

Full-length article

Orexin A promotes histamine, but not norepinephrine or serotonin, release in frontal cortex of mice¹Zong-yuan HONG^{2,3}, Zhi-li HUANG^{3,4,5}, Wei-min QU³, Naomi EGUCHI³

²School of Life Science, University of Science and Technology of China, Hefei 230027, China; ³Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Furuedai, Suita, Osaka 565-0874, Japan; ⁴Department of Pharmacology, Shanghai Medical College of Fudan University, Shanghai 200032, China

Key words

orexin A; histamine; noradrenaline; serotonin; microdialysis; frontal cortex

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⁵ Correspondence to Zhi-li HUANG, MD, PhD.
Phn 81-6-6872-4851.
Fax 81-6-6872-2841.
E-mail huangzl@obi.or.jp

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Abstract

Aim: To investigate the effects of orexin A on release of histamine, norepinephrine, and serotonin in the frontal cortex of mice. **Methods:** Samples for measuring histamine, norepinephrine, and serotonin contents were collected by *in vivo* microdialysis of the frontal cortex of anesthetized mice. The histamine, noradrenaline, and serotonin content in dialysates were measured by HPLC techniques. **Results:** Intracerebroventricular injection of orexin A at doses of 12.5, 50, and 200 pmol per mouse promoted histamine release from the frontal cortex in a dose-dependent manner. At the highest dose given, 200 pmol, orexin A significantly induced histamine release, with the maximal magnitude being 230% over the mean basal release. The enhanced histamine release was sustained for 140 min, and then gradually returned to the basal level. However, no change in norepinephrine or serotonin release was observed under application of the same dose of orexin A. **Conclusion:** These results suggest that the arousal effect of orexin A is mainly mediated by histamine, not by norepinephrine or serotonin.

Introduction

The neuropeptides orexin A and B (also called hypocretins 1 and 2) have recently been isolated from rat hypothalamic extracts, and have been reported to be involved in sleep-wake regulation^[1–3]. Intracerebroventricular (icv) application of orexin A strongly enhances arousal in rats and mice^[4–6]. Furthermore, c-fos expression in orexin neurons and prepro-orexin mRNA levels show a diurnal variation, with the strongest expression being observed during waking^[7,8]. Mice lacking either the orexin gene (*preproorexin* knock-out mice) or orexin neurons (*orexin/ataxin-3* transgenic mice) have phenotypes remarkably similar to the human sleep disorder narcolepsy^[9–11], a disabling neurological disorder characterized by symptoms including excessive daytime sleepiness, sleep attacks, sleep fragmentation, cataplexy, and sleep-onset periods of rapid eye movement^[12]. Lesions of the lateral hypothalamus by orexin-2-saporin produce narcoleptic-like sleep behavior in rats^[13]. Consistent with these findings, recent reports suggest that human narcolepsy is accompanied by a

loss of orexin neuropeptide production and specific destruction of orexin neurons^[14–17]. These results suggest that the orexinergic system is involved in sleep-wake regulation and mainly contributes to arousal.

Orexin neurons are located specifically in the lateral hypothalamic area and project to almost all parts of the brain except the cerebellum^[2,10,18]. Particularly dense projections of these neurons are observed in monoaminergic nuclei, such as the noradrenergic locus ceruleus (LC), serotonergic raphe nuclei (DRN), and histaminergic tuberomammillary nucleus (TMN). These monoaminergic nuclei expressing orexin receptors (OX1R and/or OX2R)^[19] play important roles in the promotion of wakefulness. Electro-physiological studies have revealed that orexins had mainly excitatory effects on all monoaminergic neurons *in vitro*^[20–24], suggesting that the arousal effect of orexins is mediated by these monoaminergic systems. However, which type(s) of these monoaminergic systems is involved in orexin-induced arousal remains to be elucidated.

In the present study, we investigated the effects of orexin A on the release of the monoaminergic neurotransmitters histamine, norepinephrine (NE), and serotonin (5-HT) in the frontal cortex (FrCx) of mice by using *in vivo* microdialysis to further clarify the mechanism underlying the arousal effects of orexin A.

