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Modulation of miniature inhibitory postsynaptic currents by isoflurane in rat dissociated neurons with glycinergic synaptic boutons

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Abstract

The effects of a volatile anesthetic, isoflurane, on glycinergic miniature inhibitory postsynaptic currents (IPSCs) were investigated in mechanically dissociated rat trigeminal nucleus neurons with intact glycinergic interneuronal presynaptic nerve terminals. The nystatin-perforated patch recording configuration was used to record the miniature IPSCs under voltage-clamp conditions. Isoflurane shifted in a parallel fashion the glycine (Gly) concentration–response curve of enzymatically dissociated neurons to the left without changing the maximum response. Isoflurane reversibly increased the frequency of the miniature IPSCs and prolonged the decay time constant without affecting the mean amplitude. The increase in the frequency of miniature IPSCs in the presence of isoflurane was also observed in Ca^{2+}-free external solution. Thapsigargin prohibited the facilitatory effect of isoflurane on the miniature IPSC frequency. It is concluded that isoflurane increases the Ca^{2+} concentration in the glycinergic presynaptic nerve terminal by enhancing the release and/or suppressing the uptake of Ca^{2+} into stores. © 2001 Published by Elsevier Science B.V.

Keywords: Trigeminal nucleus neuron; Mechanical dissociation; Isoflurane; Glycinergic synaptic bouton preparation; Inhibitory postsynaptic current

1. Introduction

γ-Aminobutyric acid (GABA) and glycine (Gly) are important inhibitory neurotransmitters in the central nervous system (CNS), and a major function of glycine is to suppress electrical excitation of neurons in the spinal cord and brain stem (Betz, 1991). Volatile anesthetics, including isoflurane, may suppress CNS activity in several ways. Investigators have reported that synaptic transmission is more sensitive to general anesthetics than axonal conduction is (Larrabee and Posternak, 1952; Arimura and Ikemoto, 1986) and that both excitatory and inhibitory postsynaptic responses are modulated by volatile anesthetics (Jenkins et al., 1996; Narimatsu et al., 1999; Raines and Zachariah, 1999; Yamashita et al., 1999). Enhancement of inhibitory postsynaptic currents (IPSCs) through ligand-gated chloride channels is generally thought to be the predominant mode of general anesthetic action in the CNS. Some authors have reported, however, that a major effect of volatile anesthetics is to reduce excitatory synaptic transmission (Schlame and Hemmings, 1995; MacIver et al., 1996). Although it is clear that anesthetics have substantial effects on postsynaptic receptors, presynaptic effects of general anesthetics are not well characterized. A number of studies using brain slice preparations have shown that volatile anesthetics reduce the release of glutamate and acetylcholine evoked by nerve stimulation, suggesting that there is a decrease in Ca^{2+} influx during the action potential (Ouanounou et al., 1998; Shichino et al., 1998). The voltage-gated Ca^{2+} channels are currently labeled T, L, N, P/Q and R types. The N- and P/Q-type channels are reported to be directly involved in the presynaptic release of neurotransmitters in the CNS (Takahashi and Momiyama, 1993; Moreno, 1999). Although volatile anesthetics depress Ca^{2+} currents (Study, 1994; McDowell et al., 1999), the inhibition of N- and P/Q-type channels at clinically relevant concentrations is relatively minor (Hall et al., 1994; Kameyama et al., 1999).

Few data are available, however, concerning the change in Ca^{2+} concentration in the presynaptic terminal at rest

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caused by general anesthetics. To address this issue, we recorded action potential-independent glycinergic miniature IPSCs in the presence of tetrodotoxin. In this study, we used mechanically dissociated rat trigeminal nucleus neurons, to which presynaptic boutons of glycinergic interneurons remain attached (namely, 'synaptic bouton preparation'), and we examined the influence of isoflurane on the Ca^{2+} concentration in the presynaptic terminal ($[Ca^{2+}]_i$) by measuring the frequency and the amplitude of the glycinergic miniature IPSCs. The present results clearly show that the $[Ca^{2+}]_i$ of the presynaptic terminal at rest could be increased by isoflurane.

