Pathophysiologic Basis of Anorexia: Focus on the Interaction between Ghrelin Dynamics and the Serotonergic System

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Anorexia is an important issue in the management of elderly patients with cancer because it contributes to the development of malnutrition, increases morbidity and mortality, and negatively affects patients’ quality of life. This review summarizes the potential mechanisms of the development of anorexia in three animal models that mimic the situations commonly seen in elderly patients receiving chemotherapy. Cisplatin-induced anorexia is attributable to a decrease in peripheral and central ghrelin secretion caused by the stimulation of serotonin (5-hydroxytryptamine; 5-HT) 2B and 5-HT 2C receptors via 5-HT secretion. Age-associated anorexia is caused by an increase in plasma leptin, which results from disturbed reactivity of ghrelin in the hypothalamus and regulation of ghrelin secretion. Environmental change causes the activation of central 5-HT1B and 5-HT 2C receptors and the melanocortin-4 receptor system, resulting in a decrease in circulating ghrelin levels which lowers food intake. New therapeutic approaches based on these pathophysiological mechanisms are warranted for the treatment of anorexia in cancer patients, especially elderly ones.

Key words anorexia; ghrelin; serotonin; cisplatin; aging; stress

1. INTRODUCTION

With increasing life expectancy and the higher risk of cancer with aging, cancer treatment of elderly patients is becoming an increasingly important concern for clinical oncologists. Anorexia and reduced food intake are also important issues in the management of elderly patients with cancer because they contribute to the development of malnutrition, increase morbidity and mortality, and negatively affect patients’ quality of life. Various factors may contribute to decreased food intake among elderly cancer patients, including social, psychologic, medical, and pharmacologic factors. Gastrointestinal peptide hormones play a major role in the appetite regulatory system. They can be classified as satiety (e.g., the peptides tyrosine, glucagon-like peptide-1, pancreatic polypeptide, oxyntomodulin, and cholecystokinin) or orexigenic hormones (e.g., ghrelin). Although the control of appetite is not fully understood, it is reasonable to assume that these hormones play an important role in the development of anorexia and malnutrition in cancer patients. In this review, we tried to determine the pathophysiological basis of cancer-related anorexia. Specifically, we focused on ghrelin dynamics and regulation by the serotonergic system in both human and animal studies.

2. GHR ELIN AS AN APPETITE-STIMULATING HORMONE

Ghrelin is a peripherally active orexigenic gut hormone consisting of 28 amino acids, and the third N-terminal amino acid serine (Ser) residue is octanoylated.1–3) Ghrelin is involved in the hypothalamic regulation of energy homeostasis by increasing food intake and reducing fat utilization.4,5) Plasma levels of ghrelin rise while fasting and fall upon eating, which has led to the suggestion that ghrelin is a meal-initiating hormone.6) Plasma levels of ghrelin are inversely correlated with body weight in humans and rise after weight loss.7) In addition to the regulation of energy homeostasis, ghrelin mediates increases in gastric motility, induces a positive inotropic effect on the heart, and causes vasodilatation.8,9)

3. CISPLATIN-INDUCED ANOREXIA AND GHR ELIN

3.1. Human Studies Cancer patients treated with cytotoxic drugs such as cisplatin often experience undesirable adverse events including nausea, vomiting, dyspepsia, and anorexia.8,9) Recent clinical evidence has demonstrated the relationship between chemotherapy-induced gastrointestinal side effects and plasma ghrelin levels.10–13) Initially, Shimizu et al.11) reported that an increase in plasma ghrelin concentra-

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tions occurred in patients with reduced food intake after the start of anticancer chemotherapy. On the other hand, a recent study by Ohno et al. showed that the plasma concentration of acylated ghrelin was decreased in patients with gastric cancer receiving combined chemotherapy with S-1 plus cisplatin. Similar results were obtained in a study where patients with esophageal cancer were treated with cisplatin-based neoadjuvant chemotherapy. More recently, the same study group has conducted a prospective, randomized trial to evaluate the effects of exogenous ghrelin during cisplatin-based chemotherapy for patients with esophageal cancer, showing that food intake and appetite scores were significantly higher in the ghrelin-treated group than in the placebo group.\(^\text{13}\)

### 3.2. Animal Studies

We and others reported that circulating ghrelin concentrations are reduced in cisplatin-treated rats for 6h during the early stage of anorexia.\(^\text{14,15}\) In other studies, it was found that the plasma level of acylated ghrelin returned to the normal level 24h after a single administration of cisplatin, although the decrease in food intake lasted for more than 48h. Malik et al.\(^\text{17}\) reported an increase in the plasma level of acylated but not des-acylated ghrelin in rats treated with cisplatin. They suggested that an increase in circulating ghrelin in cisplatin-treated rats may be an adaptive response to protect them against a toxic challenge to the gut.

