Neuropeptides 43 (2009) 179–185

Contents lists available at ScienceDirect

Neuropeptides

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

Activation of orexin signal in basal forebrain facilitates the emergence from sevoflurane anesthesia in rat

Hailong Dong [1], Jiyuan Niu [1], Binxiao Su, Zhenghua Zhu, Yan Lv, Yizhi Li, Lize Xiong *

Department of Anesthesiology, Xijing Hospital, Fourth Military Medical University, Xi'an, 710032 Shaanxi, China

ARTICLE INFO

Article history:
Received 23 December 2008
Accepted 29 April 2009
Available online 22 May 2009

Keywords:
Orexins
Basal forebrain
Inhalation anesthetic
Emergence time

ABSTRACT

Orexinergic system may play an important role in the regulation of anesthesia–arousal. However, which region or which pathway mediated the effect of orexins was still unclear. In current study, we investigated whether activation of orexin signals in basal forebrain (BF) may alter electroencephalographic activity, induction and emergence time to sevoflurane anesthesia in rats. Either orexin-A or orexin-B was injected into the BF while measuring electroencephalogram (EEG) under 1.0 minimum alveolar concentration (2.4%) sevoflurane anesthesia. The induction and emergence time of sevoflurane anesthesia were measured respectively after an injection of orexin receptor agonist (orexin-A or orexin-B) or antagonist (SB-334867A) into the BF also. We found that the administration of orexin-A (30, 100 pmol) and orexin-B (100 pmol) changed the burst and suppression patterns to arousal EEG in rat under sevoflurane anesthesia. Comparing with orexin-B, injection of lower dose of orexin-A induced more arousal EEG. Intrabasalis microinjection of orexin-A shorted the emergence time, whereas intrabasalis microinjection of SB-334867A (5 μg, 20 μg) delayed the emergence time to sevoflurane anesthesia, without changing anesthetic induction. These findings indicate that the orexin signals in basal forebrain, a middle region of the cholinergic ventral ascending arousal system, plays a crucial role in the anesthesia–arousal regulation.

1. Introduction

The orexins are a recently identified peptide family consisting of two peptides (orexin-A and orexin-B). In a series studies, orexins have been reported to regulate many physiological and neurological functions including appetite, sleep and arousal (Chemelli et al., 1999; Piper et al., 2000). Although there are some differences between sleep and the anesthetized state, the similarities have led to speculation that anesthesia and sleep share a common neural substrate (Nelson et al., 2002, 2003). In a previous study, we demonstrated that orexin-A induced electroencephalogram (EEG) arousal in the isoflurane-anesthetized rat (Dong et al., 2006). The pivotal role of orexin in regulating anesthesia was further supported by Kelz's study. They found that genetic ablation of orexinergic neurons delays emergence from anesthesia and without changing anesthetic induction (Kelz et al., 2008). The findings support the hypothesis that general anesthesia depends, in part, on recruitment of orexins system and stabilization of wake–active regions of brain. However, which region or which pathway mediated the effect of orexins was not elucidated from previous study.

The cholinergic ascending arousal system is one of the cortical activating systems among the various arousal systems in the brain. The system have two pathways; a dorsal pathway from the pedunculopontine tegmentum nuclei (PPTg) to the cortex through the thalamus and a ventral pathway through the hypothalamus toward the basal forebrain (BF) (Jones, 2003). Of the two pathways, the ventral ascending activating system has been shown to have a predominant role on cortical activation (Detari et al., 1999; Dringenberg and Olmstead, 2003). In fact, the cholinergic ventral ascending activating system, which is composed of the posterior hypothalamus, the BF and the cortex, regulates the state of consciousness during natural sleep–wake cycle (Villablanca, 2004). Among neuronal systems which relay input to the BF, the role of orexins on the maintenance of arousal or anesthesia remains to be well defined. In our previous study, an arousal pattern change of EEG was observed after orexins microinjection into BF under isoflurane anesthesia (Dong et al., 2006). However, it remains uncertain whether this phenomenon can be reproduced in other common used volatile anesthetics, such as sevoflurane. As a central region of the cholinergic ventral ascending activating system, BF receives the projections from orexin neurons (Taheri et al., 1999). It is important to clarify the role of orexin signal in BF in the regulating emergence and induction to general anesthesia.

Thus, in the present study, we first examined whether intrabasalis injection of orexin-A or orexin-B might influence the EEG in

* Corresponding author. Tel.: +86 2984775337; fax: +86 2984775005.
E-mail addresses: lxiong@fmmu.edu.cn, hldong6@hotmail.com (L. Xiong).
[1] These authors are co-first authors.

0143-4179/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.npep.2009.04.006

the cortex under sevoflurane anesthesia in the rat. In addition, we examined whether intrabasalis injection of orexins agonists (orexin-A and orexin-B) or orexin-1 receptor antagonist (SB-334867A), alters the induction and emergence time to sevoflurance anesthesia. The hypothesis of current study is to clarify the involvement of orexins system on the regulating of sevoflurane anesthesia and to demonstrate that the regulation is mediated by the orexin signaling in the basal forebrain.

2. Material and methods

2.1. Animals

Male Sprague-Dawley (SD) rats, weighing 270–320 g, were provided by Animal Center of Fourth Military Medical University (Xi'an, China). They were housed at a constant temperature (24 ± 0.5 °C) with a relative humidity (60 ± 2%) on a light-controlled schedule (light on between 6:00 AM and 6:00 PM), and had free access to food and water. The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation and was conducted according to the Guidelines for Animal Experimentation of our institutes.