Materials and methods

Animals Male C57BL/6 mice (Shizuoka Laboratory Animal Center, Shizuoka, Japan) weighing 24–28 g (11–13 weeks old) were housed at a constant temperature (24 ± 0.5 °C) and relative humidity ($60\%\pm 2\%$), an automatically controlled 12:12 h light/dark cycle (light on at 8 AM), and *ad libitum* access to food and water. All animal experiments used in this study were approved by the Animal Care Committee of Osaka Bioscience Institute.

Microdialysis procedure The microdialysis was performed as previously described^[5]. As shown in Figure 1, under urethane anesthesia (1.8 g/kg, ip), a micro-dialysis probe (CUP7, membrane length of 2-mm; Carnegie Medicin, Stockholm, Sweden) was inserted in the FrCx of mice at a position 1.8 mm anterior and 0.8 mm lateral to the bregma and 2.3 mm depth from the dura. One stainless steel cannula (outer diameter, 0.2 mm) was stereotaxically placed at the site of 2.0 mm lateral to the bregma and inserted to a depth of 2.2 mm from the surface of the cortex at an angle of 25° from the midsagittal plane according to the atlas of Franklin and Paxinos^[25]. The microdialysis probe was perfused with Ringer's solution (NaCl 147 mmol/L, KCl 4.0 mmol/L, and CaCl_2 2.3 mmol/L; pH 7.3) at a flow rate of 2 $\mu\text{L}/\text{min}$ to stabi-

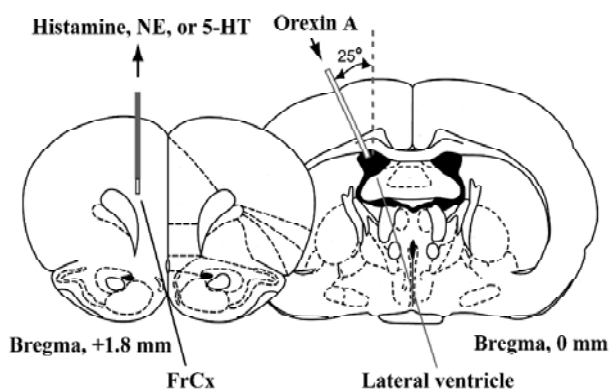


Figure 1. Schematic representation of the implantation sites for the microdialysis probe in the FrCx and the stainless steel cannula in the lateral ventricle of mice. Orexin A was infused into the lateral ventricle through the stainless steel cannula; and dialysate samples for monitoring histamine, NE, or 5-HT levels were collected from the microdialysis probe. FrCx, frontal cortex.

lize the release of histamine, NE, and 5-HT. Two hours after insertion of the microdialysis probe, dialysates were continuously collected from the FrCx at 20-min interval (40 μL each) for 1 h as the basal value before the orexin A injection, and until 4 h after administration of the peptide.

Determination of histamine, NE, and 5-HT levels by HPLC The histamine levels in the dialysates were measured by using a fluorometric HPLC system^[26], and NE and 5-HT levels were determined by using HPLC with electrochemical detection^[27]. Since the absolute basal release of histamine, NE, and 5-HT varied between subjects, the mean of the first 3 fractions before administration of orexin A was defined as the mean basal release, and subsequent fractions were expressed as a percentage of the mean basal release.

Drugs Orexin A (Peptide Institute, Osaka, Japan) was diluted in saline to the concentrations needed and was injected into the lateral ventricle of mice from the cannula at doses of 200, 50, or 12.5 pmol per mouse in 2 μL of saline at a speed of 2 $\mu\text{L}/\text{min}$. Fluoxetine (Sigma-Aldrich, St Louis, MO, USA), a 5-HT reuptake inhibitor which has been reported to also increase NE release through activation of postsynaptic 5-HT_{1A} receptors by increased 5-HT^[28,29], was dissolved in saline and injected ip (20 mg/kg).

Statistical analysis Data were expressed as mean \pm SD. Differences between groups were analyzed by analysis of variance (ANOVA) followed by the *post-hoc* Newman-Keuls test. The significant level of difference was set at $P<0.05$.

