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Review

The effects of volatile anesthetics on synaptic and extrasynaptic GABA-induced neurotransmission

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ABSTRACT

Examination of volatile anesthetic actions at single synapses provides more direct information by reducing interference by surrounding tissue and extrasynaptic modulation. We examined how volatile anesthetics modulate GABA release by measuring spontaneous or miniature GABA-induced inhibitory postsynaptic currents (mIPSCs, sIPSCs) or by measuring action potential-evoked IPSCs (eIPSCs) at individual synapses. Halothane increased both the amplitude and frequency of sIPSCs. Isoflurane and enflurane increased mIPSC frequency while sevoflurane had no effect. These anesthetics did not alter mIPSC amplitudes. Halothane increased the amplitude of eIPSCs, with a decrease in failure rate (Rf) and paired-pulse ratio. In contrast, isoflurane and enflurane decreased the eIPSC amplitude and increased Rf, while sevoflurane decreased the eIPSC amplitude without affecting Rf. Volatile anesthetics did not change kinetics except for sevoflurane, suggesting that presynaptic mechanisms dominate changes in neurotransmission. Each anesthetic showed somewhat different GABA-induced response and these results suggest that GABA-induced synaptic transmission cannot have a uniformly common site of action as suggested for volatile anesthetics. In contrast, all volatile anesthetics concentration-dependently enhanced the GABA-induced extrasynaptic currents. Extrasynaptic receptors containing α4 and α5 subunits are reported to have high sensitivities to volatile anesthetics. Also, inhibition of GABA uptake by volatile anesthetics results in higher extracellular GABA concentration, which may lead to prolonged activation of extrasynaptic $GABA_A$ receptors. The extrasynaptic GABA-induced receptors may be major site of volatile anesthetic-induced neurotransmission.

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Contents

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1. Introduction

The pharmacologic actions of volatile anesthetics were long considered as a perturbation of the fluidity of the membrane lipid bilayer. This concept derives from the correlation between the olive oil/water partition coefficient for the anesthetic and its anesthetic potency (Meyer–Oveton correlation) but was upset by the demonstration of chemically specific binding to anesthetic recognition sites (Campagna et al., 2003). However recent electrophysiological studies on interaction with membrane channels of the central nervous system suggest that general anesthetics act thorough hydrophilic regions of receptors in neurons. The most common candidate for target of general anesthetics is the $GABA_A$ receptor, a ligand-gated chloride receptor-channel complex for the GABA, which usually inhibits neuronal excitability. In fact, halothane (Mihic et al., 1997; Wakamori et al., 1991), enflurane (Mihic et al., 1997; Siegwart et al., 2003; Wakamori et al., 1991), isoflurane (Mihic et al., 1997), and sevoflurane (Sebel et al., 2006; Wu et al., 1994, 1996), all potentiated GABA-induced postsynaptic currents at their clinically relevant concentration.

However, growing evidence suggesting that $GABA_A$ receptors are not main site of volatile anesthetic action has been also reported. For example, volatile anesthetic-induced anesthesia cannot be antagonized by GABA antagonists (Pittson et al., 2004). GABA-mediated neurons change to excitatory neurotransmission in immature animals (Jang et al., 2001; Kahraman et al., 2008; Kakazu et al., 1999). The changes of chloride cotransporters from sodium-potassium-chloride cotransporter to potassium-chloride cotransporter decrease the intracellular chloride concentration and the switch from the chloride influx to efflux (Jang et al., 2001; Kakazu et al., 1999). Although molecular cloning studies have identified the ligand-binding molecules of the $GABA_A$ receptors, each volatile anesthetic has different sensitivity to various target protein (McCracken et al., 2010; Olsen and Li, 2011).

