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General anesthetic modulation of neuronal nicotinic acetylcholine receptors

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Abstract. Inhalational anesthetics are known to modulate various neurotransmitter systems in the brain. Recently, it is becoming abundantly clear that the neuronal nicotinic acetylcholine receptor (nAChR) system is an important target site of anesthetics. However, detailed mechanism of action of anesthetics on the nAChRs remained to be elucidated. We embarked on the patch-clamp analysis of anesthetic interactions with nAChRs at both whole-cell and single-channel levels. Halothane, isoflurane and sevoflurane inhibited the activity of the $\alpha4\beta2$ nAChRs in rat cortical neurons in primary culture and in HEK cells expressing the receptors. The inhibition was observed at clinically relevant concentrations of <1 MAC, and occurred in the resting and activated states of the receptors. The recovery after washing required opening of the channels by ACh. Since nAChRs modulate the activity of various other neurotransmitter systems, anesthetic inhibition of nAChRs is expected to be amplified via a cascade of multisynaptic events in the brain. It is concluded that the nAChRs, especially the $\alpha4\beta2$ nAChRs, constitute an important target site of inhalational anesthetics responsible for a variety of behavioral changes associated with general anesthesia.

Keywords: Inhalational anesthetic; Halothane; Isoflurane; Sevoflurane; Nicotinic acetylcholine receptor

1. Introduction

Inhalational general anesthetics are known to potentiate the activity of $GABA_A$ receptors, and this action is deemed to account at least in part for the anesthetic action [10,17]. The activity of glutamate receptors and neuronal nicotinic acetylcholine receptors (nAChRs) is also suppressed by general anesthetics [4,7,15]. nAChRs are located in the

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postsynaptic membrane and in the presynaptic and preterminal areas of interneurons [16], and those in the latter areas, when activated, stimulate the release of various transmitters including GABA, glutamate, ACh, dopamine, and norepinephrine [3]. Thus, it is possible that anesthetic modulation of nAChRs will have profound effects on brain function via a cascade of multisynaptic events. We have embarked on detailed analysis of general anesthetic action on nAChRs, as many features of the interactions of anesthetics with nAChRs remain to be seen.

2. Halothane modulation of nAChRs in rat cortical neurons

Rat cortical neurons in long-term primary culture (2–9 weeks) were used for the whole-cell patch-clamp experiments [8]. At least two types of currents could be recorded in response to ACh application through a U-tube [1]. One type was quickly desensitized during application of ACh, showed a low affinity for ACh (EC_{50}=300 μM), and was sensitive to the blocking action of α-bugarotoxin (α-BuTX). The other type was non-desensitizing or slowly desensitizing, showed a high affinity for ACh (EC_{50}=3 μM), and was insensitive to α-BuTX. Data in the literature point out that the former is the α7 type and the latter is the α4β2 type nAChRs.

Dose–response analyses of halothane inhibition of nAChRs showed an IC_{50} of 550 μM for the α7 type (2 MAC) and an IC_{50} of 100 μM for the α4β2 type (0.5 MAC). A receptor could be blocked in its resting state, activated state, or in both states. It was demonstrated that halothane blocks the α4β2 type nAChRs in both resting and activated states, and that recovery from block occurs when the channels are opened by ACh. It was also found that halothane block of the α4β2 type nAChRs is not due to receptor desensitization [8].

3. Isoflurane and sevoflurane modulation of nAChRs expressed in HEK cells

Isoflurane and sevoflurane are widely used clinically, and are known to act on neuroreceptors somewhat differently from halothane. Furthermore, there have been some controversies between Flood et al. [7] and Violet et al. [15] regarding the dependence of anesthetic inhibition on ACh concentration. Therefore, we performed further patch-clamp experiments to elucidate the mechanism of action of isoflurane, sevoflurane and halothane on the human α4β2 nAChRs expressed in HEK cells. Analyses were extended to those at the single-channel level [18].

The IC_{50}s for isoflurane, sevoflurane and halothane in blocking the α4β2 nAChRs were estimated to be 67 μM (0.24 MAC), 183 μM (0.61 MAC) and 40 μM (0.21 MAC), respectively. When the anesthetic inhibition as a function of ACh concentration was compared with the equivalent concentration, some differences among those anesthetics were noted. The isoflurane and sevoflurane inhibition decreased with the increasing concentration of ACh in agreement with the results by Flood et al. [7] on the isoflurane inhibition in the chicken α4β2 receptor expressed in *Xenopus* oocytes. The halothane inhibition was almost independent of ACh concentration in agreement with the data by Violet et al. [15] in the rat α4β2 receptor expressed in *Xenopus* oocytes. Thus the controversy of data previously reported was likely due to different anesthetics being used.

Using the same protocols as those used for halothane, isoflurane was shown to inhibit the α4β2 receptors in both resting and activated states. To further pursue the mechanism of