Orally Administered Zinc Increases Food Intake via Vagal Stimulation in Rats1–3

Kousaku Ohinata,4,5* Masami Takemoto,4 Makoto Kawanago,4 Shuya Fushimi,4 Hitoshi Shirakawa,4 Tomoko Goto,4 Akihiro Asakawa,6 and Michio Komai4

4Department of Science of Food Function and Health, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555 Japan; 5Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan; and 6Department of Behavioral Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka Kagoshima 890-8520, Japan

Abstract

We investigated the role of zinc in food intake regulation using rats during early-stage zinc deficiency without decreased zinc concentrations in plasma and tissues. Plasma, liver, and hypothalamic zinc concentrations were not affected in male Sprague-Dawley rats fed a zinc-deficient (Zn-Def) diet for 3 d compared with the pair-fed control group, which was fed a zinc-sufficient diet to the intake of the Zn-Def diet. Zinc sulfate at a dose of 19 μmol/kg body weight was orally or intraperitoneally (i.p.) administered to rats fed a Zn-Def diet for 3 d and food intake was measured. We found that zinc stimulated food intake after oral but not i.p. administration. The mRNA expression of neuropeptide Y (NPY) and orexin in the hypothalamus significantly increased 3 h after oral but not i.p. administration of zinc. Pretreatment with an antagonist for the NPY Y1 receptor or the orexin OX1 receptor blocked orexigenic activity after oral administration of zinc. The stimulation of food intake by oral administration of zinc was abolished by vagotomy. Taken together, orally administered zinc may stimulate food intake via orexigenic peptides coupled to the afferent vagal stimulation in rats after short-term treatment with a Zn-Def diet. J. Nutr. 139: 611–616, 2009.

Introduction

Zinc, one of the essential trace elements, is required in humans and animals for many physiological functions such as growth, immune function, and reproduction (1,2). Zinc deficiency induces a number of physiological problems, including anorexia, growth retardation, dermatitis, taste disorder, and hypogonadism (1–8). Within 3–5 d after feeding a zinc-deficient (Zn-Def)7 diet, food intake is suppressed (1,3). This suppression of food intake is the first sign of zinc deficiency and thereafter, other symptoms associated with zinc deficiency occur (1), suggesting that zinc plays an important role in food intake regulation. In the current study, we found that orally but not intraperitoneally (i.p.) administered zinc stimulated food intake in rats during early-stage zinc deficiency, which did not reduce zinc concentrations in plasma and tissues. A number of hypothalamic peptides are known to regulate food intake (9–12). We investigated whether they were involved in orexigenic activity after oral administration of zinc. Furthermore, we performed a vagotomy to examine whether orexigenic activity of zinc was mediated by the afferent vagal stimulation.

Materials and Methods

Rats and diets. Four-week-old male Sprague-Dawley rats (SLC) were used. Each rat was individually housed under regulated conditions (22°C on a 12-h-light/12-h-dark cycle, lights on 0800–2000) and consumed ad libitum a commercial pelleted diet (F-2, Funabashi Farms; protein, 21%; fat, 5%; carbohydrate, 58%) and drinking water. After a 3-d acclimation, the rats were divided into 3 groups: Zn-Def, zinc-sufficient control (Zn-Suf), and pair-fed control. The experimental diets were prepared as previously described (Supplemental Table 1) (7,8). The Zn-Suf group consumed the Zn-Suf diet ad libitum. The pair-fed control was fed the Zn-Suf diet to each intake of Zn-Def rats the day before. Zinc concentrations in the Zn-Def and Zn-Suf diets were 11 μmol/kg (0.7 mg/kg) and 0.50 mmol/kg (33 mg/kg), respectively, measured by an atomic absorption spectrophotometer SAS-727 (SEIKO DenshiKogyo). Biotin was added to the basal diet, because egg white contains avidin, which combines tightly with dietary biotin and reduced biotin absorption from the intestine (7,8). Three or five days after beginning the experimental diets, rats were food-deprived for 3 h and killed by decapitation. Zinc concentrations were measured in plasma, liver, and hypothalamus. Food intake and body weight were measured every day. All procedures involving animals were performed and the animals were cared for in conformity with the institutional guidelines of Tohoku University.
Zinc administration. Three hours before the beginning of the dark period and 3 d after starting the Zn-Def diet, the rats were food deprived but given free access to water. One hour before the dark phase, each rat was orally i.p. administered with 19 μmol/kg (3.0 mg/kg) body weight of zinc sulfate (ZnSO₄) in saline. A gastric tube was used for oral administration of zinc. At the beginning of the nocturnal period (2000 h), the preweighed Zn-Def diet was given to each rat and the weight of the diet was measured at 20 min and 1, 2, and 3 h after administration. To compare increases in food intake 20 min after oral administrations of equimolar divergent cations (19 μmol/kg) in Zn-Def rats on d 3, ZnSO₄, MgSO₄, CaSO₄, FeSO₄, and CuSO₄ were orally administered at a dose of 3.0, 2.2, 2.5, 2.8, and 3.0 mg/kg body weight, respectively, and food intake was measured. Furthermore, to investigate the effect of zinc administration on mRNA expressions of hypothalamic peptides, rats were fed a Zn-Def diet for 3 d, zinc sulfate in saline was administered after food deprivation for 2 h, and the rats were then killed by decapitation to prepare RNA from the hypothalamus 3 h after zinc administration.

