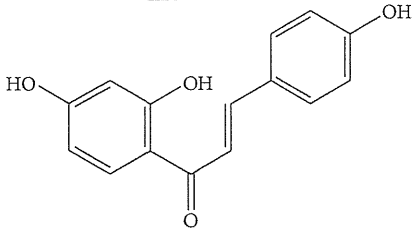
TABLE 1: The inhibitory activity for 5-HT_{2B} receptor.

Compound	IC_{50} values for 5-HT_{2B} receptor (μmol/L)	
	Binding	Cell function
 Isoliquiritigenin	6.3 ± 0.0	2.1 ± 0.2

Each value indicated the mean ± SEM of 3 samples.

The effects of drugs on anxiety responses in animal models are generally evaluated by the open field test in novelty environments [22]. In addition, novelty stress models are one of the established methods for evaluating feeding behavior, and novel environmental research using decreased food intake as an index has previously been conducted [7, 23]. Using this methodology, we reported that the acute novel environmental change caused by conversion from group housing to individual housing significantly suppressed feeding behavior in both young [14] and aged mice [24].

During fasting, secretion of acylated ghrelin by the stomach was enhanced, increasing the circulating levels [25]. However, in this study, no increase was observed in the fasting plasma levels of acylated ghrelin in mice exposed to novelty stress. In a previous study, it was clearly demonstrated that exogenous acylated ghrelin supplementation negated decreased food intake in the same model as that used in this study [14]. These results and findings suggest that the transmission of ghrelin signals to the hypothalamic feeding center under the fasting condition is decreased during stress responses caused by novel environmental changes. Although plasma des-acyl ghrelin (a metabolite of acylated ghrelin) levels after 24 h of fasting were significantly enhanced in mice exposed to novelty stress, the intensity of the increase was much lower than that in the nonstress-exposed mice. In addition, novel environmental changes did not cause any significant changes in the expression of gastric preproghrelin or ghrelin-*O*-acyltransferase gene (data are not shown). Therefore, novel environmental stress suppresses the secretion of acylated as well as des-acyl ghrelin, whereas it does not affect the biosynthesis of acylated ghrelin in the stomach during fasting.

NPY and AgRP gene expression in the hypothalamus tended to decrease in the novel environmental change group relative to that in the control group during the 3 h after stress exposure, although no statistically significant difference was observed. Ghrelin is secreted from X/A-like cells in the gastric mucosa and acts on ghrelin receptors in vagus nerve endings, then activating NPY/AgRP neurons in the hypothalamic arcuate nucleus via the vagus nerve [16]. The results of this study did not convincingly verify the attenuation of ghrelin signaling caused by stress when upregulation of hypothalamic NPY/AgRP gene expression was used as an index for ghrelin signaling. The reason why NPY and AgRP mRNA expression were not significantly decreased in mice exposed to novelty stress is unknown. With regard to NPY, there may be interference from mRNA expression in hypothalamic tissue other than the arcuate nucleus. To this end, the *in situ* hybridization technique is required for accurate evaluation of NPY and AgRP mRNA in the arcuate nucleus.

RKT, a Japanese Kampo medicine, is known to increase levels of peripheral acylated ghrelin in humans [26], rodents [13, 27], and dogs [28] as well as increase hypothalamic acylated ghrelin in rodents [29]. RKT also enhances the binding of ghrelin to ghrelin receptors [18, 30], resulting in enhanced and prolonged ghrelin signaling. We have previously reported that RKT administration to mice exposed to novelty stress suppresses a reduction in food intake 1 and 3 h after isolation, and the effects by RKT are abolished by coadministration of RKT with a ghrelin receptor antagonist [14]. The present data regarding the effect of RKT on food intake is almost in agreement with previous findings. In our experiment, RKT significantly reversed the decrease in peripheral acylated ghrelin levels caused by exposure to novelty stress. In contrast, no obvious effect of RKT was observed on des-acyl ghrelin levels after stress. On the basis of these results, we conclude that the increase in peripheral acylated ghrelin level associated with RKT may be mediated through enhanced acylated ghrelin secretion [13] in addition to the inhibition of acylated ghrelin metabolism [27].

In this study, enhanced hypothalamic preproghrelin and orexin mRNA expression and a tendency toward increased ghrelin receptor mRNA expression were observed following RKT administration. Activation of orexin neurons occurs downstream in the ghrelin signaling pathways, and the signal to increase appetite is transmitted to higher-order neurons via orexin gene expression. Enhancement of orexin mRNA by RKT may suggest ghrelin signal-enhancing effects. In addition, RKT promoted the secretion of ghrelin in the hypothalamus in cisplatin-induced hypophagia models [29] and ghrelin receptor gene expression [30]. These results may be supported by other studies indicating enhanced expression of preproghrelin mRNA [26] and ghrelin receptor mRNA

[30], despite differences in models. In addition, no significant changes in the expression of these genes can be confirmed in mice exposed to novelty stress. Our results were obtained 3 h after exposure to stress, but because hypophagia was actually observed 1 h after exposure, it may be necessary to reexamine sampling times.

It is well known that a stress model exhibits higher levels of central 5-HT and expression of 5-HTR, leading to activation of the serotonergic signal [6, 24]. We previously established the involvement of central 5-HT_{2C}R activation in decreased food intake during exposure to novel environmental stress and demonstrated that abnormalities in ghrelin dynamics may partially contribute to this reaction [14]. Contrary to 5-HT_{2C}Rs, 5-HT_{2B}Rs are sparsely expressed in discrete subregions of the central nervous system (CNS) [31], whereas they are heavily expressed in the periphery [32]. In a stomach, 5-HT_{2B}Rs are distributed throughout the gastric submucosa and smooth muscle, and their activation is known to result in contraction of the gastric fundus strip [33]. Although there have been several reports on the association between stress and 5-HT in gastrointestinal organs [34, 35], direct relationship between peripheral 5-HTR activation and novelty stress has not been proven. In the current study, we found that 5-HT_{2B}R antagonism inhibited the decrease in food intake after novelty stress. Because there is no information available on the 5-HT_{2B}R antagonists used in this study in terms of blood-brain barrier permeability, we could not determine whether 5-HT_{2B}R antagonism was effective in the CNS or the peripheral in the present study. Further investigation is required to determine the 5-HT_{2B}R activating site under stress.

5-HT_{2B}R activation by peripheral administration of a 5-HT_{2B}R agonist has been shown to cause a decrease in food intake [12] and inhibition of ghrelin secretion [13]. We also found that peripheral administration of BW723C86, a 5-HT_{2B}R agonist, decreased plasma acylated and des-acyl ghrelin levels in normal rats (see Supplemental Material available online at http://dx.doi.org/10.1155/2013/792940). These findings suggest that 5-HT_{2B}R activation is associated with abnormal ghrelin dynamics.

In the present study, the effects of certain RKT components on food intake in stress models were examined. Administration of isoliquiritigenin (4 mg/kg) significantly improves novelty stress-induced hypophagia. We also found that isoliquiritigenin inhibited binding between 5-HT and 5-HT_{2B}R and confirmed that it has an obvious antagonistic effect on 5-HT_{2B}R using a cell function assay. We previously reported that glycycoumarin, which has an antagonistic effect on 5-HT_{2B}R [13], suppressed decreased food intake 3 h after the application of stress [14]. It seems likely that glycycoumarin may inhibit the decrease in food intake after exposure to novelty stress via 5-HT_{2B}R antagonism. Therefore, multiple RKT ingredients having an antagonistic effect on 5-HT_{2B}R act and are considered to be responsible for its effects.

5. Conclusion

In conclusion, RKT has an ameliorating effect on decreased food intake caused by novel environmental changes. This effect appears to be mediated through improvement of abnormal ghrelin dynamics by 5-HT_{2B}R antagonism.