2.2. Surgical operations

Under pentobarbital anesthesia (50 mg/kg, i.p.), rats underwent surgery for implantation of electrodes for EEG recording and placement of a guide cannula for the injection needle. A guide cannula (outer diameter, 0.6 mm) for microinjection needle (14 mm) was directed stereotaxically at the BF. The coordinates of the microinjection needle tip according to the atlas of Paxinos and Watson (1998) were as follows: the BF (nucleus basalis, substantia innominata): anteroposterior; −1.4 mm, lateral; 2.5 mm, dorsoventral; −8.5 mm from bregma. The injection needle for the BF was 2 mm longer than the guide cannula. The microinjection canula into the BF were inserted and fixed with dental cement 5–7 days before the experiment.

Five stainless steel screws for epidural EEG recording were implanted. The EEG screws were placed bilaterally over the frontal (anterior 3.9 mm, lateral ±2.0 mm to the bregma) and the occipital cortex (posterior 7.4 mm, lateral ±5.0 mm to the bregma). The leads were connected to a socket, which was fixed to the skull together with the electrodes and the guide cannula using dental cement.

2.3. Experiment protocol

2.3.1. Experiment I: orexins microinjection under sevoflurane anesthesia

The rats were anesthetized again with 3% sevoflurane one day before the experiments. A heparin saline-filled polyethylene catheter (Fishersci Co., USA) was inserted into the femoral artery, for the measurement of arterial blood pressure. The tip of the arterial catheter was positioned in the abdominal aorta. The catheter was exteriorized on the back of the rat.

After 5–7 days for recovery from the surgical operations for implantation of EEG electrodes and insertion of microinjection cannula, the rats were transferred from their own home cage to a cube cage (internal diameter 25 cm, height 30 cm) for injection and electrical recordings. They were connected to the EEG recording cable for adaptation to the experimental conditions. One minimum alveolar concentration (MAC 2.4%) sevoflurane (Baxter, IL, USA) was added to the gas mixture for 30 min after a control period. The concentration reached a plateau within 1–2 min and became stable, because the outlet of the gas tubing was placed at the bottom of the cage and high flow rate (3 l/min) of oxygen containing sevoflurane immediately filled with the atmosphere around the rat. The concentration of sevoflurane was continuously measured by a side-stream capnometer (Capnox; Dex-ohmeda, USA). The 2.4% sevoflurane was considered to be 1.0 MAC as reported previously (Obal et al., 2001). According to the solutions that injected into the BF, the animals were divided into two groups. Orexin-A group ($n = 5$): 0.3 μl of 30, 100 pmol orexin-A (American Peptide, CA, USA) or the Ringer's solution (control) was injected into the BF at a random sequence under 1.0 MAC sevoflurane anesthesia. Orexin-B group ($n = 6$): 30, 100 pmol orexin-B (American Peptide, CA, USA) or the Ringer's solution (control) were injected into the BF using the same procedures as the orexin-A group described above. The interval between injections of the solutions was 60 min. Microinjections were performed with a Hamilton syringe (1 μl, Hamilton, Reno, NV, USA) connected to polyethylene tubing (PE-10, 0.1 × 0.4, 50 cm, USA) using a micropump (Pump 11plus, Harvard Apparatus, MA, USA). The injection speed of the solution with the micropump was 0.06 μl/min. The mean arterial pressure, heart rate, blood gas and EEG were monitored during the experiment.

2.3.2. Experiment II: pharmacologic activation or inhibition of orexin signaling and righting reflex studies

To determine the effect of the intrabasalis injection of orexins on induction of anesthesia, 25 SD rats were evaluated for the latency to loss of righting reflex. Fifteen minutes before sevoflurane induction, orexin-A (0.3 μl of 30, 100 pmol, $n = 5$ in each) or orexin-B (0.3 μl of 30, 100 pmol, $n = 5$ in each) were injected into the BF in the rats in orexin-A group or orexin-B group, respectively. The Ringer's solution was injected into the BF in control groups ($n = 5$ in each). All groups of rats were then anesthetized by 2.4% sevoflurane 15 min after injection. Righting reflex was checked every 15 s. To determine the intrabasalis microinjection of orexins on the emergence of sevofluane anesthesia, the recovery of righting reflex (emergence $time_{RR}$) were evaluated in the same rats three days later. Thirty minutes after 2.4% sevoflurane anesthesia, orexin-A (0.3 μl of 30, 100 pmol, $n = 5$ in each) or orexin-B (0.3 μl of 30, 100 pmol, $n = 5$ in each) were injected into the BF in the rats in orexin-A group or orexin-B group, respectively. The Ringer's solution was injected into the BF in control groups as described above. Fifteen minutes after microinjection, anesthetic gases were discontinued and emergence $time_{RR}$ was recorded.

To determine the effect of the orexin-1 receptor antagonist, SB-334867A (Tocris Bioscience), on induction of anesthesia, 15 SD rat were evaluated for the latency to loss of righting reflex. The 0.3 μl of the Ringer's solution ($n = 5$) or SB-334867A (5 μg or 20 μg, $n = 5$ in each), a selective orexin-1 receptor antagonist, dissolved in the Ringer's solution was injected into the BF through the injection needle 15 min before the sevoflurane induction. The Ringer's solution was injected into the BF as a control. All the rats in three groups were anesthetized by 2.4% sevoflurane. Righting reflex was checked every 15 s. To determine the intrabasil microinjection of orexin-1 receptor inhibitor on the emergence of sevofluane anesthesia, the recovery of righting reflex were evaluated in the same animals three days later. Thirty minutes after 2.4% sevoflurane anesthesia, 0.3 μl of the Ringer's solution or SB-334867A were injected into the BF in the rats. Fifteen minutes after microinjection, anesthetic gases were discontinued and emergence $time_{RR}$ was recorded.

