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Research report

Alpha-fluoromethylhistidine, a histamine synthesis inhibitor, inhibits orexin-induced wakefulness in rats

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ABSTRACT

Orexins A and B are involved in the regulation of feeding and arousal state. Previously, we reported that third intracerebroventricular (icv) infusion of both orexins A and B induced a significant arousal effect in rats. We determined the effects of intraperitoneal (i.p.) injection of alpha-fluoromethylhistidine (α-FMH), a histamine synthesis inhibitor, on orexin-induced wakefulness in freely behaving rats. Male Sprague–Dawley rats were chronically implanted with cortical electroencephalogram (EEG) and neck electromyogram (EMG) electrodes, and a cannula for icv infusion. EEG and EMG were monitored for three consecutive days during continuous icv saline infusion at a rate of 10 μl/h. For a 5-h diurnal period, orexin-B (10 nmol/50 μl saline) replaced the icv infusion of saline. α-FMH (100 mg/kg, i.p.) was administered 6 h before icv infusion of orexin-B.

Orexin-B at a dose of 10 nmol/h markedly increased the amount of wakefulness by 99.4% ($p < 0.05$) over the baseline value, whereas α-FMH decreased orexin-B-induced wakefulness by 48.8%. Orexin-B-induced suppression of non-REM sleep was reversed by α-FMH treatment. Pretreatment with α-FMH, significantly inhibited orexin-B-induced wakefulness in rats. The findings of this study therefore suggest that arousal-state regulation by orexin neurons is possibly mediated via the histaminergic system in the tuberomammilary nucleus.

1. Introduction

Orexin receptors are broadly expressed in the central nervous system (CNS) and are reported to play an important role in regulating and maintaining sleep–wakefulness states and energy homeostasis. A previous report showed that when rats were treated with saponin–orexin conjugates to selectively produce lesions in orexinergic neurons in the lateral hypothalamus, a marked decrease in locomotor activity was observed with hypersomnolence, in addition to some features of narcolepsy [7]. Similarly, mice lacking orexin peptide were reported to display an increase in rapid eye movement (REM) and non-REM sleep with a decrease in wake activity time during the active period [4]. Further study also showed that canine narcolepsy is caused by a mutation in the orexin-2-receptor gene [9]. The importance of orexin-B in promoting the arousal state is clearly demonstrated in all of these studies.

Orexin-containing cells project widely throughout the entire neuroaxis [20]; their abundant projections are found particularly in monoaminergic cell groups that include histaminergic cells of the tuberomammilary nucleus (TMN) [4,14], where orexin-2-receptors are highly abundant [10]. Previously, we reported that intracerebroventricular (icv) infusion of both orexins A and B induced a significant arousal effect in rats [2]. Histamine-containing neurons of the TMN have been implicated in facilitating wakefulness [15]. Yamanaka et al. reported that orexins activate histaminergic neurons via the orexin-2-receptor in rats and showed that immunohistochemical and electron microscopic techniques revealed direct synaptic interaction between orexin-immunoreactive neurons in the TMN [21]. Alpha-fluoromethylhistidine, a histamine synthesis inhibitor, is reported to significantly decrease brain histamine content and locomotor activity after intraperitoneal administration [13,16,17]. Therefore, in the present study we investigated the effects of intraperitoneal (i.p.) injection of alpha-fluoromethylhistidine (α-FMH), a histamine synthesis inhibitor [11,18], on orexin-B induced wakefulness in rats.

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2. Materials and methods

2.1. Animals

Between 16 and 18 male Sprague–Dawley rats (260–300 g) obtained from Crea Japan, Inc. (Tokyo, Japan) were housed in cages for 1 week. The room was maintained at a temperature of 25 ± 1 °C, relative humidity of 54 ± 6%, and a light–dark cycle of 12:12 h (lights on at 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 orexin-B and α-FMH

Orexin-B (Peptide Institute, Inc., Osaka, Japan) and α-FMH (Merck Sharp & Dohme Research Laboratory, Rahway, NJ, USA) were dissolved in normal saline solution. Orexin-B or vehicle was continuously infused icv at a rate of 10 μl/h while α-FMH was administered intraperitoneally (i.p.) for the analysis of sleep–wake states.

2.3. Electroencephalogram (EEG) and electromyogram (EMG)

Male Sprague–Dawley rats (60–70 days old) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus; EEG and EMG recordings were performed simultaneously as described previously [1,3,8]. In brief, a stainless-steel cannula (outer diameter, 0.35 mm) inclined at 20° from the vertical line, 2 mm posterior, 3.4 mm lateral to the bregma, 8.5 mm deep from the surface of the 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 the EEG and EMG, respectively; 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 IU, subcutaneously) and were allowed to recover for 10 days.

Following the recovery period, animals were transferred to individual experimental cages 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 as described above. Polyethylene tubing (PE10, 0.28 mm, inner diameter) was used to connect the cannula to an infusion pump (ESP-32, Eicom, Kyoto, Japan) 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.

During measurement, the electrode leads were connected to an EEG/EMG amplifier (MEG-6116, Nihon-Kohden, Tokyo, Japan) via a 5-strand cable with a slip-ring that enabled the rats to move freely. The amplifier was connected to a personal computer with an AD converter and software (SleepSign, Kissei Comtec, Nagano, Japan) for acquiring and processing data. Data were sampled at 128 Hz and subjected to online spectral analysis by Fast Fourier Transformation over 8-s epochs. Data were stored on a magnetic optical disk and subsequently analyzed visually offline after auto-analysis of the sleep–wake stages. EEG and EMG were monitored for three consecutive days [(Day 1, Baseline; Day 2, Experiment (orexin-B or α-FMH + orexin-B); Day 3, Recovery)] during continuous icv saline infusion at a rate of 10 μl/h. For a 5-h diurnal period, orexin-B (10 nmol/50 μl saline) replaced the icv infusion of saline. α-FMH (100 mg/kg, i.p.) [6,11] was administered 6 h before icv infusion of orexin-B [19].

