

FIGURE 4: Effects of obestatin on the gastroduodenal motility. (a) Effects of i.v. injection of obestatin on the fed motor activity of the antrum and duodenum. I.v. injection of obestatin prolongs the time between the initiation of phase III-like contractions and injection of obestatin in the duodenum. (b) The elongation of the time between injection of obestatin and initiation of phase III-like contractions in the duodenum induced by i.v. injection of obestatin is reversed by i.c.v. injection of selective CRF type 1 receptor antagonist NBI-27914 and also by selective CRF type 2 receptor antagonist antisauvagine-30. (c) The density of *c-Fos*-positive cells in the PVN is increased by i.v. injection of obestatin compared to saline-injected control. CRF-positive or urocortin 2-positive neurons are overlapped with *c-Fos*-positive neurons in the PVN. (d) Summary diagram of the effects of obestatin on the gastroduodenal motility and brain mechanism mediating its action.

two CRF receptor subtypes, CRF type 1 receptor is highly involved in anxiety-related behavior and CRF type 2 receptor is involved in regulating food intake and peripheral functions such as gastric acid secretion or gastric emptying. CRF is a relatively selective ligand for CRF type 1 receptor, whereas

urocortin 2 is a ligand more selective for CRF type 2 receptor [28, 29]. The density of *c-Fos*-positive cells in the PVN was significantly increased by i.p. injection of des-acyl ghrelin compared to vehicle-injected controls [7] (Figure 3(c)). These data suggest that peripherally administered des-acyl

ghrelin may activate neurons in the PVN by crossing the BBB and exert inhibitory effects on the antral motility via CRF type 2 receptor in the brain (Figure 3(d), Table 1).

6. Obestatin and Gastroduodenal Motility

Zhang et al. first reported that i.p. injection of obestatin suppressed cumulative food intake, decreased body weight gain, and inhibited gastric emptying and jejunal muscle contraction in mice [2]. Since then, however, the inhibitory effects of obestatin on food intake and gastrointestinal motility have remained controversial [8–13]. Most of the previous studies which showed the negative effects of obestatin on the gastrointestinal motility have only measured the gastric emptying or MMC cycle time as indices for motor activity. In our recent study, for more precise analysis, motor activity in both fed and fasted states was quantified by the %MI, and we measured the time taken to the initiation of phase III-like contractions in the antrum and duodenum of conscious rats [14].

We showed that motor activity in the antrum and duodenum was inhibited when obestatin was given i.v. to conscious rats in the fed state but not when it was given in the fasted state [14]. I.v. injection of obestatin decreased the %MI of fed motility in the antrum and prolonged the time before the return of fasted motility in the duodenum [14] (Figure 4(a)). Such inhibitory actions were the opposite of those obtained with ghrelin [16]. The results showed that the inhibitory action of obestatin appeared 30–90 minutes after i.v. injection [14], which is consistent with the timing of the effects of i.v. injection of ghrelin (~30 minutes) on gastroduodenal motility [16]. I.v. injection of obestatin induced a significant increase in the number of *c-Fos*-positive cells in the PVN compared to saline-injected controls [14] (Figure 4(c)). Immunofluorescence overlap staining showed that the PVN neurons activated by i.v. injection of obestatin contain CRF or urocortin 2 [14] (Figure 4(c)). The involvement of CRF type 1 and type 2 receptors in the action of obestatin on the gastroduodenal motility was examined [14]. Results showed that the inhibitory actions of i.v. injection of obestatin on the motor activities in the antrum and duodenum were blocked by i.c.v. injection of CRF type 1 and type 2 receptor antagonists, suggesting that both types of CRF receptors in the brain may mediate the action of peripherally injected obestatin on gastroduodenal motility [14] (Figure 4(b)). The results showed that vagal afferent nerve blockade by capsaicin reverses the inhibitory effects of obestatin on duodenal motility but does not alter the inhibitory effects of obestatin on antral motility [14]. These results suggest that vagal afferent pathways might be involved partially, but not entirely, in the action of obestatin. Involvement of vagal afferent pathways was confirmed by the finding that the number of *c-Fos*-positive neurons in the NTS was increased by i.v. injection of obestatin [14]. In addition to vagal afferent pathways, it is possible that circulating obestatin acts on brain targets directly by crossing the BBB, because a previous study has shown that there is a rapid influx of i.v.-injected ¹²⁵I-labeled obestatin from the blood to the brain [30]. Therefore the lack of effects of obestatin

on antral motility during capsaicin treatment might be explained by direct action of peripherally injected obestatin on brain targets by crossing the BBB, similar to what has been observed for des-acyl ghrelin. We further examined whether obestatin can antagonize the stimulatory effects of ghrelin on gastroduodenal motility [14]. We found that obestatin failed to antagonize the ability of ghrelin either to stimulate the %MI in the antrum or to accelerate the initiation of fasted motility in the duodenum when administered in the fed state [14]. These results were consistent with previous studies in which obestatin failed to antagonize the ability of ghrelin to stimulate gastric emptying or to shorten the MMC cycle time [8].

