Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Brain Research 1048 (2005) 138 – 145
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

# Selective stimulation of orexin receptor type 2 promotes wakefulness in freely behaving rats

Moses A. Akanmu[1], Kazuki Honda*

*Department of Biosystem Regulation, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan*

Accepted 22 April 2005
Available online 24 May 2005

**Abstract**

Orexins A and B are a pair of neuropeptides implicated in the regulation of feeding and arousal behavior mediated through two orexin receptors type 1 and type 2. We have determined the arousal effects of newly developed selective orexin receptor type 2 agonist, [Ala$^{11}$]orexin-B, on the sleep–wake cycle in rats. The effects of third ventricle intracerebroventricular (ICV) infusion of the novel orexin receptor type 2 selective agonist, [Ala$^{11}$]orexin-B, on the sleep–wake cycle were investigated. ICV infusion of [Ala$^{11}$]orexin-B (1, 10 and 40 nmol) during the light period (11:00–16:00) dose-dependently resulted in a significant increase in wake duration by 46.9% ($n$ = 5, $P$ < 0.05), 159.2% ($n$ = 4, $P$ < 0.01) and 163.6% ($n$ = 7, $P$ < 0.01)), respectively, and a significant decrease in rapid eye movement (REM) ($P$ < 0.01) and non-REM sleep ($P$ < 0.01) for all doses. In contrast, ICV infusion of orexin B at the same doses (1, 10 and 40 nmol) caused a 16.6% ($n$ = 6, non-significant), 99.8% ($n$ = 6, $P$ < 0.05) and 72.0% ($n$ = 4, $P$ < 0.05) increase in wakefulness, respectively. Moreover, orexin-A, which exerts its effects through orexin receptor type 1 and orexin receptor type 2 with similar potency, resulted in a significant increase in wakefulness duration by 17.1% ($n$ = 6, $P$ < 0.05), 184.0% ($n$ = 6, $P$ < 0.01) and 228.6% ($n$ = 6, $P$ < 0.01) at doses of 0.1, 1 and 10 nmol, respectively. Further, the enhancement of wakefulness was accompanied by a marked reduction in REM and non-REM sleep. These findings suggest that orexin receptor type 2 plays an important role in the modulation of sleep–wake state and behavioral responses.
© 2005 Elsevier B.V. All rights reserved.

*Theme:* Neural basis of behavior
*Topic:* Biological rhythms and sleep

*Keywords:* Orexin; Orexin-B agonist; Behavior; Non-REM sleep; REM sleep; Wakefulness

## 1. Introduction

Orexins, also called hypocretins, are a pair of neuropeptides expressed by a specific population of neurons in the lateral hypothalamic area (LHA), a region of the brain implicated in feeding and arousal [7,9,32,44]. Orexin-A (hypocretin-1) and orexin-B (hypocretin-2) are derived from a common precursor peptide [8,32]. The actions of orexins are mediated by two G protein-coupled receptors termed orexin receptor type 1 ($OX_1R$) and orexin receptor type 2 ($OX_2R$) [32]. $OX_1R$ is expressed in the locus coeruleus (LC), ventral tegmental area (VTA), dorsal raphe nucleus (DRN), laterodorsal tegmental nuclei (LDT),

*Abbreviations:* ICV, intracerebroventricular; REM, rapid eye movement; non-REM, non-rapid eye movement; LHA, lateral hypothalamic area; $OX_1R$, orexin receptor type 1; $OX_2R$, orexin receptor type 2; LC, locus coeruleus; VTA, ventral tegmental area; DRN, dorsal raphé nucleus; LDT, laterodorsal tegmental nuclei; PPT, pedunculopontine tegmental nuclei; TMN, tuberomammilary nucleus; GABA, gamma amino butyric acid; 5-HT, 5-hydroxyltryptamine; VLPO, ventrolateral preoptic nucleus; EEG, electroencephalogram; EMG, electromyogram; CSF, cerebrospinal fluid

* Corresponding author. Fax: +81 3 5280 8098.
*E-mail address:* khonda.bsr@tmd.ac.jp (K. Honda).
[1] Present address: Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.04.064

pedunculopontine tegmental nuclei (PPT) and hypothalamus, while $OX_2R$ is expressed in the tuberomammilary nucleus (TMN), VTA, DRN, LDT, PPT and hypothalamus [19,42].