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Current Topics

Recent Advances in 5-Hydroxytryptamine (5-HT) Receptor Research: How Many Pathophysiological Roles Does 5-HT Play *via* Its Multiple Receptor Subtypes?

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-HT_{1B} 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 pathophysiologic basis of cancer-related anorexia. Specifically, we focused on ghrelin dynamics and regulation by the serotonergic system in both human and animal studies.

2. GHRELIN 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.[3]

3. CISPLATIN-INDUCED ANOREXIA AND GHRELIN

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.*[10] 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.[12] 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.[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.[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.[15,16] Malik *et al.*[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.[14] 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-$HT_{2B/2C}$ receptor stimulation inhibits the release of gastric ghrelin into the circulation.[14] 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.[18] However, 5-HT_3 as well as 5-HT_4 agonists had no effect on ghrelin dynamics in cisplatin-treated rats.[14] Moreover, a 5-HT_3 receptor antagonist (granisetron) inhibited delayed gastric emptying after cisplatin treatment but failed to improve cisplatin-induced anorexia.[14] 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.[19] 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.[15] It was shown that hypothalamic ghrelin started to decline 24h after cisplatin administration and continued to decrease at least until 48h.[14,15] 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.[15] 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.[20,21] 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.[20,21]

Although the levels of many peripheral anorexigenic hormones including cholecystokinin, leptin, and insulin have been found to increase with age,[22,23] findings on ghrelin are controversial.[1] Most of the human studies indicated that ghrelin secretion and ghrelin-induced gastric hormone secretion decreased in elderly people compared with younger ones.[1] In a recent study, Schneider *et al.*[24] 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.[25] Moreover, Serra-Prat *et al.*[26] 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.[27,28] Contrary to those findings, Wolden-Hanson[29] 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.[30] 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,[30] 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.[30] Leptin is reported to inhibit ghrelin secretion from the stomach into the circulation,[31] 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.[32] Leptin activates the phosphoinositide 3-kinase (PI3K)—phosphodiesterase 3 (PDE3) pathway in NPY neurons[33,34] and it counteracts the adenylate cyclase-cAMP-protein kinase A system implicated in the effect of ghrelin.[32] 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.[30] 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.[35] The mechanism underlying these opposing behavioral responses to similar stressors has not been determined, but high stress levels appear to lead to decreased eating.[36]

Conflicting data are available regarding the effect of stress on ghrelin secretion. Acute psychosocial stress[37,38] or cold exposure[39] 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[40] 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.[41]

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.[1,42,43] In contrast, exposure to immune, visceral, or strenuous physical stressors causes a reduction in the plasma ghrelin level.[1] For example, intraperitoneally administered lipopolysaccharide was reported to decrease circulating ghrelin,[44] which is mediated by interleukin (IL)-1β-, prostaglandin-, and 5-HT_{2C} receptor-dependent mechanisms.[45,46] In another model, abdominal surgery induced a rapid, long-lasting decrease in fasted plasma acylated and des-acylated ghrelin levels.[47] Ochi *et al.*[42] 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.*[43] 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.[48] 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),[49] and hence it is believed that CRFR2 plays a major role in satiety.[50] There are several reports showing that the administration of Ucn1 to humans and rodents reduces plasma ghrelin concentrations.[51–53] In addition, Ucn1-induced decreases in plasma ghrelin and food intake were restored by CRFR2 but not CRFR1.[53]

We have recently determined that CRFR1 is also involved in the regulation of ghrelin secretion. Using a novelty stress model,[54] we found that 3 h after the novelty stress, appetite reduction was associated with a decrease in the plasma ghrelin level.[55] 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-HT_{1B} and 5-HT_{2C} receptor antagonists and melanocortin-4 (MC4) receptor antagonist alleviated the novelty stress-induced hypophagia and the reduction in circulating ghrelin levels.[55] Moreover, intracerebroventricular administration of the 5-$HT_{1B/2CR}$ agonist mCPP suppressed the plasma acylated ghrelin level and food intake.[55] 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-$HT_{1B/2C}$ 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 chemotherapy. Cisplatin-induced anorexia is attributable to a decrease in peripheral and central ghrelin secretion caused by stimulation of 5-HT_{2B} and 5-HT_{2C} 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-HT_{1B} and 5-HT_{2C} 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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抗癌剤に伴うcachexiaとその治療

Anticancer treatment and cachexia

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SUMMARY

シスプラチンによる急性の嘔吐にはセロトニン3（5-HT_3）受容体が，遅延性の嘔吐にはニューロキニン1（NK_1）受容体が関与することが明らかにされ，それぞれの拮抗薬が臨床応用されている。一方，シスプラチンによる食欲不振は，5-HT_{2B}/5-HT_{2C}受容体を介したグレリン分泌の低下によって生じることが最近明らかにされ，その対策としてグレリンや六君子湯に期待が寄せられている。

KEY WORDS

■ シスプラチン
■ 食欲不振
■ セロトニン（5-HT）受容体
■ グレリン
■ 六君子湯

I
はじめに

近年の癌化学療法の進歩は著しい。しかしながら，非小細胞肺癌や消化器癌をはじめとする多くの固形癌に対する化学療法のkey drugであるシスプラチンは，吐き気ならびに嘔吐，食欲不振といった消化管機能障害を引き起こし，患者のQOLを著しく低下させ，cachexiaを引き起こす可能性がある。シスプラチンによる消化管機能障害には，投与24時間以内に生じる急性ならびに24時間以降に生じる遅延性の障害が知られている。急性の障害は，主に小腸に存在するエンテロクロマフィン（enterochromaffin；EC）細胞から遊離したセロトニン（5-hydroxytryptamine；5-HT）が，求心性迷走神経末端に存在する5-HT_3受容体を刺激して，求心性迷走神経経由で嘔吐中枢を刺激することが明らかにされている[1)2)]。一方，シスプラチンによる遅延性嘔吐ならびに予測性嘔吐に関しては，中枢に存在するニューロキニン（neurokinin；NK）1受容体の役割が明らかにされ，NK_1受容体拮抗薬はわが国でも使用可能となっている[1)2)]。しかしながら，遅発性の副作用としての食欲不振の完全な制御はいまだ困難である。

II
シスプラチンによる食欲不振の機序

最近，癌化学療法に伴う消化器系の副作用とグレリンの関係が明らかにされてきた。グレリンは主に胃より分泌される28個のアミノ酸よりなる消化管ホルモンであり，成長ホルモン分泌促進作用のほかに，強力な食欲増進作用を有することが知られている[3)]。グレリンならびにその受容体は胃のほかに視床下部弓状核のニューロンでも発現ならびに産生されており，げっ歯類へのグレリンの末梢ならびに脳室内投与は，それぞれ消化管運動の亢進と摂餌量の増加を引き起こす[3)]。

Shimizuら[4)]は，癌化学療法開始後に摂食量が低下した患者では血漿グレリン値が上昇しているが，低下しない患者では血漿グレリン値の変動がみられないことを報告した。一方この報告とは反対に，最近，シスプラチンを含むレジメンによる術前化学療法を受けた食道癌患者では，治療開始後3日目および8日目の血漿グレリン値は有意に低下し，血漿グレリン値の低下と食欲不振がよく相関していることが報告された（**図1**）[5)]。同様の結果は，TS-1＋シスプラチンによる化学療法を受けた胃癌患者を対象とした臨床研究でも報告されている[6)]。