Intraperitoneal injection of serotonin (5-hydroxytryptamine; 5-HT) decreased the 24-h food intake and plasma acylated ghrelin level in a dose-dependent manner.\(^\text{19}\) This result suggests that the cisplatin-induced reduction in the plasma level of acylated ghrelin may be mediated *via* the release of 5-HT from the gastrointestinal tract mucosa. It was shown that the 5-HT\(_{2B}\) receptor agonist BW723C86 and 5-HT\(_{2C}\) agonist m-chlorophenylpiperazine HCl (mCPP) markedly decreased plasma acylated ghrelin levels and increased the intragastric ghrelin content, suggesting that 5-HT\(_{2C}\) receptor stimulation inhibits the release of gastric ghrelin into the circulation.\(^\text{20}\) These results strongly suggest that the activation of 5-HT\(_{2B}\) and 5-HT\(_{2C}\) receptors plays an important role in the decrease in plasma ghrelin levels in cisplatin-induced anorexia.

It is well known that the activation of 5-HT\(_3\) receptors in the gastrointestinal mucosa is involved in the generation of emesis after the administration of cisplatin. However, 5-HT\(_3\) as well as 5-HT\(_2\) agonists had no effect on ghrelin dynamics in cisplatin-treated rats.\(^\text{21}\) Moreover, a 5-HT\(_3\) receptor antagonist (granisetron) inhibited delayed gastric emptying after cisplatin treatment but failed to improve cisplatin-induced anorexia.\(^\text{22}\) These results indicate that cisplatin-induced emesis and anorexia may develop by different mechanisms, where the 5-HT\(_3\) receptor may be involved in cisplatin-induced emesis, whereas 5-HT\(_{2B/2C}\) receptors may be involved in anorexia.

While the expression of 5-HT\(_{2C}\) receptors is restricted to the central nervous system, 5-HT\(_{2B}\) receptors are mainly distributed peripherally. In the gastrointestinal tract, 5-HT\(_{2B}\) receptors are located in the longitudinal and circular smooth muscle layers and in the myenteric nerve plexus in a variety of species, including humans.\(^\text{23}\) The precise localization of 5-HT\(_{2B}\) receptors involved in the regulation of ghrelin secretion is currently unknown and needs to be determined in future experiments.

In addition to peripheral ghrelin, hypothalamic ghrelin is also reported to be involved in chemotherapy-induced delayed anorexia.\(^\text{24}\) It was shown that hypothalamic ghrelin started to decline 24h after cisplatin administration and continued to decrease at least until 48h.\(^\text{25}\) Hypothalamic 5-HT\(_{2C}\) receptor gene expression in cisplatin-treated rats increased significantly, and the intracerebroventricularly administered 5-HT\(_{2C}\) antagonist SB242084 prevented a decrease in the secretion of hypothalamic ghrelin in cisplatin-treated rats.\(^\text{26}\) These results indicate that the reduced ghrelin secretion in the hypothalamus secondary to 5-HT\(_{2C}\) receptor activation may be involved in cisplatin-induced anorexia. It was demonstrated that hypothalamic ghrelin receptor (GHS-R1a) gene expression was significantly reduced after cisplatin or mCPP treatment, and this change was reversed by the administration of a 5-HT\(_{2C}\) receptor antagonist. From these results, it was suggested that delayed-onset anorexia induced by cisplatin may be mediated by the activation of the hypothalamic 5-HT\(_{2C}\) receptor and the resultant suppression of hypothalamic GHS-R1a gene expression as well as decreased ghrelin secretion in the hypothalamus.

