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Effects of peripherally administered cholecystokinin-8 and secretin on feeding/drinking and oxytocin-mRFP1 fluorescence in transgenic rats

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ABSTRACT

Peripheral administration of cholecystokinin (CCK)-8 or secretin activates oxytocin (OXT)-secreting neurons in the hypothalamus. Although OXT is involved in the regulation of feeding behavior, detailed mechanism remains unclear. In the present study, we examined the central OXTergic pathways after intraperitoneally (i.p.) administration of CCK-8 and secretin using male OXT-monomeric red fluorescent protein 1 (mRFP1) transgenic rats and male Wistar rats. I.p. administration of CCK-8 (50 μg/kg) and secretin (100 μg/kg) decreased food intake in these rats. While i.p. administration of CCK-8 decreased water intake, i.p. administration of secretin increased water intake. Immunohistochemical study revealed that Fos-Like-Immunoreactive cells were observed abundantly in the brainstem and in the OXT neurons in the dorsal division of the parvocellular paraventricular nucleus (dpPVN). We could observe marked increase of mRFP1 fluorescence, as an indicator for OXT, in the dpPVN and mRFP1-positive granules in axon terminals of the dpPVN OXT neurons in the nucleus tractus solitarius (NTS) after i.p. administration of CCK-8 and secretin. These results provide us the evidence that, at least in part, i.p. administration of CCK-8 or secretin might be involved in the regulation of feeding/drinking via a OXTergic pathway from the dpPVN to the NTS.

1. Introduction

The neurohypophysial hormone, oxytocin (OXT), is mainly synthesized in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus. OXT-expressing neurons in the SON and the magnocellular division of the PVN (mPVN) project their axon terminals to the posterior pituitary (PP) and secrete OXT into the systemic circulation. Plasma OXT plays an essential role in mammalian labor and lactation through its peripheral actions. However, it has also been reported that OXT is released from the dendrites of magnocellular OXT-expressing neurons into the central nervous systems (CNS) (Ludwig and Leng, 2006; Pow and Morris, 1989), and parvocellular OXT-expressing neurons in the dorsal parvocellular division of the PVN (dpPVN) project their axon terminals to brainstem areas such as the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus nerve (DMV) (Sofroniew, 1980). In addition, previous studies have revealed that the effects of OXT on the CNS, such as maternal bonding, social affiliation, and feeding behavior (Higashida et al., 2010; Kublaoui et al., 2008; Leng et al., 2008; Pedersen et al., 2006).

Peripheral administration of cholecystokinin (CCK)-8, a gastrointestinal hormone, decreases food/water intake in rats (Ebenezer, 1996; Verbalis et al., 1986). Administration of CCK-8 activates neurons in the NTS and hypothalamic OXT-expressing neurons, resulting in an elevation of plasma OXT levels in rats (Katoh et al., 2014; Luckman, 1992; Olson et al., 1992; Renaud et al., 1987; Verbalis et al., 1986). Peripheral administration of secretin, which is also a gastrointestinal hormone, activates neurons in the NTS (Velmurugan et al., 2010; Yang et al., 2004) and OXT-secreting neurons in the SON with an elevation of plasma OXT level in rats (Velmurugan et al., 2010; Yang et al., 2004). Another group has reported that peripheral administration of secretin activated

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neurons in the PVN and decreased food intake in mice (Cheng et al., 2011). In addition, intracerebroventricularly (i.c.v.) administration of OXT suppressed food intake (Arletti et al., 1989, 1990; Olson et al., 1991). I.c.v. administered OXT receptor antagonist into the lateral or fourth ventricle contributes to the improvement of anorexia (Blevins et al., 2003; Ho et al., 2014; Olson et al., 1991; Rinaman and Rothe, 2002). Ong et al. have reported that OXT receptor in the medial NTS affected food intake (Ong et al., 2015). Taken together, it is considered that peripheral administration of CCK-8 and secretin may suppress food intake, at least in part, via a central OXTergic pathway, such as dpPVN-NTS.

We have previously generated transgenic rats bearing an OXT-monomeric red fluorescent protein 1 (mRFP1) fusion gene (Katoh et al., 2011). Because robust mRFP1-positive granules can be observed not only in the cell bodies but also in the axon terminals in the spinal cord (Matsuura et al., 2015), this transgenic animal is a powerful tool for elucidating physiological OXT mechanisms.

The effects of peripheral administration of secretin on food intake have not been invested in rats, though they have been invested in mice. In addition, although peripheral administration of CCK-8 and secretin up-regulated OXT neurons in the hypothalamus, neuronal pathways which induce anorexia have not been elucidated.

Thus, in the present study, we first examined the effects of intraperitoneally (i.p.) administered CCK-8 and secretin on food/water intake using adult male Wistar and OXT-mRFP1 transgenic rats. Next, using immunohistochemistry, induction of Fos-Like-Immunoreactive (LI)-cells in the hypothalamus and the brainstem were evaluated. mRFP1 fluorescence intensity, which is a quantitative indicator for OXT, was also measured in the SON and the PVN. Finally, mRFP1-positive granules in the NTS, which were presented in the axon terminals from the dpPVN OXT neurons, were counted manually to demonstrate the up-regulation of a OXTergic pathway from the hypothalamus to the brainstem.

