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Assessment of the effect of etomidate on voltage-gated sodium channels and action potentials in rat primary sensory cortex pyramidal neurons

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ABSTRACT

Although it is known that general anesthetics can suppress cortical neurons' activity, the underlying mechanisms are still poorly understood, especially the kinetic changes of voltage-gated Na^+ channels, which are mostly related to neuronal excitability. Some general anesthetics have been reported to affect the voltage-gated Na^+ channels in cell culture derived from humans and animals. However no one has ever investigated the effects of etomidate on voltage-gated Na^+ channels in pyramidal neurons using a brain slice. The present study uses a whole cell patch-clamp technique to investigate the changes of voltage-gated Na^+ channels on primary somatosensory cortex pyramidal neurons under the influence of etomidate. We found that etomidate dose-dependently inhibited Na^+ currents of primary somatosensory cortex pyramidal neurons, while shifted the steady-state inactivation curve towards the left and prolonged the recovery time from inactivation. Conversely, etomidate has no effects on the steady-state activation curve. We demonstrated the detailed suppression process of neural voltage-gated Na^+ channels by etomidate on slice condition. This may offer new insights into the mechanical explanation for the etomidate anesthesia. Finding the effects of anesthetics on primary somatosensory cortex also provides evidence to help elucidate the potential mechanism by which tactile information integrates during general anesthesia.

1. Introduction

Every year, ten million patients receive surgery under general anesthesia. However, the mechanisms by which general anesthetics produce unconsciousness, amnesia and immobility have yet to be defined despite nearly 200 years of research. Etomidate, an ultrashort-acting nonbarbiturate intravenous anesthetic, is widely used for general anesthesia induction. The standard induction dose rapidly produces hypnosis and immobility, while lower doses provide sedation (Nyman et al., 2011). Although substantial electrophysiological evidence indicates that etomidate acts by enhancing the response of gamma-aminobutyric acid type A receptors (GABAARs) to GABA, or by directly activating these receptors (Atucha et al., 2009; Meera et al., 2009; Zhang et al., 2002), there are a few studies that look into the voltage-gated ion channels, which are the substrates of neuronal electrophysiological activity (Ray et al., 2006).

Research indicates that general anesthetics modulate many members of the voltage-gated ion channel superfamily, including voltage-gated Na^+, K^+, and Ca^{2+} channels (Lazarenko et al., 2010; Todorovic et al., 2000; Zhou et al., 2011), which may affect the cell signaling mechanisms, vesicular exocytotic mechanisms and transmitter uptake mechanisms. The contributions of these voltage-gated ion targets to the synaptic actions of general anesthetics have not been clearly defined.

The voltage-gated Na^+ channel is the main molecular basis of action potential (AP) firing. This voltage-gated ion channel modulates neuronal excitability, especially the AP-conducted neuronal signal transmission (Liu et al., 2009). Previous study (Ouyang et al., 2003) had shown the effects of another two representative general anesthetics, isoflurane and propofol, on voltage-gated Na^+ currents in nerve terminals isolated from rat neurohypophysis using patch-clamp electrophysiological analysis. Both isoflurane and propofol inhibited Na^+ currents in a dose-dependent and reversible manner. Inhibition of voltage-gated Na^+ channels may contribute

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to the presynaptic effects of general anesthetics on nerve terminal excitability and neurotransmitter release.

In order to investigate if etomidate has similar effects on voltage-gated Na^+ channels, the current study applied a whole-cell recording technique to observe, under the in vitro brain slice condition, the detailed dynamic change of voltage-gated Na^+ channels on the primary sensory cortex (S1) pyramidal neurons under etomidate perfusion. S1 is well innervated by the thalamus ventral posteromedial nucleus (VPM) and the main brain area processed integration of facial sensory feeling information (Schubert et al., 2007). Our early work had indicated that the thalamocortical pathway of S1 may be interrupted during general anesthesia (Tu et al., 2011). Therefore, the electrophysiological changes of S1 neurons may provide new data that will help elucidate the variation of sense perception under general anesthesia.

2. Materials and methods

All experimental and surgical procedures were approved by Committees on Investigations Involving Animals in Zunyi Medical College, China. All animals received humane care in compliance with the "Guide for the care and use of laboratory animals" in China (No. 14924, 2001). The rats were housed in a standard animal care room with a 12:12 h light–dark cycle at 22 °C and with free access to rodent chow and water.

2.1. Materials

Etomidate was obtained from Nhwa Pharma. Corp. (XuZhou, China). Tetraethyl-ammonium chloride and tetrodotoxin were purchased from Sigma-Aldrich. Male Sprague–Dawley rats (280–320 g) were purchased from animal center of third military medical university (Chongqing, China). Recording pipettes (tip diameter < 1 μm) were made from borosilicate glass capillaries (Sutter Instruments, Novato, CA) using the P-97 micropipette puller (Sutter Instruments, Novato, CA).

2.2. Slices

The rats were anesthetized with 2% isoflurane and decapitated. A rapid craniotomy was operated to allow the total brain mass to be detached quickly with a metal spatula. The brain was then immersed and refrigerated in cold (0 °C) artificial cerebrospinal fluid (ACSF) containing (mM) 115 NaCl, 5.6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 11 glucose, 1 NaH_2PO_4, and 25 $NaHCO_3$ (pH 7.4 when bubbled with 95% O_2–5% CO_2). A block tissue containing S1 was then isolated from the brain. The block was affixed with cyanoacrylate to the bottom of a cutting chamber. The tissue was again immersed in ACSF and further sectioned with the HM 650 V Vibroslicer (Thermo Instruments, US) to manufacture about six 350-μm-thick VPM/S1 slices. Before whole cell recording, the slices were incubated at room temperature in ACSF for about 1 h. All ACSF were bubbled with a mixture of 95% O_2 and 5% CO_2.

