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Chapter 3

ABNORMAL GHRELIN DYNAMICS IN PSYCHOLOGICAL STRESS-INDUCED EATING DISORDERS: INFLUENCE ON GENDER DIFFERENCE AND AGING

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ABSTRACT

Humans are exposed to various psychological stresses through social activities. For instance, changes in the living environment and social networks induce psychological stress, which can subsequently lead to eating disorders as one of biological responses. However, the detailed mechanism underlying this process remains to be fully elucidated. In recent years, it has been reported that dynamic and functional abnormalities of the appetite-related peptides are closely related to stress-induced eating disorders. Among them, the discovery of ghrelin, an appetite-stimulating hormone, has provided a better understanding of the pathogenesis of stress-induced anorexia. Additionally, it is well known that both the degree and

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quality of the stress-induced biological reaction are affected by gender and aging. In this review, we focus on abnormalities in eating behavior induced by novel environmental change stress (novelty stress), which is primarily caused by acute environmental changes and chronic isolation stress. In this section, we address the effects of gender differences and aging on psychological stress-dependent eating disorders and discuss the involvement of endogenous ghrelin, the serotonin 2c receptor (5-HT_{2C}R), and the estrogen alpha receptor (ERα). Our findings indicate that food intake in young male mice after exposure to novelty stress is significantly reduced and plasma acylated ghrelin levels are simultaneously decreased. In aged male mice, exposure to a similar stress resulted in further sustained reductions in both food intake and ghrelin levels. Administration of a 5-HT_{2C}R antagonist or the ghrelin enhancer rikkunshito has been shown to mitigate these decreases. Furthermore, aged female mice have been found to be less susceptible to novelty stress. These results suggest that aging leads to vulnerability to acute novelty stress in male, but not female, mice. The excessive anorexia observed in aged male mice is considered to contribute by the sustained reduction of peripheral ghrelin secretion through activation of ERα and 5-HT_{2C}R. In contrast, food intake in chronically isolated young mice is significantly increased; however, this increase is not observed in aged mice. While peripheral ghrelin levels in young mice increases following exposure to chronic stress, the degree to which it increases is attenuated in aged mice. Under chronic isolation stress, an absence of stress coping due to increased ghrelin secretion is observed in aged mice. Collectively, evidence suggest that psychological stress causes a disturbance in the regulation of endogenous ghrelin, which is significantly impacted by aging and gender and can result in eating disorders.

Keywords: psychological stress, aging, 5-HT_{2C}R, estrogen receptor, rikkunshito, mice

INTRODUCTION

In modern society, stress has long been a social problem [1, 2]. In humans, exposure to stress often leads to the occurrence of various abnormal physiological responses. It is known that psychological stress in daily life through social activities is caused by “environmental changes”. Even healthy individuals cannot adequately adapt to novel environmental changes and often exhibit various physical abnormalities. Furthermore, in the aging

society in particular, changes in the living environment specific to the elderly, such as a separation from family and the lack of social networks, may be an underlying cause of the mental and physical symptoms observed in medical practice.

Among the common stress symptoms, loss of instinctual needs, appetite, is frequently observed, with long-term malnutrition in the elderly causing increased morbidity and mortality, and serious problems related to life maintenance [3, 4]. While this process is considered complex, activation of the hypothalamus-pituitary-adrenal (HPA) axis after exposure to a stressor (i.e., the stress response) has been reported to have gender differences [5-7]. Indeed, it has been reported that aging and gender both affect the stress response and subsequent phenotypes.

In recent years, appetite-related hormones that convey peripheral nutritional status to the feeding center have attracted attention and are currently being targeted for drug discovery. The majority of these hormones are peptides that act on the brain as appetite suppressant signals (CCK, Leptin, GLP-1 etc.); however, ghrelin is a very unique orexigenic peptide that is secreted from the stomach and serves to transmit a fasting signal into the brain [8].