動物実験においてもシスプラチン投与のグレリンに与える影響が検討されている。われわれ[7)]およびほかのグループ[8)]は，シスプラチン投与ラットにおいて，シスプラチン投与後6時間までの早期において血漿グレリン値が低下し，それに伴って摂餌量が低下することを報告した。またこのとき，シスプラチンによってEC細胞から放出された5-HTが5-HT_{2B}および5-HT_{2C}受容体を刺激して，胃粘膜からのグレリン分泌を低下させることも明らかになっている[7)]。さらに，Yakabiら[8)]は，シスプラチン投与後12時間には血漿グレリン値は正常レベルまで回復するものの，摂餌量の低下は48時間まで持続することを示している。彼らはシスプラチン投与後24時間以降48時間まで視床下部からのグレリン分泌が減少することを見出し，これが遅発性の摂餌量低下に関与していることを示唆した。

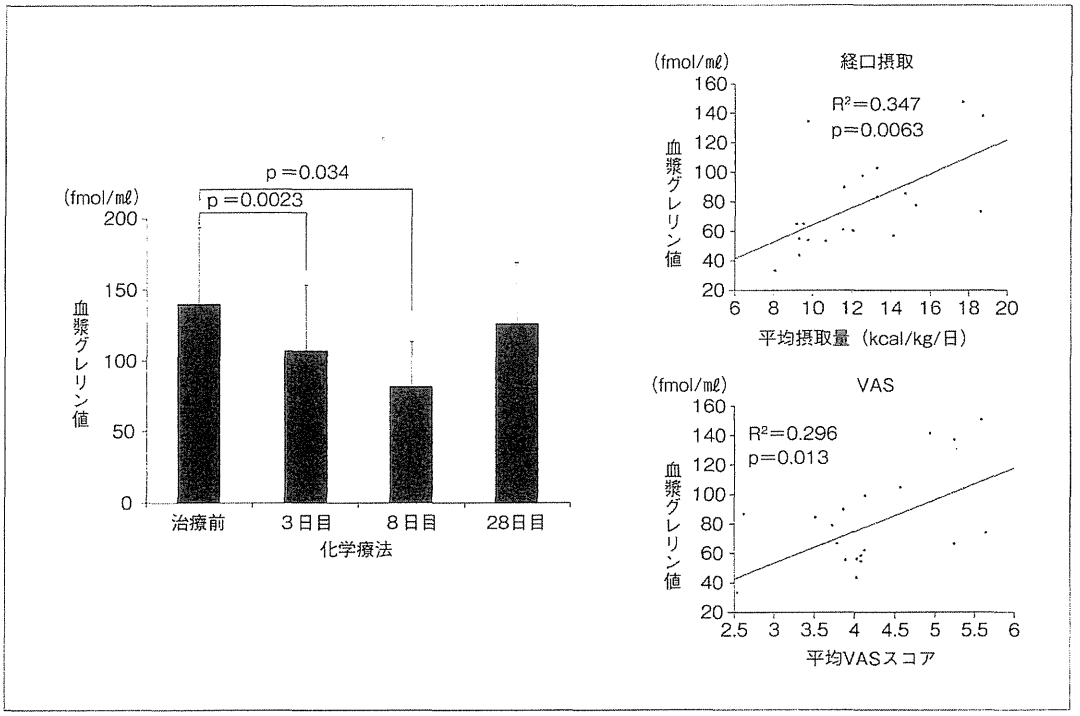

図1．進行食道癌患者に対する化学療法は血漿グレリンを低下させる[5]

Ⅲ シスプラチンによる食欲不振に対する治療

これまでの動物モデルを用いた研究により，外因性グレリンの投与がシスプラチンによる摂食量低下や嘔吐を抑制することが示されている[9)10)]。さらに臨床研究においても，Hiuraら[11)]はシスプラチンベースの術前化学療法を受ける食道癌患者に対するグレリンの効果についてランダム化比較試験を行い，食事摂取量とVisual Analogue Scale（VAS）で評価した食欲スコアがグレリン投与群で有意に高いことを示した。

また，最近われわれは機能性ディスペプシアに対して広く使用されている漢方薬（六君子湯）が，シスプラチン誘発食欲不振モデルラットにおいて血中グレリンの低下を抑制し摂餌量を回復させることを明らかにした（図2）[7)12)13)]。さらに，六君子湯に含まれる生薬成分のうち，heptamethoxyflavone, isoliquiritigenin, hesperidinをはじめとする13種類の成分が，5-HT_{2B}受容体もしくは5-HT_{2C}受容体に対する拮抗作用を有することで，グレリン分泌を回復させることも明らかになっている。

臨床研究においても，六君子湯の投与がシスプラチンを含む化学療法後の血中グレリン低下を抑制し，有害事象共通用語基準（CTCAE）v3.0による評価で食欲不振症状が改善もしくは改善傾向を示すことが示されている（図3）[14)]。このように，動物実験で明らかにされた六君子湯の食欲改善効果とその作用メカニズムは臨床現場においても確認されつつあり，今後のさらなる展開が期待されている。

Ⅳ おわりに

シスプラチンによる食欲不振がグレリン低下によって引き起こされることは明確になった。しかしながら，グレリン自体を治療薬として臨床現場で広く使用することは困難な状況にあり，六君子湯や開発中のグレリン受容体作動薬のようなグレリンシグナルを増強する薬剤の臨床応用に期待が寄せられている。

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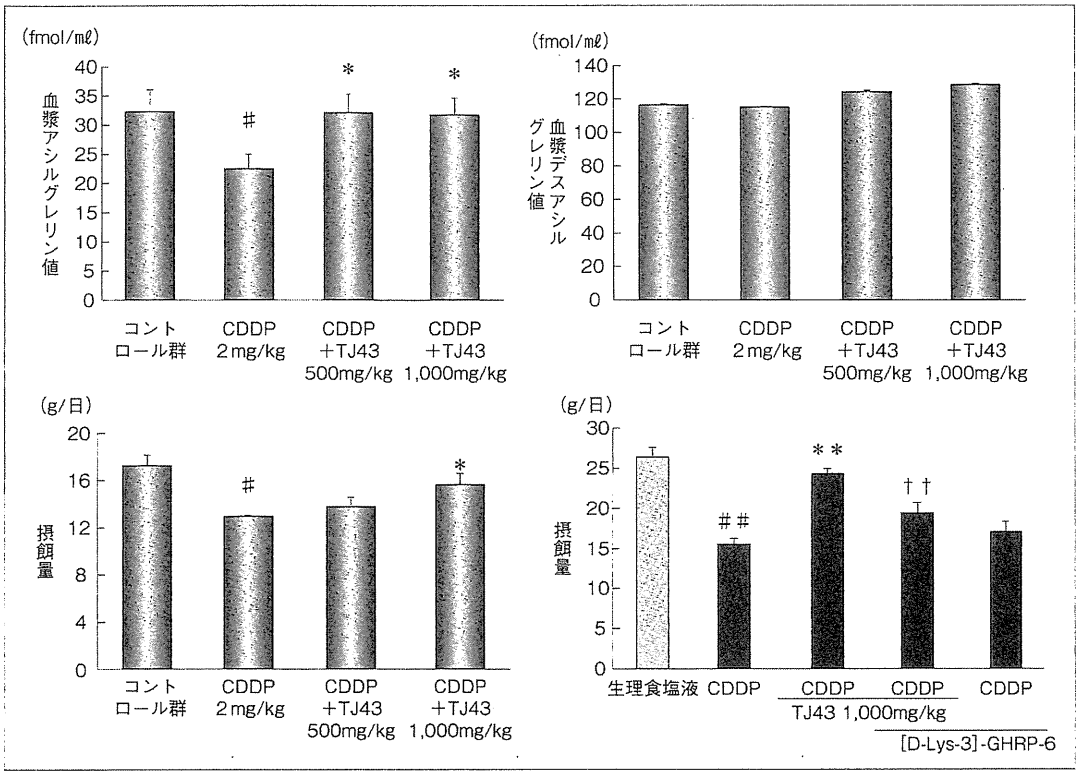

