—Review—

Actions of Propofol on Neurons in the Cerebral Cortex

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Propofol is primarily a hypnotic, and is widely used for induction and maintenance of anesthesia, as well as for sedation in various medical procedures. The exact mechanisms of its action are not well understood, although its neural mechanisms have been explored in *in vivo* and *in vitro* experiments. Accumulating evidence indicates that one of the major targets of propofol is the cerebral cortex. The principal effect of propofol is considered to be the potentiation of $GABA_A$ receptor-mediated inhibitory synaptic currents, but propofol has additional roles in modulating ion channels, including voltage-gated Na^+ channels and several K^+ channels. We focus on the pharmacological actions of propofol on cerebrocortical neurons, particularly at the cellular and synaptic levels. (J Nippon Med Sch 2017; 84: 165–169)

Key words: neocortex, synaptic transmission, ion current, anesthetics

Introduction

General anesthetics modulate neural activities in a variety of brain regions. Among these, the cerebral cortex is considered a major target, because it is involved in higher functions of the brain that play crucial roles in consciousness, learning and memory, regulation of motor behavior, integration of sensory information, etc. Indeed, electroencephalograms (EEGs), which reflect cortical neural activities, correlate well with the level of consciousness of an indivisual (Alkire et al., 2008). Therefore, the mechanisms by which general anesthetics regulate cortical activities have been a central focus of studies aiming to clarify how general anesthetics induce unconsciousness.

The cerebral cortex consists of glutamatergic excitatory and GABAergic inhibitory neurons, and the latter represent 10–20% of cortical neurons (Gabbott and Somogyi, 1986). In the primary sensory cortex, glutamatergic neurons are classified into pyramidal cells located in layers II, III, V, and VI, and spiny stellate cells located in layer IV. GABAergic neurons are classified into more than ten subtypes, depending on their firing and morphological properties (Peters and Regidor, 1981). We must understand the effects of general anesthetics on the cortical local circuit that involves both glutamatergic and GABAergic neurons to elucidate the mechanism for the induction of unconsciousness by general anesthetics. However, the complexity of the local circuit prevents researchers from obtaining a comprehensive understanding of the mechanisms by which general anesthetics modulate cortical function.

In this review, we describe the regulation of cortical activities by propofol with a particular focus on ion channels.

Propofol Enhances $GABA_A$ Receptor-mediated Currents

$GABA_A$ and $GABA_B$ are the major subtypes of GABAergic receptors expressed in the cerebral cortex. $GABA_A$ receptors are pentameric Cl^- channel receptors that induce fast hyperpolarization by promoting Cl^- influx. On the other hand, $GABA_B$ receptors are 7 membrane spanning G-protein-coupled receptors that activate K^+ currents and induce slow hyperpolarization.

Application of GABA to cortical neurons induces Cl^- currents that are mediated by $GABA_A$ receptors. Propofol enhances the GABA-evoked Cl^- currents in isolated corti-

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cal neurons (Concas et al., 1990, 1991; Hales and Lambert, 1991; Peduto et al., 1991) and cerebrocortical slice preparations (Buggy et al., 2000), suggesting that propofol increases Cl^- currents via postsynaptic mechanisms. In addition, presynaptic actions of propofol on GABA release have been reported using purified synaptosomes with [^{3}H]GABA: propofol facilitates GABA release from cortical nerve terminals (Murugaiah and Hemmings, 1998). Presynaptic GABA transporters are insensitive to propofol (Westphalen and Hemmings, 2003).

Inhibitory postsynaptic currents (IPSCs) mediated by $GABA_A$ receptors were recorded in cortical slice preparation that, at least, partially maintain the integrity of presynaptic GABAergic and postsynaptic neurons to examine the effects of propofol. Postsynaptic effects of propofol on $GABA_A$ receptors have been reported by Kitamura et al. (2003), who showed that propofol prolongs the decay phase of miniature IPSCs (mIPSCs) without affecting the frequency of mIPSCs. Moreover, propofol had little effect on EPSC kinetics (Kitamura et al., 2003). Based on these findings, propofol selectively modulates postsynaptic $GABA_A$ receptors in the cortex. Single channel recordings revealed a propofol-induced increase in the open probability of $GABA_A$ receptors by reducing the slow phase of closed receptors (Kitamura et al., 2004).