Acylated ghrelin, which is the active form of ghrelin, promotes appetite and gastrointestinal motility, plays an important role in energy storage, and stimulates the secretion of growth hormone. In human and rodent studies, it has been reported that the eating disorders often observed in various diseases are mediated by abnormalities of the ghrelin system [9-14]. However, only a few studies have examined the association between stress-induced anorexia and orexigenic peptides in the elderly and none have elucidated the mechanism underlying the stress response as it relates to aging and gender. Therefore, in this review, we discuss the involvement of feeding behavior due to stress and ghrelin with the aim of clarifying the effects of aging and gender, while focusing on the mechanism of psychological stress-induced feeding suppression.

NOVEL ENVIRONMENTAL STRESS AND GHRELIN DYNAMICS

It is well known that stress elicits a number of physiological responses and causes various changes in appetite and digestive tract motility [15, 16]. Stress can lead to either increased or decreased appetite, and several basic studies have suggested the involvement of appetite-related hormones as one of a possible mechanism. In rodent studies, water avoidance stress and social defeat stress result in increased plasma ghrelin concentration [17], with social defeat stress also leading to increased feeding [18]. Conversely, hypophagia induced in various models, including stress-like models through the intracerebroventricular (icv) administration of urocortin [19], the restraint stress model [20], and the immune stress model [21], appears to be partly mediated by a decrease in plasma acylated ghrelin concentrations. Therefore, it is likely that changes in peripheral ghrelin and abnormalities in eating behavior differ depending on the type and intensity of stress. However, the degree of the stress load in these animal studies is often far from the levels of daily stress that are routinely experienced by humans. Thus, there is still controversy regarding the relationship between stress and eating behavior.

In our social life, exposure to environmental changes and loneliness can often occur and we experience psychological stress as a result on a daily basis. Behavioral evaluations using environmental changes such as the open field test are frequently used in animal experiments and can evaluate characteristic anxiety and stress behavior in mice subjected to novel environments. In a previous study, we demonstrated that novel environmental stress (novelty stress) results when mice are transferred from their home cage, in which multiple mice (n = 3–5) are kept, to an individual cage with new bedding [22]. Under these conditions, it has been reported that food consumption in the mice decreases simultaneously with decreased plasma ghrelin secretion [12, 23]. In addition, the direct administration of acylated ghrelin to novelty stressed mice has been shown to suppress the declines in food intake [12, 23]. Thus, it is appeared that the decrease in

ghrelin secretion is involved in the decrease in eating behavior under novelty stress.

GHRELIN DYNAMICS AND NEUROTRANSMITTERS

Serotonin (5-HT) is a multitargeted neurotransmitter with 14 receptor subtypes. Recently, it has been shown that 5-HT_{2C} or 5-HT_{2B} receptor activation induces anxiety-like behavior and appetite inhibition in rodents [9, 24-29]. The localization of these two subtypes is different, with the 5-HT_{2C}R widely distributed in the brain [30], while the 5-HT_{2B}R is distributed in the gastrointestinal tract, stomach fundus [31], vascular smooth muscle, and uterus. We have previously shown that the reduction in food consumption and ghrelin concentration due to novelty stress are mediated by the activation of 5-HT_{1B}R, 5-HT_{2C}R, and 5-HT_{2B}R [12, 23]. In addition, the administration of 5-HT_{2C}R and 5-HT_{2B}R agonists to naïve animals has been shown to cause a decrease in peripheral ghrelin concentration simultaneously with a decrease in feeding behavior [32]. Furthermore, the interaction of the ghrelin receptor with 5-HT_{2C}R has also been shown. In another study, expression of the ghrelin receptor was shown to be colocalized with 5-HT_{2C}R in primary cultured hypothalamic and hippocampal rat neurons [33]. Moreover, it has been demonstrated that inhibition of 5-HT_{2C}R signaling potentiates the orexigenic effects caused by ghrelin. In contrast, the specific 5-HT_{2C}R agonist lorcaserin, which was recently approved for the treatment of obesity, attenuates ghrelin-induced food intake [33]. Conversely, administration of the 5-HT_{1A}R agonist 8-OH-DPAT to the brain further promotes the ghrelin-induced orexigenic action [34]. These results support the notion that the interaction of several 5-HT receptors and ghrelin, not only in the center but also in the periphery, is closely related to the decreased appetite caused by psychological stress. However, further studies are needed to determine how psychological stress activates 5-HT receptors and which subtypes are dominant.