Results

Effect of orexin A on histamine release The mean basal release of histamine was 0.06 ± 0.01 pmol per 20 min. Compared with the control, orexin A at doses of 12.5 and 50 pmol produced a rapid and significant elevation of histamine release, with the maximal magnitude being 150% and 175% over the mean basal release, respectively; and these higher levels maintained for approximately 1 and 2 h (Figure 2A), respectively. At the highest dose (200 pmol) tested, orexin A markedly promoted histamine release, and the release reached its maximal level of 230% over the mean basal level. The significant increase lasted 140 min.

For comparison of the differences between different dosage groups, we calculated the total amount of histamine released over a 3-h period after the administration of orexin A. The total amounts of histamine released were 0.55 ± 0.08 , 0.74 ± 0.09 , and 0.79 ± 0.11 pmol per 3 h in the groups treated with orexin A at doses of 12.5, 50, and 200 pmol, respectively. In the latter 2 groups the release was significantly higher than that of the control (0.43 ± 0.05 pmol per 3 h, $P<0.05$) (Figure

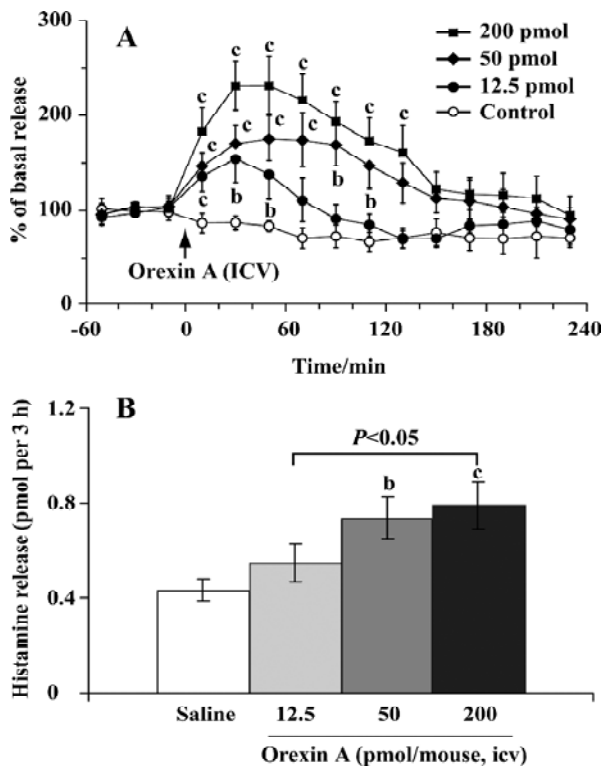


Figure 2. Effect of orexin A on histamine release in the FrCx of anesthetized mice. Time-courses of histamine release (A) and the total amounts of histamine released over 3 h after the administration of orexin A (B) in the FrCx are shown. $n=5-6$ mice. Mean \pm SD. ^b $P<0.05$, ^c $P<0.01$ vs the control.

2B). These results indicated that orexin A induced histamine release in a dose-dependent manner.

Effects of orexin A on NE and 5-HT release The mean basal release of NE and 5-HT was 1.75 ± 0.21 and 16.9 ± 2.33 pg per 20 min, respectively. No difference was observed in NE or 5-HT release between the group treated with orexin A (200 pmol) and the control. As the positive control, fluoxetine, significantly elevated the extracellular levels of NE and 5-HT, with the maximal magnitude being approximately 200% over the mean basal release at approximately 1.5 h and 1 h after administration (20 mg/kg, ip), respectively. Compared with the control, the increase in NE and 5-HT lasted about 180 and 160 min, respectively (Figure 3).