RNA preparation from hypothalamus and quantitative RT-PCR. The hypothalamic tissues were cut using an ice-cold brain slicer (RBS-1, Aster Industries). We prepared 4-mm-thick slices centered at about −3.1 mm (anterior to bregma) and the hypothalamic tissues were dissected as rectangles from the slices, based on a rat brain atlas (13). The dissected hypothalamus was immediately soaked in RNAlater RNA Stabilization reagent (Qiagen) according to the instruction manual and kept at −20°C until RNA extraction. RNA preparation and quantitative RT-PCR were performed as described previously (14). Total RNA was isolated from the hypothalamus using the guanidine-isothiocyanate–based reagent Isogen (Nippon Gene). For cDNA synthesis, 2 μl of total RNA was incubated in RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L dithiothreitol) containing 50 units of SuperscriptIII RT, 40 units of RT buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, and 5 mmol/L CaCl₂, 0.6 mmol/L NaHCO₃, 0.6 mmol/L NaH₂PO₄, 5.6 mmol/L glucose, pH 7.4) was administered 30 min before zinc administration [19 μmol/kg, per os (p.o.),] and thereafter food intake was measured. After the experiment, cannula placement was confirmed by i.c.v. administration of dye. SB334867 (31 μmol/kg, i.p.) in 0.5% methylcellulose saline was administered 30 min before zinc administration (19 μmol/kg, p.o.) to rats fed the Zn-Def diet for 3 d and food intake was similarly measured.

Truncal vagotomy. To test whether feeding behavior induced by zinc administration was mediated by the afferent vagus nerve, we used vagotomized rats (16). Rats were anesthetized with sodium pentobarbital at a dose of 0.20 mmol/kg i.p. After incising the midline of the abdominal wall, the lower part of the esophagus was exposed, and the anterior and posterior branches of the vagal nerve were cut above the hepatic and celiac branches. In sham-operated rats, the vagal trunks were similarly exposed but not cut. At the end of the operation, the abdominal wall was sutured. After a 3-d postoperative recovery, rats were fed the Zn-Def diet for 4 d. Zinc sulfate at a dose of 19 μmol/kg was orally administered 4 d after feeding the Zn-Def diet and food intake was measured.

Statistical analysis. Values are expressed as means ± SEM. Statistical comparisons between each group were performed using 1-way ANOVA followed by Tukey’s multiple test unless otherwise mentioned. The effect of i.p. or p.o. administration of zinc sulfate on food intake was tested by 2-way repeated-measures ANOVA with Tukey’s multiple test when the interaction was significant. The effect of vagotomy or an antagonist for NPY or orexin on orexigenic activity after zinc administration was assessed by 3-way repeated-measures ANOVA followed by Tukey’s multiple test. P < 0.05 was considered significant.