図2．シスプラチン誘発食欲不振ラットの血漿アシルグレリンおよび食欲に対する六君子湯の影響

CDDP：シスプラチン，TJ43：六君子湯

＃：p<0.05vsコントロール群（t-test），＊：p<0.05vsCDDP（Dunnett），The mean ±S.E. of 10～12 rats

＃＃：p<0.01vs生理食塩液，＊＊：p<0.01vsCDDP，††：p<0.01vsCDDP＋TJ43（t-test，n = 8）

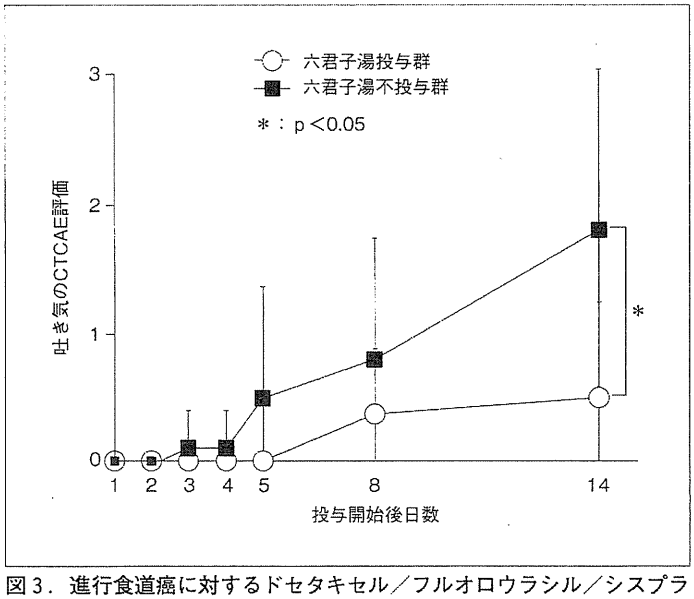

図3．進行食道癌に対するドセタキセル／フルオロウラシル／シスプラチン（DFP）療法における悪心に対する六君子湯の抑制効果[14]

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The gene expression of the hypothalamic feeding-regulating peptides in cisplatin-induced anorexic rats

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ABSTRACT

Cisplatin has been widely used; however, various disadvantageous side effects afflict patients. Rikkunshito (RKT), a traditional Japanese herbal medicine, has been widely prescribed in Japan to improve anorexia; but the mechanisms are unknown. Here we studied whether RKT could improve anorexia induced by cisplatin and changes in feeding-regulating peptides in the hypothalamus in rats. Adult male rats were divided into 4 groups: water + saline (WS), water + cisplatin (WC), RKT + saline (RS), and RKT + cisplatin (RC) groups. Water or RKT (1 g/kg) was intragastrically administered for 4 days, from day −1 to day 2, and saline or cisplatin (6 mg/kg) was intraperitoneally (i.p.) administered at day 0. After i.p. administration, cumulative food intake, water intake, urine volume and body weight were measured. The rats were then decapitated, followed by removal of the brain, and feeding-regulating peptides in the hypothalamus were measured by *in situ* hybridization histochemistry. In the three-day measurements, there were no significant changes in cumulative water intake and urine volume. The body weight and cumulative food intake in WC significantly decreased compared to WS, whereas these were not observed in RC. *Pro-opiomelanocortin* (*POMC*) and *cocaine and amphetamine-regulated transcript* (*CART*) in the arcuate nucleus (ARC) in WC significantly increased, and *neuropeptide Y* (*NPY*) in the ARC decreased compared to WS, whereas those in RS and RC were comparable to WS. These results suggest that RKT may have therapeutic potential for anorexia induced by cisplatin.

1. Introduction

Cisplatin is widely used for a variety of malignant tumors. It demonstrates anti-tumor effects by inhibiting the replication of DNA [7], but it has various side effects, such as loss of appetite, nausea and vomiting. It has been suggested that serotonin receptors are involved in the occurrence of nausea and vomiting from the use of cisplatin [17]. 5-HT3 receptor antagonist, steroids and metoclopramide have been used for the treatment of nausea and vomiting caused by cisplatin. However, in Japan, Rikkunshito (RKT) has also been used empirically for patients who suffer from anorexia caused by cisplatin.

RKT, a traditional Japanese herbal medicine, or "kampo", is widely prescribed in Japan for the treatment of the various disorders, such as upper gastrointestinal symptoms in patients with functional dyspepsia, gastroesophageal reflux disease, dyspeptic symptoms in postgastrointestinal surgery patients, and chemotherapy-induced dyspepsia in cancer patients [6,15,16,20,23]. The largest component of RKT is "Hesperidin" [22], which is a polyphenol that is contained in the peels of some kinds of oranges. It has been reported in *in vitro* experiments that RKT could act as an antioxidant [4].

Recent studies have revealed that RKT administration stimulates peripheral ghrelin secretion [3] or selective serotonin reuptake inhibitor [3,19] in rats with anorexia induced by cisplatin. Yakabi et al. demonstrated that cisplatin-induced anorexia is due to reduced ghrelin secretion in the hypothalamus of rats [26]. However, there are few studies about the mechanism of RKT for cisplatin-induced anorexia, and details of its actions have not been elucidated.

Here we studied the effects of RKT on cisplatin-induced anorexia in rats. We also assessed the impact of RKT and cisplatin on the feeding-regulating peptides in the hypothalamus.

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2. Materials and methods

2.1. Animals

Adult male Wistar rats (260–290 g body weight) were individually housed and maintained in temperature controlled (23–25 °C) conditions under a 12.12 h light/dark cycle (lights on 07.00 h). All experiments were performed in strict accordance with guidelines on the use and care of laboratory animals issued by the Physiological Society of Japan, and were approved by the Ethics Committee of Animal Care and Experimentation of University of Occupational and Environmental Health.

2.2. Test substance

RKT (Tsumura & Co., Tokyo, Japan) includes eight crude herbs (*Atractylodis lanceae rhizome, Ginseng radix, Pinelliae tuber, Hoelen, Zizyphi fructus, Aurantii nobilis pericarpium, Glycyrrhizae radix* and *Zingiberis rhizoma*). These were mixed and extracted with hot water and then spray-dried to make a RKT powdered extract. The RKT was dissolved in tap water (0.1 g/mL) for intragastrical administration. Cisplatin (Sigma–Aldrich Japan Co. LLC., Tokyo, Japan) was dissolved in 0.9% sterile physiological saline (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) (0.6 mg/1 mL).

2.3. Experimental procedure

All the rats had access to food and water *ad libitum* throughout the experiments. The rats were divided into four groups: water + saline (WS, $n = 7$), water + cisplatin (WC, $n = 7$), RKT + saline (RS, $n = 8$), and RKT + cisplatin (RC, $n = 8$). Water (1 mL/100 g bode weight) or RKT (0.1 g/1 mL/100 g body weight) were administered directly to the stomach using sondes per os. These were administered from day −1 to day 2 (16.00–18.00 h). Saline (1 mL/100 g body weight) or cisplatin (0.6 mg/1 mL/100 g body weight) were administered intraperitoneally only one time 1 h after administration of water or RKT on day 0. Body weights were measured from day −1 to day 3 every 24 h. Food and water intake were measured from day 0 to day 3 every 24 h.

After the treatment, at day 3, the rats were decapitated immediately without being anesthetized, followed by removal of the brain promptly onto dry ice, then storing at −80 °C. Trunk blood samples were taken during decapitation, and were collected into chilled reaction tubes (Greiner Bio-One) containing an aprotinin/EDTA mixture. Blood samples were centrifuged for 10 min at 4 °C, 3000 rpm. After the blood was centrifuged, a 15 μL sample of plasma was taken for measuring plasma osmolality (P-Osm) using a ONE-TEN osmometer (FISKE, Norwood, MA, USA), 10 μL for measuring plasma glucose using a Medisafe Reader GR-101 (TERUMO, Tokyo, Japan), 500 μL for measuring plasma corticotrophin (SRL, Tokyo, Japan), and 500 μL for measuring plasma active and desasyl ghrelin.

