

論 文 要 旨

学 位 論 文 (要約)

表 題 社会的敗北ストレスにおけるオキシトシンの働き
Roles of Oxytocin-Oxytocin Receptor Systems in Physiological
Responses to Social Defeat Stress

申 請 者 氏 名 Naranbat Nasanbuyan

担当指導教員氏名 尾仲 達史 教授

所 属 自治医科大学大学院医学研究科
人間生物学系 専攻
生体制御医学 分野
神経生理学

論 文 要 旨

氏名 Naranbat Nasanbuyan

表題

社会的敗北ストレスにおけるオキシトシンの働き

Roles of Oxytocin-Oxytocin Receptor Systems in Physiological

Responses to Social Defeat Stress

1 研究目的

Social stress has deteriorating effects on various psychiatric disorders. In animal models, exposure to socially dominant conspecifics, i.e., social defeat stress, evokes species-specific defeat posture and freezing behavior. Underlying mechanisms are unknown.

Previous studies have suggested that the ventromedial hypothalamus and midbrain periaqueductal gray region have facilitative roles in the control of defensive behaviors in response to various threatening stimuli. However, there are heterogeneous neurons in the ventromedial hypothalamus and periaqueductal gray region. Details of physiological functions of each subtype in the ventromedial hypothalamus and periaqueductal gray region were poorly understood. Furthermore, neural pathways underlying expression of defensive behaviors remain unclear.

Oxytocin neurons are located mainly in the hypothalamic paraventricular nucleus, supraoptic nucleus and the bed nucleus of the stria terminalis. Oxytocin neurons have been shown to be activated by various types of stressful stimuli. Oxytocin plays an important role not only in uterine contraction during parturition and in milk-ejection reflex, but also in the control of stress or anxiety-related responses, social behavior and energy metabolism. Oxytocin administration has been shown to have pro-social, anxiolytic and anorexic actions, although controversial data have also been reported. For examples, oxytocin facilitates aggressive behavior and augments stress responses in some conditions. Underlying mechanisms of actions of oxytocin are largely unclear. Physiological roles of oxytocin during social defeat stress are not known.

I examined in mice whether social defeat stress activates oxytocin neurons and oxytocin receptor-expressing neurons. I also examined projecting fibers of oxytocin neurons of the paraventricular hypothalamic nucleus by using a viral tracer. I then examined roles of the oxytocin receptor during social defeat stress by using oxytocin receptor-deficient mice.

2 研究方法

Animals

Adult male C57BL/6J mice (CLEA Japan, Shiga, Japan), retired CD1 mice (Charles River Laboratories Japan, Kanagawa, Japan), oxytocin receptor-Venus knock-in mice (backcrossed with C57BL/6J mice), oxytocin-Ires Cre knock-in mice (backcrossed with C57BL/6J mice), Rosa-CAG-LSL-tdTomato-WPRE::deltaNeo knock-in reporter mice (C57BL/6J background), and oxytocin receptor-deficient mice (backcrossed with C57BL/6J mice) were used in this study. Oxytocin receptor-Venus knock-in mice express a variant of yellow fluorescent protein (Venus) under the control of the endogenous regulatory region of the oxytocin receptor gene.

The animals were housed in rooms with controlled temperature (20 - 24 °C) and humidity (40 - 70%) under a 12-hour light/dark cycle (lights on at 7:30 AM - 7:30 PM). Food and water were available *ad libitum*. 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 defeat stress

Social defeat stress was applied by 10 min exposures to aggressive CD1 mice. A mouse was placed as the intruder in the chamber of the aggressive resident male CD1 mouse and kept there for 10 min. The behavior of the intruder mouse during 10 min confrontation with the resident aggressor was videotaped. Defensive behaviors during social defeat stress were analyzed. The duration of defeat posture (an upright posture with the belly exposed toward the resident) and duration of freezing behavior (no movements except for respiration with four paws on the ground) were measured.

Immunohistochemical examination

For detection of neurons activated after social defeat stress, mice were exposed to aggressive CD1 mice for 10 min. One hundred minutes after the end of social defeat stress, mice were anesthetized with Avertin (200 mg/kg body weight, i.p.; tribromoethanol) and were perfused transcardially with heparinized saline (20 U/ml) followed by 4% paraformaldehyde. Expression of c-Fos, a marker of neuronal activation, in oxytocin neurons and in oxytocin receptor-expressing neurons was investigated in mice by immunohistochemical examination.

Brain sections were cut coronally at 30 µm intervals with a freezing sledge microtome and processed for immunohistochemical detection of c-Fos and oxytocin or Venus.