2.4. EEG recording and analysis

EEG was measured continuously before and after orexin-A, orexin-B, or SB-334867A microinjection. EEG signal was on-line digitized at a sampling rate of 200 Hz, and subjected to off-line

spectral analysis. In current study, the EEG global frequency band (0–30 Hz) was divided into four frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz) and beta (13–30 Hz) bands. If EEG was exhibiting a burst suppression pattern, the burst suppression ratio (BSR: percentage of total sum of isoelectric EEG time for 60 s) was measured. In the experiment 1, the time of EEG arousal was measured after intrabasalis microinjection of orexin-A or orexin-B. EEG arousal was defined as the arousal pattern which is characterized by low-amplitude, high-frequency signals. In experiment 2, EEG was continuously monitored for referring.

2.5. Loss and return of righting reflex

Induction to and emergence from sevoflurane were defined behaviorally as the respective loss and return of the righting reflex. The time courses were evaluated as follows. Rats were placed in cylindrical gas-tight, controlled-environment chambers arrayed in parallel. After 90 min of habituation with 100% oxygen each day on two successive days, anesthesia was induced with a Drager model 19.1 sevoflurane vaporizer by increasing the concentration of anesthetic gas dissolved in 100% oxygen. The volatile anesthetic concentration was 2.4% sevoflurane. At the 15 s interval, the cylindrical chambers were rotated 180°. A rat was considered to have lost the righting reflex if it did not to turn itself prone onto all four limbs. Rat temperature was maintained between 37.0 ± 0.5 °C by control the environment temperature.

2.6. Histological verification

Correct placement of the tip of the guide cannula for the microinjection in the nucleus was confirmed by histological examination for each animal. At the end of the experiment, Evans blue (0.3 μl) was injected into the BF to verify the site of orexin administration through the microinjection needle. Then, the animals were further anesthetized with excess sodium pentobarbital, and perfused transcardially with the saline followed by the 10% formalin in 0.1 M phosphate buffer (pH 7.4). Fifty-micrometer sections were collected on a cryostat microtome. Sections containing the track of the guide cannula were stained with neutral red. Digitized sections were compared with coronal plates from a rat brain atlas (Paxinos and Watson, 1998) to determine the stereotaxic coordinates for each microinjection sites. The photographies of the representative brain sections showing the guide cannula track, microinjection location is shown in Fig. 5.

2.7. Statistical analysis

One way ANOVA was used for the comparisons of BSR in electroencephalogram and the time of electroencephalogram arousal. Induction and emergence time data generated immediately after each anesthetic exposure are reported as a mean ± standard error. Induction and emergence time were analyzed with one-way ANOVA with post hoc Bonferroni multiple comparison testing where appropriate. All statistical analyses were undertaken using the SPSS 11.5 program (SPSS, Chicago, IL, USA) and a 5% probability of Type I errors was used to determine statistical significance. In all cases, $P < 0.05$ was taken as the level of significance.

3. Results

3.1. Effects of intrabasalis microinjection of orexins on EEG in the cortex under sevoflurane anesthesia

The changes in BSR, number of EEG aroused animals and time of EEG arousal (min) induced by intrabasalis microinjection of orexins are shown in Tables 1 and 2. The burst and suppression patterns in EEG were observed in all animals under 1.0 MAC sevoflurane anesthesia before injection of the orexins. A representative illustration of the EEG changes after microinjection of 100 pmol orexin-A or orexin-B is shown in Fig. 1A and B. Microinjection of 100 pmol orexin-A resulted in a change in EEG from the burst and suppression patterns to the arousal patterns in five out of six animals used in the group (Fig. 1A), whereas in four of six animals the same dose of orexin-B did not shift the EEG into arousal patterns (Fig. 1B). No significant difference in BSR was found between the basal condition and the conditions after microinjection of the Ringer's solutions. The BSR after 30 pmol orexin-A microinjection was significantly smaller than that in the basal condition ($P < 0.05$; Table 1). Three of the six animals after 30 pmol microinjection and five of the six animals after 100 pmol orexin-A microinjection showed the arousal pattern's changes in EEG under 1.0 MAC sevoflurane anesthesia. The time of EEG arousal after intrabasalis microinjection of 100 pmol orexin-A were significantly longer than that observed after microinjection of 30 pmol orexin-A ($P < 0.05$; Table 1).

No significant difference in BSR was observed for the basal conditions, or following microinjection of the Ringer's solution and 30 pmol of orexin-B into the BF. The BSRs after intrabasalis microinjection of 100 pmol orexin-B were significantly smaller than those at basal condition ($P < 0.05$; Table 2). Microinjection of 100 pmol orexin-B changed the burst and suppression patterns to arousal EEG pattern under 1.0 MAC isoflurane anesthesia in two of six animals (Table 2). No animal's EEG changed to arousal pattern after 30 pmol orexin-B microinjection. No significant difference in the mean arterial pressure, heart rate and blood gas was observed after each dose of orexin-A, orexin-B and the Ringer's solution.

3.2. The effects of intrabasalis microinjection of orexins on the induction and emergence of sevoflurane anesthesia

Loss of righting reflex and return of righting reflex were used to determine induction and emergence of anesthesia (Campagna et al., 2003). To explore the intrabasalis microinjection of orexins on the induction of sevoflurane anesthesia, rats were treated with vehicle or two different doses of the orexin-A or orexin-B prior to 2.4% sevoflurane inhalation and found no difference in time to induction of anesthesia (Fig. 2A and B).

Although intrabasalis microinjection of orexin-A failed to alter induction of sevoflurane anesthesia, it induced dramatic differences in emergence from sevoflurane anesthesia. Rats injected with orexin-A (30 and 100 pmol) showed markedly shorted emergence from anesthesia (40% less time to emerge) ($P < 0.05$, $P < 0.01$, respectively) (Fig. 3A). However, intrabasalis microinjection of orexin-B failed to alter the emergence to sevoflurane anesthesia (Fig. 3B).