2.4. In situ hybridization histochemistry

The removed brains were cut into 12 μm thickness, and thaw mounted on gelatin/chrome alum-coated slides. The locations of the hypothalamic areas, including the paraventricular nucleus (PVN), arcuate nucleus (ARC) and lateral hypothalamic area (LHA), were determined according to coordinates of the rat brain atlas. ^{35}S 3′-end-labeled deoxyoligonucleotide complementary to transcripts encoding *oxytocin, corticotrophin releasing hormone* (*CRH*), *pro-opiomelanocortin* (*POMC*), *cocaine and amphetamine-regulated transcript* (*CART*), *neuropeptide Y* (*NPY*), *agouti-related protein* (*AgRP*), *melanin-concentrating hormone* (*MCH*) and *orexin* were used (*oxytocin* probe sequence, 5′-CTC GGA GAA GGC AGA CTC AGG GTC GCA GGC-3′; *CRH* probe sequence, 5′-CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC-3′; *POMC* probe sequence, 5′-TGG CTG CTC TCC AGG CAC CAG CTC CAC ACA TCT ATG GAG G-3′; *CART* probe sequence, 5′-TCC TTC TCG TGG GAC GCA TCA TCC ACG GCA GAG TAG ATG TCC AGG-3′; *NPY* probe sequence, 5′-CAA ATG GAT GAT TGG TCA TTT CAA CAT AGA GTT GGG GGC TTG CT-3′; *AgRP* probe sequence, 5′-CGA CGC GGA GAA CGA GAC TCG CGG TTC TGT GGA TCT AGC ACC TCT GCC-3′; *MCH* probe sequence, 5′-CCA ACA GGG TCG GTA GAC TCG TCC CAG CAT-3′; and *orexin* probe sequence, 5′-TCC TCA TAG TCT GGA GGC AGG TGG AAG GGT TCC CCA CTG CTA GTG-3′).

The probe was 3′-end-labeled using terminal deoxynucleotidyl transferase and [^{35}S] dATP. The *in situ* hybridization protocol has been previously described in detail [24]. Briefly, sections were fixed in 4% (w/v) formaldehyde for 5 min and incubated in saline containing 0.25% (v/v) acetic anhydride and 0.1 M triethanolamine for 10 min and then dehydrated, delipidated in chloroform, and partially rehydrated. Hybridization was carried out overnight at 37 °C in 45 μL of hybridization buffer under a Nescofilm (Bando Kagaku, Osaka, Japan) cover slip. A total count of 1×10^5 c.p.m. for oxytocin transcripts and 1×10^6 c.p.m. for CRH, POMC, CART, NPY, AgRP, MCH and orexin transcripts and per slide were used. After hybridization, sections were washed 4 times with SSC (150 mM NaCl and 15 mM sodium citrate) for 1 h at 55 °C and for an additional hour with two changes of SSC at room temperature. Hybridized sections containing hypothalamus were exposed for autoradiography (Hyperfilm, Amersham, Bucks, UK) for 6 h for oxytocin probe, 5 days for MCH and orexin probe, and 1 week for CRH, POMC, CART, NPY and AgRP probe. The resulting images were analyzed by computerized densitometry using a MCID imaging analyzer (Imaging Research Inc., Ontario, Canada). The mean optical densities (OD) of the autoradiographs were measured by comparison with simultaneously exposed ^{14}C-labeled microscale samples (Amersham, Bucks, UK) and represented in arbitrary units setting the mean OD obtained from control rats.

2.5. Statistical analysis

The mean ± SEM was calculated from the results of the body weight change, cumulative water and food intake, cumulative urine volume, and *in situ* hybridization histochemistry studies. In the results of *in situ* hybridization, the expression levels of the genes were expressed as a percentage of WS. All data were analyzed by one-way ANOVA followed by a Bonferroni-type adjustment for multiple comparisons (Origin Pro version 8.5J, Lightstone, Tokyo, Japan). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Changes in body weight

The body weight of each group was measured from day 0 to day 3 (Fig. 1A). The body weight gradually increased during the experiments, except for WC (Fig. 1A). The body weight in WC at day 3 was significantly difference in comparison with all the other groups. Data are also presented as percentage from day 0 (Fig. 1B). The results of body weight in WC presented as percentage was also significantly different in comparison with all the other groups.

3.2. Water intake, urine volume, food intake

Cumulative water intake and cumulative urine volume were measured from day −1 to day 3 (Fig. 2A and B). There were no significant differences in cumulative water intake (Fig. 2A) or cumulative urine volume (Fig. 2B) among all the experimental groups.

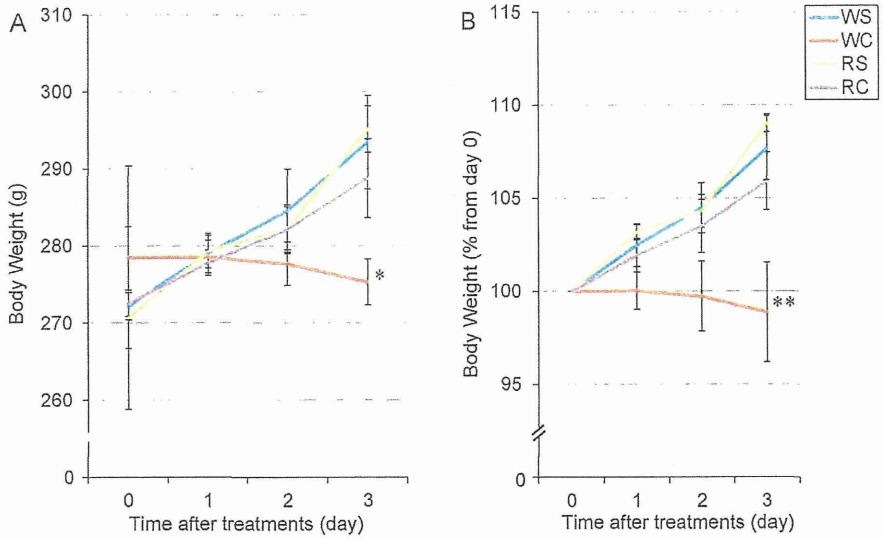

Fig. 1. Changes in body weight. (A) Changes in body weight from day −1 to day 3. (B) Changes in body weight from day 0 (percentage comparison from day 0). Body weight of WS, RS and RC gradually increased during the experiment, whereas that of WC (red line) gradually decreased. Data are presented as mean ± SEM. $^{*}P < 0.05$ *vs.* all other groups. $^{**}P < 0.01$ *vs.* all other groups.

Cumulative food intake was measured from day −1 to day 3 (Fig. 2C). At day 1, food intake in RC (purple line) and WC (red line) significantly decreased compared to the other two groups (Fig. 2C). After day 2, food intake in WC significantly decreased compared to all the other groups (Fig. 2C). Food intake per day from day 0 to day 3 is also shown in the graph (Fig. 2D). Food intake per day in WC significantly decreased compared to WS. There were no statistical differences among WS, RS and RC.