### 4. ANOREXIA OF AGING AND GHRELIN

#### 4.1. Human Studies

Protein energy malnutrition in the elderly is a frequent, clinically important problem, which leads to increased morbidity, mortality, disability, and health costs in this growing population. One of the most important causes of the reduction in energy intake is anorexia. The causes of the anorexia of aging have not yet been fully defined but are probably multifactorial and include sensory impairment, social isolation, and psychologic and physiologic factors, in addition to the presence of disease.\(^\text{27,28}\)

Although the levels of many peripheral anorexigenic hormones including cholecystokinin, leptin, and insulin have been found to increase with age, findings on ghrelin are controversial. Most of the human studies indicated that ghrelin secretion and ghrelin-induced gastric hormone secretion decreased in elderly people compared with younger ones. In a recent study, Schneider et al.\(^\text{29}\) have found no increase in ghrelin levels in malnourished elderly individuals compared with well-nourished ones, which suggests that hunger may be suppressed during the postprandial period in the aged population. In another study, it was found that fasting acylated ghrelin levels were lower in the elderly, and the postprandial acylated ghrelin curve remained low and flat after a meal. Moreover, Serra-Prat et al.\(^\text{30}\) found that advanced age determines a poorer ghrelin postprandial recuperation phase, a reduced cholecystokinin postprandial response, and an exaggerated postprandial insulin release. All of these findings suggest that the disturbance of the regulation of ghrelin secretion and reduced production during hunger and satiety may cause “anorexia of aging” in elderly people.

#### 4.2. Animal Studies

In contrast to human data, several lines of animal studies revealed that plasma ghrelin concentrations in aged rats are significantly higher than in young rats. Contrary to those findings, Wolden-Hanson reported that fasting failed to induce increased ghrelin in aged animals. The reason for these conflicting data seems to owe to differences in the experimental conditions (fasting or freely fed, daytime or night) under which the plasma ghrelin concentration was measured. Our group found that plasma ghrelin in aged C57BL/6 mice did not increase under fasted conditions but was higher than that in young mice under freely fed con-
ditions. This suggests that the regulation of ghrelin secretion from the stomach may be disturbed in older mice. We also found that exogenously administered ghrelin (33 µg/kg) failed to increase food intake in 75-week-old mice, whereas the same dose of ghrelin had an orexigenic effect in young mice, suggesting that aging is associated with decreased ghrelin signaling. It seems that a dysregulation of ghrelin secretion as well as ghrelin resistance in the appetite control system occurs in aged mice.

Although the detailed mechanisms of disturbed ghrelin dynamics remain unclear, one of the possible causes appears to be leptin. We have found that plasma leptin levels in aged mice are significantly higher compared with those in young ones. Leptin is reported to inhibit ghrelin secretion from the stomach into the circulation, and hence elevated leptin in the elderly may contribute to the inhibition of ghrelin secretion. Moreover, it was reported that leptin suppresses the ghrelin-induced activation of neuripeptide Y (NPY) neurons. Moreover, it was reported that leptin suppresses the ghrelin-induced activation of neuripeptide Y (NPY) neurons. Leptin activates the phosphoinositid 3-kinase (PI3K)—phosphodiesterase 3 (PDE3) pathway in NPY neurons and counteracts the adenylyl cyclase-cAMP-protein kinase A system implicated in the effect of ghrelin. In agreement with these findings, we found that administration of either the PI3K inhibitor LY-294002 or the PDE3 inhibitor cilostamide improved anorexia in aged mice. These results suggest that plasma leptin, which increases with age, may induce resistance to ghrelin reactivity via cAMP downregulation.

5. STRESS AND GHRELIN

5.1. Human Studies Stress and negative emotions have been associated with both increased and decreased food intake. The mechanism underlying these opposing behavioral responses to similar stressors has not been determined, but high stress levels appear to lead to decreased eating.

Conflicting data are available regarding the effect of stress on ghrelin secretion. Acute psychosocial stress or cold exposure increased plasma ghrelin levels. However, there are several reports showing that plasma ghrelin levels did not change or even decreased with exposure to stresses. For example, a recent study by Zimmerman and colleagues has revealed that plasma ghrelin levels of men of normal weight subjected to the Trier Social Stress Test did not change when cortisol levels increased. Moreover, ghrelin levels decreased after drinking alcohol. Another recent study has shown that strenuous physical cycling exercise in healthy individuals results in a decline in fasting levels of acylated ghrelin while no decline occurred in des-acylated and total ghrelin plasma levels.

