Review Article

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Ghrelin: The Gastrointestinal Peptide Hormone That Regulates Energy Balance

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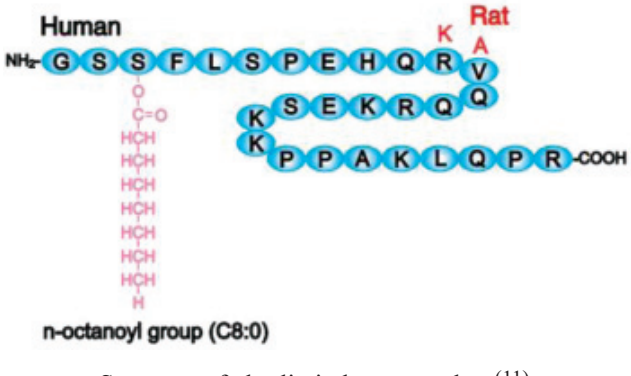
What is ghrelin?

Ghrelin, which is known as a twenty-eight-amino-acid peptide with an essential n-octanoyl modification on the third amino acid, is an orexigenic peptide hormone that formed by X/A-like cells in the stomach and playing a crucial role in inducing appetite activities. Ghrelin was first discovered in 1999 by Kujima and colleagues. The name "ghrelin" is derived from "ghre", meaning "grow", to indicate the ability of this hormone that is to stimulate growth hormone (GH) release and increase appetite[1]. It is later believed that ghrelin is also deeply involved in the regulation of feeding behavior and energy homeostasis[2-6]. Nonetheless, it has been reported that both expression and secretion of this hormone are mainly influenced by changes in energy balance, for example, there is ghrelin increasing during periods of fasting and decreasing after food intake[7-10].



Structure of ghrelin in human and rat[11].

Distribution of ghrelin

There are two major forms of ghrelin are found in tissues and plasma. Those forms are n-octanoyl-modified and des-acyl ghrelin. The normal ghrelin concentration of plasma samples in humans is 10 -20 fmol/ml forn-octanoyl ghrelin and the total is 100 -150 fmol/ml, including both acyl-modified and des-acyl ghrelins. Plasma ghrelin concentration is built up in fasting conditions and reduced after habitual feeding[7,9], suggesting that an initiation signal for food intake or ghrelin secretion is controlled by some nutritional factors in blood may be ghrelin.

Stomach and gastrointestinal organs, ghrelin is mainly produced in the stomach in all vertebrate species[12]. In the stomach, ghrelin-which contains cells are more abundant in the fundus than in the pylorus. In the analyses of situ hybridization and immunohistochemical let us know that ghrelin-containing cells are a distinct endocrine cell type found in the mucosal layer of the stomach[13].

Hypothalamus and pituitary, as the ghrelin receptor GHS-R is mainly expressed, the existence of its endogenous ligand has been thought to be mainly in

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the hypothalamic regions[14]. The hypothalamic arcuate nucleus, an important region for controlling appetite, is where ghrelin has been found[1]. In addition, a late study has reported the presence of ghrelin in previously uncharacterized hypothalamic neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei[15]. All of these ghrelin-containing neurons send efferent fibers to neurons that contain neuropeptide Y (NPY) and agouti-related protein (AgRP) and also may stimulate the release of these orexigenic peptides.

Other tissues, the study indicate that plasma ghrelin concentration was significantly correlated with the serum creatinine level and was increased 2.8-fold in patients with end-stage renal disease compared with those in normal renal function patients[16]. This result gives us important information about that the kidney is an important site for clearance or degradation of ghrelin.

Ghrelin-producing cells, there are several cultured cell lines that express ghrelin. Ghrelin is produced in a human thyroid medullary carcinoma cell line, known as TT cells[17]. TT cells express ghrelin mRNA, and ghrelin peptides are contain in both conditioned medium and cellular extracts of TT cells. Being in the stomach, cellular extracts of TT cells contain both n-octanoyl ghrelin and des-acyl ghrelin.