GPR39 was initially proposed as the receptor for obestatin [2], and GPR39 expression has been detected in peripheral organs such as the duodenum and kidney but not in the pituitary or hypothalamus [4]. However recent publications indicate that obestatin is unlikely to be the endogenous ligand for GPR39 on the basis of a lack of specific binding of obestatin to GPR39 receptor-expressing cells [2, 4, 5, 31]. Nevertheless, although binding of obestatin to the receptor GPR39 remains controversial, the functional effect of obestatin on gastrointestinal motility has been clearly demonstrated in our study.

Our study indicates that obestatin inhibits gastroduodenal motility in the fed state but not in the fasted state of conscious rats. In the brain, CRF- and urocortin 2-containing neurons might be activated by i.v. injection of obestatin, and at the level, CRF type 1 and type 2 receptors might be involved in the inhibitory action of obestatin on antral and duodenal motility (Figure 4(d), Table 1). Vagal afferent pathways might be involved partially, but not entirely, in these actions of obestatin (Figure 4(d), Table 1).

7. Conclusion

Although ghrelin, des-acyl ghrelin, and obestatin are derived from a common prohormone, originating from endocrine cells in the stomach, their roles on the gastrointestinal motility are quite different each other. Ghrelin stimulates the gastroduodenal motility in both fed and fasted states, des-acyl ghrelin inhibits the stomach motility in the fasted state, and obestatin inhibits the gastroduodenal motility in the fed state of animals (Table 1). Different hypothalamic peptides are involved in these actions, NPY Y2 and Y4 receptors may mediate the action of ghrelin, CRF type 2 receptor may mediate the action of des-acyl ghrelin, and CRF type 1 and type 2 receptors may mediate the action of obestatin (Table 1). The regulatory roles of ghrelin, des-acyl ghrelin, and obestatin on the gastrointestinal motility might give us the therapeutic strategies for the functional disorders of the gastrointestinal tracts

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Modulation of Ingestive Behavior and Gastrointestinal Motility by Ghrelin in Diabetic Animals and Humans

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Acyl ghrelin, a 28-amino acid peptide hormone, is the endogenous cognate ligand for the growth hormone secretagogue receptor. Ghrelin is involved in stimulating growth hormone release, eliciting feeding behavior, inducing adiposity and stimulating gastrointestinal motility. Ghrelin is unique for its post-translational modification of *O*-*n*-octanoylation at serine 3 through ghrelin *O*-acyltransferase, and is the only peripheral signal to enhance food intake. Plasma ghrelin levels manifest "biphasic changes" in diabetes mellitus (DM). In the early stage of DM, the stomach significantly increases the secretion of ghrelin into the plasma, and elevated plasma ghrelin levels are correlated with diabetic hyperphagic feeding and accelerated gastrointestinal motility. In the late stage of DM, plasma ghrelin levels may be lower, which might be linked with anorexia/muscle wasting, delayed gastrointestinal transit, and even gastroparesis. Therefore, the unique ghrelin system may be the most important player compared to the other hindgut hormones participating in the "entero-insular axis". Further studies using either knockdown or knockout of ghrelin gene products and ghrelin *O*-acyltransferase may unravel the pathogenesis of DM, and show benefits in combating this disease and metabolic syndrome. [*J Chin Med Assoc* 2010;73(5):225-229]

Key Words: acyl ghrelin, diabetes mellitus, feeding, gastrointestinal motility, ghrelin *O*-acyltransferase

Introduction

Acyl ghrelin, a 28-amino acid peptide hormone, has been identified as the endogenous cognate ligand for the growth hormone secretagogue receptor (GHS-R).¹ It was discovered by "reverse pharmacology".^{1,2} After acyl ghrelin binds to GHS-R, it induces the release of growth hormone.³ Ghrelin is mainly synthesized in specific endocrine cells, designated X/A-like cells, in the gastric oxyntic glands.^{1,4} Des-acyl ghrelin, the major form of ghrelin in plasma,² may be acylated into acyl ghrelin through ghrelin *O*-acyltransferase (GOAT) in the stomach.⁵ In addition to inducing growth hormone release, acyl ghrelin enhances food intake, and it is the only peripheral signal to increase meal size.⁶

Acyl ghrelin also stimulates adiposity, which is independent of its hyperphagic effects.⁷ Therefore, ghrelin is an interesting molecule of high clinical relevance to human obesity and metabolic syndrome.⁸ With regard to the gastrointestinal tract, acyl ghrelin accelerates gastric emptying⁹ and elicits gastroduodenal phase III-like contractions¹⁰ in rats.

