gastrin[50]. However, production of somatostatin appears not to be affected by gastric mTOR. No mTOR activity is detected in somatostatin positive cells. Furthermore, inhibition of gastric mTOR signaling by rapamycin demonstrates no effect on the synthesis and secretion of somatostatin[20].

All of this evidence supports that mTOR signaling selectively modulates the production of gastric hormones. The differential regulation of gastric hormones by mTOR signaling may provide an alternative strategy for the development of novel therapeutics for obesity and other disorders of energy metabolism.

GASTRIC FUEL SENSING AND ENERGY METABOLISM

In the central nervous system, fuel substrates such as glu-

cose, fatty acids and amino acids, or hormones including leptin and insulin, act on the hypothalamic neurons to inform the energy metabolism regulating center of the energy status[3]. Specific populations of "glucosensing" neurons have been identified[51]. In the hypothalamic arcuate nucleus, pro-opiomelanocortin neuron is the glucose-excited neuron, while NPY neuron is inhibited by glucose. These neurons form the neuronal circuits to monitor and integrate the quantitative and temporal changes in glucose concentration[51]. By regulating their activity and neurotransmitter release, these neurons coordinate the central glucose level with the peripheral glucose production and utilization to maintain the glucose homeostasis[3,51].

How hypothalamic neurons sense the energy supply is being actively explored. Studies by Cota *et al*[9] strongly support the notion that mTOR is a critical intracellular molecule within hypothalamic neurons to coordinate the energy supply with food intake and energy metabolism. Although mTOR and S6K1 are widely expressed in a variety of tissues within the CNS, the phosphorylated form of these two kinases is abundantly localized in the hypothalamus, particularly in the NPY/AgRP neurons. Activity of the mTOR pathway in the hypothalamus is tightly linked with energy supply. mTOR activity decreases during fasting and its activity conversely increases during re-feeding. Central administration of leucine, a branch chained amino acid, decreases food intake and body weight by activation of the hypothalamic mTOR signaling. Leptin stimulates hypothalamic mTOR activity and inhibition of mTOR signaling blunts the anorectic effect of leptin[9]. Hypothalamus specific expression of dominant negative S6K results in an increase in food intake, whereas expression of constitutively active S6K decreases food intake[52]. These observations suggest that mTOR is a critical fuel sensor in the hypothalamus.

Inhibition of mTOR signaling by rapamycin has been demonstrated to increase food intake. Such an orexigenic effect of rapamycin may be mediated by ghrelin. Intraperitoneal injection of rapamycin stimulates ghrelin secretion and expression. Ghrelin receptor antagonist D-Lys-3-GH-releasing peptide-6 or ghrelin receptor deletion abolishes the rapamycin-induced increment in food intake despite that plasma ghrelin remains elevated[5]. Together with the observation that mTOR is selectively expressed in a subpopulation of gastric endocrine cells and its activity is reciprocally related with the energy level, we propose that gastric mTOR is a peripheral fuel sensor integrating the energy supply with the food intake and energy metabolism by alteration of ghrelin production. Defining the mTOR signaling pathway to inhibit the production of acyl ghrelin, the active form of ghrelin, would shift therapeutic focus to gastric targets.

CONCLUSION

The fuel sensing mechanism in the central nervous system is critical for energy homeostasis. However, the anatomical structure and location of the hypothalamus pose significant hurdles for therapy targeting this organ. Searching for peripheral targets is appealing. Novel evidence suggests that mTOR is a critical regulatory molecule in gastric ghrelin cells and that its activity is linked to energy supply through modulation of the production of acyl ghrelin. Further studies will aim to advance our understanding of intracellular processes in the production of ghrelin and to provide new information on the integration of cellular activities of gastric endocrine cells with overall nutrient availability. Results of these new investigations will yield new insights relevant to treatment strategies for human obesity.

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