3.3. The effects of intrabasalis microinjection of SB-334867A, an orexin-1 receptor antagonist, on the induction and emergence of sevoflurane anesthesia

To explore the disruption of orexin signaling on the induction of sevoflurane anesthesia, rats were treated with vehicle or two different doses of the selective orexin-1 receptor antagonist, SB-334867A. Similar as found in above experiment, no differences in time to induction of anesthesia were detected at both doses of SB-334867A intrabasalis microinjection (Fig. 4A). Although intrabasalis microinjection of SB-334867A failed to alter induction of sevoflurane anesthesia, it induced dramatic differences in emergence from sevoflurane anesthesia. Rats injected with SB-334867A (5 and 20 μg) showed markedly delayed emergence from anesthesia (20–40% more time to emerge) ($P < 0.01$) (Fig. 4B).

Table 1
EEG changes caused by orexin-A intrabasalis microinjection under 1.0 MAC sevoflurane.

Group	BSR	EEG arousal number	EEG arousal duration (min)
1.0 MAC Sev.	69.0 ± 2.24	0	0
Ringer	70.2 ± 1.84	0	0
30 pmol orexin-A	57.5 ± 2.58*	3	16.2 ± 3.78
100 pmol orexin-A	45.3 ± 0.00	5	25.5 ± 5.32**

* $P < 0.05$ vs 1.0 MAC Sev.
** $P < 0.05$ vs 30 pmol orexin-A.

Table 2
EEG changes caused by orexin-B intrabasalis microinjection under 1.0 MAC sevoflurane.

Group	BSR	EEG arousal number	EEG arousal duration (min)
1.0 MAC Sev.	72.1 ± 3.85	0	0
Ringer	71.5 ± 4.33	0	0
30 pmol orexin-B	63.1 ± 3.85	0	0
100 pmol orexin-B	51.5 ± 1.95*	2	19.2 ± 5.64

* $P < 0.05$ vs 1.0 MAC Sev.

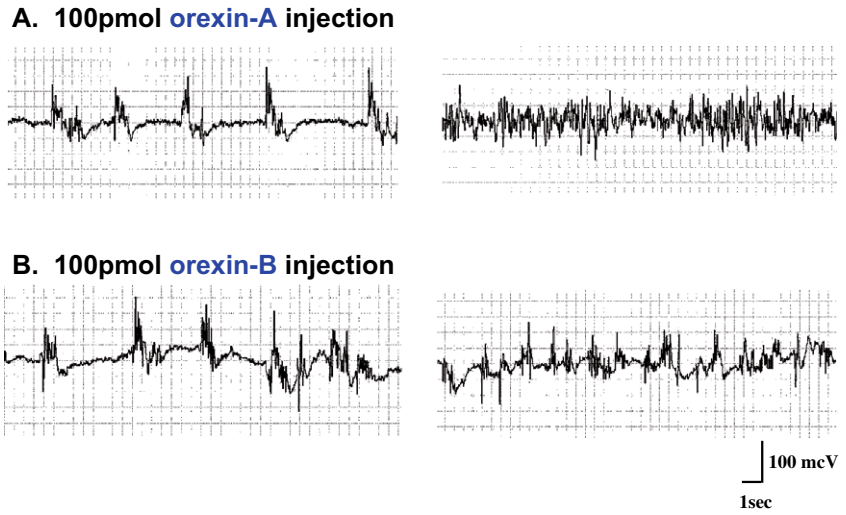

Fig. 1. Typical tracings of an electroencephalogram before and after intrabasalis microinjection of orexin-A or orexin-B. (A) Basal electroencephalogram showed burst and suppression patterns (*upper left panel*). Microinjection of orexin-A (100 pmol) changed a burst and suppression pattern to an arousal pattern (*upper right panel*) of the electroencephalogram during 1.0 minimum alveolar concentration (MAC) sevoflurane anesthesia. (B) In two of six animals, microinjection of orexin-B (100 pmol) did not influence a burst and suppression pattern in electroencephalogram during 1.0 MAC sevoflurane (*lower panel*).

Administration of 20 μg of SB-334867A increased more emergence time from sevoflurane anesthesia than 5 μg of SB-334867A administration ($P < 0.001$).

4. Discussion

The main finding of our study was that microinjection of orexin-A and orexin-B into the BF induced the sevoflurane-induced anesthetic arousal by changing EEG. Microinjection of orexin-A into the BF was more potent than orexin-B in producing an arousal EEG shift. We demonstrated that intrabasalis microinjection of orexin-A shorted the emergence time to sevoflurane anesthesia, but did not change induction time. The intrabasalis administration of orexin-B did not altered induction and emergence of anesthesia. Intrabasalis microinjection of orexin-1 receptor antagonist (SB-334867A) delayed the emergence time to sevoflurane anesthesia, without changing anesthetic induction. These findings suggest the involvement of an orexinergic pathway in anesthesia–arousal regulation and the orexin signals in basal forebrain plays a crucial role in this regulation.

As we know, the essential goals of the anesthetic state are immobility, unconsciousness and amnesia. Kendig et al. put forward the hypothesis that different components of general anesthesia arise from the effects of a single drug in different parts of the CNS (Kendig, 1993; Eger et al., 1997), and it is now widely accepted that general anesthetics cause immobility by depressing spinal neurons, and amnesia and hypnosis by acting on neurons in the brain (Campagna et al., 2003; Sonner et al., 2003). At higher, hypnotic concentrations, subcortical structures, including the thalamus, midbrain reticular formation and possibly the hypothalamus, are also affected. It has been proved that propofol-induced hypnosis is through a mechanism of action on GABAergic system (Alkire and Haier, 2001). However, the pattern of depressed brain areas varies between intravenous and volatile anesthetics, such as isoflurane and sevoflurane, indicating that they act on different molecular targets.