Diabetes mellitus (DM) is a common clinical problem with increasing prevalence in the world. There are 2 main types of DM. Both types are caused by derangement of insulin's function and activity in the body.¹¹ Type 1 DM most often develops in childhood or adolescence and causes hyperglycemia due to insufficient production of insulin, while over 90% of all DM cases are type 2 DM. DM may manifest many



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gastrointestinal symptoms such as nausea, vomiting, diarrhea, constipation, abdominal pain,¹² and even hyperphagia.^{13,14} Some symptoms can be attributed to gastrointestinal dysmotility. Acyl ghrelin dose-dependently inhibits insulin secretion in mice,¹⁵ and the relationship between ghrelin and glucose metabolism has been previously discussed in our review.⁸ A recent study revealed that MK-677, an acyl ghrelin mimetic, increases blood glucose and HbA1c levels in healthy aged volunteers after 1 year of administration.¹⁶ Mice lacking acyl ghrelin demonstrate lower fasting blood glucose, a better insulin-induced blood-glucose-lowering effect, as well as higher plasma insulin and lower blood glucose levels after intraperitoneal glucose injection.¹⁷ These *acyl ghrelin* knockout mice are protected against hyperinsulinemia and hyperglycemia induced by a high-fat diet.^{18,19} *GHS-R* knockout mice exhibit lower blood glucose and serum insulin levels,^{20,21} and greater "metabolic flexibility" under diet-induced metabolic stress.²² Furthermore, *acyl ghrelin* and *GHS-R* double knockout mice show a greater blood glucose drop during 50% caloric restriction.²¹ Inhibition of GOAT⁸ and *GOAT* knockout²³ is proposed to have potential for anti-diabetic therapeutics. Since acyl ghrelin plays an important role in inducing food intake, eliciting gastroduodenal phase III-like contractions, accelerating gastric emptying, and glucose homeostasis, the present mini-review will focus on the influence of ghrelin on ingestive behavior and gut motility in diabetic animals and humans.

Regulation of Ghrelin Secretion and the Influence of Ghrelin on Ingestive Behavior in Diabetes

Ghrelin-immunoreactive cell density was found to be reduced in type 1 non-obese and type 2 obese diabetic mice.²⁴ This observation could explain the slow gastric emptying and slow intestinal transit encountered in human diabetic gastroenteropathy, based on the fact that ghrelin has gastrointestinal prokinetic effects. However, the correlation between ghrelin levels and diabetic gastrointestinal dysmotility needs to be further investigated before drawing a definite conclusion. In streptozotocin-induced diabetic rats, the number of ghrelin-immunoreactive cells in the gastric fundus is consistently found to be decreased, whereas insulin treatment reversed this finding, implying that a decrease in ghrelin-immunoreactive cells reflects a decrease in ghrelin content in X/A-like cells but not a decrease of ghrelin-producing cells.²⁵ Body weight and serum insulin levels in the streptozotocin-induced

rats was decreased, whereas plasma acyl ghrelin and total ghrelin levels and gastric preproghrelin mRNA expression levels were significantly increased.²⁵ When considered together, these results indicate that DM, a negative energy balance condition, may enhance preproghrelin mRNA expression in the stomach and ghrelin secretion into the bloodstream. Acyl ghrelin and des-acyl ghrelin have been demonstrated to inhibit apoptosis and stimulate proliferation of pancreatic β cell lines and human islets of Langerhans.²⁶ This finding indicates that acyl ghrelin, as well as des-acyl ghrelin, might protect β cells against apoptosis and increase β cell survival. A subsequent study revealed that acyl ghrelin treatment for 21 days increases pancreatic insulin, pancreatic and duodenal homeobox 1 gene (*Pdx1*) mRNA and the number of replicating cells in streptozotocin-treated neonatal rats.²⁷ This finding showed that acyl ghrelin and des-acyl ghrelin promote regeneration of β cells in streptozotocin-treated animals. Collectively, in addition to the effects compensatory for the loss of body weight and serum insulin levels in streptozotocin-induced rats, the increases in plasma acyl ghrelin and total ghrelin levels and gastric preproghrelin mRNA expression levels could prevent further β cell damage and facilitate β cell regeneration. Therefore, early administration of acyl ghrelin might prevent or ameliorate the development of DM in disease-prone subjects after β cell destruction.²⁷

