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Research article

Involvement of central nesfatin-1 neurons on oxytocin-induced feeding suppression in rats

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HIGHLIGHTS

- Ip administration of OXT and OXT analog suppressed feeding in rats.
- Ip administration of OXT and OXT analog activated nesfatin-1 neurons in hypothalamus.
- Nesfatin-1 neurons in hypothalamus may be sensitive to peripheral OXT and analog.
- Feeding suppression induced by OXT and analog was attenuated by antisense nesfatin-1.

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ABSTRACT

Peripheral anorectic hormones, such as peptide YY (PYY) and oxytocin (OXT), suppress food intake. A newly identified anorectic neuropeptide, nesfatin-1, is synthesized in both peripheral tissue and the central nervous system, particularly by various nuclei in the hypothalamus and brainstem. Here, we examined the effects of intraperitoneal (ip) administration of PYY_{3-36}, OXT, and OXT analog, on nesfatin-1-immunoreactive (ir) neurons in the rat hypothalamus and brainstem, using Fos double fluorescence-immunohistochemistry. The ip administration of OXT and OXT analog significantly increased the number of nesfatin-1-ir neurons expressing Fos-ir in the paraventricular nucleus, the arcuate nucleus, and the nucleus tractus solitarius, but not in the supraoptic nucleus, the lateral hypothalamic area, and the area postrema. No differences in the percentage of nesfatin-1-ir neurons expressing Fos in the nuclei of the hypothalamus and brainstem were observed, between rats treated with vehicle or those treated with PYY_{3-36}. The decreased food intake, induced by OXT and OXT analog, was attenuated significantly by pretreatment with intracerebroventricular administration of antisense nesfatin-1. These results suggested that nesfatin-1-expressing neurons in the hypothalamus and brainstem may play a role in sensing the peripheral level of OXT and its suppression of feeding in rats.

1. Introduction

To date, numerous peptides that mediate feeding behavior have been identified. Peptide YY (PYY), secreted from endocrine L cells of the ileum, belongs to the neuropeptide Y (NPY) family and reduces food intake in rodents [1,2]. PYY_{3-36} is a selective agonist for the NPY-Y2 receptor located in the intestine (vagal-brainstem afferent signaling) and hypothalamus [1,2]. Oxytocin (OXT) is a nine-amino acid neuropeptide produced by hypothalamic OXT neurons; it is released locally in the pituitary. The systemic action of OXT is

Abbreviations: AP, area postrema; ARC, arcuate nucleus; Asu1,6l-OXT, deamino-dicarba-oxytocin; CRH, corticotrophin releasing hormone; FIHC, fluorescence immunohistochemistry; icv, intracerebroventricular; ip, intraperitoneal; ir, immunoreactive; mPVN, magnocellular division of the paraventricular nucleus; LHA, lateral hypothalamic area; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; OXT, oxytocin; PB, phosphate buffer; PBS, phosphate buffered saline; PFA, paraformaldehyde; pPVN, parvocellular division of the paraventricular nucleus; PVN, paraventricular nucleus; PYY, peptide YY; SON, supraoptic nucleus.

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known to mediate reproductive activities of females, including labor and lactation [3]. OXT is now recognized as having a central role in the neural circuits involved in social behavior, appetite, anxiety, and stress [4,5]. Recent studies have shown that intraperitoneal (ip) administration of OXT suppresses food intake [6–8].

Nesfatin-1 is a recently identified 82-amino acid anorectic neuropeptide, which is generated via post-translational cleavage from a precursor protein, nucleobindin-2, by proteolytic prohormone convertases [9]. Nesfatin-1 expression is modulated by starvation and refeeding [9]. Numerous studies have demonstrated that, in rodents, the central administration of nesfatin-1 rapidly and significantly decreases food intake. Further, the central administration of nesfatin-1 anti-serum results in increased food intake and weight gain in rodents [9–12]. Moreover, a peripheral injection of nesfatin-1 also decreases food intake [9,13,14].

In the present study, the effects of ip administration of PYY_{3-36}, OXT and OXT analog on nesfatin-1 neurons, were first examined in rats, using Fos with double-fluorescence immunohistochemistry (FIHC). Second, the effect of intracerebroventricular (icv) administration of antisense nesfatin-1 on the decreased food intake, which was induced by ip administration of OXT and OXT analog, were examined in rats.

2. Materials and methods

2.1. Animals

Nine-week-old adult male Wistar rats weighing 200–250 g were housed in plastic cages, under standard conditions, at 23–25 °C, with a 12:12 h light: dark cycle (lights on at 7:00 a.m.). Animals were fed a standard rat diet and had access to tap water *ad libitium.* All procedures in the present study were performed in accordance with the guidelines on the use and care of laboratory animals established by the Physiological Society of Japan and were approved by the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health, Japan.

2.2. Surgical procedures

For icv administration, animals were implanted with stainless steel cannulae targeting the lateral ventricle. They were anaesthetized (sodium pentobarbital, 50 mg/kg body weight, ip administration) and placed in a stereotaxic frame. Stainless steel guide cannulae (550 μm outer diameter and 10 mm length) were stereotaxically implanted at the following coordinates: 0.8 mm posterior to the Bregma, 1.4 mm lateral to the midline, and 2.0 mm below the surface of the left cortex, such that cannula tips were 1.0 mm above the left cerebral ventricle [15]. Two stainless steel anchoring screws and acrylic dental cement were used to secure cannulae in place. After the surgical procedure, animals were handled daily, individually housed in a plastic cage, and allowed to recover for at least 7 days. Thereafter, animals were handled daily and individually housed in metabolic cages for 2 days prior to the start of experiments.