It has been widely argued whether sleep and general anesthesia are share common neural circuit or not. Recently, accumulating evidences indicate sleep and general anesthesia use common neuronal and genetic substrates. Tung et al. found that sleep

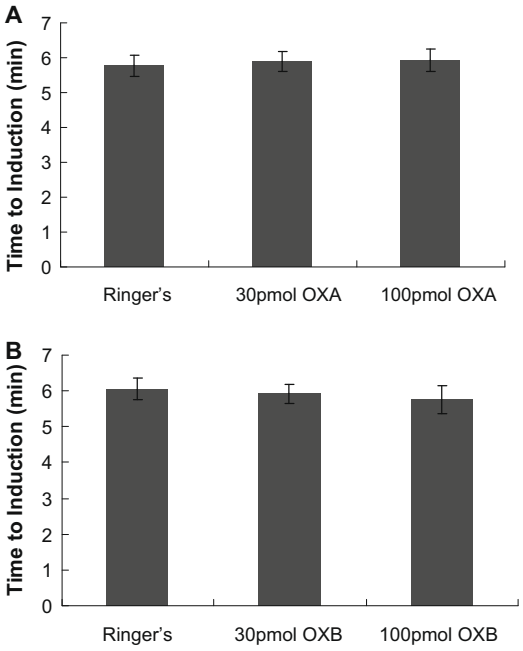

Fig. 2. Pharmacologic activation of orexin signaling in BF on the change of induction to sevoflurane anesthesia. Induction to anesthesia was determined by the time from sevoflurane inhalation (2.4%, 1 MAC) to loss of the righting reflex. Microinjection of orexin-A (A) or orexin-B (B) into BF did not change the induction time of sevoflurane. OXA: orexin-A; OXB: orexin-B.

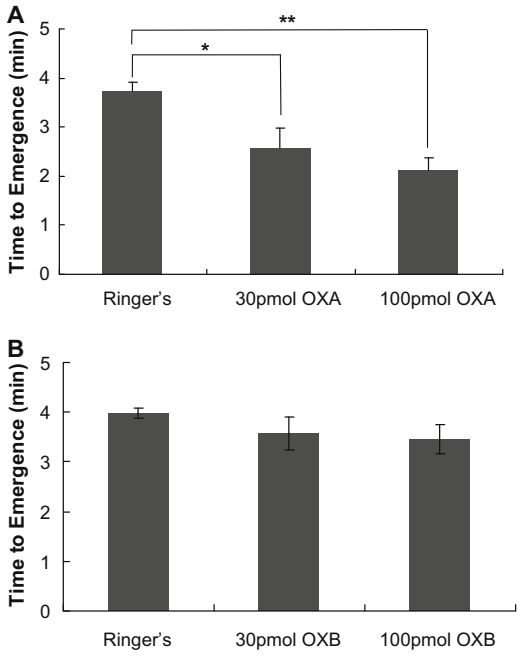

Fig. 3. Pharmacologic activation of orexin signaling in BF on the change of emergence from sevoflurane anesthesia. Emergence from anesthesia was determined by the time elapsed from discontinuation of sevoflurane inhalation (2.4%, 1 MAC) until the return of the righting reflex. Microinjection of orexin-A (OXA, 30 and 100 pmol) showed markedly shorted emergence from anesthesia ($P < 0.05$, $P < 0.01$, respectively) (A). Intrabasalis microinjection of orexin-B (OXB) failed to alter the emergence to sevoflurane anesthesia (B). $^{*}P < 0.05$; $^{**}P < 0.01$.

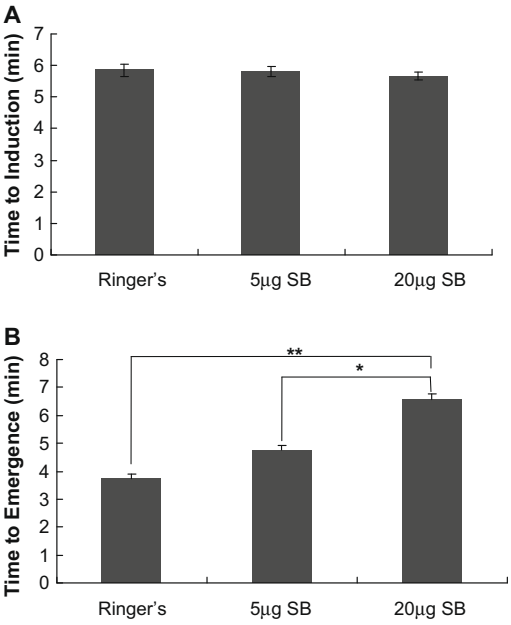

Fig. 4. Pharmacologic inhibition of orexin signaling in BF on the change of induction and emergence to sevoflurane anesthesia. Microinjection of SB-334867A (A) into BF did not change the induction time of sevoflurane. Intrabasalis microinjection of SB-334867A (5 and 20 μg) showed markedly delay of emergence from anesthesia ($P < 0.01$, $P < 0.001$, respectively) (B). Administration of 20 μg of SB-334867A increased more emergence time from sevoflurane anesthesia than 5 μg of SB-334867A administration ($P < 0.001$). SB: SB-334867A. $^{*}P < 0.01$; $^{**}P < 0.001$.