2. Materials and methods

2.1. Acutely dissociated neurons with and without synaptic boutons

Wistar rats (10–14 days old) were decapitated under pentobarbital anesthesia. The midbrain was quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF). Slices including the trigeminal nucleus were cut at a thickness of 400 μm with a microslicer (DTK-1000, Dosaka, Kyoto, Japan). The brain slices were kept in ACSF saturated with 95% O_2 and 5% CO_2 at room temperature (22–25 °C) for at least 1 h. Thereafter, the slices were transferred to a 35-mm culture dish (Primaria 3801, Becton Dickinson, NJ, USA) and the midbrain was identified under a binocular microscope (SMZ-1, Nikon, Tokyo, Japan). A fire-polished glass pipette was touched lightly on the surface of the dorsal horn and was vibrated horizontally at 3–5 Hz for about 2 min using an apparatus built in our laboratory. The slices were removed from the dish and the remaining dissociated neurons adhered to the bottom of the dish within 10 min. These neurons, which were dissociated without using enzymes, also retained some of their original morphological features such as proximal dendritic processes. In the present study, the dissociated trigeminal nucleus neurons had oval, fusiform or triangular somata and were large neurons with a somatic diameter of over 15–20 μm and a thick proximal dendrite.

The technique for the dissociation of the trigeminal nucleus neurons without boutons was as previously described (Wakamori et al., 1991). Briefly, the midbrain slices (400 μm thick) containing the trigeminal nucleus neurons were incubated in ACSF containing 0.2 mg/ml pronase for 15 min at 31 °C and subsequently with 0.2 mg/ml thermolysin for 15 min at 31 °C. After enzyme treatment, the trigeminal nucleus region was identified under a binocular microscope (SMZ-1, Nikon) and micropunched out with an electrically polished injection needle. The micro-punch pieces were mechanically triturated in a 35-mm culture dish (Primaria 3801, Becton Dickinson) with a fire-polished Pasteur pipette under a phase-contrast microscope (TMS-1, Nikon). The dissociated neurons adhered to the bottom of the dish within 20–30 min.

2.2. Electrical measurements

Electrical measurements were performed using the nystatin-perforated patch recording mode at a holding potential of −50 mV under voltage-clamp conditions. Patch pipettes were made from borosilicate glass tubes (1.5 mm o.d., 0.9 mm i.d.; G-1.5, Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PB-7, Narishige). The resistance of the recording electrode was 5–7 MΩ. Neurons were visualized with phase-contrast equipment on an inverted microscope (Diapot, Nikon). Current and voltage were measured with a patch-clamp amplifier (CEZ-2300, Nihon Kohden, Tokyo, Japan), monitored on both an oscilloscope (Textronix 5111A, Sony, Tokyo, Japan) and a pen recorder (Recti-Horiz 8K, Nippondenki San-ei, Tokyo, Japan), and stored on videotapes after digitization with a pulse-coded modulation processor (PCM-501 ES, Sony). The membrane currents were filtered at 1 kHz (E-3201A Dicade Filter, NF Electronic Instruments, Tokyo, Japan) and data were digitized at 4 kHz. All experiments were performed at room temperature (22–25 °C).

2.3. Data analysis

Events were counted and analyzed using DETECTiVENT (Ankri et al., 1994) and IGOR PRO software (Wavemetrics, Lake Oswego, OR). Further analysis of the miniature IPSCs was performed using cumulative probability plots. Cumulative amplitude and frequency probability plots were compared using the Kolmogorov–Smirnov test for significant difference. A difference was assumed to be statistically significant at $P < 0.05$. Numerical values are provided as means ± S.E.M. Differences in mean amplitude and frequency were tested by paired two-tailed *t*-test.

2.4. Solutions

The ionic composition of ACSF was (mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 24 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$ and 10 glucose. The pH of the incubation medium was adjusted to 7.4 with 95% O_2 and 5% CO_2. The ionic composition of the external standard solution was (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES. Ca^{2+}-free external solution contained (mM): 150 NaCl, 5 KCl, 3 $MgCl_2$, 10 glucose, 10 HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid) and 2 EGTA [ethyleneglycol-*bis*-(β-aminoethyl)-*N*,*N*,*N*′*N*′-tetraacetic acid].

For recording the miniature IPSCs, the external solution routinely contained 300 nM tetrodotoxin to inhibit voltage-dependent Na channels, 3 μM bicuculline to block

γ-aminobutyric acid type A receptor ($GABA_A$) responses, and 1 μM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and 10 μM DL-AP5 (DL-2-amino-5-phosphovaleric acid) to block glutamatergic responses. The ionic composition of the internal (patch-pipette) solution for nystatin-perforated patch recording was (mM): 100 Cs-methanesulfonate, 50 CsCl and 10 HEPES. The pH of the internal solution was adjusted to 7.2 with Tris[hydroxymethyl]amino methane–OH. Nystatin was dissociated in acidified methanol at 10 mg/ml. This stock solution was diluted in the internal solution just before use to a final concentration of 100–200 μg/ml.