The sleep–wake state was classified as wakefulness (W), REM sleep, and non-REM sleep from the EEG and EMG recordings, and the results were verified visually according to the standard criteria [3,8]: wakefulness (high EMG amplitude, low EEG amplitude); non-REM sleep (low EMG amplitude, high EEG amplitude with high power density in the delta band (0.5–4.0 Hz)); and REM sleep (silent low EMG amplitude, low EEG amplitude with high values in the theta band (4.0–8.0 Hz)). The analyzed sleep variables included the amount of non-REM and REM sleep, and the number and duration of each sleep parameter episode. The durations of wakefulness, non-REM sleep, and REM sleep were determined for each hour of the 24-h recording.

2.4. Statistical analysis

Statistical analyses were performed using repeated measures analyses of variance (ANOVA) followed by post hoc analysis of significance using the Student–Newman–Keuls test. Probability (p)-values less than 0.05 were considered to indicate statistical significance.

3. Results

Sleep–wake activity was evaluated and EEG data were analyzed at baseline (vehicle-treated) during the icv administration of orexin-B and during pretreatment with α-FMH i.p. 6 h before the administration of orexin-B. The effects on sleep–wake stages are as shown in Fig. 1 for wakefulness, non-REM sleep and REM sleep on the time-course study with continuous icv saline (vehicle) infusion at a rate of 10 μl/h administration, orexin-B (10 nmol/50 μl saline), and pretreatment with α-FMH (100 mg/kg, i.p.) administered 6 h before icv infusion of orexin-B. The results show that orexin-B significantly ($p < 0.05$) increased wakefulness during the infusion period and that α-FMH significantly ($p < 0.05$) blocked this effect of orexin. Regarding non-REM sleep, the results showed that orexin significantly decreased this sleep stage and that this orexin-B effect was blocked with prior administration of α-FMH. Fig. 2 presents further analysis regarding the total time spent during the 5-h infusion of orexin-B (10 nmol) and the influence of α-FMH on the effect of orexin-B for wakefulness [$F(2,23) = 213.25$, $p = 0.0001$], non-REM

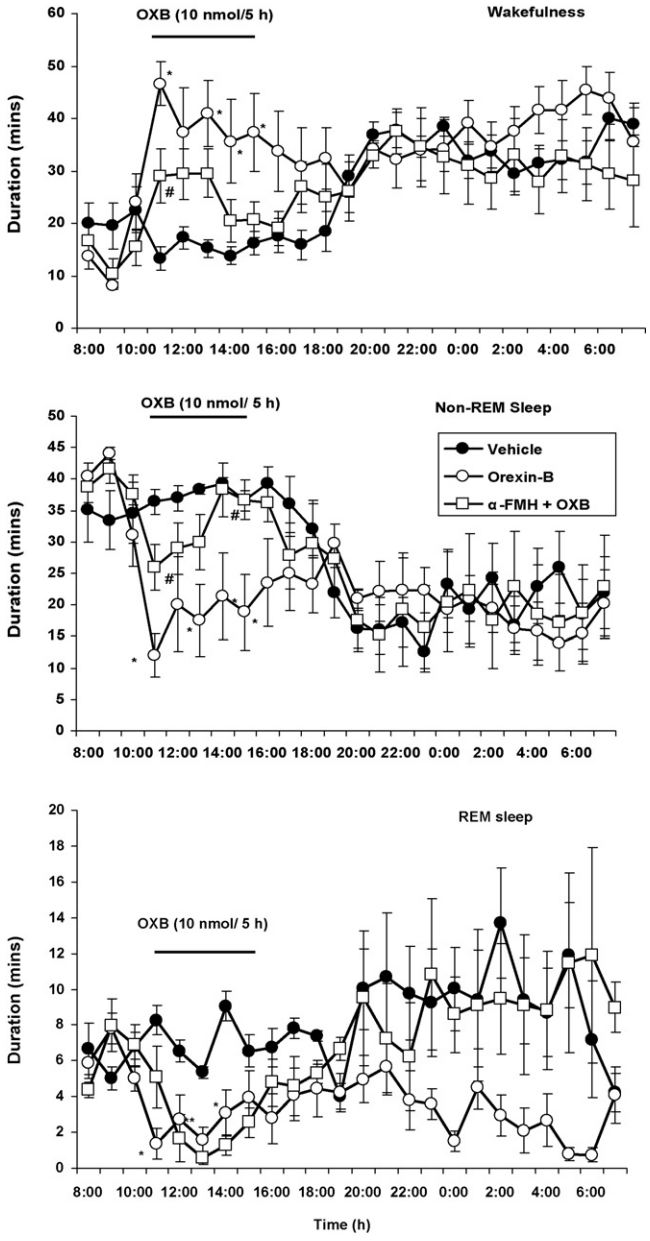

Fig. 1. Effects of vehicle (saline), orexin-B (10 nmol), and α-FMH + OXB on the time-course of wakefulness, non-rapid eye movement (non-REM) sleep and rapid eye movement (REM) sleep in rats. *$p < 0.05$; compared with vehicle-treated rats and #$p < 0.01$ compared with orexin-B. Vehicle, baseline; OXB, orexin-B; α-FMH, alpha-fluoromethyhistidine. Values are presented as mean ± SEM [vehicle, $n = 12$; orexin-B, $n = 6$; and α-FMH + OXB, $n = 6$]. The horizontal black bar denotes the onset and duration of icv infusion of orexin-B.