The production of corticotropin-releasing factor (CRF) as first step of the stress response, and the resultant activation of the CRF receptor

following its release, also affects appetite, gastrointestinal motility, and even emotional response [35, 36]. Icv administration of a CRF1 receptor antagonist to novelty stressed mice has been shown to suppress the reduction in plasma acylated ghrelin concentrations and hypophagia [12]. Thus, novelty stress may cause the activation of CRF neurons and the CRF1 receptor, with resulting hypophagia. In addition, several studies have demonstrated that CRF interacts with 5-HT to regulate stress responses. Icv administration of CRF to naïve mice reduces plasma ghrelin concentrations, and this effect is reversed by the combined administration of 5-HT_{1B}R or 5-HT_{2C}R antagonists [12]. Therefore, it is believed that the CRF1 receptor is activated in the early stages of novelty stress, with the subsequent activation of the CRF1 receptor inducing 5-$HT_{1B/2C}$R activation. While, drug-induced acute intracerebral 5-HT depletion elicits anxiety behavior [37], and activation of 5-HT_{2C}R on CRF neurons and 5-HT_{1A}R in the amygdala regulate anxiety behavior [26, 38, 39]. There may also be a pathway in which 5-HT receptor activity affects CRF receptor activity [38, 40].

Taken together, it is possible to hypothesize that CRF neurons, 5-HT neurons, and/or 5-HT receptors are interactively activated by psychological stress and that this process is involved in decreased gastric ghrelin secretion via the vagus nerve [12]. However, the relationship between 5-HT receptors and signal transmitters in the brain, along with the associated ghrelin dynamics, is complex, thus further research is warranted.

EFFECT OF AGING ON GHRELIN DYNAMICS

Within the aging society, it is anticipated that complaints of depression and anxiety in the elderly will continue to increase. Indeed, in the elderly, amounts of food intake itself is reduced due to decreased energy consumption via the decrease in activity; as such, the nutritional base is weakened. Furthermore, stress symptoms may be prolonged or worsened with aging due to impairments in avoidance behaviors and reduced adaptations against stress. Stress exposure in the elderly sometimes causes significant malnutrition, which in turn promotes increased morbidity in

conjunction with various diseases and decreased physical function [3, 4]. Therefore, the prevention of frailty in the elderly through the improvement of nutritional status is an important consideration for the aging society [41]. Anorexia in the elderly is known to be closely related to psychological factors such as social factors and environmental changes, in addition to those caused by various disorders and diseases [42, 43]. In particular, the lack of social networks and a separation/bereavement from family members and close relatives have all been reported to increase the possibility of the onset and recurrence of depression [44]. Similarly, as an approach from basic research, novelty stress in young mice results in transient declines in food intake; however, in aged mice (79 weeks), the reduction in food intake is markedly sustained [45]. Interestingly, stress-induced reductions in plasma ghrelin concentrations are also markedly persistent [12, 45]. While the detailed mechanism of sustained hypophagia in aged mice remains unclear, 5-HT_{2C}R antagonist administration or supplementation with exogenous acylated ghrelin suppresses the hypophagia. In addition, 5-HT_{2C}R antagonist administration significantly suppresses the decreased plasma ghrelin concentrations observed as a result of stress [46].

These findings suggest the involvement of ghrelin dynamics and the 5-HT receptor in aged mice as well as young. With aging, 5-HT concentrations reportedly decrease in the hypothalamus [47] and amygdala [48]. Novelty stress reportedly increases 5-HT concentrations in the brain through inhibiting 5-HT turnover in aged mice compared with young mice [48]. This increase in central 5-HT concentrations in aged mice after exposure to novelty stress may play a role in 5-HT_{2C}R activation. In recent studies, increased 5-HT_{2C}R gene expression in the hypothalamic paraventricular nucleus and activation of the positive neurons by stress exposure in aged male mice has been observed [45, 46]. In addition, the administration of the 5-HT_{2C}R agonists mCPP or CP-809101 to aged mice causes a marked and sustained reduction in food intake as compared with young mice [45, 46]. These results indicate that elevated sensitivity of 5-HT_{2C}R is involved in the hypophagia observed in aged mice. As a result, it is considered that decreased ghrelin secretion and suppressed feeding occur more significantly in aged mice than in young mice.