These propofol-induced facilitatory effects on GABAergic synaptic transmission contribute to behavioral symptoms, including a loss of consciousness. An antagonist of $GABA_A$ receptors, gabazine, diminishes the loss of the righting reflex induced by propofol in rats (Nelson et al., 2002).

Connections from Fast-spiking Neurons to Pyramidal Cells Are the Most Sensitive to Propofol

Electrophysiological spike firing properties are good criteria for classifying cortical GABAergic neurons: fast-spiking (FS), late-spiking, low threshold spike (LTS), and non-pyramidal regular spiking cells (Kawaguchi and Kubota, 1997; Koyanagi et al., 2010). Another popular classification of GABAergic neurons uses immunohistochemistry: calcium binding proteins, including parvalbumin (PV) or calretinin, neuropeptide Y, somatostatin, cholecystokinin (CCK), and vasoactive intestinal peptide (VIP). These neurons are further named according to their morphological features, i.e., axonal and dendritic arborization patterns. Basket cell axons innervate pyramidal neurons with a reticulated pattern that resemble a "basket". Chandelier cells show a characteristic axonal pattern of vertically descending axon terminals that make contact with the axonal initial segment (DeFelipe et al., 1985). Most of these basket and chandelier cells are PV-immunopositive and exhibit nonadaptive repetitive spike firing with an extremely high firing rate; therefore, they are electrophysiologically classified as FS cells.

Interestingly, the decay kinetics of IPSCs depends on the presynaptic GABAergic neural subtypes. Presynaptic FS and LTS neurons exhibit the fastest and slowest decay time constants, respectively (Li et al., 2009). The differences in the decay kinetics are due to differences in the expression of $GABA_A$ receptor subunits. For example, LTS neurons express a lower density of β2/β3-containing $GABA_A$ receptors, suggesting that β1 expression plays a major role in shaping IPSC decay kinetics (Bacci et al., 2003). The main target of propofol is considered to be the β subunits of $GABA_A$ receptors (Rudolph and Antkowiak, 2004). According to the study by Jurd et al. (2003), the propofol-induced hypnotic and immobilizing response occurs via β3 subunits. Therefore, propofol may induce different effects on IPSCs according to the composition of $GABA_A$ receptor subunits.

This possibility was examined using paired whole-cell patch-clamp recordings that are used to identify both the presynaptic and postsynaptic neurons. As shown in the study by Koyanagi et al. (2014), FS→pyramidal cell connections showed the largest increase in the conductance of $GABA_A$ receptor-mediated IPSCs. Because FS neurons potently suppress the activities of postsynaptic neurons, this mechanism may contribute to the efficient induction of alpha oscillation rhythms.

Propofol-induced Modulation of Intrinsic Membrane Properties

In addition to the enhancement of $GABA_A$ receptor-mediated IPSCs, propofol modulates intrinsic neural properties by hyperpolarizing the resting membrane potential and changing the input resistance and action potential kinetics. These modulatory effects are likely to be mediated, not only by increasing $GABA_A$ receptor-mediated Cl^- conductance, but also by changing the kinetics of various ion channels in the cerebral cortex. Taking into account the diversity of various ionic channels in the cortical neurons, the effect of propofol may be dependent on neural subtypes in the cerebral cortex. Among these channels, we focus on the mechanisms by which propofol modulates voltage-gated Na^+ channels, I_h channels, and K^+ channels.

Propofol is known to inhibit the induction of action potentials in cortical pyramidal neurons (Martella et al.,

2005; Kaneko et al., 2016), suggesting that propofol suppresses voltage-gated Na^+ channels, which are required to generate action potentials. Indeed, several studies have described the direct effects of propofol on Na^+ influx by regulating voltage-gated Na^+ channels in rat cerebrocortical synaptosomes (Ratnakumari and Hemmings, 1997) and cortical neurons (Martella et al., 2005).

I_h is mediated by cation conductance that is activated by hyperpolarization of the membrane potential and plays a critical role in pacemaker depolarization during rhythmic oscillatory activity (Pape, 1996). In addition, I_h regulates the resting membrane potential and afterhyperpolarization that is induced immediately after an action potential (Pape, 1996). Propofol suppresses I_h in cortical pyramidal neurons (Chen et al., 2005; Kaneko et al., 2016). These studies have reported the propofol-induced attenuation of the depolarizing sag potential and the rebound potential.