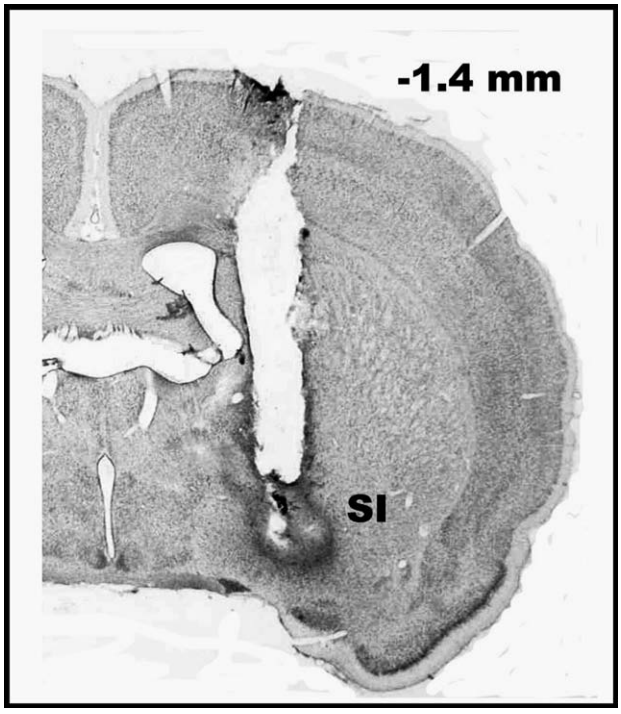

Fig. 5. Representative histological determination of the microinjection location in the brain slices. The substantia innominata (SI, microinjection location) is shown in the photo. Numbers at the *top right* indicate distance from bregma along the anteroposterior axis.

deprivation significantly potentiated the ability of inhaled and intravenous anesthetic agents to induce a loss of righting reflex (Tung et al., 2002). The result supports that neuronal networks active in sleep are also involved in the anesthetized state. Functional-brain-imaging study also revealed general anesthetics act on sleep–wake neural circuitry (Alkire et al., 2000). However, among the numerous neural loci related to sleep, which one plays the fundamental role on the regulation of anesthesia–arousal?

As a specific neural locus of sleep–wake regulation, orexin system is presumed to alter the anesthesia–arousal regulation. The orexinergic system was found in 1998 by two independent groups (Chicurel, 2000; Samson and Resch, 2000). The peptides were named orexins, because they increased food intake in nonfasted rats. The system includes the 33- and 28-aa peptides orexin-A and orexin-B and the orexin-1 and orexion-2 receptors. Both orexins were found preferentially in the rat brain and more specifically in the hypothalamus (Sakurai et al., 1998). One of the most prominent and well-demonstrated effect of orexins is regulation of sleep/wakefulness (Sutcliffe and de Lecea, 2000; Kilduff and Peyron, 2000; Willie et al., 2001). A numbers of studies have demonstrated that orexin and orexin neurons are important regulators of sleep and awake (de Lecea and Sutcliffe, 2005; Jones, 2008). However, the effect of orexins on the anesthesia–arousal regulation remained unclear for a long time. Kushikata et al. (2003) have found that orexinergic neurons may be an important target for barbiturates, and GABAA, orexin-1 and orexion-2 receptors may not be involved in this interaction. In a pilot study to recognize a role for orexins in modulating anesthetic action, Yasuda et al. (2003) demonstrated that intracerebroventricular delivery of orexin-A induced signs of EEG arousal in rats during isoflurane anesthesia. Orexins appear to partially antagonize anesthetic-induced hypnosis by activation of the neural substrates required for arousal. Base on this finding, we further demonstrated that intrabasalis injection of orexin-A elicited an arousal EEG change under isoflurane anesthesia (Dong et al., 2006). Kelz et al. (2008) proved that disruption of orexin signaling selectively affects emergence from, but not induction of, volatile general anesthesia. These evidences further solidify the connection between anesthesia and sleep and indicates that orexin signaling is involved in the wake-promoting neural circuitry which selectively contributing to emergence from anesthesia.

The results of current study support the role of orexinergic system in the anesthetic–arousal regulation under volatile anesthesia. Our results showed that intrabasalis orexin-A microinjection induced arousal EEG patterns change under 1.0 MAC sevoflurane anesthesia. However, microinjection of low dose of orexin-B (30 pmol) into the basal forebrain failed to induce arousal EEG patterns change. The arousal EEG patterns shift could be observed just after 100 pmol orexin-B intrabasalis microinjection in two of six animals under 1.0 MAC sevoflurane anesthesia. These findings suggest that sevoflurane anesthesia might be partially reversed by microinjection of orexins into the BF, especially when orexin-A was used. The results of experiment done in the unanesthetized animals have found intracerebroventricular injection of orexin-A induced more potent effects than orexin-B on behavioral and electrophysiological measures of arousal (Espana et al., 2001). The results were consistent with our present study done in sevoflurane anesthetized animals in the aspects that both orexins activated the cortical activation and that orexin-A induced more potent influences than orexin-B. It has been previously reported that endogenous orexins can be increased by forced motor (swim) activity (Martins et al., 2004), fasting and insulin induced hypoglycemia (Lopez et al., 2000), and corticotropin-releasing hormone (CRH) (Winsky-Sommerer et al., 2004). A recent study has proved that maternal deprivation (MD) induces increase of orexin level in the neonatal rat brain (Feng et al., 2007). All of these findings suggest that endogenous orexins can be elevated at some physiological or pathological conditions.

Kelz et al. (2008) found that 1.0 MAC isoflurane resulted in a 30% reduction and 1.0 MAC sevoflurane resulted in a 50% reduction in c-Fos-expression in orexinergic neurons. The difference of reduction ratio between isoflurane and sevoflurane may reflect that the pharmacologic effects are different in two volatile anesthetics. This may partially explain the different EEG arousal ratio between isoflurane anesthesia and sevoflurane anesthesia to intrabasalis injection of orexins that found in current and our previous study. Whether the inhibition of orexinergic neurons is caused by a direct action of volatile anesthetics on orexinergic neurons themselves or on their afferents need to be elucidated.