AGING, GENDER, AND GHRELIN

It is well known that there are gender differences in terms of stress sensitivity. Indeed, the incidences of major depressive disorder [49] and anorexia nervosa [50] are higher in young women. Likewise, activation of the HPA axis by stress in rodent also tends to be higher in females compared with males [51]. On the other hand, symptoms of anorexia in the elderly are reported more frequently in men than in women [52, 53]. However, limited data exist regarding gender differences, stress sensitivity, and food consumption in the elderly.

Gender differences in a number of physiological responses are often affected by sex hormones. The actions of estradiol are mediated by the estrogen receptors (ER) α and β. ERα activates the HPA axis and negatively regulates feeding behavior, while ERβ is its counterpart [5, 54]. Sex differences in terms of stress are closely related to various neurotransmitters and the sex hormones that mediate stress responsiveness [5, 6]. As described above, aged male mice exposed to novelty stress show more markedly decreased ghrelin concentrations and food intake than younger mice. Furthermore, in aged male mice, gene expression of aromatase, which produces estrogen from testosterone, is increased in the hypothalamus, and aromatase inhibitors have been shown to be effective in improving anorexia due to stress.

Hypothalamic ERα gene expression in aged mice has also been shown to be increased. Similarly, decreased feeding behavior by ERα stimulation is suppressed by 5-HT_{2C}R antagonist administration [46]. Also, estradiol has been reported to enhance 5-HT_{2C}R protein synthesis in the brain [55, 56]. Therefore, it may be that the synthesis of both aromatase and ERα are enhanced with aging, and the concentration of estrogen in the brain is increased, which stimulates ERα signaling in aged male mice. As a result, this process induces an increase in and hyperfunctioning of 5-HT_{2C}R, with stress-induced reductions in ghrelin concentrations and hypophagia in aged mice more significantly pronounced than in younger mice.

On the other hand, a transient decline in food intake has been observed in aged female mice to the same extent as young mice after novelty stress,

and no significant declines in plasma ghrelin concentrations are observed [46]. Aged female mice, unlike aged males, do not exhibit activation of 5-HT_{2C}R positive cells in the hypothalamus after stress exposure [46]. In addition, in aged female mice, the stress-induced transient anorexia is not restored and increased plasma ghrelin concentrations are not observed by administration of a 5-HT_{2C}R antagonist [57]. Decreased food intake from the administration of an ERα stimulant in aged female mice is attenuated in comparison with aged male mice [46]. Therefore, in aged female mice, it was inferred that the action mechanism was different from that in aged males. Furthermore, novelty stress results in significantly increased plasma corticosterone concentrations in aged male mice, but not aged female mice [46]. These findings suggest that aged male mice may be vulnerable to stress, which is consistent with the observed gender differences in elderly people with anorexia, which is more pronouced in men than in women [52, 53]. However, clinically, young women have a higher incidence of depression and anorexia nervosa than men [49, 50]. Similarly, in animal studies, there are reports that support it [51] and females may exhibit more anxiety behaviors compared with males [58, 59]. However, the contrary results has also been reported [7, 60] and a complete conclusion has not been reached. There are also reports that the social breeding environment influences both male and female responses to stress [61, 62]. The observed differences between research studies (that use feeding as an indicator) and epidemiological clinical data may be due to the evaluation index, the type of stress applied and its intensity, measurement timing, and/or feeding conditions. But the effects of aging are likely to be significant in terms of gender differences and stress responsiveness. Further studies are expected to provide insight into what hormones mediate stress-induced transient hypophagia in aged female mice.

CHRONIC ISOLATION STRESS

The novelty stressors described so far have included acute psychological stress; however, the response to chronic psychological stress may be