2.5. Drugs

Drugs used in the present study were DL-AP5, bicuculline, CNQX, EGTA, and nystatin (Sigma, USA), isoflurane (Dinabot, Japan) and tetrodotoxin (Wako, Japan). CNQX was dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution.

Drugs were applied by "Y-tube system" (Murase et al., 1990), which enables solution exchange within 20–30 ms.

2.6. Preparation of isoflurane in aqueous solution

For preparing a solution with a desired isoflurane concentration, an amount of the agent calculated from the respective specific gravity (1.5) and molecular weight (184.5) was injected into 100 ml of external solution in a tightly capped glass flask, using a 10-μl syringe. Thereafter, the solution was sonicated for a few minutes until the isoflurane droplet had completely dissolved. After the isoflurane solution was prepared, care was taken not to allow evaporation of isoflurane. The concentrations of isoflurane used in this study were approximately equivalent to 0.3 and 3 times the minimum alveolar concentration, based on the solution/air partition coefficient and the temperature coefficient of solubility (Franks and Lieb, 1996; Kira et al., 1998).

3. Results

3.1. Augmentation of glycine-mediated current by isoflurane

Enzymatically dissociated trigeminal nucleus neurons were voltage clamped at a holding potential (V_H) of −50 mV, and various concentrations of glycine were applied using the "Y-tube system". The glycine-induced current was increased by co-application of isoflurane as shown in the inset of Fig. 1. In some cells, isoflurane itself evoked a small inward current, which was not further investigated. The averaged concentration–response relationship for the glycine-induced current is illustrated in Fig. 1, in the absence and presence of isoflurane. Isoflurane produced a parallel shift of the glycine concentration–response curve to the left without affecting the maximum response. In the presence of 1 mM isoflurane, the concentration corresponding to the half-maximal response (EC_{50}) decreased from 32.4 to 13.4 μM and the Hill coefficient did not change (0.94 and 0.93 with and without isoflurane, respectively). Similar results for the effects on the concentration–response curve were obtained with mechanically dissociated neurons (data not shown).

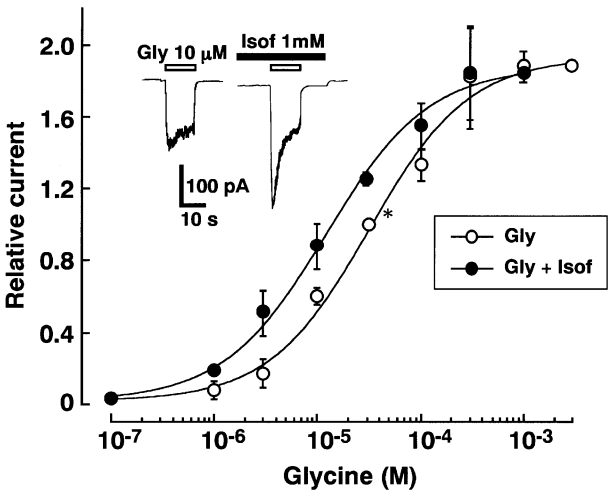

Fig. 1. Augmentation of the glycine response by isoflurane in enzymatically dissociated trigeminal nucleus neurons. Holding potential was −50 mV. Peak currents were normalized to that evoked by 30 μM Gly (asterisk) and plotted against the concentration. Each circle and the associated vertical bar indicate the mean ± S.E.M. for six to eight neurons. Inset: Gly (10 μM) evoked an inward current. Preapplied isoflurane (1 mM) generated a small inward current and augmented the glycine response.

3.2. Miniature IPSCs mediated by glycine receptors

Spontaneous miniature postsynaptic currents were recorded with neurons acutely dissociated from the trigeminal nucleus of the rat midbrain. These dissociated neurons have their "native presynaptic nerve endings" attached and are termed "synaptic bouton" preparations (Rhee et al., 1999). Miniature postsynaptic currents were recorded using the nystatin-perforated patch mode at a holding potential of −50 mV in the presence of 300 nM tetrodotoxin, 1 μM CNQX, 10 μM DL-AP5 and 3 μM bicuculline. The miniature currents were dose dependently inhibited by strychnine and blocked completely by 1 μM of the agent (Fig. 2A). This finding indicates that the currents were glycine-mediated miniature IPSCs.

