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Brain Research 1044 (2005) 116–121
BRAIN RESEARCH
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Research report

Orexin-A (hypocretin-1) is possibly involved in generation of anxiety-like behavior

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Accepted 1 March 2005

Abstract

Orexins (hypocretins) are neuropeptides expressed specifically in neurons in the lateral hypothalamic area and are known to be involved in the regulation of vigilance and feeding behavior. However, the relationship between orexin and emotional behaviors like anxiety is still poorly understood. Therefore, in this report we evaluated the effect of intracerebroventricular injection of orexin-A in two major anxiety tests, the light–dark exploration test (mouse) and the elevated plus-maze test (mouse, rat). Orexin increased time spent in the dark compartment in the light–dark test and time spent on the closed arms in the elevated plus-maze test. These results were not caused by a hypothetical sedative or activity-inducing effect of orexin-A because spontaneous locomotor activity did not alter upon orexin-A application under novel conditions. We therefore suggest an anxiogenic effect of orexin-A. To our knowledge, this is the first report about a relationship between orexin-A and anxiety.

Theme: Neural basis of behavior
Topic: Neuropeptides and behavior

Keywords: Orexin; Anxiety; Light–dark exploration test; Elevated plus-maze test; Mouse; Rat

1. Introduction

Orexin-A (hypocretin-1) and orexin-B (hypocretin-2) were initially identified as endogenous peptide ligands for two orphan G protein-coupled receptors [20]. Mammalian orexin-A is a 33-amino acid peptide with two sets of intrachain disulfide bonds and mammalian orexin-B is a 28-amino acid peptide. Two orexin receptor subtypes named orexin receptor-1 (OX_1R) and orexin receptor-2 (OX_2R) have been identified in mammals [20]. OX_1R has almost 50 times greater affinity for orexin-A as compared to orexin-B, while OX_2R has comparable affinities for both orexin-A and orexin-B [20].

Orexin-producing neurons are localized to the lateral hypothalamic area and posterior hypothalamus [4,11,15,17,20]. These neurons project widely to various brain regions; among them cerebral cortex, olfactory bulb, hippocampus, amygdala, septum, diagonal band of Broca, bed nucleus of the stria terminals, thalamus, anterior and posterior hypothalamus, midbrain, brainstem and spinal cord have been described [4,11,15,17,20]. This wide distribution of orexin projections suggests that orexin peptides play a substantial role in various physiological functions. Indeed, it has been reported that at least orexin-A is involved in the control of vigilance and feeding behavior [2,6,20].

Recently, reports indicating an involvement of orexin in response to stress are emerging. For example, intracerebroventricular (icv) injection of orexin-A increased stress-like behaviors such as face washing, grooming, searching and burrowing [8,9]. However, until now there have been only speculations about an effect of orexin on anxiety. The purpose of the present study was to investigate the central effect of orexin-A on the anxiety level of rodents by using light–dark exploration and elevated plus-maze paradigms.

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doi:10.1016/j.brainres.2005.03.002

2. Materials and methods

2.1. Materials

All animal experiments were approved by the Animal Care and Use Committee of Eisai. Male C57BL/6NCrj mice (25–30 g, Charles River, Japan) were housed 10 per cage, and used for light–dark exploration test, elevated plus-maze test and measurement of spontaneous locomotor activity. Male WISTAR rats (300–400 g, Charles River, Japan) were housed two per cage and used for elevated plus-maze test and measurement of spontaneous locomotor activity. All animals were maintained under a 12:12-h light–dark cycle (lights on at 7:00). Food and water were available ad libitum.

2.2. Surgery

Mice were anesthetized with ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip), and rats were anesthetized with ketamine (80 mg/kg ip) and xylazine (8 mg/kg ip). A 10 mm long guide cannula for mice was placed into the lateral ventricle (0.3 mm posterior to bregma, 0.9 mm lateral from mid line and 2.3 mm ventral to the cortical surface) and fixed to the skull with Ketac™ Cem Maxicap™ dental cement (3 M ESPE, Seefeld, Germany). A 17 mm long stainless steel guide cannula for rats was placed into the lateral ventricle (0.8 mm posterior to bregma, 1.4 mm lateral from mid line and 3.0 mm ventral to the cortical surface) and fixed to the skull with dental cement (GC Co., Tokyo, Japan). For icv injection of orexin-A, animals were surgically equipped with a single cannula. Animals were allowed at least a 1-week recovery period before testing.

