Regulatory Peptides 194–195 (2014) 16–22

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Orexin administration to mice that underwent chronic stress produces bimodal effects on emotion-related behaviors

Hye-Seung Chung [a,1], Jae-Gon Kim [a,1], Jae-Won Kim [a], Hyung-Wook Kim [b], Bong-June Yoon [a,*]

[a] Division of Life Sciences, College of Life Sciences and Biotechnology, Korea University, Anam-Dong, Seoul 136-701, Korea
[b] College of Life sciences, Sejong University, Seoul 143-747, Korea

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 4 November 2014
Accepted 10 November 2014
Available online 20 November 2014

Keywords:
Orexin
Stress susceptibility
Anxiety
Social defeat stress

ABSTRACT

Orexin plays diverse roles in regulating behaviors, such as sleep and wake, reward processing, arousal, and stress and anxiety. The orexin system may accomplish these multiple tasks through its complex innervations throughout the brain. The emerging evidence indicates a role of orexin in emotional behaviors; however, most of the previous studies have investigated the function of orexin in naïve animals. Here, we examined a functional role of orexin in mice that had been exposed to repeated stress. Chronic social defeat stress produced differential social interaction behaviors in mice (susceptible versus resilient) and these two groups of mice displayed different levels of prepro-orexin in the hypothalamus. Exogenously added orexin A to the brain induced an antidepressant-like effect in only the susceptible mice but not in the resilient mice. In contrast, orexin A and orexin B infused together produced an anxiogenic effect in only the resilient mice and not in the susceptible mice. Furthermore, we found that the antidepressant-like effect of orexin A is mediated by the bed nucleus of the stria terminalis (BNST) after exposure to chronic restraint stress. These findings reveal a bimodal effect of the orexin system in regulating emotional behavior that depends on stress susceptibility.

1. Introduction

Orexins are neuropeptides that are expressed and released from a small number of neurons that are localized to specific areas of the hypothalamus including the perifornical hypothalamus (PeF), the dorsomedial hypothalamus (DMH), and the lateral hypothalamus (LH) [1,2]. These neurons have extensive afferent and efferent projections throughout the brain, suggesting that they are involved in multiple functions of the brain. Among efferent targets, particularly dense projections are present in the locus caeruleus (LC), the dorsal raphe nucleus (DRN), the tuberomammillary nucleus (TMN), the substantia nigra (SN), the ventral tegmental area (VTA), the bed nucleus of the stria terminalis (BNST), the central amygdala (CeA), the periaqueductal gray (PAG), and the paraventricular nucleus of the hypothalamus (PVN) [3,4]. Many of these areas are also the sites of afferent projections to the orexin system [5,6], indicating that the orexin neurons are reciprocally interconnected with these areas to form certain feedback loops.

The orexins were initially found to regulate feeding behavior, earning the name "orexin" [7]; however, it was discovered that the loss of orexin function caused narcolepsy, a neurological disorder that affects the control of sleep-wake cycles [8,9]. Further studies revealed the multifunctional roles of the orexin system in promoting arousal [10], regulating energy homeostasis [11], reward seeking [12], and addiction [13]. Another emerging role of the orexin system is its involvement in emotional responses, such as depression- and anxiety-related behavior [14,15]. Indeed, many of the areas that are connected to the orexin system are known to be involved in the stress and arousal systems (LC, DRN, TMN, and PVN) and anxiety/emotion centers (BNST and CeA) [2]. However, experimental findings regarding the role of orexin function in emotional behavior are inconsistent. Exogenously added orexins, either by intracerebroventricular (icv) injection or directly to emotion centers, such as BNST or CeA, have been shown to increase anxiety-related behavior [16–18]. Conversely, an endogenous increase in orexin gene expression induced by acute calorie restriction has been shown to have an antidepressant effect [14].

Stress is one of the most important precipitating factors for major depression. However, an individual's susceptibility to a specific level of stress will vary partly because of differences in their genetic predisposition. Behavioral resilience to stress has recently attracted a great deal of attention [19] because understanding the nature of the susceptibility to

Abbreviations: Orx_1R, orexin receptor-1; Orx_2R, orexin receptor-2; SI, social interaction; RS, restraint stress; OFT, open field test; FST, forced swim test; icv, intracerebroventricular; PeF, perifornical hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus.

* Corresponding author. Tel.: +82 2 3290 3159; fax: +82 2 927 9028.
E-mail addresses: creya@naver.com (H.-S. Chung), worhs2@korea.ac.kr (J.-G. Kim), onlyhope57@naver.com (J.-W. Kim), kimhyung@sejong.ac.kr (H.-W. Kim), bjyoon69@korea.ac.kr (B.-J. Yoon).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.regpep.2014.11.003
0167-0115/

stress may contribute to unraveling the pathophysiology of stress-related mental disorders.

Here, we demonstrate that the orexins have bimodal functions, depending on the animal's stress susceptibility. Mice that received social defeat stress could be divided into a susceptible group and a resilient group, which is similar to the natural variation of social interaction behavior observed after repeated exposure to restraint stress [20]. These mice displayed different levels of prepro-orexin mRNA in the hypothalamus and responded differently to the infusion of orexin A alone and the infusion of orexin A and orexin B together (orexin A/B). Furthermore, we also found that the antidepressant effect of orexin A is exerted through the activation of orexin receptors in the BNST. Our results suggest that stress coping and the activation of the orexin system are closely interrelated and that the functional role of orexins is bimodal depending on stress susceptibility.