Fig. 2Ba shows sample traces of typical glycinergic miniature IPSCs at various holding potentials and Fig. 2Bb shows the corresponding *I–V* relationship. The current reversed its polarity at −22.0 mV, which was close to the

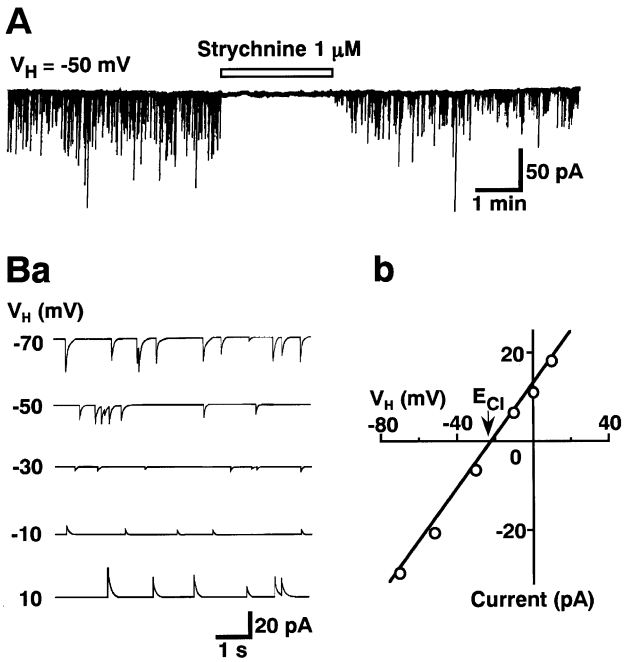

Fig. 2. Glycinergic miniature IPSCs in a mechanically dissociated trigeminal nucleus neuron. A: Holding potential was −50 mV. In the presence of tetrodotoxin (300 nM), DL-AP5 (10 μM), bicucullin (3 μM) and CNQX (3 μM), the glycinergic miniature IPSCs were completely and reversibly blocked by 1 μM strychnine. B: (a) Original traces of miniature currents, which reversed their polarity between −10 and −30 mV. (b) The average of the peak current is plotted against the membrane potential, giving the current–voltage relationship. The reversal potential was −22 mV.

theoretical Cl^- equilibrium potential (E_{Cl}) of −27.7 mV calculated from the Nernst equation using extracellular (161 mM) and intracellular (50 mM) Cl^- concentrations.

3.3. Modulation of the miniature IPSCs by isoflurane

We examined the effects of isoflurane on the glycinergic miniature IPSCs in 41 cells and all recordings were performed in the presence of 300 nM tetrodotoxin, 1 μM CNQX, 10 μM AP5 and 3 μM bicuculline. A 5-min application of isoflurane increased the frequency of the miniature IPSCs in all 41 cells without affecting their miniature IPSC amplitude distribution. Isoflurane (1 mM) increased the miniature IPSC frequency from 5.87 to 7.14 Hz without affecting the amplitude distribution (Fig. 3A). Fig. 3Ab and c shows cumulative probability plots for the amplitude and frequency of the miniature IPSCs, in the presence and absence of isoflurane. The amplitude was not affected, whereas the frequency was increased, indicating that the probability of glycine release was enhanced. After the washout of isoflurane, the facilitatory effect gradually disappeared with a variable time course and the facilitation had always completely disappeared within 20 min. Fig. 3B illustrates collected data showing the effects of two concentrations (0.1 and 1 mM) of isoflurane on the amplitude and the frequency. The amplitude was not changed significantly by either concentration. The frequency was significantly increased to 1.34 ± 0.05 ($n = 7$) by the lower concentration (0.1 mM) and to 1.89 ± 0.08 ($n = 10$) by the higher concentration (1 mM) of the anesthetic.