mediated by completely different mechanisms. The effects of food intake due to stress loading have been reported for both food intake suppression [19, 45, 63] and overfeeding [18, 64-66]. In particular, with respect to chronic stress (such as social defeat stress), increased peripheral ghrelin concentrations and subsequent feeding behavior have been observed. Unlike acute novelty stress, chronic isolation for two weeks after a novel environmental change increases food intake in young male and female mice [67]. Indeed, plasma ghrelin concentrations in young male mice at one week after isolation are significantly increased as compared with group-housed mice. In young female mice, a one week isolation did not alter plasma ghrelin concentrations, but it did result in a significant increase in preproghrelin gene expression in the hypothalamus. This mechanism remains to be elucidated. Although the relationship between central ghrelin concentrations and food consumption is not completely understood, icv administration of ghrelin has been found to promote feeding in rodents [68]. One hypothesis is that, in young male mice, increased feeding during isolation is primarily the result of increased plasma ghrelin, while in young female mice, it is believed that chronic isolation causes an increase in the secretion of acylated ghrelin in the brain leading to enhance feeding. Interestingly, there were no differences in the body weights or weight gain rates, despite increased food intake in the isolated group compared with the control group (group-housed mice) in the both young male and female mice. Unlike acute psychological stress, the continuation of isolation breeding may increase energy consumption, which increases food intake by promoting the secretion and/or synthesis of ghrelin as a compensation reaction for it.

On the other hand, aged male and female mice kept in continuous isolation display a completely different response from young mice. In the aged male and female mice, the increased feeding due to isolation decreases and the rate of body weight gain decreases [67]. As an explanation, plasma ghrelin concentrations during isolation in aged male mice have been shown to increase more mildly than in young mice, and neither plasma ghrelin concentrations nor the gene expression of preproghrelin in the brain are observed in aged female mice [67]. Therefore, in aged mice, the secretion

and/or synthetic function of ghrelin may be lower than in young mice. In addition, it is believed that there is an abnormality in the balance between energy consumption and supply in aged mice, with no increase in food intake corresponding with consumption; thus, the rate of weight gain is not maintained.

POTENTIAL TREATMENT FOR STRESS-INDUCED ANOREXIA

Rikkunshito is a kampo and traditional Japanese medicine prescribed for anorexia and approved by the Japanese Ministry of Health and Welfare. In clinical studies, rikkunshito has been shown to significantly improve dyspeptic symptoms (as determined by the Gastrointestinal Symptom Rating Scale) and increase plasma acylated ghrelin concentrations in patients with functional dyspepsia (FD) [69]. It also has been shown to improve chemotherapy-induced anorexia and leads to no decline of the plasma acylated ghrelin concentration caused by chemotherapy in patients with gastric cancer [13]. Furthermore, in a randomized, double-blind study of FD patients, rikkunshito significantly increased overall treatment efficacy and improved upper gastrointestinal symptoms compared with placebo [70]. Rikkunshito has been widely reported in basic research to support clinical evidence, and has been shown to promote peripheral ghrelin secretion in humans [71], rodents [9, 72, 73], and dogs [74]. In addition, it is known that continuous ghrelin signaling can be potentiated via the enhanced binding of ghrelin to its receptor [10, 11]. Basic research studies using mice have shown that rikkunshito improves the decrease in food intake and elevates plasma ghrelin concentrations after exposure to novelty stress [12, 23]. The action mechanism of rikkunshito is considered to be ghrelin receptor-mediated, because it can be abolished by a ghrelin receptor antagonist [12]. Rikkunshito contains many herbal ingredients that inhibit the binding of 5-HT to 5-$HT_{2B/2C}$R [9], and it is considered to increase ghrelin concentrations through the binding inhibition of the 5-HT receptor.

In turn, the elevation of CRF in the brain also affects peripheral ghrelin secretion. Icv administration of urocortin, a CRF receptor activator, causes a reduction in plasma ghrelin concentrations via sympathetic activation [19]. Rikkunshito also suppresses the decreased ghrelin concentrations in this model. It is likely that rikkunshito contains receptor binding inhibitory actions that affect not only 5-HT, but also CRF [75]. These findings suggest that ghrelin potentiation by rikkunshito may have multiple pathways.

It has also been demonstrated that rikkunshito significantly improves acid-related dysmotility symptoms (as determined by the Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease) in the elderly (≥ 65 years) in patients with PPI-refractory non-erosive reflux disease. These findings suggest that it may be particularly effective for gastrointestinal symptoms affecting the elderly [76]. Rikkunshito also improves the sustained hypophagia observed in aged male mice after exposure to novelty stress via increased ghrelin concentrations [46].

