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PROPOFOL-INDUCED SPIKE FIRING SUPPRESSION IS MORE PRONOUNCED IN PYRAMIDAL NEURONS THAN IN FAST-SPIKING NEURONS IN THE RAT INSULAR CORTEX

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Abstract—Propofol is a major intravenous anesthetic that facilitates $GABA_A$ receptor-mediated inhibitory synaptic currents and modulates inward current (I_h), K^+, and voltage-gated Na^+ currents. This propofol-induced modulation of ionic currents changes intrinsic membrane properties and repetitive spike firing in cortical pyramidal neurons. However, it has been unknown whether propofol modulates these electrophysiological properties in GABAergic neurons, which express these ion channels at different levels. This study examined whether pyramidal and GABAergic neuronal properties are differentially modulated by propofol in the rat insular cortical slice preparation. We conducted multiple whole-cell patch-clamp recordings from pyramidal neurons and from GABAergic neurons, which were classified into fast-spiking (FS), low threshold spike (LTS), late-spiking (LS), and regular-spiking nonpyramidal (RSNP) neurons. We found that 100 μM propofol hyperpolarized the resting membrane potential and decreased input resistance in all types of neurons tested. Propofol also potently suppressed, and in most cases eliminated, repetitive spike firing in all these neurons. However, the potency of the propofol-induced changes in membrane and firing properties is particularly prominent in pyramidal neurons. Using a low concentration of propofol clarified this tendency: 30 μM propofol decreased the firing of pyramidal neurons but had little effect on GABAergic neurons. Pre-application of a $GABA_A$ receptor antagonist, picrotoxin (100 μM), diminished the propofol-induced suppression of neural activities in both pyramidal and FS neurons. These results suggest that GABAergic neurons, especially FS neurons, are less affected by propofol than are pyramidal neurons and that propofol-induced modulation of the intrinsic membrane properties and repetitive spike firing are principally mediated by $GABA_A$ receptor-mediated tonic currents

Key words: anesthetics, GABAergic, interneurons, nociception, tonic current.

INTRODUCTION

Propofol, a popular intravenous anesthetic, is considered to suppress cortical activities by facilitating $GABA_A$ receptor-mediated inhibitory synaptic currents (Koyanagi et al., 2014). In addition to the potentiation of GABAergic currents, propofol modulates other ionic currents in pyramidal neurons in the cerebral cortex. First, propofol hyperpolarizes the resting membrane potential by suppressing the hyperpolarization-activated inward current (I_h) (Higuchi et al., 2003; Chen et al., 2005; Ying et al., 2006), which is involved in depolarization of the resting membrane potential (Pape, 1996). Second, the voltage-dependent Na^+ currents are suppressed by propofol, which may result in decreased spike firing frequency in response to injection of depolarizing current pulses (Ratnakumari and Hemmings, 1997; Martella et al., 2005). Third, propofol also suppresses K^+ currents (Song et al., 2011; Zhang et al., 2016). This propofol-induced modulation of ionic currents is likely to diminish the electrical activities of cortical pyramidal neurons (Martella et al., 2005).

Pyramidal neurons are a major type of excitatory neurons in the cerebral cortex, and another 10–20% of cortical neurons are GABAergic neurons, which are classified into several types based on their firing and morphological profiles (Kawaguchi and Kubota, 1997; Koyanagi et al., 2010; Kobayashi et al., 2012). Characterizing the effects of propofol on these GABAergic neurons is critical to understanding the mechanisms of the propofol-induced suppression of cortical activity because pyramidal and GABAergic neurons have opposing effects

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Abbreviations: ACSF, artificial cerebrospinal fluid; ATP, adenosine triphosphate; DMSO, dimethyl sulfoxide; FS, fast-spiking; GTP, guanosine triphosphate; IC, insular cortex; I_h, hyperpolarization-activated inward current; LS, late-spiking; LTS, low threshold spike; RSNP, regular-spiking nonpyramidal; TASK, TWIK-related acid-sensitive K^+.

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on postsynaptic neurons, i.e., excitation and inhibition, respectively. However, it has remained unknown how propofol modulates membrane properties of GABAergic neurons in the cerebral cortex.

The propofol-mediated modulation of GABAergic neurons in other parts of the brain may be referenced to predict its effect on cortical GABAergic neurons. However, interestingly, propofol shows a wide variety of effects on GABAergic neurons. Propofol treatment of GABAergic neurons in the reticular thalamic nucleus produces increased firing frequency and input resistance due to blocking of SK channels (Ying and Goldstein, 2005). On the other hand, propofol treatment of GABAergic neurons in the ventrolateral preoptic nucleus produces increases in the firing frequency and in the frequency and amplitude of spontaneous EPSCs by blocking NKCC1 (Li et al., 2009). In contrast, in hippocampal CA1 GABAergic neurons, propofol potentiates GABAergic tonic currents, thereby hyperpolarizing the membrane potential and decreasing input resistance, which results in suppression of spike firing (Bieda and MacIver, 2004).