2.3. Whole-cell recording

After incubation, individual slices were transferred to a thermoregulated (32.8 °C) recording dish. Sites for voltage-gated Na^+ currents recording in rat coronal slices were taken according to the stereotaxic coordinates of S1 (Bregma coordinates: 2.5 mm posterior, 5.0–5.5 mm lateral, and 0.8–1.5 mm depth, Paul Halasz & Lewis Tsalis 5th, Fig.1A and B) and previously published atlases plotting (Datwani et al., 2002). Distinct S1 pyramidal neurons for whole-cell recordings were selected under a BX51WI microscope (Olympus, Japan) with an infrared camera (Fig.1C). Slices were

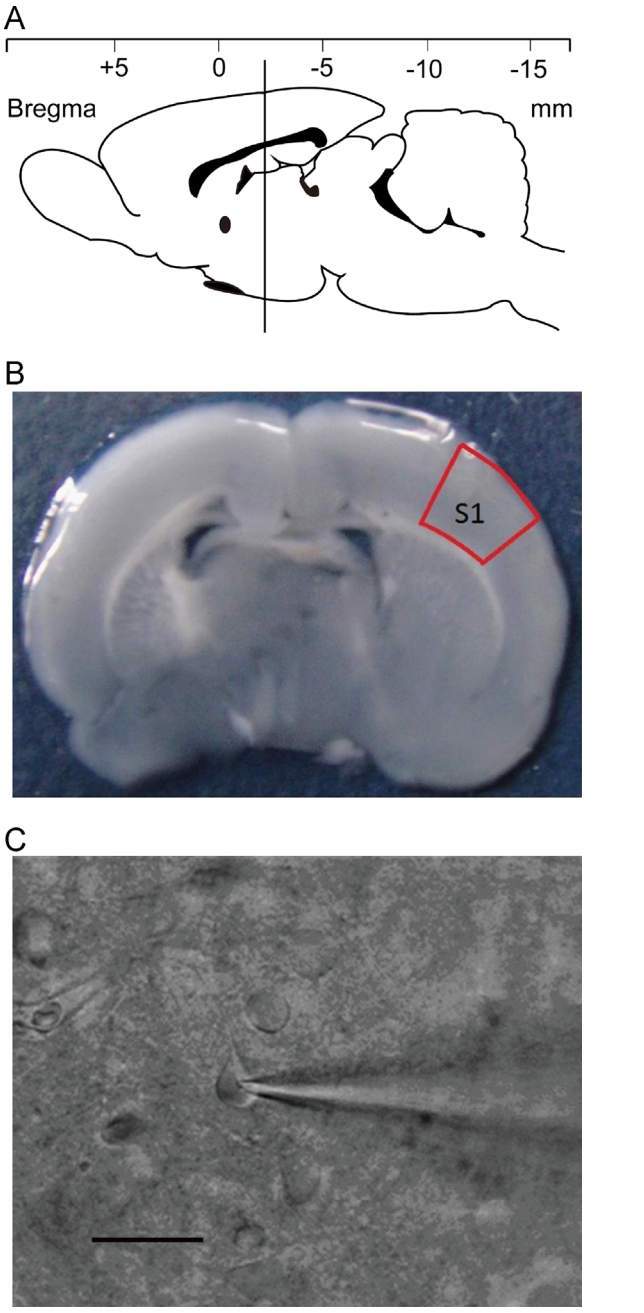

Fig. 1. Presentation of recording site. (A) Bregma coordinates of the primary somatosensory cortex (S1). (B) Coronal brain slice of 300 μm thickness and S1 recording area. (C) Pyramidal neuron with whole-cell recording electrode in place. Only one dendrite is visible in the focal plane. Scale bar, 20 μM.

maintained immersed and continuously perfused at 0.5–2 ml/min with ACSF. Tetraethyl-ammonium (TEA) containing ACSF used for Na^+ current recordings contained (mM) 95 NaCl, 5.6 KCl, 0.1 $CaCl_2$, 5 $MgCl_2$, 11 glucose, 1 NaH_2PO_4, 25 $NaHCO_3$ and 20 TEA-Cl (pH 7.4 when bubbled with 95% O_2–5% CO_2). The Na^+ currents were measured using high-Cs containing pipette solution (mM) 5.8 NaCl, 134 CsCl, 1 $MgCl_2$, 3 EGTA, and 10 HEPES (pH 7.3 adjusted with 9.2 mM NaOH). The pipette solution used for action potential recordings contained (mM) 5 NaCl, 144.4 KCl, 1 $MgCl_2$, 3 EGTA, and 10 HEPES (pH 7.3 by 10.6 mM KOH). Capacitance and 60–80% series resistance were routinely compensated. Voltage-gated Na^+ currents were sampled at 10 kHz and filtered at 2.9 kHz using an HEKA EPC10 amplifier and PatchMaster software (HEKA Instruments, Inc., Lambrecht, DE).