Uncontrolled DM is characterized by marked behavioral perturbations, such as severe hyperphagia and increased circulating ghrelin levels could cause the development of diabetic hyperphagia.^{12,13} In streptozotocin-induced rats, plasma total ghrelin levels are increased well before the onset of hyperphagic feeding, supporting the hypothesis that increased ghrelin signaling contributes to the stimulatory effect on food intake in the early stage of DM.¹³ A subthreshold dose of intracerebroventricular administration of acyl ghrelin was found to increase food intake by 357% in diabetic rats compared with that in controls, indicating increased behavioral sensitivity to acyl ghrelin in the absence of the opposing effects of leptin and insulin in DM.¹³ Similarly, plasma fasting acyl ghrelin levels are increased, whereas des-acyl ghrelin levels are decreased in patients with obesity-related type 2 DM compared with lean subjects.²⁸ Metformin therapy was found to prolong the postprandial fall in total plasma ghrelin levels, and thus had concomitant effects on appetite in type 2 DM, contributing to its actions in promoting weight loss and attenuating weight gain in these patients.²⁹ A recent study demonstrated that barley intake dose-dependently decreases plasma glucose and insulin levels, whereas postprandial reduction of plasma

des-acyl ghrelin is suppressed by barley intake in a dose-dependent manner, compared with glucose and white rice.³⁰ Since des-acyl ghrelin might have anorexigenic^{31,32} and insulin-mimetic³³ effects, either through binding to an additional as-yet unidentified receptor⁸ or buffering³³ of acyl ghrelin's actions, it has been advocated that a combination of white rice and barley may play a beneficial role in preventing and treating human type 2 DM.³⁰ However, total plasma ghrelin levels are negatively correlated with HbA1c in diabetic patients, suggesting that long-term poor glycemic control might impair ghrelin secretion,³⁴ and that plasma ghrelin levels could be lower in the late stage of DM. Consistently, fasting total plasma ghrelin levels are decreased in insulin-resistant obese adults compared with those in equally obese insulin-sensitive controls, implying that insulin resistance and compensatory hyperinsulinemia are independently associated with suppression of ghrelin.³⁵ In addition, salivary levels of acyl ghrelin and des-acyl ghrelin are similarly decreased in obese diabetic subjects in comparison with non-obese diabetic and healthy controls.³⁶ These alterations may have a causal role in the development and severity of disease.

Impacts of Ghrelin on Gastrointestinal Motility in Diabetes

Circulating acyl ghrelin levels fluctuate and the peaks are associated with the gastric migrating motor complex cycle,³⁷ indicating the indispensable role of endogenous acyl ghrelin in modulating gastrointestinal motility. Experiments with a streptozotocin-induced DM rat model showed elevated plasma acyl ghrelin levels in diabetic rats, and the elevated levels were accompanied with accelerated solid gastric emptying and enhanced postprandial antro-pyloric coordination.³⁸ Treatment with anti-acyl ghrelin antibodies suppressed the accelerated gastric emptying and stimulated antro-pyloric coordination. An elevated plasma acyl ghrelin level-induced accelerated gastric emptying could predispose to overeating, which would, in turn, exacerbate DM in the diabetic early stage. In contrast, gastric emptying becomes slow in the late stage of DM, and severe gastroparesis sometimes occurs. These findings have clinical implications in the prevention for the development of complications in DM, such as diabetic gastroparesis, as in the late stage of DM. Sham feeding is characterized by an increase in pancreatic polypeptide and ghrelin in normal healthy humans, whereas changes in pancreatic polypeptide and ghrelin levels in diabetic gastroparesis are significantly less than those in normal

subjects.³⁹ Ghrelin subsequent to lunch significantly decreases in patients without gastroparesis, but not in gastroparetic patients.⁴⁰ Taken together, these findings suggest that decreased plasma ghrelin levels are linked with a slow gastrointestinal transit in the late stage of DM. Loss of rhythmicity in ghrelin levels of diabetic gastroparesis highlights the importance of integrity of the neurohumoral-intestinal axis.³² Patients with diabetic gastroparesis show no decrease of plasma acyl ghrelin after glucose loading, unlike patients without gastroparesis or healthy controls,⁴¹ indicating that diabetic gastroparesis might be related to ghrelin-associated neurohormonal abnormalities. Conceivably, intravenous infusion of acyl ghrelin improves impaired gastric emptying in patients with diabetic gastroparesis, and this effect is independent of vagal tone.⁴² Therefore, we propose that analogs of acyl ghrelin may represent a new class of prokinetic agents in future treatment for patients with diabetic gastroparesis.

Conclusions and Future Perspectives

Obesity has replaced cigarette smoking as a severe new burden on public health.⁴³ Obesity-related metabolic syndrome, and DM, which negatively affects quality of life and life expectancy, also cannot be overlooked. Ghrelin is an exceptionally intriguing gastric hormone, and actively participates in the modulation of ingestive behavior and gastrointestinal motility. Plasma ghrelin levels are elevated in the early stage of DM, which correlates with hyperphagic feeding and accelerated gastrointestinal motility. In contrast, plasma ghrelin levels can be decreased in the late stages of DM, which may be linked with poor appetite, body weight loss and gastroparesis. The "entero-insular axis" has clinical implications for the treatment of human DM.⁴⁴ Hindgut hormones, such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1, hold great promise. However, a recent study indicated that selective bypass of the proximal intestine by an endoluminal sleeve, mimicking human Roux-en-Y gastric bypass (the only way to resolve DM), reduces body weight and food intake, and improves fasting hyperglycemia and glucose tolerance in rats with diet-induced obesity.⁴⁵ These results suggest that the "foregut theory" may be preferable to the "hindgut theory". Therefore, ghrelin deserves more attention in the pathogenesis of DM. Two recent studies showed that measurement of total ghrelin did not adequately reflect acyl ghrelin and des-acyl ghrelin levels.^{46,47} Therefore, in contrast to the original concept, levels of total ghrelin are not an ideal surrogate for those of