Discussion

In the present study we found that orexin A activated a histaminergic system in mice. An increasing body of evidence indicates the interaction between the orexinergic and histaminergic systems. For example, with respect to neuro-

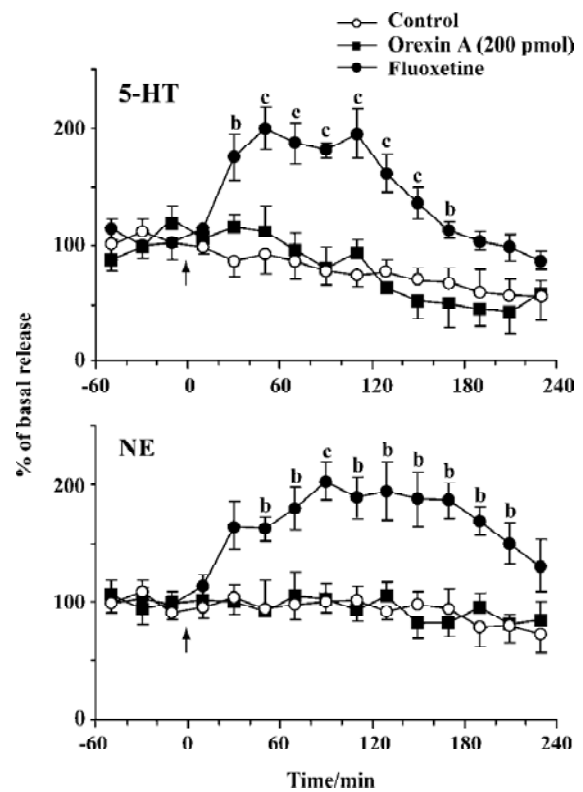


Figure 3. Effects of orexin A on 5-HT and NE release in the FrCx of anesthetized mice. Time-courses of 5-HT and NE release in the FrCx are shown. The arrow stands for the time point of administration. $n=5-6$ mice. Mean \pm SD. ^b $P<0.05$, ^c $P<0.01$ vs the control.

anatomy, orexin cells densely innervate the histaminergic TMN^[18,30], a nucleus enriched in orexin 2 receptors^[19]. Most human narcolepsy is caused by a loss of orexin neurons^[16] and a consequent reduction in orexin levels^[15,31]. Gliosis accompanies this loss of orexin neurons and is most intense in the posterior hypothalamus where the histaminergic TMN is located^[16,17], suggesting that the orexinergic terminals are lost in this region and that the consequent loss of orexinergic innervation of histaminergic cells is an important component of the pathology of narcolepsy. Based on neurochemical studies, Nishino *et al*^[31] reported that the histamine content was markedly decreased in the cortex of orexin-2 receptor-mutated narcoleptic Dobermans and that the decrease was due to a lack of excitatory input of orexin neurons caused by a loss of function of orexin-2 receptors in histaminergic TMN cell groups. Furthermore, Lin *et al*^[32] found that orexin A and B contents were significantly lower in histamine H₁ receptor knockout mice. These results indicate that there exist functional connections between histaminergic and orexinergic systems.

The FrCx is a brain region that has higher EEG frequency during waking^[33], and which receives projections of monoaminergic neurons such as the noradrenergic, serotonergic, and histaminergic neurons that originate from the LC, DRN, and TMN, respectively. Microdialysis studies have revealed that extracellular levels of histamine and 5-HT in the FrCx showed typical changes across the sleep-wake cycle, with their highest levels during the waking period^[34-37]. Thus, orexin-activated release of these neurotransmitters in the FrCx reflects their contributions to the arousal effect of orexin. In the present study, we found that orexin A significantly promoted histamine release in the FrCx in a dose-dependent manner, but not release of NE or 5-HT, although orexin A excites noradrenergic and serotonergic neurons *in vitro*^[20-22]. We previously reported that a prostaglandin E₂ receptor subtype EP₄ agonist enhanced histaminergic neuron activity with an increase in histamine release in the FrCx to produce arousal^[38]. Together with our previous observations that orexin induced wakefulness in wild-type mice but not all in histamine H₁ receptor knockout mice^[5], these findings suggest that the arousal effect of orexin is largely mediated by histaminergic systems and activation of H₁ receptors. In contrast to the histaminergic activity linked to the maintenance of wakefulness, John *et al*^[39] found that noradrenergic and serotonergic neurons were more tightly coupled to the maintenance of muscle tone during wakefulness and its loss during rapid eye movement sleep and cataplexy.

Taken these findings together, we conclude that the arousal effect of orexin A is mainly mediated by the activation of histaminergic systems.

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