2.3. Peptides

Orexin-A (Peptide Institute Inc., Osaka, Japan) and corticotropin releasing factor (CRF; Bachem AG, Bubendorf, Switzerland) were dissolved in artificial cerebrospinal fluid (aCSF: glucose 10 mM, KCl 2 mM, NaCl 115 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25 mM, KH_2PO_4 2.2 mM, pH 7.4). Peptide solutions were always prepared freshly before each experiment.

2.4. Intracerebroventricular injection

Peptide solution or vehicle solution (aCSF) were icv administered via a stainless steel needle placed into and projecting 0.5 mm below the tip of the guide cannula into the lateral ventricle. Injection volume was 2 μl for mice and 5 μl for rats, respectively. A microsyringe (705 N 50 μl, Hamilton, Nevada, USA) connected to an infusion pump (CMA100, BAS Inc., Tokyo, Japan) ensured an accurate and constant volume delivery (2 μl/2 min for mice or 5 μl/2 min for rats). Injection needle was left in place for an additional 1 min after injection to allow for diffusion.

Correct placement of the guide cannula was verified by ink injection after experiments.

2.5. Behavioral tests

2.5.1. Light–dark exploration test (mouse)

The apparatus consisted of two compartments: dark compartment (10 cm width (W), 15 cm depth (D), 20 cm height (H)) and light one (20 cm W, 15 cm D, 20 cm H). The dark compartment had a lid on top and consisted of black Plexiglas, the light compartment was open at the top and consisted of white Plexiglas. A black Plexiglas tunnel (7 cm W, 10 cm D, 4.5 cm H) separated the dark box from the light box. Light intensity in the experimental room was 150 lx. Mice were transferred to and habituated to 10 lx light intensity at least 3 h before the test. All tests were performed between 13:00 and 16:00. Animals were icv injected 15 min before the test and kept in the 10-lx area until experiment was started by placing the mouse in the dark compartment. Behavior was recorded on videotape over a 5-min period, and the time spent in the light compartment was measured after experiments by two observers blinded as to treatment. A mouse with all four paws in the light compartment was considered to be fully in the light compartment.

2.5.2. Elevated plus-maze test (mouse)

We basically followed the procedure of the elevated plus-maze test described by Pellow et al. [16]. The apparatus comprised of two open arms (30 cm length (L), 5 cm W) and two closed arms (30 cm L, 5 cm W, 15 cm H) that extended from a common central platform (5 × 5 cm). A small raised lip (0.3 cm) around the perimeter of the open arms prevented mice from falling. The apparatus consisted of Plexiglas, with gray floor and walls, and elevated to a height of 45 cm above floor level. Mice were transferred to a standby room (20 lx) that was separated from the test room at least 3 h before the test. Experiments were performed between 13:00 and 16:00. In the standby room, orexin-A was icv injected 15 min before the test. At the beginning of an experiment, a mouse was placed into a transparent box (7.8 cm L, 7.8 cm W, 18 cm H) on the central platform. One minute later, the test was started by removing the box. Mice were allowed to freely explore the apparatus under overhead fluorescent lighting (10 lx) for 5 min. Behavior was recorded by an overhead video camera and time spent in the open arms was measured after experiments by two observers blinded as to treatment. A mouse with all four paws in an open arm was considered to be fully on the open arm.

2.5.3. Elevated plus-maze test (rat)

We adopted basically the same procedure as for mice; with open arms (50 cm L, 10 cm W), closed arms (50 cm L, 10 cm W, 40 cm H), central platform (10 × 10 cm) and a small raised lip (0.5 cm) around the perimeter of the