The cholinergic ascending arousal pathway is one of the most powerful cortical activation systems. The ventral ascending activating system of this pathway, comprising the posterior hypothalamus, the BF and the S1BF, has been known to regulate the state of consciousness during the natural sleep–wake cycle (Dringenberg and Olmstead, 2003; Villablanca, 2004) and contain orexin fibers (Taheri et al., 1999). Among these nuclei, the BF is believed to play the most dominant role (Detari et al., 1999; Dringenberg and Olmstead, 2003).

To clarify the role of orexin signal in BF on regulating of inhalation anesthesia, loss of righting reflex (LORR) and return of righting reflex were used to determine induction and emergence of anesthesia after intrabasalis injection of orexin agonists and antagonist, respectively. The emergence time to sevoflurane anesthesia was shorted by intrabasalis microinjection of orexin-A and was delayed by intrabasalis microinjection of orexin-1 receptor antagonist SB-334867A. SB-334867A produced a dose dependent delay in emergence of animals exposed to sevoflurane, and significantly delayed emergence at 20 μg intrabasalis injection. The induction time to sevoflurane anesthesia was unaffected either by intrabasalis microinjection of orexin-A or SB-334867A. From the results mentioned above, we speculated that BF is the most important transduction region of orexins signaling for regulating anesthesia–arousal. The previous anatomical studies also support that orexin neurons contribute to hypothalamo-basal forebrain pathway (Schofield and Harrison, 2005).

Two receptor types have been identified for orexin-A and orexin-B: orexin-1 and orexin-2 receptors (Trivedi et al., 1998). It has been demonstrated that orexin-1 and orexin-2 receptors are co-expressed in the neurons in BF (Marcus et al., 2001). Orexin-A has higher affinity for orexin-1 receptor than orexin-B, whereas both orexin-A and orexin-B almost equally bind orexin-2 receptor at high affinity (Kilduff and Peyron, 2000). Thus, orexin-1 receptor is considered to be a selective receptor for orexin-A, and orexin-2 receptor is considered to be a nonselective receptor for both orexin-A and orexin-B. In the current study, orexin-A was more potent than orexin-B in causing EEG activation, suggesting that the orexin-1 receptor may have major contribution to the activity at the BF neurons under sevoflurane anesthesia. The emergence time delay induced by intrabasalis SB-334867A in current study also proved that orexin-1 receptor is a significant mediator of neurotransmission in the BF.

In summary, we demonstrated that intrabasalis administration of orexin-A and B elicited anesthetic arousal under sevoflurane anesthesia in terms of EEG pattern. Orexin-A is more potent in the cholinergic BF neurons than orexin-B. The emergence time to sevoflurane anesthesia was altered by intrabasalis injection of orexin-A and SB-334867A. It indicated that the arousal activation of orexin might be mediated mainly through the afferents in the BF and the orexin-1 receptor. Taken together, the activation of cholinergic ventral ascending pathways may contribute, at least in part, to the emergence to sevoflurane anesthesia through the orexinergic system.

Acknowledgement

This work was supported in part by the National Natural Science Foundation of China (Grant 30725039 to Lize Xiong, Grant 30772075 to Hailong Dong, Grant 30600582 to Zhenghua Zhu).

References

Alkire, M.T., Haier, R.J., 2001. Correlating in vivo anaesthetic effects with ex vivo receptor density data supports a GABAergic mechanism of action for propofol, but not for isoflurane. Br. J. Anaesth. 86, 618–626.