acyl ghrelin.⁸ Further studies, particularly using state-of-the-art techniques to separately measure acyl ghrelin, des-acyl ghrelin, and obestatin, are necessary to clarify the differential roles of ghrelin gene products in the pathogenesis of DM. Ghrelin manifests “biphasically” in DM. GOAT enhancers, acyl ghrelin and/or des-acyl ghrelin, and GHS-R agonists, may rescue damaged β cells and even endothelial progenitor cell function in individuals with type 2 DM,⁴⁸ while GOAT inhibitors, immunization against acyl ghrelin/acyl ghrelin antibodies, des-acyl ghrelin, and GHS-R antagonists, may be useful in the treatment of hyperphagic feeding and accelerated gastrointestinal motility in the early stage of DM. Conversely, GOAT enhancers, acyl ghrelin, as well as its mimetics and GHS-R agonists, may provide therapeutic targets in the treatment of diabetic anorexia-cachexia and gastroparesis in the late stage of DM. In conclusion, manipulating the unique GOAT/ghrelin/GHS-R system may provide relevant approaches to prevent, ameliorate and treat disturbance of ingestive behavior and gastrointestinal motility in human DM.

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Centrally Administered Neuromedin S Inhibits Feeding Behavior and Gastroduodenal Motility in Mice

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Key words

- ◊ neuromedin S
- ◊ gastroduodenal motility
- ◊ gastric emptying
- ◊ feeding behavior
- ◊ mice

Abstract

Neuromedin S (NMS) was recently identified as an endogenous ligand for the FM-4/TGR-1 receptor in the rat hypothalamus. No previous studies have examined the effect of NMS on gut motility. We examined the effects of intracerebroventricular administration of NMS on food intake in food-deprived and free-feeding mice, and on

gastroduodenal motility by using a manometric method, and gastric emptying in mice. We found that NMS decreased food intake and the gastric emptying rate. It also disrupted the motor activity in the antrum and duodenum of conscious food-deprived mice. These results suggest that NMS influences gut motility as well as feeding behavior.

Introduction

Neuromedin S (NMS), a novel 36-amino acid peptide, was recently isolated from rat brain and identified as an endogenous ligand for 2 orphan G-protein coupled receptors, FM-3/GPR66 and FM-4/TGR-1 [1], which are also known as neuromedin U (NMU) receptor 1 (NMUR-1) and 2 (NMUR-2), respectively [2]. NMUR-1 is located in a wide range of peripheral tissues such as the intestine, testis, pancreas, uterus, lung, and kidney. On the other hand, NMUR-2 expression is limited to discrete brain areas such as the paraventricular nucleus and arcuate nucleus [2–8]. Neuromedin U has been reported to be involved in various functions, including adrenocortical regulation and energy balance [9–11]. With regard to feeding behavior, centrally administered NMU has been reported to decrease food intake in rats [2, 12, 13]. Neuromedin S and NMU are encoded by 2 different genes [1]. Neuromedin S is homologous to the C-terminal 7-amino acid region of NMU, and a high NMS expression is found in the suprachiasmatic nucleus (SCN) [1]. The SCN is known as the pacemaker site to master circadian rhythm in mammals and is important for the regulation of energy balance [14–17]. Thus far, NMS has been known to be involved in feeding behavior [5, 11, 18], circadian rhythm [1, 5, 11], and stress responses [19]. However, little is known about the effects of NMS on gut motility. In the present study, we aimed to exam-

ine the effects of NMS on cumulative food intake in fed and fasting states and on gastrointestinal motility by using a manometric method and gastric emptying rate in mice.

Materials and Methods

Animal experiments

Male mice of the C57BL/6J mice (20–25 g, 8–10 weeks old; CLEA Japan, Tokyo, Japan) were used. They were individually housed in a regulated environment ($22 \pm 2^\circ\text{C}$, $55 \pm 10\%$ humidity, 12:12h light: dark cycle with lights on at 7 AM). Food and water were available ad libitum unless otherwise indicated. All experiments were approved by our university animal care committee. Mouse neuromedin S was purchased from Phoenix Pharmaceuticals Inc (CA, USA). Just before administration, drug was diluted with 4 μl of artificial cerebrospinal fluid (ACSF: NaCl, 138.9 mM; KCl, 3.4 mM; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.26 mM; NaHCO_3 , 4.0 mM; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.6 mM; and glucose, 5.6 mM) for intracerebroventricular (ICV) administration. Cannula implantation for ICV administration was performed as previously described [20–22].