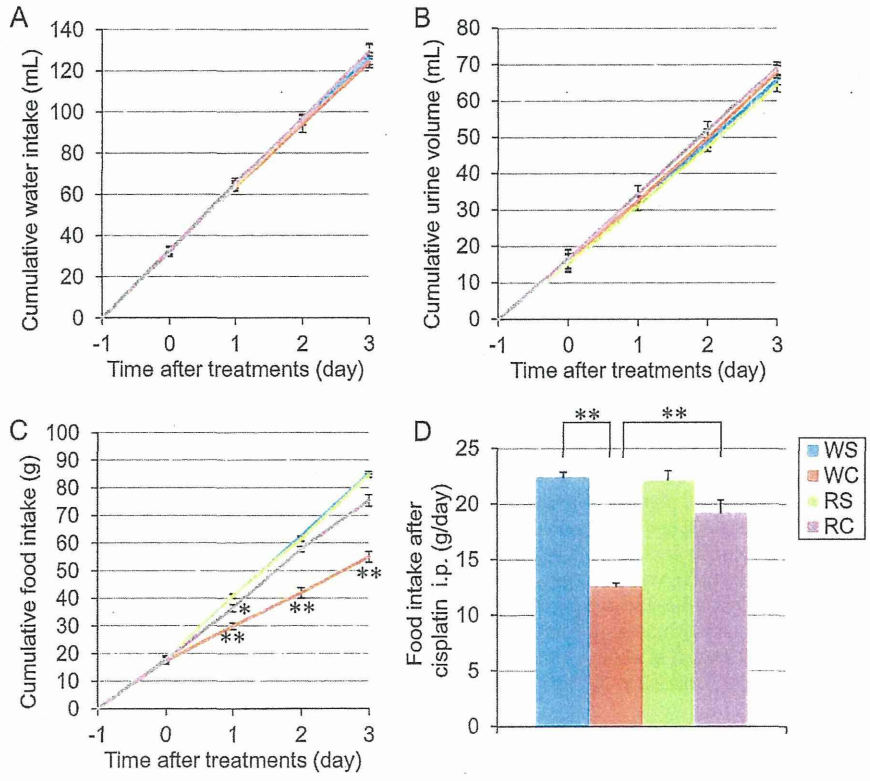

Fig. 2. Cumulative water intake, urine volume and food intake. (A) Cumulative water intake from day −1 to day 3. (B) Cumulative urine volume from day −1 to day 3. No statistical differences were seen in cumulative water intake and cumulative urine volume among all experimental groups. (C) Cumulative food intake from day −1 to day 3. (D) Food intake per day after administering cisplatin (day 0). Data are presented as mean ± SEM. $^{*}P < 0.05$ *vs.* all other groups. $^{**}P < 0.01$ *vs.* all other groups.

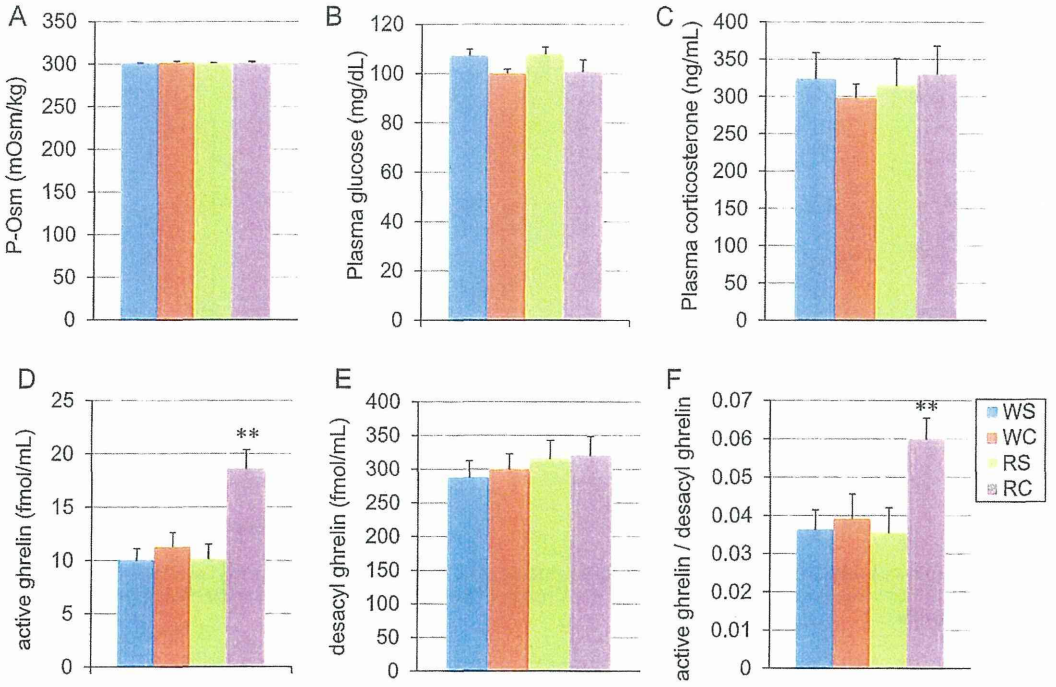

Fig. 3. Plasma measurements. (A) Plasma osmolality, (B) plasma glucose, (C) plasma corticosterone, (D) plasma active ghrelin, (E) plasma desacyl ghrelin, and (F) the ratio of plasma active and desacyl ghrelin. $^{**}P < 0.01$ *vs.* all other groups. Data are presented as mean ± SEM.

3.3. Plasma measurement

We did plasma measurements of P-Osm (Fig. 3A), plasma glucose (Fig. 3B), plasma corticotrophin (Fig. 3C), plasma active ghrelin (Fig. 3D), and plasma desasyl ghrelin (Fig. 3E). The ratio of active and desasyl ghrelin is demonstrated in Fig. 3F. There were no significant differences in P-Osm, plasma glucose and plasma corticotrophin among all the experimental groups. Plasma active ghrelin dramatically increased only in RC (Fig. 3D). There were no statistical differences in desacyl ghrelin in all among the groups (Fig. 3E). Understandably, the ratio of active and desasyl ghrelin dramatically increased only in WC (Fig. 3F).

3.4. Feeding-regulating peptides in the hypothalamus

Feeding-regulating peptides in the hypothalamus were measured by *in situ* hybridization histochemistry followed by quantification using MCID. The expression of *oxytocin* mRNA signals in the PVN was comparable among all the experimental groups (Figs. 4A-a–A-d and 5). *CRH* mRNA in the PVN in WC significantly decreased compared to all the other groups (Figs. 4B-a–B-d and 5). *POMC* and *CART* mRNA in the ARC in WC significantly increased compared to all the other groups (Figs. 4A, C-a–C-d, D-a–D-d and 5). *NPY* mRNA in the ARC in WC significantly increased compared to all the other groups (Figs. 4E-a–E-d and 5), whereas that of *AgRP* in the ARC in WC was comparable (Figs. 4F-a–F-d and 5). *MCH* and *orexin* mRNA in the LHA in WC significantly increased compared to all the other groups (Figs. 4G-a–G-d, H-a–H-d and 5). There were no statistical differences among WS, RS and RC in all the peptides that were examined in this study.

4. Discussion

Traditional Japanese herbal medicines (kampo) are widely prescribed, and RKT is one of the most popularly prescribed kampo in Japan. However, although most kampo are used empirically, their mechanism is unclear. Although there has been no exception regarding RKT, its mechanism has been gradually proposed in recent years. In this study, we confirmed the efficacy of RKT on cisplatin-induced anorexia, and examined the feeding-regulating peptides in the hypothalamus in cisplatin-treated and cisplatin-RKT-treated rats.

As shown in Figs. 1 and 2C, the results of body weight and cumulative food intake showed a gradual increase in WS, RS and RC, whereas cumulative food intake in WC was less than those three groups and body weight decreased. These were observed at least three days after administering cisplatin or saline. Ghrelin is produced in the stomach and localized to neurons in the hypothalamic ARC [8,11]. It has been revealed that RKT is a ghrelin enhancer [3,19,26], thus indicating increased food intake and body weight. Cisplatin-induced anorexia may also be associated with reduced hypothalamic ghrelin secretion [26]. Taken together with our results, this is true under cisplatin-administered conditions but not under saline-treated conditions. In other words, administration of RKT alone does not increase body weight and food intake. It is indicated that even though ghrelin secretion is increased by RKT, food intake does not increase unless under pathological conditions.