Results

Food intake and body weight during early-stage zinc deficiency. Daily food intake began to decrease 2 d after feeding the Zn-Def diet and a temporary decrease occurred on d 3 (Fig. 1). Thereafter, food intake in the Zn-Def group recovered nearly to that in the Zn-Suf control group. Anorexia from zinc deficiency is characterized by a cyclical 3- to 4-d pattern of decreased food consumption (1,3) and we confirmed this typical cyclic pattern of food intake in Zn-Def rats in a previous study (17) as well as in this study (Fig. 2). Body weight decreased on d 3, accompanied by the suppression of food intake (Fig. 1). Although body weight decreased in Zn-Def rats compared with Zn-Suf control rats, body weight did not differ between Zn-Def and pair-fed control rats, suggesting that the decrease in body weight of the Zn-Def rats could be explained by suppression of food intake by zinc deficiency.

FIGURE 1 Effect of zinc deficiency on food intake (A) and body weight (B) in rats. Values are presented as means ± SEM, n = 6. *Different from Zn-Suf, P < 0.05.

Antagonist study. To investigate the mechanism underlying the stimulation of food intake after oral administration of zinc, we used the NPY Y₁ receptor antagonist, BIBO3304, which was generously supplied by Boehringer-Ingelheim Pharma (Rheinland Pfalz), and the orexin OX1 receptor antagonist, SB334867 (Tocris Bioscience) (15). For intracerebroventricular (i.c.v.) administration of BIBO3304, rats were stereotaxically implanted with guide cannulas (AG-S, Eicom) under sodium pentobarbital anesthesia (0.20 mmol/kg, i.p.) in the 3rd cerebral ventricle (antero–0.8 mm; lateral, 0.0 mm; ventral, −4.8 mm from bregma). The 5μL artificial cerebrospinal fluid (ACSF; 138.9 mmol/l NaCl, 3.4 mmol/l KCl, 1.3 mmol/l CaCl₂, 4.0 mmol/l NaHCO₃, 0.6 mmol/l NaH₂PO₄, 5.6 mmol/l glucose, pH 7.4) was administered 30 min before zinc administration [19 μmol/kg, per os (p.o.),] and thereafter food intake was measured. After the experiment, cannula placement was confirmed by i.c.v. administration of dye. SB334867 (31 μmol/kg, i.p.) in 0.5% methylcellulose saline was administered 30 min before zinc administration (19 μmol/kg, p.o.) to rats fed the Zn-Def diet for 3 d and food intake was similarly measured.

Truncal vagotomy. To test whether feeding behavior induced by zinc administration was mediated by the afferent vagus nerve, we used vagotomized rats (16). Rats were anesthetized with sodium pentobarbital at a dose of 0.20 mmol/kg i.p. After incising the midline of the abdominal wall, the lower part of the esophagus was exposed, and the anterior and posterior branches of the vagal nerve were cut above the hepatic and celiac branches. In sham-operated rats, the vagal trunks were similarly exposed but not cut. At the end of the operation, the abdominal wall was sutured. After a 3-d postoperative recovery, rats were fed the Zn-Def diet for 4 d. Zinc sulfate at a dose of 19 μmol/kg was orally administered 4 d after feeding the Zn-Def diet and food intake was measured.
Plasma zinc concentrations were not significantly decreased in the Zn-Def group compared with pair-fed control group on d 3, but they were decreased on d 5 (Fig. 3), suggesting that food intake was suppressed on d 3 without decreasing plasma zinc concentrations and that these rats may be in the very early stage of zinc deficiency. Liver and hypothalamic zinc concentrations in each group did not change on d 3 and 5, although the decrease in food intake had already occurred. These results suggest that food intake on d 3 and 5 are independent of changes in both plasma and hypothalamic zinc concentrations. To investigate the role of zinc in food intake regulation, we used rats fed a Zn-Def diet for 3 d during early-stage zinc deficiency without decreasing zinc concentrations in plasma and tissues.

Oral but not i.p. administration of zinc stimulates food intake. Orally administered zinc sulfate at a dose of 19 μmol/kg significantly stimulated food intake in rats fed a Zn-Def diet for 3 d (Fig. 4); interestingly, an i.p. injection had no effect under our experimental conditions. Both oral and i.p. administration of zinc sulfate similarly increased plasma zinc concentrations (data not shown). We also confirmed that a Zn-Suf diet containing zinc chloride acutely stimulated food intake after short-term zinc deprivation (data not shown). Other divalent cations, including Mg^{2+}, Ca^{2+}, Fe^{2+}, and Cu^{2+}, at the same molar concentrations as Zn^{2+} did not stimulate food intake after oral administration after zinc deprivation for 3 d (Fig. 5). These results suggest that the orexigenic activity of zinc after short-term zinc deficiency is specific to zinc.