Fig. 4A shows the decay phase of a representative miniature IPSC in the presence and absence of isoflurane. The miniature IPSC decay was fitted with a single exponential function. Isoflurane (1 mM) prolonged the decay phase, increasing the time constant from 15.4 to 34.1 ms. The averaged time constants in the presence and absence of 1 mM isoflurane are depicted in Fig. 4B. The anesthetic

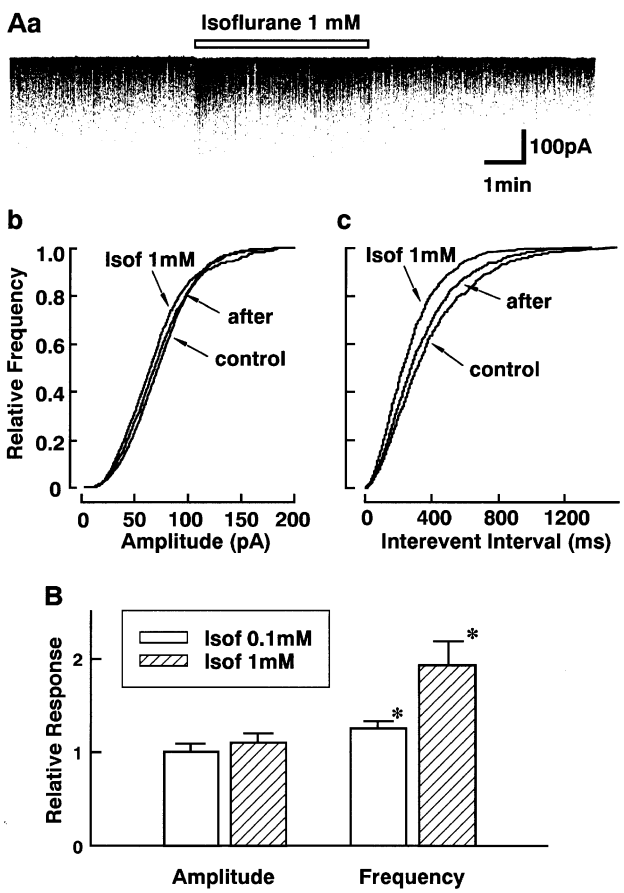

Fig. 3. Effects of isoflurane on the glycinergic miniature IPSCs. A: (a) A current trace recorded before, during and after application of 1 mM isoflurane in the normal external solution. (b) Cumulative probability distributions of the amplitude are plotted for the miniature IPSCs in the presence and absence of isoflurane. Isoflurane (1 mM) did not affect the amplitude distribution. (c) Cumulative probability distributions of the interval are plotted for the miniature IPSCs in the presence and absence of isoflurane. The frequency of miniature IPSCs increased. The number of events for the cumulative plots was 2483 for control and 2187 for isoflurane. B: IPSC amplitude and frequency in the presence of two concentrations (0.1 and 1 mM) of isoflurane relative to the control values. Neither concentration of isoflurane altered the amplitude significantly, while the anesthetic increased the frequency of the miniature IPSCs dose dependently. Asterisks indicate a significant change at $P < 0.05$ using Kolmogorov–Smirnov test.

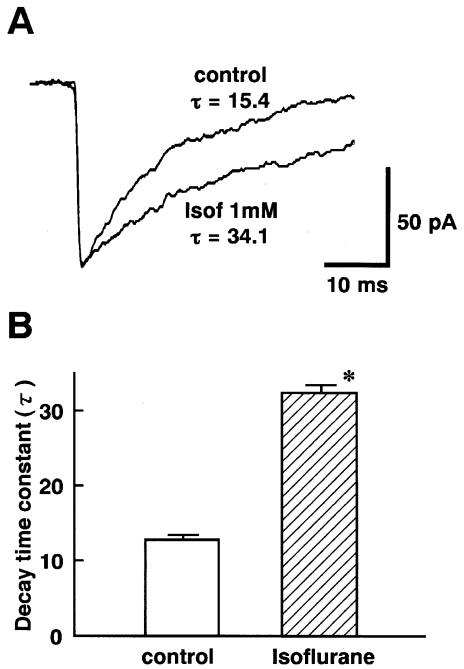

Fig. 4. Prolongation of the miniature IPSC decay phase by isoflurane. A: Superimposed traces of single miniature IPSCs in the presence and absence of isoflurane (1 mM). The decay phase of the miniature IPSCs was fitted with a single exponential function. B: In a single neuron, the decay time constants were measured and averaged for 50 miniature IPSCs in the presence and absence of isoflurane. The columns illustrate the mean time constant for six neurons for the control and in the presence of isoflurane. Isoflurane significantly increased the mean time constant. Each column and vertical bar indicates the mean ± S.E.M. for six neurons.

significantly increased the decay time constant from 14.4 ± 0.28 to 34.6 ± 0.95 ms ($n = 5$).

