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Activation of Supraoptic Oxytocin Neurons by Secretin Facilitates Social Recognition

Yuki Takayanagi, Masahide Yoshida, Akihide Takashima, Keiko Takanami, Shoma Yoshida, Katsuhiko Nishimori, Ichiko Nishijima, Hirotaka Sakamoto, Takanori Yamagata, and Tatsushi Onaka

ABSTRACT

BACKGROUND: Social recognition underlies social behavior in animals, and patients with psychiatric disorders associated with social deficits show abnormalities in social recognition. Oxytocin is implicated in social behavior and has received attention as an effective treatment for sociobehavioral deficits. Secretin receptor–deficient mice show deficits in social behavior. The relationship between oxytocin and secretin concerning social behavior remains to be determined.

METHODS: Expression of c-Fos in oxytocin neurons and release of oxytocin from their dendrites after secretin application were investigated. Social recognition was examined after intracerebroventricular or local injection of secretin, oxytocin, or an oxytocin receptor antagonist in rats, oxytocin receptor–deficient mice, and secretin receptor–deficient mice. Electron and light microscopic immunohistochemical analysis was also performed to determine whether oxytocin neurons extend their dendrites into the medial amygdala.

RESULTS: Supraoptic oxytocin neurons expressed the secretin receptor. Secretin activated supraoptic oxytocin neurons and facilitated oxytocin release from dendrites. Secretin increased acquisition of social recognition in an oxytocin receptor–dependent manner. Local application of secretin into the supraoptic nucleus facilitated social recognition, and this facilitation was blocked by an oxytocin receptor antagonist injected into, but not outside of, the medial amygdala. In the medial amygdala, dendrite-like thick oxytocin processes were found to extend from the supraoptic nucleus. Furthermore, oxytocin treatment restored deficits of social recognition in secretin receptor–deficient mice.

CONCLUSIONS: The results of our study demonstrate that secretin-induced dendritic oxytocin release from supraoptic neurons enhances social recognition. The newly defined secretin-oxytocin system may lead to a possible treatment for social deficits.

Keywords: Dendritic release, Medial amygdala, Oxytocin, Secretin, Social recognition, Supraoptic nucleus

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Oxytocin, a nonapeptide hormone, is primarily synthesized in magnocellular and parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) and in magnocellular neurons of the supraoptic nucleus (SON) in the hypothalamus, and it is modestly expressed in the bed nucleus of the stria terminalis (1,2). Oxytocin in magnocellular neurons is released from the neurohypophysis into the systemic circulation and has roles in parturition and lactation (3). Oxytocin is also released from axon terminals of parvocellular oxytocin neurons and from dendrites or cell bodies of magnocellular oxytocin neurons (4,5). The oxytocin receptor is distributed widely within the brain, including the olfactory bulb, basal ganglia, hypothalamus, limbic areas, and brainstem (3,6), suggesting that oxytocin has various functions in the central nervous system. Growing evidence has shown that oxytocin modulates social recognition and social behavior. Animal studies showed that oxytocin increases social interactions and is involved in partner preference and parental behavior (7–10). Oxytocin-deficient and oxytocin receptor–deficient mice show sociobehavioral deficits (11,12). Oxytocin has also been implicated in disorders associated with dysfunctional social behaviors, including autism spectrum disorders (10,13–15). For improving social deficits in humans, activation of endogenous oxytocin neurons, rather than exogenous oxytocin administration, the reported effects of which are controversial, has been proposed to be effective (16).

Secretin, a 27–amino acid peptide hormone that belongs to the vasoactive intestinal peptide/glucagon/pituitary adenylate cyclase–active polypeptide family (17), has also been suggested to play a role in the control of social behavior. Secretin is synthesized in various brain regions, including the hypothalamus, hippocampus, cerebellum, cerebral cortex, and brainstem (18–21). The secretin receptor is also distributed widely in the brain, including the brainstem, cerebellum, cerebral cortex, hypothalamus, and hippocampus (21–24). Secretin receptor–deficient mice have been suggested to have deficits in social behavior (25).

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Central administration of secretin increases oxytocin messenger RNA expression in the hypothalamus (21). However, the relationship between secretin and oxytocin in the regulation of social behavior is unknown. In this study, we demonstrated that secretin activated oxytocin neurons selectively in the SON and potentiated dendritic oxytocin release. We further investigated whether dendritically released oxytocin from the SON facilitated social recognition via the oxytocin receptor in the medial amygdala, where oxytocin has been shown to facilitate social recognition (26,27).

