Title

Intracerebroventricular Administration of C-Type Natriuretic Peptide Suppresses Food Intake via Activation of the Melanocortin System in Mice.

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Intracerebroventricular Administration of C-Type Natriuretic Peptide Suppresses Food Intake via Activation of the Melanocortin System in Mice


C-type natriuretic peptide (CNP) and its receptor are abundantly distributed in the brain, especially in the arcuate nucleus (ARC) of the hypothalamus associated with regulating energy homeostasis. To elucidate the possible involvement of CNP in energy regulation, we examined the effects of intracerebroventricular administration of CNP on food intake in mice. The intracerebroventricular administration of CNP-22 and CNP-53 significantly suppressed food intake on 4-h refeeding after 48-h fasting. Next, intracerebroventricular administration of CNP-22 and CNP-53 significantly decreased nocturnal food intake. The increment of food intake induced by neuropeptide Y and ghrelin was markedly suppressed by intracerebroventricular administration of CNP-22 and CNP-53. When SHU9119, an antagonist for melanocortin-3 and melanocortin-4 receptors, was coadministered with CNP-53, the suppressive effect of CNP-53 on refeeding after 48-h fasting was significantly attenuated by SHU9119. Immunohistochemical analysis revealed that intracerebroventricular administration of CNP-53 markedly increased the number of c-Fos-positive cells in the ARC, paraventricular nucleus, dorsomedial hypothalamus, ventromedial hypothalamic nucleus, and lateral hypothalamus. In particular, c-Fos-positive cells in the ARC after intracerebroventricular administration of CNP-53 were coexpressed with α-melanocyte-stimulating hormone immunoreactivity. These results indicated that intracerebroventricular administration of CNP induces an anorexigenic action, in part, via activation of the melanocortin system. Diabetes 62:1500–1504, 2013

RESEARCH DESIGN AND METHODS

Animals and diets. Male C57BL/6J mice (6 weeks old) obtained from Japan SLCL (Shizuoka, Japan) were housed in plastic cages in a room kept at a room temperature of 23 ± 1°C and a 12:12-h light–dark cycle (lights turned on at 9:00 a.m.). The mice had ad libitum access to water and food (CE-2; CLEA Japan, Tokyo, Japan). All experiments were performed at 10 weeks of age in accordance with the guidelines established by the Institutional Animal Investigation Committee at Kyoto University and the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to optimize comfort and to minimize the use of animals.

Peptides. CNP-22, CNP-53, ghrelin, and NPY were purchased from Shanghai Peptide Institute (Osaka, Japan). SHU9119 was purchased from Bachem AG (Bubendorf, Switzerland).

Intracerebroventricular injection. Intracerebroventricular injection was performed according to our previous report (6).

Measurement of food intake

Fasting-refeeding. Mice were fasted for 48 h and then refed for 4 h. Water was available ad libitum during the experiments. The intracerebroventricular or intraperitoneal administration of CNP-22 or CNP-53 was performed just before refeeding. Food intake was measured for 4 h of refeeding. At the end of the experiments, the hypothalamus was collected for examination of the expression of mRNA for neuropeptides (7).

Nocturnal food intake. To assess the effect of intracerebroventricular administration of CNP-22 or CNP-53 on nocturnal food intake, peptides were injected intracerebroventricularly 1 h before the beginning of the dark phase. Food intake was measured for 15 h after intracerebroventricular injection. Water was available ad libitum during the experiments.

Food intake induced by NPY and ghrelin. The experiments were performed from 11:00 a.m. to 3:00 p.m. CNP-22 or CNP-53 was intracerebroventricularly administered just before intracerebroventricular injection of NPY (5 nmol/mouse) or intraperitoneal injection of ghrelin (100 nmol/kg). Food intake was measured for 4 h after peptide injection. In these experiments, food and water were available ad libitum.

PCR. The extraction of mRNA and quantitative real-time RT-PCR were performed according to our previous report (8). Primers for preproenkephalin, cocaine and amphetamine-related peptide, NPY, agouti gene-related peptide (AgRP) and glyceroldehyde 3-phosphate dehydrogenase are shown in Supplementary Table 1.

Immunohistochemistry for c-Fos and α-MSH in the hypothalamus. The immunohistochemical methods and the stereotaxic coordinates for the hypothalamic nuclei were based on our previous report (6). Briefly, mice were anesthetized with pentobarbital at 1 h after intracerebroventricular injection of CNP-53 (1.5 nmol/mouse) and perfused with 50 mL 0.1 mol/L PBS, followed by 50 mL ice-cold 4% paraformaldehyde in 0.1 mol/L PBS. Sections of 30-μm thickness were cut with a cryostat. According to the mouse brain atlas (9), cross-sections were selected in correspondence to −1.70 mm (ARC, lateral hypothalamus (LH), dorsomedial hypothalamus (DMH), ventromedial hypothalamic...
RESULTS