3.4. Extracellular Ca^{2+} is not needed for the frequency increase

To examine the role of extracellular Ca^{2+} in the effects of isoflurane, we applied the anesthetic in Ca^{2+}-free solution. The Ca^{2+}-free solution itself reduced the miniature IPSCs frequency from 0.42 ± 0.11 to 0.20 ± 0.06 Hz without causing any significant change in the amplitude distribution ($n = 5$). Fig. 5 shows the effect of isoflurane on miniature IPSCs in Ca^{2+}-free external solution. In Ca^{2+}-free solution, isoflurane still clearly increased the miniature IPSC frequency (Fig. 5Aa). Fig. 5Ab and c shows cumulative probability plots for the same recording, showing that the frequency was markedly increased without there being a large effect on the amplitude distribution. After the washout of isoflurane, the facilitatory effect disappeared within 20 min. Fig. 5B shows that isoflurane caused a significant increase in the mean miniature IPSC frequency (2.19 ± 0.04 of control; $P < 0.05$ by Kolmogorov–Smirnov test) but not in the mean amplitude (1.08 ± 0.06 of control).

3.5. Effects of isoflurane in the presence of thapsigargin

We next examined the role of intracellular Ca^{2+} stores in the facilitation of miniature IPSC frequency caused by isoflurane. In the external standard solution, 1 μM of thapsigargin (a sulfhydryl alkylating agent) itself enhanced the spontaneous miniature IPSC frequency to 3.27 ± 0.47 of the control value ($P < 0.05$, paired two-tailed t-test, $n = 5$) but had no effect on the distribution of the amplitude (Fig. 6A), indicating that thapsigargin markedly increased the probability of glycine release. A higher concentration (10 μM) further increased the frequency, showing that no occlusion occurred in the presence of 1 μM of thapsigargin. Isoflurane had little effect on either the miniature IPSC frequency or its amplitude (Fig. 6Ab and

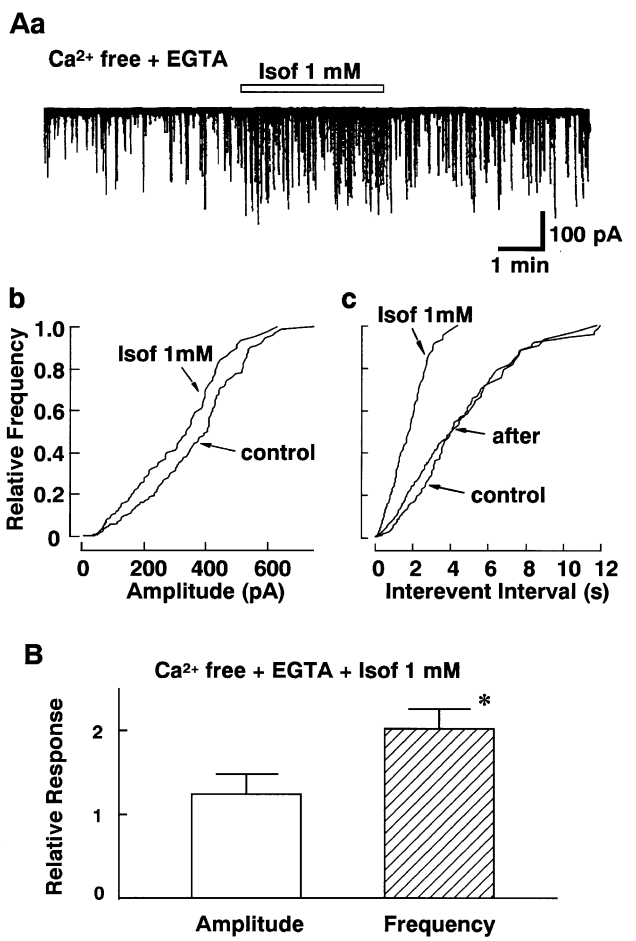

Fig. 5. Increase in IPSC frequency by isoflurane in Ca^{2+}-free external solution. A: (a) A current trace recorded before, during and after application of 1 mM isoflurane in Ca^{2+}-free external solution. The miniature IPSC appeared sparsely in the Ca^{2+}-free solution. Isoflurane increased the IPSC frequency in the absence of external Ca^{2+}. (b and c) Cumulative amplitude and frequency distributions. The number of events for the cumulative plots was 255 for the control and 186 in the presence of isoflurane. B: IPSC amplitude and frequency in the presence of isoflurane relative to the control values. Isoflurane did not alter the amplitude significantly, while the agent increased the frequency of the miniature IPSCs significantly (asterisk).