Feeding tests

The effects of ICV administration of NMS on cumulative food intake were examined in food-deprived and free-feeding mice. Experiments

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were performed during light and dark phases [23]. Experiments on food-deprived mice in light phase were started at 9 AM. Before the experiments, mice were deprived of food for 16 h with free access to water. Experiments for dark phase food intake were started at 7 PM. Mice were given free access to food and water before the experiment. A standard diet (CE-2 containing 59 kcal % carbohydrate, 30 kcal % protein and 11 kcal % fat; CLEA Japan, Tokyo, Japan) was used. NMS (0.1, 0.3, and 1.0 nmol/mouse) was administered ICV and then the cumulative food intake was calculated for 20 min, 1 h, 2, 4, 8, 12, and 24 h in light phase, and for 20 min, 1 h, 2, 8, 12, and 24 h in dark phase.

Operation for motility

Mice were anesthetized with intraperitoneal (IP) administration of pentobarbital sodium (50 mg/kg body weight) and implanted with catheters for manometric recordings of antrum and duodenum. A polyurethane tube (I.D. 0.30×O.D. 0.84 mm, Eicom, Kyoto, Japan) was inserted through the gastric fistula and the tip was placed at the gastric antrum, and at the same time, a polyurethane tube was inserted through the duodenal wall and the tip was placed at 7 mm from the pylorus. The tube was fixed at the gastric wall and duodenal wall by purse-string suture, run subcutaneously to emerge at the top of the neck, and then secured at the neck skin of the mice. Mice were allowed to recover for 1 week before the experiment of measuring the gastroduodenal motility.

Measurement of gastroduodenal motility

Mice were deprived of food, but not water for 16 h before the experiment. On the day of the experiment, the manometric catheters from the stomach and duodenum were connected to the infusion swivel (375/D/20, Instech Laboratories, PA, USA) on the single-axis counter-weighted swivel mount (TBS-23, Eicom, Kyoto, Japan) to allow free movement, and then joined to a pressure transducer (TP-400T, Nihon Koden Kogyo, Tokyo, Japan). The manometric catheters were continuously infused with bubble-free distilled water at the rate of 0.15 ml/h by an infusion pump (NE-1600, New ERA Pump System, Inc. NY, USA) so that the system used infused manometry, not solid-state manometry. The data were recorded and stored in a PowerLab (AD Instruments, CO, USA). The mice were placed in the black box (150×200×300 mm) with the top open. In this experiment, basal motor patterns in the antrum and duodenum were monitored for 60 min under the fasted state, then NMS (0.1 and 1.0 nmol/mouse) was administered ICV, and motility was monitored for 150 min after ICV administration. At the end of the experiments measuring gut motility, animals were euthanized by IP administration of excess doses of pentobarbital.

Gastric emptying

Before the experiments in gastric emptying, mice were deprived of food for 16 h with free access to water. The fasted mice had free access to preweighed pellets for 1 h; they were then administered ICV with NMS (0.3 and 1.0 nmol/mouse). The mice were deprived of food again for 2 h after administration. Food intake was measured by weighing the uneaten pellets. Mice were killed by cervical dislocation 3 h after the start of experiments. Immediately after, the stomach was exposed by laparotomy, quickly ligated at both the pylorus and cardia, then removed and the dry content was weighed. Contents were dried by a vacuum freeze drying system (Model 76705, Labconco Corp, MO, USA). Gastric

emptying was calculated according to the following formula: gastric emptying (%) = $[1 - (\text{dry weight of food recovered from the stomach} / \text{weight of food intake})] \times 100$.

Statistical analysis

Results are expressed as means ± SEM. Analysis of variance followed by Bonferroni's *t*-test were used to assess differences among groups. A *p*-value < 0.05 was considered to be statistically significant.

Results

Effect of ICV administration of NMS on food intake

In food-deprived mice, ICV administration of NMS (0.1, 0.3, and 1.0 nmol/mouse) significantly decreased light phase food intake in a dose dependent manner. This anorexigenic action was apparent by 20 min and continued for 24 h after ICV administration (○ Fig. 1a). In free-feeding mice, ICV administration of NMS (0.1, 0.3, and 1.0 nmol/mouse) significantly decreased dark phase food intake. This anorexigenic action was apparent by 1 h and continued for 4 h after ICV administration (○ Fig. 1b).