We investigated whether orally administered zinc changed the mRNA expression of hypothalamic peptides regulating food intake. Orally administered zinc sulfate increased mRNA expressions of orexigenic peptides such as NPY and orexin 3 h after administration (Fig. 6A). In contrast, mRNA expression of anorexigenic proopiomelanocortin decreased (Fig. 6B). The i.p.-administered zinc did not affect mRNA levels of hypothalamic peptides, which was consistent with the result that an i.p. injection of zinc sulfate did not increase food intake. These results indicate that orally administered zinc can induce mRNA expressions of the hypothalamic peptides stimulating food intake in mildly Zn-Def rats. Because food intake stimulation occurred 20 min after zinc administration, orexigenic peptides such as NPY and orexin were probably beginning to be released from the secretory granule in their neurons just after administration and their mRNA expression and peptide synthesis might have increased thereafter.

**Orexigenic activity of zinc was mediated by NPY and orexin.** We investigated whether food intake stimulation by zinc was mediated by the hypothalamic orexigenic peptides such as NPY and orexin, the mRNA expression of which was increased after oral administration of zinc. NPY is the most potent orexigenic peptide in the hypothalamus, and the NPY Y1 receptor is known predominantly to mediate the orexigenic activity of NPY among 5 receptor subtypes for NPY (11,12). An antagonist for NPY Y1 receptor (5 nmol/mouse, i.c.v.) blocked the orexigenic activity of zinc after oral administration at a dose of 19 μmol/kg (Fig. 7A). These results suggest that orally administered zinc stimulates food intake by activating the NPY Y1 receptor.
Orexin is also an orexigenic peptide in the hypothalamus, and the orexin OX1 receptor is known to mediate its orexigenic activity (15). Pretreatment with an orexin OX1 antagonist, SB334867 (31 μmol/kg, i.p.), abolished the orexigenic activity of zinc after oral administration (Fig. 7B), suggesting that zinc-induced stimulation of food intake was mediated by the OX1 receptor. Taken together, oral administration of zinc may stimulate food intake through the NPY Y1 and orexin OX1 receptors.

**Zinc signal was mediated by the vagus nerve.** The afferent vagus nerve sends peripheral energy conditions to the central nervous system (9–12). We thus tested whether the increase in food intake after oral administration of zinc was mediated by the vagus nerve using vagotomized rats. In sham-operated rats, oral administration of zinc stimulated food intake (Fig. 8); however, in vagotomized rats, zinc and saline administrations did not differ. These results suggest that orally administered zinc stimulates food intake through the afferent vagus nerve followed by activating hypothalamic orexigenic peptides.

---

**FIGURE 5** Comparison of increases in food intake 20 min after oral administrations of equimolar divalent cations in Zn-Def rats on d 3. Values are presented as means ± SEM, n = 4. *Different from saline control, P < 0.05.

**FIGURE 6** Effect of zinc administration on mRNA expressions of hypothalamic peptides associated with food intake regulation (A, orexigenic peptides; B, anorexigenic peptides). Values are presented as means ± SEM, n = 6–7. Means without a common letter differ, P < 0.05.

**FIGURE 7** Effects of antagonists for NPY Y1 receptor (A) and orexin OX1 receptor (B) on food intake after oral administration of zinc in rats fed a Zn-Def diet for 3 d. Values are presented as means ± SEM, n = 4. *Different from the ACSF + saline control group, P < 0.05; #ACSF + zinc group vs. the antagonist + zinc group, P < 0.05.