The interpretation of activation of $GABA_A$-induced postsynaptic currents by exposure to volatile anesthetics is also very problematic. A major limitation is that previous patch clump studies were performed on either slice preparations or cultured cells and that the electric responses in these studies were influenced by extrasynaptic modulations or by other surrounding structures including neuronal and glia cells. In fact, extrasynaptic $GABA_A$ receptor-mediated currents represent a much greater proportion of the total $GABA_A$ receptor-mediated current than that mediated by synaptic activity (Walker and Semyanov, 2008). It thus remains unknown how volatile anesthetics exactly act on GABA-induced neurotransmission at the single synapse level.

We established a method for isolation of single nerve-adherent-synaptic-bouton using enzyme-free mechanical dissociation (Akaike and Moorhouse, 2003; Akaike et al., 2002). This 'synaptic bouton' preparation has the advantages of single neurons that are isolated from extrasynaptic responses and surrounding neurons and glia but retain adherent function terminals. This new technique enables us to evaluate better how volatile anesthetics act on synaptic transmission at a single synapse. This approach allows use to simultaneously quantify the presynaptic and postsynaptic contributions to GABA-induced neurotransmission by measuring the amplitude and frequency of spontaneous or miniature as well as directly evoked inhibitory postsynaptic currents (sIPSC, mIPSC and eIPSC). The responses measured during volatile anesthetic exposures using the synaptic bouton preparation give new insights that are completely different from those reported previously (Kotani et al., 2012; Ogawa et al., 2011).

One of the aims in this review is to show how this new isolation method reveals synaptic function at individual synaptic boutons. Then we review our results of volatile anesthetic-induced changes in sIPSCs or mIPSCs followed by changes in IPSCs evoked from single synapses along with comparisons to previous studies including extrasynaptic GABA-induced responses. Finally, we will reconcile such results with possible mechanisms for these differences.

2. A new method for evaluation of single synaptic transmission

2.1. Experimental procedures

Our new method isolates single neurons from the central nervous system using mechanical dissociation without any enzymatic treatment while retaining adherent and functioning excitatory and inhibitory synaptic nerve terminals (boutons). The isolated neurons are free from confounding effects from extrasynaptic modulation and other neuronal and glial cells. The acute mechanical dissociation avoids possible changes in tissue and/or its function as result of either enzyme treatment or *in vitro* culture. This synaptic bouton preparation offers improvements to inquiries into the mechanisms of synaptic transmission in the central nervous system.

2.2. Acute isolation

The experimental procedure was described in detail previously (Akaike and Moorhouse, 2003; Akaike et al., 2002). Briefly, the procedure begins with slices from various mammalian brain or spinal cord slice prepared by the standard method. For mechanical dissociation, the brain slice was transferred to a culture dish containing standard external solution, and fixed to the bottom of the dish by an anchor made from a platinum frame and nylon thread (Fig. 1A). The target region for harvesting neurons was identified under a binocular microscope and the tip of a fire-polished glass pipette was lightly placed on the slice surface above the target neurons and vibrated horizontally (0.2–2 mm displacement) at 50–60 Hz using a custom assembled vibration device (Fig. 1B). The dish is typically moved horizontally along the target region by hand to harvest neurons from sub region of interest within the slice. Then, the slices were removed from the dish and the mechanically dissociated neurons left to settle and adhere to the bottom of the dish for at least 15 min before electrophysiological measurements. Electron microscopic and fluorescent studies confirm that boutons remain attached to the dissociated neurons (Fig. 1C and D). To date, we have used this approach successfully on neurons from various brain regions including the Meynert's nuclei (Arima et al., 2001), the basolateral amygdala (Koyama et al., 1999), the hippocampal CA1 (Matsumoto et al., 2002), and CA3 (Yamamoto et al., 2011), periaqueductal gray (Kishimoto et al., 2001), the ventromedial hypothalamus (Jang et al., 2001), and the spinal sacral dorsal commissural nucleus (Akaike et al., 2010).

2.3. Spontaneous, miniature and action potential-evoked postsynaptic currents

In the synaptic bouton preparation, neurotransmitter is released from the adherent terminals that give rise to spontaneous synaptic