2. Materials and methods

2.1. Animals

Male C57BL/6N mice and male CD1 mice (Orient Bio, Seoul, Korea) were housed in the Korea University animal facility under standard conditions (12:12 h light–dark cycles, 23 ± 1 °C, food and water were available *ad libitum*). The subject mice (C57BL/6N) were 7 weeks old, and the aggressors (CD1) were 2 months old at the beginning of social defeat stress episodes. Seven-week-old C57BL/6N mice were also used in the chronic restraint stress (RS) experiments. All animals were given at least 1 week of habituation upon arrival to the facility before they were used for experiments.

2.2. Experimental design

The first set of experiments was conducted to investigate the expression of the prepro-orexin gene and the effects of orexin administration in animals that had been exposed to chronic social defeat stress (Fig. 2A). After 10 days of exposure to chronic social defeat stress, the animals were subjected to a social interaction test and grouped into the susceptible group or the resilient group based on changes in their social interaction. A subset of animals from each group was sacrificed for tissue collection 12 h after the first social interaction test (SI $test_{pre}$). The remaining mice received an icv injection of orexin A, orexin A/B or saline 12 h after the SI $test_{pre}$ and were subjected to the second social interaction test (SI $test_{post}$) 12 h after the injection. Unstressed, naïve animals also received injections and underwent a social interaction test or open field test (OFT) 12 h after the injection. We could not implant cannula because most of the implanted cannulae were damaged during the social defeat stress sessions. Therefore, we decided to do the injections under anesthesia using a glass micropipette, and the behavioral tests were performed after the anesthesia had completely worn off. In the second set of experiments, animals had stereotaxic surgery for the implantation of a cannula into the BNST (Fig. 4A). After 1 week of recovery, mice were exposed to chronic restraint stress for 2 weeks. The control group received daily handling without restraint stress. Orexin A was infused 20 min prior to behavioral tests.

2.2.1. Social defeat stress

Exposure to social defeat stress was conducted as previously described [21]. Briefly, subject mice (C57BL/6N) were placed in the home cage of an aggressor mouse, and physical contact was allowed for 5 min. A perforated Plexiglas divider was then placed in the middle of the cage so that the two mice were separated but continued to have sensory contacts for the next 24 h. Stress sessions were repeated for 10 days with a new aggressor each day.

2.2.2. Restraint stress (RS)

Mice were exposed to RS for 2 h from ZT7 to ZT9 everyday for 2 weeks. Perforated acrylic restrainers (28 mm diameter, 90 mm length) were used to immobilize the animals. All restrainer apparatuses were cleaned after the daily stress session. The control mice were housed separately and were gently handled for one min while the experimental group received RS.

2.3. Behavioral tests

2.3.1. Social interaction test

The social interaction test was performed as previously described [21]. Briefly, a subject mouse was placed in an arena (30 cm × 30 cm) with a small wire mesh cage (empty, no target session) at one end and allowed to freely explore the arena for 5 min. The strip of area (6 cm wide) surrounding the target cage was designated as the interaction zone. The time spent and distance moved by the experimental animals in the interaction zone was analyzed. The social interaction ratio was defined as the ratio of time spent in the interaction zone with a target animal present over time in interaction zone without a target present. Social interaction behavior was video recorded and analyzed using Ethovision XT 7.1 software (Noldus). All of the social interaction tests were performed between ZT1 and ZT3.

2.3.2. OFT

For the OFT, a mouse was placed in the center of an activity chamber (27 × 27 × 20.3 cm) (Med Associates Inc.) under dim lighting and allowed to freely explore the chamber for 1 h. The locomotor activity of a mouse was measured by the number of laser beam breaks in the chamber. The central area that occupied 16% of the whole area was designated as the "center zone" and the locomotor activity of the animal in this area was analyzed.

2.3.3. Forced swim test (FST)

For the FST, a mouse was placed in an acrylic cylinder (44.5 cm height, 20 cm diameter) filled with 5 L of distilled water (23 ± 1 °C) and subjected to forced swimming for 6 min. Fresh distilled water was used in each trial. The amount of time the mice remained immobile during the last 4 min was measured using Ethovision XT 7.1 software (Noldus).

2.4. Real-time PCR

After the behavioral tests were completed, the hypothalamus tissue was collected within 12 h (between ZT13 and ZT14) and immediately frozen in liquid nitrogen. The RNase Away (Molecular BioProducts, cat. no. 7002) and diethylpyrocarbonate (DEPC) (SIGMA-ALDRICH, cat. no. D5758)-treated water were used to minimize the adverse effects of RNases. Total RNA was extracted from the frozen tissue samples using the RNeasy Mini Kit (Qiagen, cat. no. 74104). All of the sample preparation procedures for RNA extraction were conducted following the protocol provided by the manufacturer. Approximately 300 ng of total RNA from each tissue was used to generate cDNA using the amfiRivert cDNA synthesis master mix (GenDEPOT, cat. no. R5500).

Quantitative analysis of prepro-orexin mRNA (Forward: 5′-GCCGTC TCTACGAACTGTTG-3′, Reverse: 5′-CGAGCAGAGGGGGAAGTTTG-3′) level was conducted using an LC 480 (Roche) real-time PCR thermocycler. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative quantity of gene expression in each group, and GAPDH expression level was used as a reference. The relative expression ratio was calculated by dividing the target (prepro-orexin) crossing point (cp) by the reference cp.

2.5. Stereotaxic injections

The icv administration of orexin A (100 nM) (SIGMA-ALDRICH, cat. no. O6012) or orexin A/B (50 nM each) (SIGMA-ALDRICH, cat. no. O6262) into the lateral ventricle [from bregma: anteroposterior