**FIGURE 8** Effect of vagotomy on food intake after oral administration of zinc in rats fed a Zn-Def diet for 3 d. Values are presented as means ± SEM, n = 10–11. **Different from saline control, P < 0.01.
Discussion

We found for the first time, to our knowledge, that orally but not i.p.-administered zinc rapidly stimulates food intake through orexigenic peptides coupled to the afferent vagus nerve. Plasma zinc concentrations after oral administration of zinc did not differ from that after i.p. administration. These results suggest that the absorption process of zinc across the gastrointestinal tract is necessary for orexigenic activity after oral administration of zinc. Other divergent cations at the same molar concentrations as Zn\(^{2+}\) did not stimulate food intake after oral administration after short-term zinc deprivation (Fig. 5), suggesting that the orexigenic activity of zinc is specific to zinc. Zinc is largely bound to a number of proteins, including so-called zinc enzymes and zinc-finger proteins, for their functions in animals. Free zinc also exists in the nervous system and sometimes acts as a neurotransmitter or neuromodulator (18–20). Zinc-containing neurons have been reported (18,21). At present, the targets of zinc for activating the orexigenic system are unclear; however, zinc transporters and/or receptors present in the enteric nervous system may be candidates. Recently, it has been reported that zinc is able to activate GPR39, which is a 7-transmembrane G-protein–coupled receptor associated with food intake regulation and gastrointestinal motility in the enteric nervous system (22). Further investigations will elucidate the targets of orexigenic activity after oral administration of zinc and clarify the roles of the brain-gut axis in food intake regulation.

The vagal afferent pathways are involved in food intake regulation of peripheral peptides. For example, satiety signals, including cholecystokinin, glucagon-like peptide-1, peptide YY, and pancreatic polypeptide, during a meal originated from the gastrointestinal tract via the afferent vagus nerve, reach the nucleus tractus solitarius in the caudal brainstem (23,24). From the nucleus tractus solitarius, afferent fibers project into the arcuate nucleus (ARC), where NPY neurons exist. Ghrelin is an orexigenic peptide abundantly produced in the stomach (16,23). Orexigenic activity of ghrelin after peripheral administration was abolished by truncal vagotomy in rodents, suggesting that the ghrelin-induced orexigenic activity is mediated by the afferent vagus nerve (16,23). The orexigenic activity was also blocked by central administration of the NPY Y\(_1\) receptor antagonist (16,23). Thus, orexigenic activity of peripheral ghrelin may be mediated by the afferent vagus nerve followed by activation of the central NPY Y\(_1\) receptor (16,23). Hypothalamic NPY is primarily synthesized in the ARC neurons that project adjacent hypothalamic areas such as the paraventricular nucleus and lateral hypothalamic area. NPY neurons in the ARC project orexin neurons into the lateral hypothalamic area and i.c.v. administration of anti-orexin antisera attenuated NPY-induced feeding (25). Taken together, this suggests that food intake simulation by peripheral administration of zinc might activate the hypothalamic NPY Y\(_1\) receptor followed by the orexin OX\(_2\) system downstream of vagus nerve stimulation.

It was previously reported that NPY mRNA levels and extracellular NPY concentrations in the hypothalamus increased in Zn-Def rats on d 21 and 14, respectively, compared with pair-fed controls (5,6). In contrast, we found that the hypothalamic mRNA level of NPY was not higher in rats fed the Zn-Def diet on d 3 than that in pair-fed controls (K. Ohinata, M. Takemoto, M. Kawanaga, T. Goto, H. Shirakawa, and M. Komai, unpublished observation), suggesting that short-term zinc deprivation might not affect the hypothalamic NPY system.

Zinc deficiency in humans was initially reported by Prasad et al. (2). Many features of zinc deficiency in animal experiments were observed in anorexia nervosa (AN) patients, including anorexia, poor growth or weight loss, skin abnormalities, and amenorrhea (1). Clinical studies indicated that approximately one-half of all AN patients tested were zinc deficient and that zinc supplementation improved weight gain in AN patients (1,26). In addition to anorexia, brain dysfunctions such as memory consolidation impairment, anxiety, and depression has been reported in zinc deficiency (27,28) and the roles of dietary zinc in central functions should be clarified in animals and humans.

In conclusion, we found that orally but not i.p.-administered zinc stimulates food intake in short-term Zn-Def rats. The mRNA expressions of hypothalamic peptides, such as orexin and NPY, increased after oral administration of zinc to increase food intake. The orexigenic activity of zinc was blocked by the vagotomy operation. Taken together, our results indicate that zinc stimulates food intake in short-term Zn-Def rats through the afferent vagus nerve followed by activating the hypothalamic peptide associated with food intake regulation.

Literature Cited