METHODS AND MATERIALS

Animals

Male Wistar rats and male and female C57BL/6J mice were obtained from Japan SLC, Inc. (Shizuoka, Japan), CLEA Japan, Inc. (Tokyo, Japan), or Charles River Laboratories Japan (Kanagawa, Japan). Oxytocin receptor–deficient male mice (12) and secretin receptor–deficient male mice (25) after backcrossing with C57BL/6J mice for >11 generations were used. Adult male animals were used in the present study except where specified otherwise. Juvenile male rats and ovariectomized female C57BL/6J mice were used as stimulus animals for the social recognition test, and 4-week-old male rats were used for experiments with isolated SON. The animals were housed in a room with controlled temperature (22 ± 2°C) and humidity (40%–70%) with a 12-hour light/dark cycle (lights on 7:30 AM–7:30 PM). Food and water were available ad libitum. All animals except the ones used in the experiment, for which results are shown in Figure 1B and C, were kept in individual cages. All animal procedures were approved by the Institutional Animal Experiment Committee of Jichi Medical University and were in accordance with the Institutional Regulations for Animal Experiments and Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

Social Recognition Test

A juvenile male rat (21–35 days old) or an ovariectomized female mouse (>10 weeks old) was presented to an adult male rat or mouse, respectively, for 4 minutes (training session). After an interval (30 minutes or 45 minutes), both the same stimulus animal (familiar) and another stimulus animal (novel) were presented for 4 minutes (test session). Social recognition was estimated by using a preference index ([time investigating novel animal]/[time investigating familiar animal + time investigating novel animal] × 100) (28). As social recognition lasts <40 minutes in rodents (29), a 45-minute interval between the training and the test was used to assess facilitative effects of drugs on social recognition. A 30-minute posttraining interval was used in tests for which results are shown in Figures 1B and C and 3H, and a 45-minute posttraining interval was used in tests for which results are shown in Figures 1D–F and 3A–C and E. Drugs were injected 10 minutes before the training session, unless otherwise specified.

Surgery and Intracerebroventricular Injection

Animals were anesthetized with intraperitoneal injection of 200 mg/kg tribromoethanol (Avertin; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and placed in a stereotactic frame. Stainless steel guide cannulae (23 gauge for rats or 25 gauge for mice) were inserted into the right lateral cerebral ventricle (coordinates for rats, .6 mm caudal to the bregma, 1.6 mm lateral to the midline, and 4.5 mm below the skull; coordinates for mice, .4 mm caudal to the bregma, 1.0 mm lateral to the midline, and 2.0 mm below the skull) and secured to the skull with screws and dental cement. Animals were allowed to recover in individual cages for 1–2 weeks (rats) or for 2–3 weeks (mice). Animals were given an intracerebroventricular (i.c.v.) injection (30-gauge needle for rats or 31-gauge needle for mice) of secretin (.1 μg/5 μL, 1 μg/5 μL, 10 μg/5 μL [rats (1 μg secretin for behavioral and immunohistochemistry experiments)] or 1 μg/2 μL [mice]) (Tocris Bioscience, Ellisville, Missouri [rats], Phoenix Pharmaceuticals, Inc., Burlingame, California [mouse]), a selective oxytocin receptor antagonist (1 μg/5 μL [rats]; des Gly-NH_2-d$(CH_2)_5$ [D-Tyr^2, Thr^4] OVT; kindly provided by M. Manning, University of Toledo College of Medicine, Toledo, Ohio) (30), oxytocin (1 ng/2 μL [mice]; Peptide Institute, Inc., Osaka, Japan), or artificial cerebrospinal fluid (vehicle; 138 mmol/L sodium chloride, 5 mmol/L potassium chloride, 1.5 mmol/L calcium chloride, 1 mmol/L magnesium chloride, 11 mmol/L sodium bicarbonate, 1 mmol/L sodium phosphate [pH 7.2]). The position of the injection needle tip was verified with methylene blue injected through the cannula after experiments.

Detection of Activated Neurons After Secretin Injection

For detection of neurons activated after secretin injection, male rats, male secretin receptor–deficient mice, or male oxytocin receptor–deficient mice were given an i.c.v. injection of secretin (1 μg/5 μL [rats] or 1 μg/2 μL [mice]) or the vehicle, anesthetized with intraperitoneal injection of 50 mg/kg pentobarbital (Nembutal; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) 100 minutes after the injection, and perfused transcardially with heparinized saline (20 U/mL) and then with 4% paraformaldehyde in .1 mol/L phosphate buffer (pH 7.4). The brains were removed, postfixed in 4% paraformaldehyde overnight, and transferred to 30% sucrose solution in .1 ml/L phosphate buffer until tissues sank. The brains were frozen on dry ice and stored at −80°C. Coronal brain sections were cut at 30 μm with a freezing sledge microtome. Every fourth (rats) or third (mice) section of the hypothalamus and medial amygdala was collected and processed for immunohistochemistry for c-Fos or oxytocin or both (2,31) (see Supplemental Methods and Materials for details).

X-gal Staining and Immunohistochemistry for Oxytocin

For detection of β-galactosidase activity in oxytocin neurons of the SON in secretin receptor–heterozygous mice, the mice were perfused transcardially with heparinized saline (20 U/mL) and then with 4% paraformaldehyde in .1 mol/L phosphate buffer. Brain sections were cut at 5 μm and processed for X-gal staining and immunohistochemical detection of oxytocin (see Supplemental Methods and Materials for details).