Physiological functions of ghrelin

Effect of GH-Releasing activity

A multifaceted peptide hormone is another term of ghrelin[11]. Ghrelin acts on the GHS-R, increasing intracellular Ca^{2+} concentration via IP_3 to stimulate GH release. The GH-releasing activity of ghrelin is similar to that of GHRH when injected intravenously into rats[1,20] in terms of both the area under the curve and mean peak GH levels. However, the maximal stimulation is effected by ghrelin is two to three times greater than that of GHRH[20]. In addition, high doses of ghrelin in humans advance ACTH, prolactin, and cortisol levels[19]. Ghrelin stimulates GH which released from primary pituitary cells, which indicates that ghrelin can act directly on the pituitary[1]. On the other hands, in ghrelin-mediated stimulation of GH release, the involvement of the hypothalamus has been strongly suggested. Patients with organic lesions in the hypothalamic region showed insufficiency of GH release even when stimulated by ghrelin. The maximal level of GH releases to be achieved by ghrelin administration[1]. Another possibility is that GHRH levels in primary pituitary cells are too low. Co-administration of ghrelin and GHRH had a synergistic effect on GH secretion; that is, co-administration results in more GH release than does either GHRH or ghrelin alone[19]. Synergistic effect on GH release was also observed by co-administration of GHSs, synthetic ghrelin agonists, and GHRH[18]. This finding implies that, for maximally effective in inducing GH release, GHRH is necessary.

Effect of ghrelin in central regulation of energy balance

Brain areas and neurons that involved in the regulation of energy balance

Energy balance is a critical homeostasis that body has to be preserved and involved with various systems. The arcuate nucleus (ARC), which is well known as the regulation center of food intake in the hypothalamus, receives many information from neurons and hormones. These signals are work together to maintain satiety and feeding equilibrium. Arcuate nucleus consist of two major feeding-related neuronal subtypes that have contrary effect on energy balance; neuropeptide Y (NPY)/agouti-related peptide (AgRP) and propriomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART), which stimulates and inhibits food intake, respectively[22]. ARC neurons subsequently send their efferent signals to the paraventricular nucleus (PVN) and the lateral hypothalamus (LH). PVN secrets several neuropeptides that responsible for satiety and catabolic action, while LH secretes anabolic peptides and orexin that stimulates food intake[23]. After that, all of these signals are transmitted to regulate the energy balance of the body via the nucleus tractus solitaries (NTS) pathway.

Central pathway of ghrelin in energy balance regulation

There are three possible pathways that ghrelin stimulates appetite neurons in hypothalamus. Firstly, the signal of ghrelin sent to food intake center in the hypothalamus via the vagus nerve, which received the signal from gastrointestinal tract. Previous studied in 7 individuals who completed truncal vagotomy showed that the appetite-stimulating effects of ghrelin required intact of vagus nerve[24]. Secondly, circulating ghrelin directly crossed the blood-brain barrier to stimulate

hypothalamic neurons[25]. Finally, ghrelin can be directly synthesized and secreted from ghrelin-containing neurons within the hypothalamus[26].

Since, growth hormone secretagogue receptor, which is the receptor for ghrelin, highly distributed in ARC, PVN, and LH[26-27], ghrelin has a potential role on energy balance regulation. Ghrelin-induced food intake via stimulating neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons in hypothalamus because of NPY/AgRP neurons in ARC showed mRNA expression of ghrelin receptor[28]. Single and chronic intracerebroventricular administration of ghrelin were shown to increased mRNA expression levels of NPY and AgRP in the ARC[29]. Moreover, peripheral ghrelin administrations demonstrates directly stimulate neuronal activities of NPY/AgRP, while indirectly inhibit POMC/CART neurons via increasing γ-aminobutyric acid (GABA) release from NPY/AgRY neurons[26,30]. The secretion of GABA from NPY/AgRP neurons; stimulated by ghrelin, resulting to hyperpolarization of POMC neurons[30] and leading to inhibition of satiety center.

The mechanism which ghrelin stimulates NPY/AgRP neurons was proposed and may be involved with AMP-activated protein kinase (AMPK) in hypothalamus. AMPK was found in several areas of hypothalamus including ARC, LH, and PVN[31]. Feeding shows to inhibit hypothalamic AMPK, whereas, fasting stimulates it[32]. Moreover, feeding and body weight gain are response to activation of AMPK in hypothalamus. In contrast, hypothalamic AMPK inhibition leads to anorexia and weight loss[33].

Central ghrelin, acting through GHS-R1a, activates hypothalamic AMPK by phosphorylating it. AMPK, then, inactivates acetyl-CoA carboxylase (ACC), resulting to decrease malonyl-CoA in cytoplasm. Several cascade reactions occur and net result of action is increase in carnitine palmitoyltransferase 1 (CPT-1) activity. Afterward, ROS levels in is increase from mitochondria fatty acid oxidation. These might be critical processes for ghrelin-induced feeding. However, the molecular events remain unclear[34].