Brain sections were incubated with a rabbit polyclonal antibody against the N-terminal region (4–17) of the c-Fos peptide at 4 °C for 2 days and with peroxidase-labeled goat anti-rabbit IgG at 4°C overnight. c-Fos immunoreactivity was visualized by incubation with

3,3'-diaminobenzidine tetrahydrochloride, nickel sulphate, glucose and glucose oxidase.

Sections for immunohistochemical detection of oxytocin were incubated with guinea pig anti-oxytocin antibody at 4°C for 2 days, biotinylated anti-guinea pig IgG at room temperature for 2 h, and avidin biotinylated horseradish peroxidase complex at room temperature for 30 min. Oxytocin immunoreactivity was visualized as a brown cytoplasmic precipitate with 3,3'-diaminobenzidine tetrahydrochloride or as a black precipitate with 3,3'-diaminobenzidine tetrahydrochloride and nickel sulphate.

Sections for immunohistochemical detection of Venus were incubated with rat monoclonal anti-green fluorescent protein antibody at 4°C for 2 days and a peroxidase-labeled polymer solution containing an anti-rat Fab' fragment, amino-acid polymer and peroxidase at room temperature for 2 h. Immunoreactivity of green fluorescent protein was visualized as a pink cytoplasmic precipitate with 0.01% Tris-aminophenylmethane and 0.07% p-cresol.

Anterograde tracing

The projection of oxytocin neurons was examined with an anterograde viral tracer, which induces selective expression of membrane-targeted palmitoylated green fluorescent protein in oxytocin neurons. Green fluorescent protein tagged with a palmitoylation signal has been shown to be sorted to the plasma membrane and has been used to trace neuronal fibers efficiently. Adeno-associated virus vectors were applied into oxytocin-Cre mice in order to label oxytocin fibers by selective expression of palmitoylated green fluorescent protein in oxytocin neurons of the paraventricular hypothalamic nucleus. The adeno-associated virus vectors contained a sequence for expressing palmitoylated green fluorescent protein under the control of the CAG promoter selectively in oxytocin cells expressing Cre recombinase activity.

Data Analysis

Data are expressed as means \pm SEM. Data were analyzed by the Mann-Whitney *U* test, repeated-measures two-way ANOVA or *t* test. $P < 0.05$ was considered statistically significant.

3 研究成果

During exposures to aggressive CD1 mice, mice showed defeat posture and freezing behavior, suggesting that that exposure to the aggressor induced social defeat stress.

Following social defeat stress, expression of c-Fos protein was increased in oxytocin neurons of the bed nucleus of the stria terminalis, supraoptic nucleus, and paraventricular hypothalamic nucleus. Expression of c-Fos protein was also increased in oxytocin receptor-expressing neurons of the insular cortex, lateral septum, amygdala, thalamic nuclei, the ventrolateral part of the ventromedial hypothalamus and midbrain periaqueductal gray.

Projecting fibers from oxytocin neurons of the paraventricular hypothalamic nucleus were

found in the ventrolateral part of the ventromedial hypothalamus and in the ventrolateral periaqueductal gray.

Oxytocin receptor-deficient mice showed reduced defeat posture during social defeat stress.

4 考察

Social defeat stress induces species-specific defeat posture in animals. Concerning neural mechanisms of social defeat, social defeat stress has been shown to activate the ventrolateral part of the ventromedial hypothalamus and ventrolateral periaqueductal gray. On the other hand, oxytocin neurons in the hypothalamus are activated by a variety of stressful stimuli. Here, we suggest that oxytocin neurons in the paraventricular hypothalamic nucleus innervate the ventrolateral part of the ventromedial hypothalamus and ventrolateral periaqueductal gray, and that social defeat stress activates oxytocin neurons in the paraventricular hypothalamic nucleus and oxytocin receptor-expressing neurons in the ventrolateral part of the ventromedial hypothalamus and ventrolateral periaqueductal gray. Oxytocin receptor-deficient mice showed reduction of social defeat posture during social defeat stress. Our results suggest that social defeat stress activates oxytocin-oxytocin receptor system in the brain and that activation of oxytocin-oxytocin receptor system facilitates social defeat posture during social defeat stress.

During acute social defeat stress, animals show species-specific submissive posture, defeat postures. Expression of submissive posture can induce suppression of aggressive behaviors by the dominant conspecific animal. It is interesting to speculate that oxytocin facilitates submissive defeat posture in an imminent confrontation with dominant conspecifics to reduce aggression of dominant conspecifics and as a result increases adaptive values in group-forming animals.

5 結論

Social defeat stress activates oxytocin-oxytocin receptor system, and activation of the oxytocin receptor possibly in the ventrolateral part of the ventromedial hypothalamus or the ventrolateral periaqueductal gray facilitates social defeat behavior. This newly defined oxytocin pathway may lead to treatments for stress-related disorders.