The present study aimed to examine whether pyramidal and GABAergic neurons in the rat insular cortex (IC) are differentially modulated by propofol. We compared propofol-induced modulation of the passive electrophysiological properties, including the resting membrane potential and input resistance, and repetitive firing properties between pyramidal and GABAergic neurons.

EXPERIMENTAL PROCEDURES

The Institutional Animal Care and Use Committee at Nihon University approved the study protocol, and all experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used as well as their suffering.

Slice preparations

The techniques for slice preparation and maintenance were similar to those described previously (Koyanagi et al., 2014; Yamamoto et al., 2015). Briefly, vesicular GABA transporter (VGAT)-Venus line A transgenic rats of either sex (postnatal days 18–35) were deeply anesthetized with isoflurane (5%) and decapitated. Tissue blocks including the IC were rapidly removed and stored for 3 min in ice-cold modified artificial cerebrospinal fluid (ACSF) (in mM: 230 sucrose, 2.5KCl, 10$MgSO_4$, 1.25NaH_2PO_4, 26$NaHCO_3$, 2.5$CaCl_2$, and 10 D-glucose). Coronal slices were cut to a thickness of 350 μm using a microslicer (Linearslicer Pro 7, Dosaka EM, Kyoto, Japan). Slices were incubated at 32 °C for 40 min in a submersion-type holding chamber that contained 50% modified ACSF and 50% normal ACSF (in mM: 126NaCl, 3KCl, 2$MgSO_4$, 1.25NaH_2PO_4, 26$NaHCO_3$, 2$CaCl_2$, and 10 D-glucose). Modified and normal ACSF were continuously aerated with a mixture of 95% O_2 and 5% CO_2. Slices were then placed in normal ACSF at 32 °C for 1 h and thereafter maintained at room temperature until used for recording.

Cell identification and paired whole-cell patch-clamp recording

The slices were transferred to a recording chamber that was continuously perfused with normal ACSF with 0.1% dimethyl sulfoxide (DMSO) at a rate of 2.0 ml/min. Whole-cell patch-clamp recordings were obtained from Venus-positive fluorescent neurons and Venus-negative pyramidal neurons identified in layer V of the IC using a fluorescence microscope equipped with Nomarski optics (× 40, ECLIPSE FN1, Nikon, Tokyo, Japan) and an infrared-sensitive video camera (IR-1000, DAGE-MTI, Michigan City, IN, USA). Electrical signals were recorded by amplifiers (Multiclamp 700B, Molecular Devices, Sunnyvale, CA, USA), and then digitized (Digidata 1440A, Molecular Devices), observed online, and stored on a computer hard disk using Clampex (pClamp 10, Molecular Devices).

The composition of the pipette solution for recordings unless otherwise specified was (in mM) as follows: 145 potassium gluconate, 5 KCl, 5 *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 2 $MgCl_2$, 5 magnesium adenosine triphosphate (ATP), and 5 EGTA. The pipette solution had a pH of 7.3 and an osmolarity of 300 mOsm. As an internal solution including high concentration of Cl^-, the following pipette solution was used: 85 potassium gluconate, 70KCl, 10 HEPES, 0.5EGTA, 2$MgCl_2$, 2 magnesium ATP), and 0.3 sodium GTP. The liquid junction potentials of the former and latter pipette solutions were −13 mV and −9 mV, respectively. The voltage was not corrected in the present study. Thin-wall borosilicate patch electrodes (2–5 MΩ) were pulled on a Flaming-Brown micropipette puller (P-97, Sutter Instruments, Novato, CA, USA).

Recordings were obtained at 30 ± 1 °C. The seal resistance was > 5 GΩ, and only data obtained from electrodes with access resistance of 6–20 MΩ and < 20% change during recordings were included in this study. The voltage responses of cells were recorded by the application of long hyperpolarizing and depolarizing current pulse (300 ms) injections to examine basic electrophysiological properties, including input resistance, spike threshold, and repetitive firing patterns. Hyper- to depolarizing ramp current pulse (1 s) was injected to measure the latency of spike firing (Fig. 1Ca). Propofol (2,6-Diisopropylphenol; Sigma–Aldrich, St. Louis, MO, USA) was dissolved in DMSO at a concentration of 100 mM and diluted to 100 μM or 30 μM in the perfusate. Picrotoxin (100 μM) was used to antagonize $GABA_A$ receptors.

Data analysis

Clampfit (pClamp 10, Molecular Devices) was used for analyses of electrophysiological data. Input resistance was calculated from the relationship between the voltage responses and the injected current intensity (up to −200 pA). The repetitive spike firing properties were evaluated by measuring the maximum spike number