Cumulative water intake and cumulative urine volume were the same extent in all the groups in our study (Fig. 2A and B). Although there have been reported cases of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) induced by cisplatin [1,5,21], it is suggested that cisplatin and/or RKT have little effect on body fluid regulation within a few days after administration. The same level of P-Osm (Fig. 3A) among the groups also supports this hypothesis.

Cumulative food intake in WS and RS did not differ at all (Fig. 2C). However, cumulative food intake in WC at day 1, day 2 and day 3 significantly decreased in comparison with all the other groups

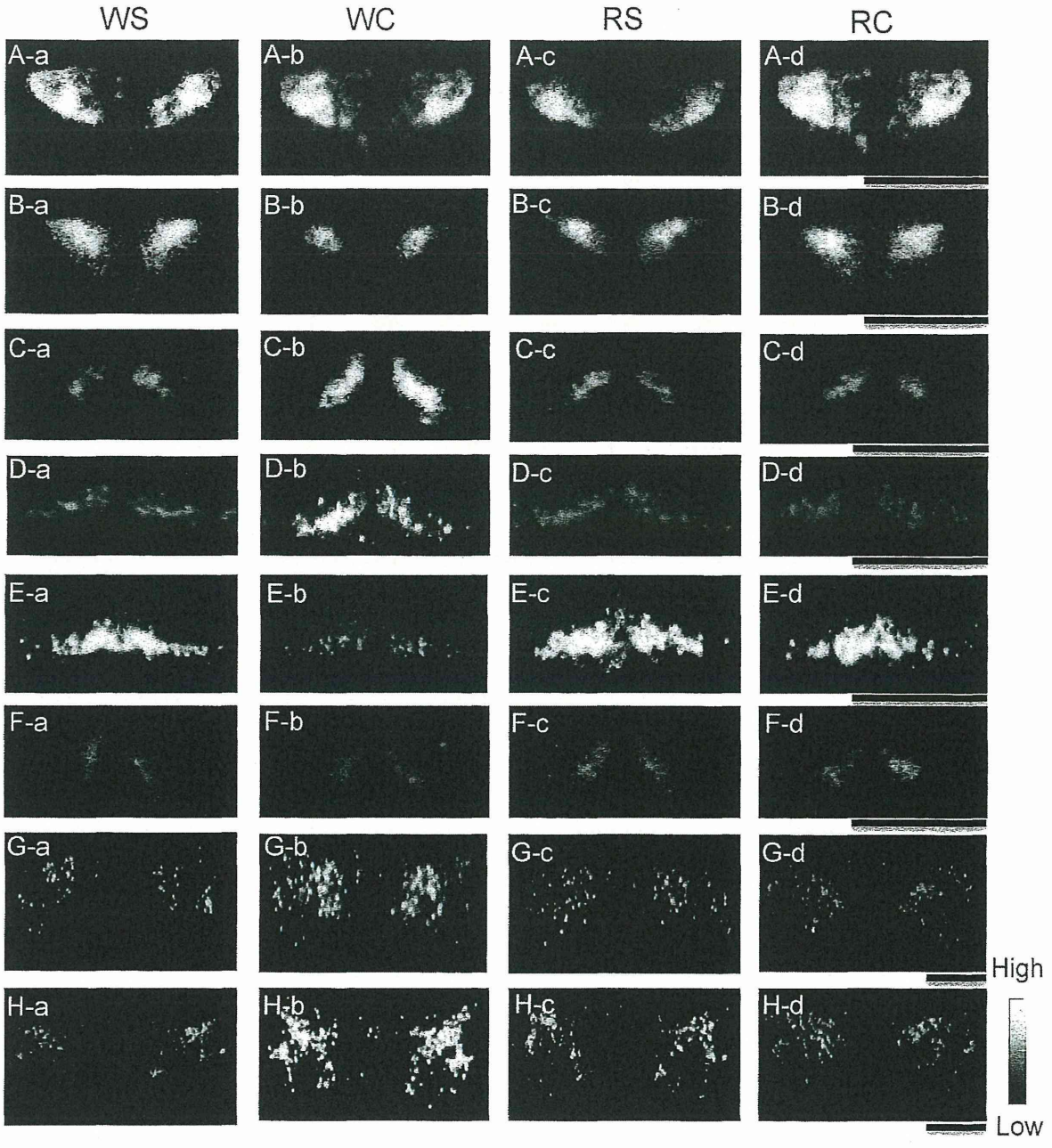

Fig. 4. Digital images of ISH of feeding-regulating peptides. (A) Digital images of *in situ* hybridization histochemistry of *oxytocin* in the PVN (A-a–A-d), *CRH* in the PVN (B-a–B-d), *POMC* in the ARC (C-a–C-d), *CART* in the ARC (D-a–D-d), *NPY* in the ARC (E-a–E-d), *AgRP* in the ARC (F-a–F-d), *MCH* in the LHA (G-a–G-d), and *orexin* in the LHA (H-a–H-d).

(Fig. 2C). The administration of RKT with cisplatin canceled the decreased food intake induced by cisplatin. This may indicate that RKT does not affect food intake in a non-pathological state.

In plasma measurements, P-Osm did not differ among the groups, as mentioned above. It is expected that plasma glucose levels were decreased as a result of reduced food intake in WC (Fig. 2D). There may be a possibility that other factors are involved in glucose metabolism, or that, as a result of stress response by administration of cisplatin, plasma glucose levels were comparable to other groups despite decreased food intake in WC. Needless to say, Li et al. isolated adrenocorticotrophic hormone from sheep pituitary [9], and it is widely recognized as a hormone which is secreted by various kinds of stress. Although it is expected that plasma corticotrophin levels might have increased in WC because of the stress which was induced by cisplatin administration, they did not differ among all the groups, whereas *in situ* hybridization of *CRH* in the PVN decreased in WC (Figs. 4B-b and 5). Perhaps there may be a very little change that cannot be captured in peripheral plasma even if there is a significant change in central nervous system. Interestingly, plasma active ghrelin neither decreased by cisplatin treatment nor by RKT treatment, however, it dramatically increased by RKT with cisplatin treatment (Fig. 3D-3F), which indicating that RKT acts only in pathological state but not in physiological condition. Although several studies have demonstrated that

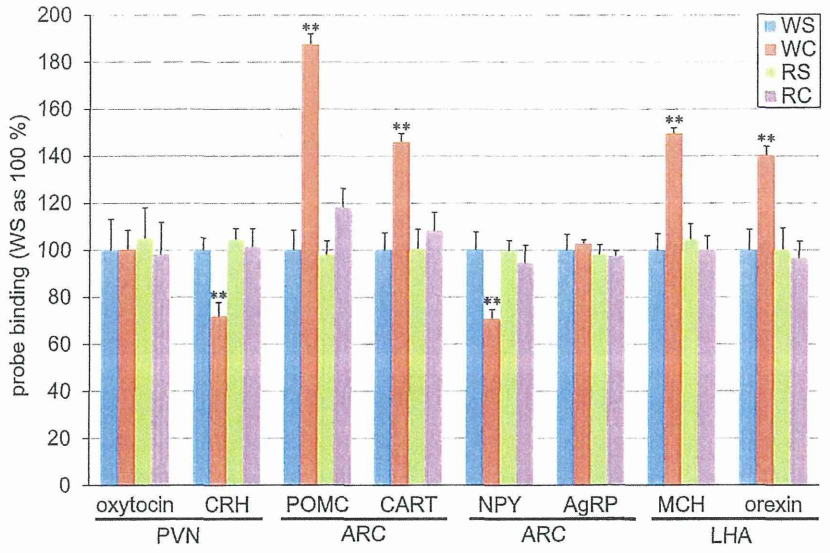

Fig. 5. Quantification of ISH of feeding-regulating peptides. Quantification of mRNA signals using MCID. WS data are presented as 100%. Signals of *oxytocin* in the PVN and *AgRP* in the ARC did not differ among all experimental groups. Signals of *POMC* in the ARC, *CART* in the ARC, *MCH* in the LHA and *orexin* in LHA in WC increased significantly compared to all other groups. On the other hand, signals of *CRH* in the PVN and *NPY* in the ARC significantly decreased in WC compared to all other groups. There were no statistical differences among WS, RS and RC in all examined peptides. Scale bar; 1 mm. Data are presented as mean ± SEM. $^{*}P<0.05$ *vs.* all other groups. $^{**}P<0.01$ *vs.* all other groups.