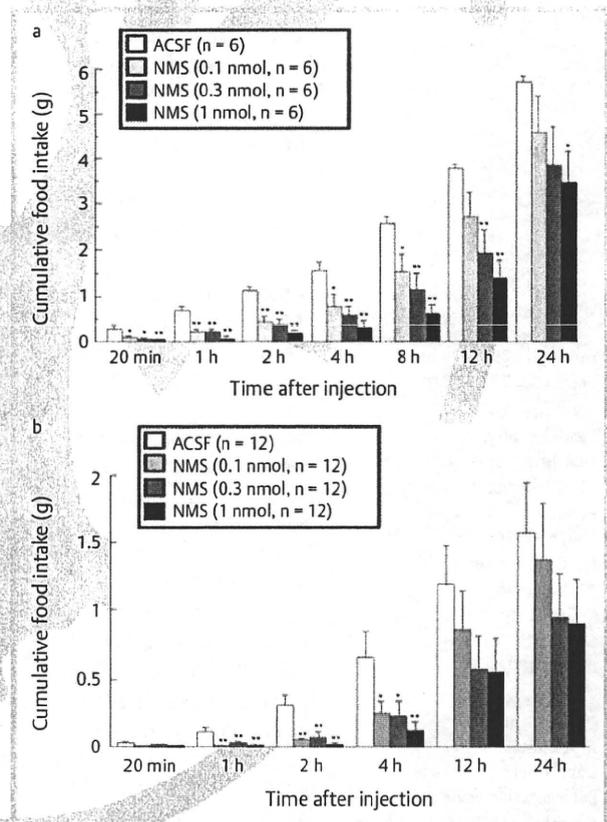


Fig. 1 a: Effects of intracerebroventricular (ICV) administration of neuromedin S (NMS) (0.1, 0.3, and 1 nmol/mouse) on cumulative food intake in food deprived mice. b: Effects of ICV administration of NMS (0.1, 0.3, and 1 nmol/mouse) on cumulative food intake in nonfood deprived mice. Results are expressed as mean ± SEM; n indicates the number of mice used. **p* < 0.05, ***p* < 0.01 compared with artificial cerebrospinal fluid (ACSF) treated control by Bonferroni's *t*-test.

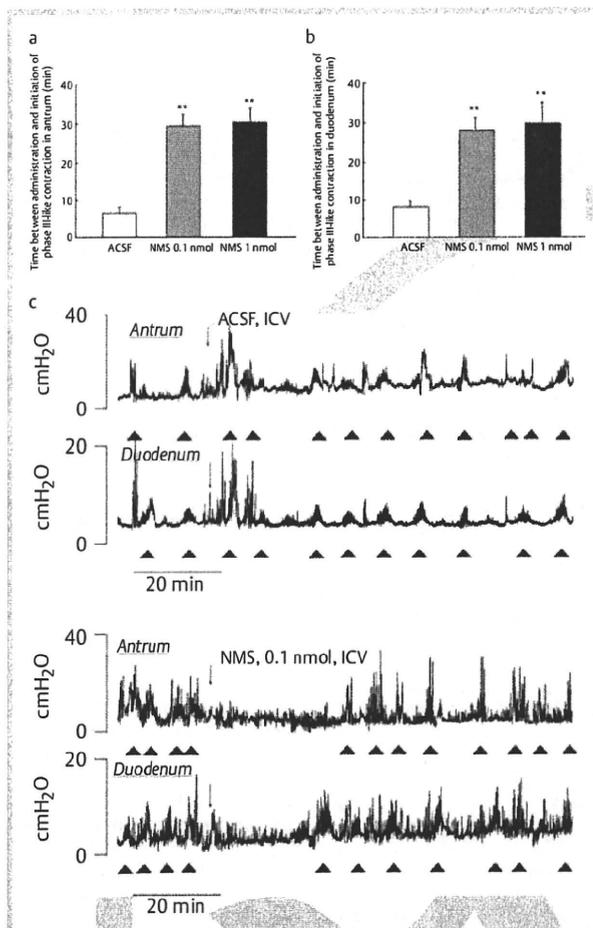


Fig. 2 Effects of intracerebroventricular (ICV) administration of neuromedin S (NMS) (0.1 and 1 nmol/mouse) on the fasted motor activity in the antrum (a) and duodenum (b). Time between the administration of increasing doses of NMS and the initiation of phase-like contractions in the antrum and duodenum. c: Representative tracing of antral and duodenal motility in fasted mice treated with ICV administration of NMS (0.1 nmol/mouse) or artificial cerebrospinal fluid (ACSF). The fasted motor pattern in the antrum and duodenum were disrupted by ICV administration of NMS. The phase-like contractions in fasted motility are indicated by arrowheads. Results are expressed as mean \pm SEM ($n = 4-9$); n indicates the number of mice used. ** $p < 0.01$ compared with ACSF treated control by Bonferroni's t -test.