Propofol has been reported to modulate currents produced by several types of K^+ channels, such as leak K^+ currents (K_{leak}) and delayed rectifier K^+ currents. K_{leak} is mediated by TWIK-related acid-sensitive K^+ (TASK) channels and is the major determinant of the resting membrane potential and input resistance (Millar et al., 2000, Sirois et al., 2000, Talley et al., 2000, Meuth et al., 2003). TASK-1 knockout mice show a prolonged propofol-induced loss of the righting reflex, suggesting an interaction between propofol and TASK-1 channels (Linden et al., 2008). In contrast, propofol has little effect on TASK-3 channels. A behavioral pharmacological study showed little difference in the propofol-induced latency and duration of the loss of the righting reflex between wild-type and TASK-3 knockout mice (Linden et al., 2007). A relatively high dose of propofol (50−200 μM) has little effect on human TASK-1 or TASK-3 channels expressed in oocytes (Putzke et al., 2007).

In addition to TASK-1 channels, propofol suppresses delayed rectifier K^+ currents, which may represent a neuroprotective action of propofol (Song et al., 2011). Kv2.1 K^+ channels are major contributors to delayed rectifier K^+ currents, and propofol suppresses the expression of the Kv2.1 K^+ channel protein and subsequently inhibits delayed rectifier K^+ currents (Zhang et al., 2016). These effects may explain the propofol-induced inhibitory effect on neural apoptosis during ischemia and hypoxia.

It is well known that benzodiazepines potentiate inhibitory synaptic transmission mediated by increasing the open probability of $GABA_A$ chloride channels (Study et al., 1981). Although some benzodiazepine derivatives modulate other ionic channels, such as KCNQ1 potassium channels (Tinel et al., 1998), little is known about the effects of benzodiazepines on ionic currents other than $GABA_A$ receptor-mediated Cl^- currents. These distinct actions on ionic channels might be one of the mechanisms for different roles between propofol and benzodiazepines in the regulation of neural functions.

Extrasynaptic $GABA_A$ Receptor Modulations Induced by Propofol

Bicuculline, a $GABA_A$ receptor antagonist, suppresses not only GABAergic synaptic transmission but also the resting conductance, and induces an inward shift of the baseline current in rat cortical pyramidal neurons (Salin and Prince, 1996). Therefore, extrasynaptic $GABA_A$ receptors play a role in tonic currents that hyperpolarize the resting membrane potential. Among various $GABA_A$ receptor subunits, the α1-subunit is likely to play a principal role in tonic current induction in supragranular and infragranular pyramidal neurons, whereas the α5-subunit is specifically involved in infragranular pyramidal neurons (Yamada et al., 2007). In addition to α1- and α5-subunits, the α4-subunit is considered to be involved in the tonic current: tonic current mediated by α4δ $GABA_A$ receptors is facilitated by ethanol exposure (Carlson et al., 2016).

According to the study by Kaneko et al. (2016), propofol hyperpolarizes the resting membrane potential, which is blocked via a $GABA_A$ receptor antagonist. Similar results have been obtained in hippocampal neurons; propofol potentiates GABAergic tonic currents, which hyperpolarizes the resting membrane potential by inducing a decrease in input resistance and a reduction of the spike firing frequency (Bieda and MacIver, 2004). Based on this evidence, propofol regulates intrinsic membrane properties by facilitating the activity of extrasynaptic $GABA_A$ receptors and potentiating $GABA_A$ receptor-mediated synaptic currents.

Propofol Has Little Effect on Gap Junctions

Several subtypes of GABAergic interneurons, represented by FS neurons, are frequently electrically coupled via gap junctions (Gibson et al., 1999; Galarreta and Hestrin, 1999). Electrical synapses contribute to the induction of synchronized neural activities such as oscillations (Deans et al., 2001). Propofol elicits frontal α-rhythms in the EEG (Ching et al. 2010). However, propofol has little effect on electrical synapses between FS neurons (Koyanagi et al., 2014).

Future Directions

Although the targets of propofol are widely distributed in the cerebral cortex, the potentiation of $GABA_A$ receptor-mediated inhibitory currents is likely to be required for the regulation of cortical neural activity. However, the mechanism by which propofol induces a loss of consciousness through these modulatory effects remains an open question. Researchers must bridge the gap between *in vivo* and *in vitro* studies to answer this question. Recent developments in calcium imaging techniques using two-photon microscopy may assist researchers in testing this idea.

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