Orexin-containing neurons project from the LHA to numerous brain regions, with the limbic system, hypothalamus, monoaminergic LC, dopaminergic VTA, serotonergic DRN, cholinergic nuclei of brainstem and histaminergic TMN receiving particularly strong innervation [8,24,29]. Amygdala is highly interconnected with a number of brain regions involved in sleep regulation, such as the basal forebrain, hypothalamus and brainstem [2] and also connected with the parabrachial region, DRN, PPT and LDT. Bilateral lesions of the amygdala produced substantial effects on sleep in monkeys [4]. Moreover, lesions of the posterior lateral hypothalamus where the tuberomammillary neurons reside and rostral midbrain in the brains of monkeys, rats and cats caused sleepiness while the lesions of lateral preoptic and basal forebrain in rats and cats caused sleep suppression [20,25,31,39,43]. Interestingly, these terminal areas such as the posterior lateral hypothalamic and preoptic and basal forebrain are involved in the homeostatic and circadian control of sleep [5,33]. Furthermore, the sleep–wake mechanism involves a positive neuronal feedback mechanism in which orexin excites other arousal systems, including serotonin (5-hydroxyltryptamine; 5HT) and noradrenaline. This ascending arousal system sends projection from the brainstem and posterior hypothalamus throughout the forebrain.

Orexin neurons in the lateral hypothalamic area innervate all of the components of ascending arousal system and may stabilize behavioral state by increasing the activity of aminergic neurons, thus maintaining consistent inhibition of sleep-promoting neurons in the ventrolateral preoptic nucleus (VLPO) and rapid eye movement (REM)-promoting neurons in the PPT-LDT [6,10,35,38]. Indeed, central orexin administration in rodents results in increases in sympathetic tone, plasma corticosterone levels [13], metabolic rate [18], food intake [32], locomotor activity [23] and wakefulness [13]. Further, mice lacking either the *orexin* gene (*prepro-orexin* knockout mice) or orexin neurons (*orexin/ataxin-3* transgenic mice), as well as mice and dogs with null mutations in the orexin receptor type 2 gene, all have phenotypes remarkably similar to those of the human sleep disorder, narcolepsy [7,14,17,44,45], and recent reports suggest that human narcolepsy is accompanied by a specific destruction of orexin neurons in the hypothalamus [30,41]. While these studies suggest that orexin stabilizes prolonged periods of wakefulness by opposing homeostatic sleep propensity [33], the differential roles of the two orexin receptors remain unclear.

Thus, the goal of the present study was to determine the effects of intracerebroventricular administration of the $OX_2R$-selective agonist, [Ala$^{11}$]orexin-B, on the sleep–wake cycle in rats.

## 2. Materials and methods

### 2.1. Animal usage

Sixteen to eighteen male Sprague–Dawley rats (330–360 g) were obtained from Crea Japan, Inc. (Tokyo, Japan) and were housed in cages for one week. The room was maintained at a temperature of 25 ± 1 °C, a relative humidity of 54 ± 6%, and a light–dark cycle of 12:12 h (lights on 8:00 h). All experimental protocols were performed in accordance with the Guidelines for Animal Experimentation of Tokyo Medical and Dental University.

### 2.2. Preparation of [Ala$^{11}$]orexin-B and orexins A- and -B

The $OX_2R$ selective agonist, [Ala$^{11}$]orexin-B, was obtained from Banyu pharmaceutical Co. Ltd., Tsukuba, and orexin-A and orexin-B were purchased from Peptide institute, Inc, Osaka. The peptides were dissolved in normal saline, and either peptides or vehicle were continuously infused intracerebroventricularly (icv) at a rate of 10 μl/h for the sleep–wake analysis.

### 2.3. Electroencephalogram (EEG) and electromyogram (EMG)

Simultaneous EEG and EMG recordings were performed as described previously [15]. Briefly, 60- to 70-day-old male Sprague–Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus. A stainless steel cannula (0.35 mm o.d., inclined at 20° from the vertical line, 2 mm posterior, 3.4 mm lateral to the bregma, 8.5 mm depth from the surface of cortex) was implanted in the third cerebral ventricle for continuous icv infusion. Three cortical gold-plated screw electrodes and paired stainless-steel electrodes were fixed to the skull with dental acrylic resin for recording of the EEG and EMG, respectively, and the cable from the electrodes was attached to a socket. After implantation of the cannula and attachment of the electrodes, animals were administered penicillin G potassium (20,000 U, subcutaneously) and were allowed to recover for 10 days.

Following the recovery period, animals were transferred to an individual experimental cage that allowed continuous icv infusion and monitoring of EEG and EMG. The experimental cages were placed in a soundproof, electromagnetically shielded room with the same environmental conditions described above. Polyethylene tubing (PE10, 0.28 mm, i.d.) was used to connect the cannula to an infusion pump (Eicom, ESP-32, Kyoto), which was set for continuous infusion of normal saline with or without the experimental peptides at a rate of 10 μl/h. During the 7 days preceding the experiment, animals underwent daily continuous infusion of normal saline in the experimental chamber to acclimatize the animals to the infusion and recording conditions. For measurements, lead wires of the

Download English Version:

https://daneshyari.com/en/article/9416208

Download Persian Version:

https://daneshyari.com/article/9416208

Daneshyari.com