On the other hand, in aged female mice, it has also been reported that rikkunshito improves food intake, although 5-HT_{2C}R antagonists do not reverse the food intake suppression elicited by novelty stress [57]. It is likely that rikkunshito may cause appetite stimulation through multiple pathways different from the central 5-HT-ghrelin system. As one possibility, it may be mediated by the action of glucagon-like peptide-1 (GLP-1). GLP-1 is a neuropeptide that is secreted postprandially from the gut, and has the effect of reducing food intake [77].

GLP-1 may be a anorectic peptide with the antagonize effect to ghrelin. Rikkunshito has also been reported to suppress increases in plasma GLP-1 in rats after gastrectomy [78]. Thus, it is possible that the various actions described above occur in combination and lead to an improvement in anorexia by rikkunshito. Although the detailed mechanisms of the action and safety of rikkunshito in the elderly need to be examined, Kampo therapies are expected to promising treatments for stress-induced anorexia in the future.

CONCLUSION

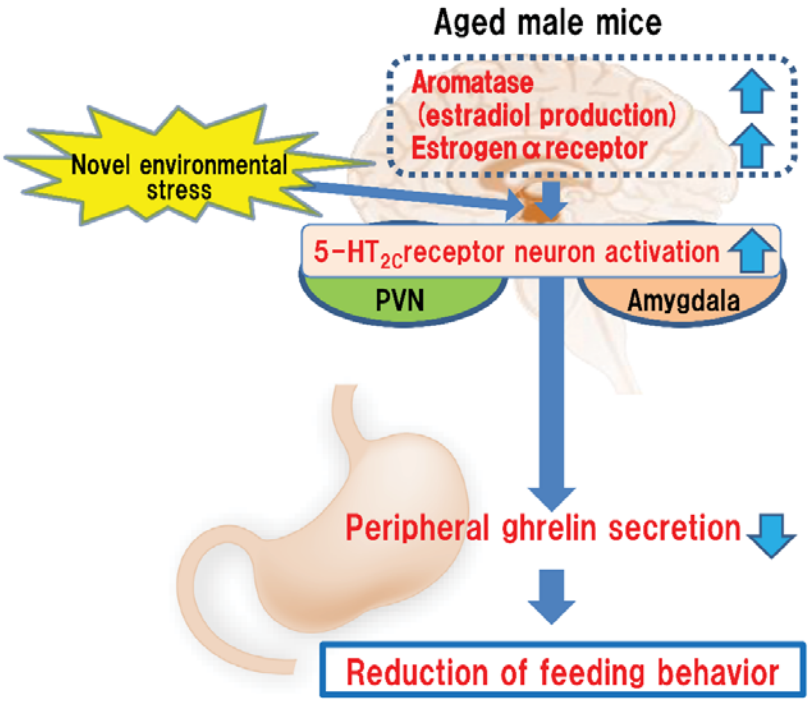

Figure 1. Scheme of decreased feeding behavior due to novel environmental stress in aged male mice. PVN: paraventricular nucleus.

In summary, exposure to novelty stress in aged male mice results in lower plasma ghrelin concentrations and sustained lower food intake compared with young and aged female mice. This suggests that decreased peripheral ghrelin concentrations caused by excessive activation of 5-HT_{2C}R positive cells in the paraventricular nucleus and amygdala are involved in the mechanism of sustained hypophagia in aged male mice. The elevated reactivity of 5-HT_{2C}R positive cells is considered to be associated with an increase in estrogen concentrations in the brain and an enhanced synthesis of ERα (Figure 1). In addition, in young mice subjected to continuous isolation for 2 weeks, increased food consumption, peripheral ghrelin concentrations, and/or intracerebral preproghrelin gene expression are observed. These changes are not observed in aged mice. It has been

suggested that stress interferes with the regulation of endogenous ghrelin, and both acute and chronic psychological stress models are affected by aging and gender differences, with results indicating differences in acylatd ghrelin production and secretion. It is expected that 5-HT_{2C}R antagonists, ghrelin supplementation, and rikkunshito may represent promising therapeutic strategies for anorexia due to psychological stress in the elderly. However, these results are just the first step in elucidating the mechanism of gender differences related to stress-induced anorexia in the elderly; further study is warranted to investigate the detailed action site and the interaction between sex hormones and ghrelin.

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