Effects of intracerebroventricular administration of CNP-22 and CNP-53 on food intake at refeeding after fasting. The intracerebroventricular administration of CNP-22 (1.5 and 4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) significantly suppressed food intake during 4-h refeeding after 48-h fasting in comparison with data from saline-treated mice (Fig. 1A). In this experiment, CNP-53 (1.5 nmol), but not other treatments, induced significant reduction of body weight compared with saline treatment (Supplementary Table 2). The mRNA expressions of preproopiomelanocortin and cocaine and amphetamine-related peptide significantly decreased, and the mRNA expressions of NPY and AgRP significantly increased after refeeding compared with control animals (Supplementary Fig. 1). The intracerebroventricular administration of CNP-53 did not influence the mRNA expressions of these neuropeptides in the hypothalamus (Supplementary Fig. 1). Next, the peripheral action of CNP on food intake was examined when a 10-fold greater dose than intracerebroventricular injection of each CNP was intraperitoneally administered. The intraperitoneal administrations of CNP-22 (1.5 μmol/kg) and CNP-53 (0.5 μmol/kg) did not change the food intake during 4-h refeeding after 48-h fasting (Fig. 1B), nor were there changes in body weight (Supplementary Table 3).

The intracerebroventricular administrations of CNP-22 (4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) at 1 h before the start of the dark phase significantly suppressed nocturnal food intake compared with saline treatment (Fig. 1C).

Effect of intracerebroventricular administration of CNP-22 and CNP-53 on NPY-induced and ghrelin-induced food intake. When CNP-22 (4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) were concomitantly administered intracerebroventricularly with NPY, they significantly suppressed the food intake induced by NPY compared with that of saline treatment (Fig. 2A). When CNP-22 (4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) were administered intracerebroventricularly with ghrelin, they significantly suppressed the food intake induced by ghrelin compared with that of saline treatment (Fig. 2B).

Effect of melanocortin receptor antagonist, SHU9119, on the anorectic effect of CNP. To examine its involvement in the anorectic effect of CNP, SHU9119 was administered intracerebroventricularly together with CNP-53 (1.5 nmol/mouse). SHU9119 (1 nmol/mouse) significantly attenuated the suppressive action of CNP-53 on the food intake during 4-h refeeding after 48-h fasting, whereas SHU9119 itself significantly enhanced the increase of food intake in comparison with mice administered saline treatment (Fig. 3).
whether c-Fos immunoreactivity coexisted with α-

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injection of CNP-53 in comparison with saline treatment 

were predominantly increased after intracerebroventricular 

of c-Fos 

tricular injection of CNP-53 (1.5 nmol/mouse). The numbers 

nohistochemical examination at 1 h after intracerebroven-

markers of neuronal activation, was monitored by immu-

rectic actions of CNP, the expression of c-Fos, one of the 

To understand the neuronal pathway involved in the ano-

B FIG. 2. Effects of CNP-22 and CNP-53 on food intake induced by NPY and ghrelin. A: Effects of intracerebroventricular administration of CNP-22 (4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) on NPY-

induced (5 nmol/mouse, intraperitoneal) food intake in mice. Food intake was observed for 4 h after coadministration of NPY and CNP. B: Effects of intracerebroventricular administration of CNP-22 

(4.5 nmol/mouse) and CNP-53 (1.5 nmol/mouse) on ghrelin-induced 

(100 nmol/kg, intraperitoneal) food intake in mice. Food intake was 

observed for 4 h after coadministration of ghrelin and CNP. Data re-

represent mean ± SEM. The number of mice is given in parentheses. Signi-

ficant differences: *P < 0.05, **P < 0.01.

c-Fos-immunoreactive cells in the hypothalamus after 

intracerebroventricular administration of CNP.

To understand the neuronal pathway involved in the ano-

erect actions of CNP, the expression of c-Fos, one of the 

markers of neuronal activation, was monitored by immu-

nolhistochemical examination at 1 h after intracerebroven-

tricular injection of CNP-53 (1.5 nmol/mouse). The numbers 

of c-Fos–immunoreactive cells in the ARC, PVN, and DMH 

were predominantly increased after intracerebroventricular 

injection of CNP-53 in comparison with saline treatment 

(Fig. 4A). The c-Fos–positive cells were also moderately 

increased in the VMH and LH (Fig. 4A). Next, we examined 

whether c-Fos immunoreactivity coexisted with α-MSH–

containing cells. In the ARC of saline-treated mice, only a 

few α-MSH–immunoreactive cells showed weak c-Fos im-

munoreactivity (Fig. 4B). However, c-Fos–immunoreactive 

cells that increased with intracerebroventricular administra-

tion of CNP-53 in the ARC expressed a large amount of 

α-MSH immunoreactivity (Fig. 4B).

DISCUSSION

The current study demonstrated that intracerebroventri-

cular administration of CNP-22 and CNP-53, but not in-

traperitoneal injection, led to significant reduction of food 

intake induced by fasting–refeeding. This reduction was 

inhibited by the melanocortin-3 receptor (MC3R)/melanocortin-

4 receptor (MC4R) antagonist SHU9119. In addition, CNP 

significantly suppressed nocturnal food intake and orexi-

genic actions induced by NPY and ghrelin. The immuno-

histochemical study revealed that intracerebroventricular 

administration of CNP-53 increased the number of c-Fos–

expressing cells containing α-MSH in the hypothalamus.

