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

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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

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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