Roles of ghrelin in energy balance and obesity

Energy homeostasis is well coordinate regulated by central nervous system and peripheral tissue such as adipose tissue and stomach. Ghrelin is the only one hormones that stimulates the appetite, whereas the other hormones that involved in energy balance regulation such as leptin or insulin cease appetite[35]. Effect of ghrelin-induced appetite is not independent from growth hormone releasing effects[36].

The secretory profile of ghrelin shows in a diurnal pattern[37], and related with meal time without cues for time or food, thus, it suggested as meal initiator[38-39]. The levels of plasma ghrelin suddenly increased before meal and shortly decreased after meal. These may occur because ghrelin secretion can be induced by fasting, whereas feeding or nutrient absorption suppress it[40]. Types of nutrients have an influence on ghrelin levels. Carbohydrates or proteins show more ghrelin suppression effect than lipid that is why high-fat diet more induced weight gain than carbohydrates or proteins[41]. Moreover, the duration and depth of postprandial suppression on ghrelin are proportional to energy intake[42], proposed that the suppression of ghrelin depend on meal size. Desacyl ghrelin might be an inhibitory effect on acylated ghrelin because it suppressed ghrelin levels in human[43].

Ghrelin is an obesity-induced hormone. Previous studies reported that ghrelin receptor antagonist decreased food intake and induced weight loss in mice[44]. Meal number was increased response to peripheral and central administration of ghrelin without changing meal size[45]. In addition, ghrelin injection in intracerebroventricular of rats demonstrated markedly increased eat motivation via D_1 receptor-dependent mechanism[46]. However, studied in ghrl$^{+/+}$ and ghrl$^{-/-}$ showed non-significant differences in feeding patterns[47], thus, ghrelin might be not critical role for feeding performance.

Ghrelin causes weight gain from fat accumulation without lean body mass changed. Although, previous researches reported that des-acyl ghrelin inhibited acylated ghrelin and exactly effect on energy balance is not be known, intracerebroventricular infusion of des-acyl with high dose also increased fat mass[48]. Moreover, subcutaneously infusion of acylated ghrelin regulated fat mass, whereas des-acyl ghrelin failed[48]. These demonstrated that des-acyl ghrelin is a ligand for growth hormone secretagogue receptor in CNS as well. In addition, repeated central injection of ghrelin reduced energy expenditure and inhibited lipid oxidation[49].

Ghrelin has a pivotal role in long-term through alteration of body weight. Although, ghrelin stimulates food intake and may be one factor that link to obesity, basal ghrelin levels in normal weight were higher than

in obesity[50]. Previous studies found that ghrelin levels are decrease in obese individuals[51] and diurnal pattern also disturbed[52]. Total ghrelin levels were inversely associated with BMI; the indicator for obesity, and body fat mass[53], while, acylated ghrelin levels were positively associated with BMI.

Although, ghrelin levels decrease in obesity, the circulating concentrations of acylated ghrelin are increase and des-acyl ghrelin levels are decreased in obese and obese-associated type 2 diabetes[54]. These indicated that AG/DAG ratio is elevated in obese and type 2 diabetes. Furthermore, obese individuals increased binding affinity of ghrelin-reactive IgG immunoglobulin to ghrelin and injected ghrelin and IgG from obese human in mice resulting increase food intake and meal frequency[55]. This might be the reason for the effect of ghrelin that stimulates food intake more sensitively in obese than in lean subjects[56].

Conversely to obese individual, patients with anorexia nervosa showed higher plasma ghrelin levels than control[57]. Overproduction of ghrelin in hypothalamus leads to food intake and associated with increases in body weight. However, this increase is attenuated after 3 weeks[58]. These suggest the compensatory response of ghrelin to the alteration of body energy reserves. Feeding was able to rapidly suppress ghrelin levels backward to the baseline in lean subjects, but not in obese subjects[59]. Therefore, ghrelin function is proposed to sustain the obesity.

Ghrelin also has a role in glucose homeostasis regulation because of the presence of GHS-R1a on pancreas. Circulating ghrelin levels reported inversely associated with insulin[38]. Acute ghrelin administration in normal weight and obese women showed that ghrelin increased glucose and decreased insulin levels only in obese women[50]. Plasma ghrelin levels in healthy non-diabetic subjects was decreased after insulin infusion and still decreased after insulin infusion discontinued[60]. Moreover, the requirement of insulin for post-prandial ghrelin suppression was reported[61]. These suggest the inhibitory feedback between ghrelin and insulin on the regulatory of energy homeostasis.

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