These findings indicated that the intracerebroventricular 

administration of CNP exhibits anorexigenic actions par-

tially via activation of the melanocortin system, although 

the doses of CNP used in the current study could be phar-

macological doses.

The hypothalamus is considered to be an important re-

gion in regulating energy homeostasis. In particular, the ARC 

in the hypothalamus contains both an orexigenic peptide, 

NPY, and an anorexigenic peptide, α-MSH, and is postu-

lated to be involved in the first-order regulation of food 

intake. Synthetic MC3R/MC4R agonists, melanotan II, and 

[Nle⁴-D-Phe⁷]–α-MSH completely blocked food deprivation–

induced increase in food intake as well as the food in-

take stimulated by intracerebroventricular administration 

of NPY (10,11). Regarding the reciprocal interactions of 

α-MSH and NPY, melanocortin neurons in the ARC 

project to the PVN (12). In the current study, intracere-

broventricular administration of CNP significantly sup-

pressed food intake after fasting, which was antagonized 

by SHU9119. Our results also showed that CNP suppressed 

NPY-induced food intake. Taken together, these findings 

indicate that CNP exhibits anorexigenic actions via acti-

vation of MC3R/MC4R downstream signaling. However, 

mRNA expressions of prepromelanocortin, cocaine and 

amphetamine-related peptide, NPY, and AgRP in the hy-

pothalamus after the intracerebroventricular injection of 

CNP-53 in fasting–refeeding experiment did not change 

compared with those after saline. The reason for this
discrepancy may lie in the experimental condition, time course, and regional specificity. To clarify this discrepancy, further examinations will be required.

This study demonstrated that the intracerebroventricular administration of CNP significantly suppressed the nocturnal food intake. Robust feeding during the nocturnal phase of the daily light–dark cycle was demonstrated to be attributed to the upregulation of NPY and its receptors (13). These findings indicate that CNP may decrease food intake in the nocturnal phase via suppression of NPY action.

In the current study, CNP significantly suppressed the increase in food intake induced by ghrelin, an orexigenic hormone secreted by the stomach (14). NPR-B, a C-type natriuretic peptide receptor, has been identified in appetite-regulating regions, such as the ARC, VMH, PVN, DMH, and LH (15). The systemic administration of ghrelin significantly increased NPY and AgRP expression in the ARC of the hypothalamus in fed and fasted rats (15), resulting in hyperphagia. The intracerebroventricular injection of melanotan II caused a significant decrease in ghrelin-induced food intake (16). These findings suggest that the actions of ghrelin are modulated by α-MSH and NPY systems. Furthermore, plasma ghrelin and hypothalamic ghrelin receptor mRNA expression are reported to be increased after fasting (17,18). These findings suggest the possibility that intracerebroventricular administration of CNP activates the melanocortin system, which subsequently inhibits the action of NPY, resulting in a reduced increase of food intake induced by ghrelin.

To assess which hypothalamic nucleus is involved in the anorexigenic action of CNP, a marker for neuronal activity, c-Fos expression in the hypothalamus was examined after intracerebroventricular administration of CNP-53. The intracerebroventricular administration of CNP-53 significantly increased the number of c-Fos–expressing cells in several hypothalamic nuclei, such as ARC, PVN, DMH, VMH, and LH, indicating that CNP-53 directly or indirectly stimulates neurons in these hypothalamic nuclei. Especially in the ARC, the result was an increased number of c-Fos–immunoreactive cells containing α-MSH immunoreactivity, indicating that CNP stimulates α-MSH–containing neurons. This possibility is supported by the finding that the suppressive action of CNP-53 on food intake was blocked by concomitant administration of SHU9119, an MC3R/MC4R antagonist.

The current study has demonstrated the anorexigenic action of intracerebroventricular administration of CNP via activation of the melanocortin system. To define the precise effect of CNP in the brain on food intake, further investigation using mice with inducible brain-specific deletion of CNP or NPR-B/NPR-C will be required.

From the present findings, we postulate the possible mechanism for anorexigenic action of exogenous CNP to be as follows: CNP directly or indirectly acts on α-MSH–containing neurons and subsequently stimulates α-MSH release, resulting in suppression of food intake induced by NPY and ghrelin. This possible mechanism may apply to the suppressive effects of CNP on food intake after fasting and in the nocturnal phase. Further work is needed to define the pathophysiological significance of brain CNP in regulation of food intake.

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No potential conflicts of interest relevant to this article were reported.

N.Y.-G. and G.K. performed experiments, contributed to discussion, and wrote the manuscript. K.E., M.I., Y.O., Y.Y., and E.K. contributed to discussion. K.N. contributed to discussion, and edited the manuscript. K.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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