2.3. Experimental procedure

In the first experiment, rats were divided into the following four groups: ip administration of vehicle (control group; n = 6), OXT (500 μg/kg; n = 6), deamino-dicarba-oxytocin [Asu1,6]-OXT; an OXT analog (500 μg/kg; n = 6), and PYY_{3-36} (60 μg/kg; n = 6) at 10:00 a.m. At 90 min after ip administration, animals were deeply anaesthetized with sodium pentobarbital (50 mg/kg body weight, ip administration) and tissues were harvested. The tissues were examined using FIHC, to evaluate the expression of Fos and nesfatin-1 in the supraoptic nucleus (SON), the paraventricular nucleus (PVN), magnocellular division of the PVN (mPVN), parvocellular division of the PVN (pPVN), the arcuate nucleus (ARC), and the lateral hypothalamic area (LHA) in the hypothalamic area, in addition to the area postrema (AP) and the nucleus tractus solitarius (NTS) in the brainstem.

In the second experiment, effects of the anorectic hormones on food intake were determined by measuring cumulative food intake after ip administration of each peptide in rats fasted for 24 h. The anorectic hormones were ip administration at 30 min before the start of the measurements.The measurements were commenced at 19.00 h, the start of the dark cycle. Food intake, water intake, and urine volumes were measured at 0.5, 1, 2, 3, 4, 5, 6, and 24 h after ip administration.

In the third experiment, we performed an icv administration of antisense nesfatin-1, as previously demonstrated, to confirm that the decreased food intake, induced by OXT (n = 6) and [Asu1,6]-OXT (n = 6), could be attenuated by inhibiting nesfatin-1 transcription. Rats that had been deprived of food for 24 h, were divided into the following four groups: icv administration of morpholino antisense RNA against nucleobindin-2 (8 μg in 4 μl saline vehicle; antisense nesfatin-1 sequence, 5′-ATGGTCCTCCACCTCATCTTCAGAG-3′; Lot No, N16K110020 Greiner Japan, Tokyo, Japan; n = 7) or morpholino missense RNA against nucleobindin-2 (8 μg in 4 μl saline vehicle; missense nesfatin-1 sequence, 5′-ATCGTGCTCCACGTCATCTACACAG-3′; Lot No, N16K110019 Greiner Japan, Tokyo, Japan; n = 6) [9,16], which were followed by 30 min before ip administration of either vehicle, OXT, or [Asu1,6]-OXT. The anorectic hormones were ip administration at 30 min before the start of the measurements. The measurements were commenced at 19.00 h, the start of the dark cycle. Food intake was then measured at 0.5, 1, 2, 3, 4, 5, 6, and 24 h after ip administration.

2.4. Fos and nesfatin-1 double-fluorescence immunohistochemistry

Animals were deeply anaesthetized, using ip administration of sodium pentobarbital (50 mg/kg), and were transcardially perfused with 100 mL of 0.1 M phosphate buffer (PB; pH 7.4), which contained heparin (1000 U/L) and 150 mL of fixative containing 4% paraformaldehyde (PFA) in 0.1 M PB. The brains were then removed and divided into blocks, including the hypothalamus and the brainstem, which were post-fixed with 4% PFA in 0.1 M PB for 48 h at 4 °C. The tissue was then cryoprotected in 20% sucrose in 0.1 M PB for 24 h at 4 °C. For immunofluorescence, serial sections (30 μm thick) were sliced using a microtome. Sections were rinsed twice with 0.1 M phosphate-buffered saline (PBS) and washed in 0.1 M Tris buffer (pH 7.6) containing 0.3% Triton X-100. For Fos FIHC, sections were incubated for 48 h at 4 °C in the primary antibody solution (rabbit anti-goat c-Fos, Santa Cruz Biotechnology; 1:500). The floating sections were then washed four times in 0.3% Triton X-100 in PBS and treated with a secondary antibody (Alexa Fluor 546 donkey anti-goat IgG; Molecular Probes, OR, USA; 1:2000 in PBS containing 0.3% Triton X-100) for 2 h at 4 °C. For nesfatin-1 FIHC, sections were incubated in a primary antibody solution (anti-rabbit nesfatin-1, Sigma-Aldrich; 1:5000) for 48 h at 4 °C. The floating sections were then washed four times in 0.3% Triton X-100/PBS and treated with a secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG; Molecular Probes, OR, USA; 1:1000 in PBS containing 0.3% Triton X-100) for 2 h at 4 °C. Fos-immunoreactive (ir) was observed as red-labelled nuclei (Fig. 1A, D), whereas nesfatin-1-ir expression was observed as green cytoplasmic precipitates (Fig. 1B, E). The sections were then washed twice in PBS, mounted using a glycerol/PBS (1:1) solution, and covered with coverslips.

Microphotographs were captured with a fluorescence microscope (Nikon) using appropriate excitation and emission filters,