Alkire, M.T., Haier, R.J., Fallon, J.H., 2000. Toward a unified theory of narcosis: brain imaging evidence for a thalamocortical switch as the neurophysiologic basis of anesthetic-induced unconsciousness. Conscious. Cogn. 9, 370–386.
Campagna, J.A., Miller, K.W., Forman, S.A., 2003. Mechanisms of actions of inhaled anesthetics. N. Engl. J. Med. 348, 2110–2124.
Chemelli, R.M., Willie, J.T., Sinton, C.M., Elmquist, J.K., Scammell, T., Lee, C., Richardson, J.A., Williams, S.C., Xiong, Y., Kisanuki, Y., Fitch, T.E., Nakazato, M., Hammer, R.E., Saper, C.B., Yanagisawa, M., 1999. Narcolepsy in orexin knockout mice. molecular genetics of sleep regulation. Cell 98, 437–451.
Chicurel, M., 2000. Neuroscience. The sandman's secrets. Nature 407, 554–556.
de Lecea, L., Sutcliffe, J.G., 2005. The hypocretins and sleep. FEBS J. 272, 5675–5688.
Detari, L., Rasmusson, D.D., Semba, K., 1999. The role of basal forebrain neurons in tonic and phasic activation of the cerebral cortex. Prog. Neurobiol. 58, 249–277.
Dong, H.L., Fukuda, S., Murata, E., Zhu, Z., Higuchi, T., 2006. Orexins increase cortical acetylcholine release and electroencephalographic activation through orexin-1 receptor in the rat basal forebrain during isoflurane anesthesia. Anesthesiology 104, 1023–1032.
Dringenberg, H.C., Olmstead, M.C., 2003. Integrated contributions of basal forebrain and thalamus to neocortical activation elicited by pedunculopontine tegmental stimulation in urethane-anesthetized rats. Neuroscience 119, 839–853.
Eger, E.I., Koblin, D.D., Harris, R.A., Kendig, J.J., Pohorille, A., Halsey, M.J., Trudell, J.R., 1997. Hypothesis: inhaled anesthetics produce immobility and amnesia by different mechanisms at different sites. Anesth. Analg. 84, 915–918.
Espana, R.A., Baldo, B.A., Kelley, A.E., Berridge, C.W., 2001. Wake-promoting and sleep-suppressing actions of hypocretin (orexin): basal forebrain sites of action. Neuroscience 106, 699–715.
Feng, P., Vurbic, D., Wu, Z., Strohl, K.P., 2007. Brain orexins and wake regulation in rats exposed to maternal deprivation. Brain Res. 1154, 163–172.
Jones, B.E., 2003. Arousal systems. Front. Biosci. 8, s438–s451.
Jones, B.E., 2008. Modulation of cortical activation and behavioral arousal by cholinergic and orexinergic systems. Ann. NY Acad. Sci. 1129, 26–34.
Kelz, M.B., Sun, Y., Chen, J., Cheng, M.Q., Moore, J.T., Veasey, S.C., Dixon, S., Thornton, M., Funato, H., Yanagisawa, M., 2008. An essential role for orexins in emergence from general anesthesia. Proc. Natl. Acad. Sci. USA 105, 1309–1314.
Kendig, J.J., 1993. Spinal cord as a site of anesthetic action. Anesthesiology 79, 1161–1162.
Kilduff, T.S., Peyron, C., 2000. The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. Trends Neurosci. 23, 359–365.
Kushikata, T., Hirota, K., Yoshida, H., Kudo, M., Lambert, D.G., Smart, D., Jerman, J.C., Matsuki, A., 2003. Orexinergic neurons and barbiturate anesthesia. Neuroscience 121, 855–863.
Lopez, M., Seoane, L., Garcia, M.C., Lago, F., Casanueva, F.F., Senaris, R., Dieguez, C., 2000. Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. Biochem. Biophys. Res. Commun. 269, 41–45.
Marcus, J.N., Aschkenasi, C.J., Lee, C.E., Chemelli, R.M., Saper, C.B., Yanagisawa, M., Elmquist, J.K., 2001. Differential expression of orexin receptors 1 and 2 in the rat brain. J. Comp. Neurol. 435, 6–25.
Martins, P.J., D'Almeida, V., Pedrazzoli, M., Lin, L., Mignot, E., Tufik, S., 2004. Increased hypocretin-1 (orexin-a) levels in cerebrospinal fluid of rats after short-term forced activity. Regul. Pept. 117, 155–158.
Nelson, L.E., Guo, T.Z., Lu, J., Saper, C.B., Franks, N.P., Maze, M., 2002. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. Nat. Neurosci. 5, 979–984.
Nelson, L.E., Lu, J., Guo, T., Saper, C.B., Franks, N.P., Maze, M., 2003. The alpha2-adrenoceptor agonist dexmedetomidine converges on an endogenous sleep-promoting pathway to exert its sedative effects. Anesthesiology 98, 428–436.
Obal, D., Preckel, B., Scharbatke, H., Mullenheim, J., Hoterkes, F., Thamer, V., Schlack, W., 2001. One MAC of sevoflurane provides protection against reperfusion injury in the rat heart in vivo. Br. J. Anaesth. 87, 905–911.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, Spiral Bound.
Piper, D.C., Upton, N., Smith, M.I., Hunter, A.J., 2000. The novel brain neuropeptide, orexin-A, modulates the sleep–wake cycle of rats. Eur. J. Neurosci. 12, 726–730.
Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J., Yanagisawa, M., 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92, 573–585.
Samson, W.K., Resch, Z.T., 2000. The hypocretin/orexin story. Trends Endocrinol. Metab. 11, 257–262.
Schofield, C.M., Harrison, N.L., 2005. Transmembrane residues define the action of isoflurane at the GABAA receptor alpha-3 subunit. Brain Res. 1032, 30–35.
Sonner, J.M., Antognini, J.F., Dutton, R.C., Flood, P., Gray, A.T., Harris, R.A., Homanics, G.E., Kendig, J., Orser, B., Raines, D.E., Rampil, I.J., Trudell, J., Vissel, B., Eger, E.I., 2003. Inhaled anesthetics and immobility: mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth. Analg. 97, 718–740.
Sutcliffe, J.G., de Lecea, L., 2000. The hypocretins: excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. J. Neurosci. Res. 62, 161–168.
Taheri, S., Mahmoodi, M., Opacka-Juffry, J., Ghatei, M.A., Bloom, S.R., 1999. Distribution and quantification of immunoreactive orexin A in rat tissues. FEBS Lett. 457, 157–161.
Trivedi, P., Yu, H., MacNeil, D.J., Van der Ploeg, L.H., Guan, X.M., 1998. Distribution of orexin receptor mRNA in the rat brain. FEBS Lett. 438, 71–75.
Tung, A., Szafran, M.J., Bluhm, B., Mendelson, W.B., 2002. Sleep deprivation potentiates the onset and duration of loss of righting reflex induced by propofol and isoflurane. Anesthesiology 97, 906–911.
Villablanca, J.R., 2004. Counterpointing the functional role of the forebrain and of the brainstem in the control of the sleep–waking system. J. Sleep Res. 13, 179–208.
Willie, J.T., Chemelli, R.M., Sinton, C.M., Yanagisawa, M., 2001. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu. Rev. Neurosci. 24, 429–458.
Winsky-Sommerer, R., Yamanaka, A., Diano, S., Borok, E., Roberts, A.J., Sakurai, T., Kilduff, T.S., Horvath, T.L., de Lecea, L., 2004. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. J. Neurosci. 24, 11439–11448.
Yasuda, Y., Takeda, A., Fukuda, S., Suzuki, H., Ishimoto, M., Mori, Y., Eguchi, H., Saitoh, R., Fujihara, H., Honda, K., Higuchi, T., 2003. Orexin a elicits arousal electroencephalography without sympathetic cardiovascular activation in isoflurane-anesthetized rats. Anesth. Analg. 97, 1663–1666.