5.2. Animal Studies Regarding ghrelin dynamics in stressed conditions, mixed results are also available in animal studies. Elevations in plasma ghrelin were observed in response to various psychological/environmental stressors, including tail pinch stress, water avoidance stress, chronic exposure to cold, repeated restraint stress, and chronic social defeat stress. In contrast, exposure to immune, visceral, or strenuous physical stressors causes a reduction in the plasma ghrelin level. For example, intraperitoneally administered lipopolysaccharide was reported to decrease circulating ghrelin, which is mediated by interleukin (IL)-1β, prostaglandin-, and 5-HT2C receptor-dependent mechanisms. In another model, abdominal surgery induced a rapid, long-lasting decrease in peaked plasma acylated and des-acylated ghrelin levels. Ochi et al. reported that although active ghrelin levels in plasma were not increased in the initial phase (until 24 h) of stress loading, they were significantly higher on day 3 than those in the control group, suggesting that a homeostatic adaptation mechanism may develop in response to repeated stress involving upregulation of gastric ghrelin secretion. In support of this notion, Lutter et al. suggested that increased ghrelin in response to stress protects against depressive reactions to stress and helps cope with stress. Collectively, it seems likely that acute or severe stress causes a reduction in circulating ghrelin levels, resulting in the suppression of appetite, whereas mild or chronic repeated stress causes an upregulation of ghrelin secretion as an adaptation to stress.

Corticotropin-releasing factor (CRF) and its family of peptides, urocortin1 (Ucn1), urocortin2 (Ucn2), and urocortin3 (Ucn3), play an important role in the control of food intake. Among them, Ucn1 was shown to have the most potent inhibitory effect on food intake. Ucn1 has a higher affinity for CRF2 receptors (CRFR2) than for CRF1 receptors (CRFR1), and hence it is believed that CRFR2 plays a major role in satiety. There are several reports showing that the administration of Ucn1 to humans and rodents reduces plasma ghrelin concentrations. In addition, Ucn1-induced decreases in plasma ghrelin and food intake were restored by CRFR2 but not CRFR1.

We have recently determined that CRFR1 is also involved in the regulation of ghrelin secretion. Using a novelty stress model, we found that 3 h after the novelty stress, appetite reduction was associated with a decrease in the plasma ghrelin level. Administering a CRF1R selective antagonist, but not a CRF2R antagonist, resolved the reduction in food intake 3 h after the novelty stress by enhancing circulating ghrelin concentrations. Interestingly, 5-HT1B and 5-HT2C receptor antagonists and melancortin-4 (MC4) receptor antagonist alleviated the novelty stress-induced hypophagia and the reduction in circulating ghrelin levels. Moreover, intracerebroventricular administration of the 5-HT1B/2CR agonist mCPP suppressed the plasma acylated ghrelin level and food intake. From these results, we hypothesize that acute appetite suppression due to CRF1R activation after a novelty stress is caused by a chain reaction of appetite control mechanisms mediated by 5-HT1B/2CR receptors in the arcuate nucleus to MC4 receptor system in the paraventricular nucleus, causing lowered peripheral ghrelin secretion.

6. CONCLUSION

In this review, we summarized the potential mechanisms for anorexia developed in three different animal models that mimic the situations commonly seen in aged patients treated with chemotheroapy. Cisplatin-induced anorexia is attributable to a decrease in peripheral and central ghrelin secretion caused by stimulation of 5-HT2B and 5-HT2C receptors via 5-HT secretion. Aging-associated anorexia is caused by an increase in plasma leptin, which results from disturbed reactivity of ghrelin in the hypothalamus and regulation of ghrelin secretion. Environmental change causes activation of central 5-HT1B and 5-HT2C receptors and the MC4 receptor system, resulting in a decrease in circulating ghrelin levels and sup-
pressed food intake. New therapeutic approaches based on these pathophysiologic mechanisms are warranted for the future treatment of anorexia in cancer patients, especially elderly ones.

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