cisplatin provoke anorexia *via* reduced hypothalamic ghrelin secretion [25,26], cisplatin alone did not reduce plasma active ghrelin according to our study. Because our result is based on whole body plasma measurements, cisplatin may reduce the hypothalamic ghrelin secretion which cannot be captured by plasma measurements.

With respect to *in situ* hybridization histochemistry of feeding-regulating peptides in the hypothalamus, as shown in Figs. 4 and 5, the *oxytocin* probe binding in the PVN were comparable among all the experimental groups. It seems likely that cisplatin and RKT have little involvement with oxytocin transcription, or that a significant change cannot be detected as a state of mRNA. Further study, such as comparison with heteronuclear RNA expression, is needed.

CRH probe binding in the PVN significantly decreased in WC, and *MCH* and *orexin* probe binding in the LHA significantly increased in WC compared to all the other groups. CRH is recognized as an anorexigenic peptide, and MCH and orexin are recognized as orexigenic peptides [12]. We believe that these changes were a result of decreased appetite induced by cisplatin.

POMC and *CART* probe binding in the ARC were significantly increased, and *NPY* probe binding in the ARC significantly decreased. POMC and CART are recognized as anorexigenic peptides, and NPY is an orexigenic peptide [12]. These results indicate that cisplatin induces anorexia by increasing *POMC* and *CART* and decreasing *NPY* in the ARC. It seems likely that RKT affects the decrease of *POMC* and *CART* and the increase of *NPY* in the ARC under cisplatin-administered conditions, however there were no differences between WS and RS in our study, which suggests that RKT does not directly affect *POMC*, *CART* and *NPY* in the ARC without cisplatin. NPY, which is located downstream of the control of a number of feeding-regulating peptides, plays an important role in controlling feeding action [14,18]. It has been reported that centrally administered ghrelin stimulates food intake and weight gain by inducing NPY production in the ARC [13]. Furthermore, cisplatin-induced anorexia is involved in reduced ghrelin secretion [26], and the *CART* in the ARC has been inhibited by ghrelin [2]. Taken together with our results, cisplatin induces anorexia not only by reducing *NPY* in the ARC by reduced ghrelin secretion but also by increasing *POMC* and *CART* transcripts in the ARC. RKT may increase ghrelin secretion in the hypothalamus [26], thus canceling these reactions. It is not clear whether RKT affects the increase of *POMC* and *CART* directly or not. Ghrelin may have a potential role in regulating these peptides which were examined in this study.

AgRP probe binding in the ARC was comparable in all the experimental groups. It has been reported that ghrelin regulates not only *NPY* but also *AgRP* in the ARC [10]. We expected that *NPY* and *AgRP* would change similarly; however, they did not change in the same way. There may be other factors regulating AgRP except ghrelin when administering cisplatin.

The reason why the feeding-regulating peptides in RS were comparable to those in WS remains unclear. These reactions were not observed by administering RKT alone. There may have been involvement of other feeding-regulating peptides which were not investigated in this study when administering RKT alone. Further study is needed to elucidate the anti-anorexigenic mechanism of RKT.

In conclusion, we confirmed that cisplatin-induced anorexia is attenuated by RKT in rats. We revealed that cisplatin increases *POMC* and *CART* and decreases *NPY* in the ARC, which may also be one of the causes of anorexia, whereas intragastric RKT administration with cisplatin i.p. attenuates these reactions. Our study suggests that RKT may have therapeutic potential for cisplatin-induced anorexia.

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ORIGINAL ARTICLE

A c-fos-Monomeric Red Fluorescent Protein 1 Fusion Transgene is Differentially Expressed in Rat Forebrain and Brainstem after Chronic Dehydration and Rehydration

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We have previously shown that an acute osmotic stimulation induces the expression of a c-*fos* and monomeric red fluorescent protein 1 (mRFP1) fusion transgene in osmosensitive rat brain areas, including the supraoptic (SON) and paraventricular nuclei (PVN). However, the effects of chronic stimuli, such as dehydration, have not been investigated. In the present study, the expression patterns of the c-*fos*-mRFP1 fusion gene in the forebrain and the brainstem of male and female transgenic rats were studied in seven experimental groups: *ad lib.* water (euhydration), water deprivation for 12, 24 or 48 h (dehydration) and water deprivation for 46 h + *ad lib.* water for 2, 6 or 12 h (rehydration). The number of cells that express nuclear mRFP1 fluorescence was quantified in the hypothalamus, the circumventricular organs and the brainstem. Compared to the euhydrated state, the number of transgene expressing cells significantly increased in all forebrain areas and in the rostral ventrolateral medulla after dehydration and 2 h of rehydration. In the nucleus of the solitary tract and area postrema, the number of mRFP1 fluorescent cells was markedly increased after 2 h of rehydration. Although the number of mRFP1 fluorescent cells in the organum vasculosum laminae terminalis, median preoptic nucleus and subfornical organ remained significantly increased after 6 h of rehydration, reaching control levels after 12 h of rehydration, the number of mRFP1 fluorescent cells in the SON and the PVN reached control levels after 6 h of rehydration. There were no significant differences between male and female rats. These results show that the expression of the c-*fos*-mRFP1 fusion gene changes in the forebrain and the brainstem not only after acute osmotic stimulation, but also after chronic osmotic stimulation. Interestingly, these studies reveal the differential activation of different neuronal groups over the time course of dehydration and rehydration.

Key words: c-*fos*, red fluorescent protein, osmotic stimulation, brain, transgenic rat

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The expression of the c-*fos* gene, assayed at the RNA or the protein level, has been widely used as a marker of the neuronal activity elicited in the central nervous system by various physiological stimuli (1–6). Previous studies have used electrophysiological methods (7) and *in situ* hybridisation histochemistry for c-*fos* mRNA (8), or immunocytochemistry for Fos and Fos-related proteins (9–13), to reveal a network of osmosensitive areas in the brain involved in body fluid regulation. Recently, we have described the generation of a transgenic rat line that expresses a c-*fos* and monomeric red fluorescent protein 1 (mRFP1) fusion gene (7). Acute osmotic stimulation of these transgenic rats induced the expression of the c-*fos*-mRFP1 fusion gene in the osmosensitive areas, including the supraoptic (SON) and paraventricular nuclei (PVN), the organum vasculosum of the lamina terminalis (OVLT), the median preoptic nucleus (MnPO) and the subfornical organ (SFO) (7). In the brainstem, acute osmotic stimulation also induced c-*fos*-mRFP1 fusion gene expression in the nucleus of the solitary tract (NTS) and rostral ventrolateral medulla (RVLM) of the brainstem but not in the area postrema (AP) (M. Y. and Y. U.'s unpublished data). Thus, in the present study, we examined the effects of the chronic osmotic