Effect of ICV administration of NMS on gastroduodenal motility

When ICV administration of NMS (0.1 and 1.0 nmol/mouse) was given in the fasted state, the fasted motor pattern observed in the antrum and duodenum was replaced by the fed motor pattern, which consisted of irregular contractions that continued for 29.3 ± 2.9 min (0.1 nmol/mouse, $n = 4$) and 30.4 ± 3.5 min (1 nmol/mouse, $n = 9$) in the antrum and for 28.1 ± 3.0 min (0.1 nmol/mouse, $n = 4$) and 29.7 ± 5.2 min (1 nmol/mouse, $n = 9$) in the duodenum. These values were significantly longer ($p < 0.01$) compared with ICV administration of ACSF in the antrum (6.5 ± 1.6 min, $n = 8$) and duodenum (8.0 ± 1.7 min, $n = 8$) (○ Fig. 2a, b). Representative tracing of antral and duodenal motility in fasted mice treated with ICV administration of NMS (0.1 nmol/mouse) is shown in ○ Fig. 2c.

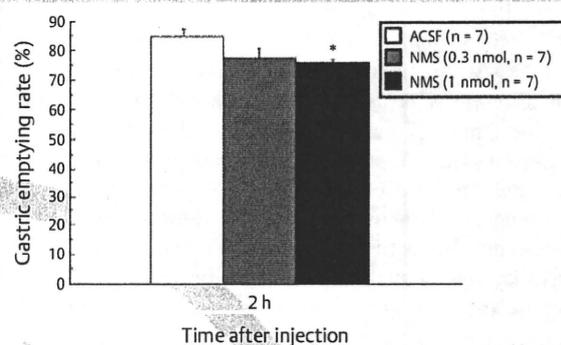


Fig. 3 Effects of intracerebroventricular (ICV) administration of neuromedin S (NMS) (0.3 and 1.0 nmol/mouse) on gastric emptying rate 2 h after administration. Results are expressed as mean \pm SEM; n indicates the number of mice used. * $p < 0.05$ compared with artificial cerebrospinal fluid (ACSF) treated control by Bonferroni's t -test.

Effect of ICV administration of NMS on gastric emptying

Neuromedin S (1.0 nmol/mouse) produced a significant decrease in gastric emptying rate 2 h after ICV administration compared with ACSF treated control [$75.8 \pm 5.2\%$ vs. $84.7 \pm 3.0\%$ (control); $p < 0.05$] (○ Fig. 3). Administration of 0.3 nmol/mouse had a tendency to decrease gastric emptying 2 h after ICV administration, although this failed to reach statistical significance ($77.5 \pm 1.3\%$; $p < 0.07$).

Discussion

Feeding behavior is regulated by various hormones, including neuropeptide Y (NPY), leptin, and ghrelin. In particular, central neuropeptides in the hypothalamus play an important role in feeding behavior. In our study, centrally administered NMS decreased the food intake in both food-deprived mice and non-food-deprived mice. These results indicate that NMS has an anorexigenic activity, similar to those of other anorexigenic peptides such as corticotropin-releasing factor (CRF) peptides.

Very recently, Yayou et al. showed that centrally administered NMS increases plasma cortisol concentrations, thereby indicating that NMS participates in the control of the hypothalamo-pituitary-adrenal axis [24]. Additionally, it has been reported that central administration of NMS decreases food intake in food-deprived mice. The effects induced by NMS on feeding behavior were absent in NMUR-2-deficient mice [11]. Moreover, repeated administration of NMS was found to decrease body weight gain [25].

It has been shown that feeding behavior and gastrointestinal motility are closely related; feeding stimulatory peptides such as NPY and ghrelin induce motor activity in the gastrointestinal tract in food-deprived mice [26,27], whereas feeding inhibitory peptides such as CRF, urocortin [28-30], bombesin [31], and cholecystokinin [32] disrupt the motor activity in the gastrointestinal tract of food-deprived mice.

Very recently, we have reported manometric methods to measure the physiologically fed and fasted motor activities in the gastrointestinal tract of conscious mice [33]. When NMS was administered ICV in the food-deprived state, the patterns of antral and duodenal motility were disrupted and motor patterns like those observed in fed state were observed. These results indicate that

NMS influences gastroduodenal motility, similar to the effect of other feeding inhibitory peptides. In addition, it has been shown that the gastric emptying rate is decreased by the ICV administration of anorexigenic peptides such as those belonging to the CRF peptide family. In our study, the ICV administration of NMS decreased gastric emptying rate. Neuromedin S at the lower dose (0.1 nmol/mouse) affected fasted motor pattern in the antrum and duodenum, whereas NMS only at the higher dose (1.0 nmol/mouse) delayed gastric emptying in conscious mice. These findings may represent different sensitivity between manometric method and solid gastric emptying in detecting the inhibitory effects of anorexigenic peptides on the upper gut motility. In conclusion, we have described here for the first time the effects of NMS on gastroduodenal motility and gastric emptying in mice. Our findings indicate that NMS influences feeding behavior and gut motility. A better understanding of the role of NMS may provide an entirely new therapeutic approach for the treatment of various diseases, including obesity, diabetes mellitus, eating disorders, and functional gastrointestinal disorders, all of which have become increasingly prevalent globally.

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