2. Materials and methods

2.1. Animals

We used adult male Wistar rats (weighing 200–240 g) in the experiment to measure food/water intake, and adult male OXT-mRFP1 Wistar transgenic rats (weighing 220–430 g) in the experiments of fluorescence immunohistochemistry and mRFP1 fluorescence. The rats were housed in plastic cages under standard conditions in an animal room at 23–25 °C with a 12:12-h light:dark cycle (lights on at 7:00 am). The animals were fed a standard rat diet and tap water ad libitum. All animals were acclimated to the experimental conditions for 7 days by handling them daily and holding them in the position used for i.p. administration. All procedures 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. OXT-mRFP1 transgenic rats were screened by polymerase chain reactions using genomic DNA extracted from rat-ear biopsies, as described previously (Katoh et al., 2011, 2014).

2.2. Test substances

CCK-8 (Peptide Institute, Osaka, Japan) was dissolved in 0.9% sterile physiological saline (saline) (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) to a concentration of 10 μg/mL. Secretin (rat) (Tocris Bioscience, Bristol, UK) was dissolved in saline to a concentration of 20 μg/mL.

2.3. Experimental procedures

2.3.1. Food and water intake after i.p. administration of CCK-8 and secretin

Wistar rats were divided into three groups ($n = 6$ in each group) receiving i.p. administration of saline as a control, CCK-8 (50 μg/kg body weight), or secretin (100 μg/kg body weight). We used metabolic cages to measure cumulative food/water intake 0.5 h, 1 h, 1.5 h, 3 h, and 6 h after i.p. administration of saline, CCK-8, or secretin to conscious Wistar rats which were fasted for 17 h.

2.3.2. Immunohistochemistry for Fos protein in the hypothalamus and brainstem

OXT-mRFP1 transgenic rats were divided into three groups ($n = 6$ in each group) receiving i.p. administration of saline as a control, CCK-8 (50 μg/kg body weight), or secretin (100 μg/kg body weight). The rats were deeply anesthetized with i.p. administration of sodium pentobarbital (50 mg/kg) 1.5 h after i.p. administration of saline, CCK-8, or secretin. The rats were perfused transcardially with 0.1 M phosphate buffer (PB, pH 7.4) containing heparin (1000 U/L), followed by 4% paraformaldehyde (PFA) in 0.1 M PB. The brains were carefully removed, and the brains were divided into three blocks including the hypothalamus and brainstem.

The blocks were post-fixed with 4% PFA in 0.1 M PB for 48 h at 4 °C. The tissues were then cryoprotected in 20% sucrose in 0.1 M PB for 48 h at 4 °C. Fixed tissues were cut at 30-μm thickness with a microtome (REM-700; Yamato Kohki Industrial Co., Ltd., Saitama, Japan) containing the SON, PVN, area postrema (AP), NTS, and DMV. The locations of the regions were determined according to coordinates given in the rat brain atlas (Paxinos and Watson, 1998). The sections were rinsed twice with 0.1 M phosphate-buffered saline (PBS) and washed in 0.1 M PBS containing 0.3% Triton X-100. The sections were incubated for 3 days at 4 °C in a primary anti-Fos antibody solution (rabbit polyclonal c-Fos, Santa Cruz Biotechnology, #sc-52; 1:500 in PBS). After washing four times in 0.1 M PBS containing 0.3% Triton X-100, floating sections were treated for 2 h at 4 °C with a secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG; Molecular Probes, OR, USA, #A11034; 1:1000 in PBS). The sections were washed twice in PBS, mounted using a glycerol/PBS (1:1) solution, and coverslipped.

The sections were examined by a fluorescence microscopy (ECLIPSE Ti-E [FL/DIC]; Nikon Corporation, Tokyo, Japan) with a GFP filter (Nikon Corporation, Tokyo, Japan). Fos-LI appeared as green-labeled nuclei using a GFP filter. The images were captured with a digital camera (DS-Qi1Mc; Nikon Corporation, Tokyo, Japan). Fos-LI cells (green-colored round shapes) were counted manually and the average number of Fos-LI cells in the SON, mPVN, parvocellular division of the PVN (pPVN), AP, NTS, and DMV were quantified.

2.3.3. Observation of Fos-LI cells and OXT-mRFP1 fluorescence in the SON and PVN

The sections were examined by a fluorescent microscopy with a RFP filter (Nikon Corporation, Tokyo, Japan) in order to examine OXT-mRFP1 expression. The OXT-mRFP1 signal appeared as a red cytoplasmic precipitate using a RFP filter. The images were captured with a digital camera. The images obtained using a GFP filter were merged with those obtained using a RFP filter. The number of mRFP1-positive cells and the number of mRFP1-positive cells expressing Fos-LI cells were counted manually. The percentage of mRFP1-positive cells with Fos-LI was quantified in the SON, mPVN, and dpPVN.

2.3.4. Measurement of mRFP1 fluorescence intensity in the SON and PVN

OXT-mRFP1 transgenic rats were divided into three groups ($n = 6$ in each group) receiving i.p. administration of saline as a control,