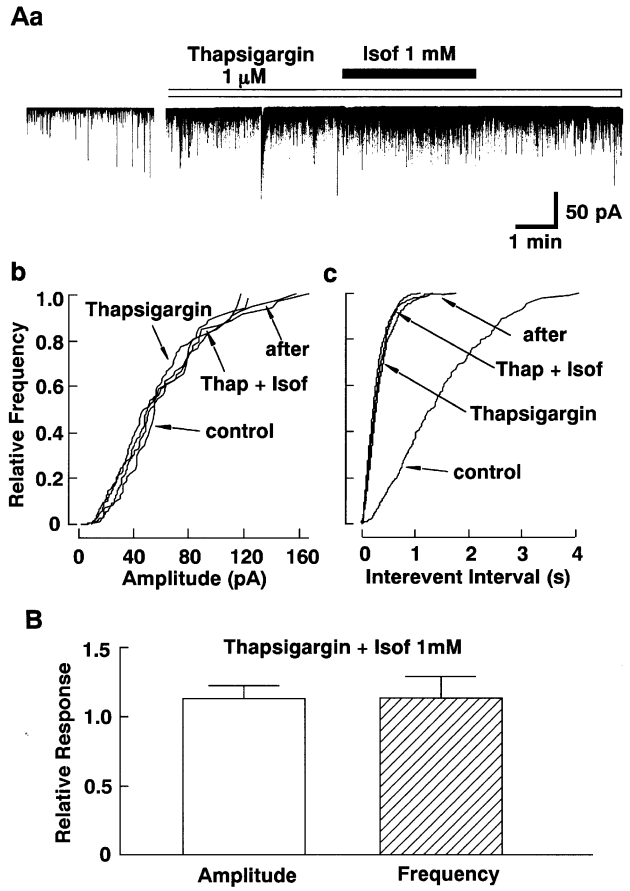

Fig. 6. Effects of isoflurane in the presence of thapsigargin. A: (a) A current trace recorded under control conditions and in the presence of thapsigargin. Isoflurane (1 mM) was applied in the presence of thapsigargin (1 μM). (b and c) Cumulative amplitude and frequency distributions. Thapsigargin itself markedly increased the miniature IPSC frequency from 0.14 to 0.31 Hz with a small change in the amplitude from 40 to 45 pA. The amplitude in the presence of thapsigargin was changed to 54 pA by isoflurane and became 50 pA following the washout of isoflurane. The frequency was altered from 0.31 to 0.30 Hz by the anesthetic and became 0.36 Hz after washout of the agent. The number of events for the cumulative plots was 130 for the control and 127 for isoflurane. B: IPSC amplitude and frequency in the presence of thapsigargin plus isoflurane relative to those in the presence of thapsigargin alone. Isoflurane did not change the amplitude or the frequency of the miniature IPSC significantly ($P > 0.05$, $n = 5$ by Kolmogorov–Smirnov test).

c). Fig. 6B shows data that clearly indicate that isoflurane had no significant effect on either the mean miniature IPSC amplitude (1.09 ± 0.06) or its frequency (1.14 ± 0.18) in the presence of thapsigargin ($n = 5$).

4. Discussion

4.1. Effects of isoflurane on postsynaptic glycine receptors

The glycine receptor and the $GABA_A$ are ligand-gated receptor chloride channel complexes located on neuronal membranes in the CNS, the former being found mainly in the brain stem and spinal cord. Both inhibitory glycine receptors and $GABA_A$ receptors are thought to largely mediate the actions of general anesthetics. In the present study, a relatively high concentration of isoflurane, a volatile anesthetic, was found to greatly enhance the glycine-induced chloride currents evoked by rapid application of the transmitter (Fig. 1). The anesthetic (1 mM) caused a parallel shift of the glycine concentration–response curve to the left, reflecting a reduction in the EC_{50} of the receptor for the agonist. This finding suggests that isoflurane increases glycine binding affinity and/or channel gating efficacy, as previously reported with various volatile anesthetics and different preparations (Wakamori et al., 1991, 1998; Downie et al., 1996; Daniels and Roberts, 1998).

The average miniature IPSC amplitude was not changed (Figs. 3,5 and 6), although isoflurane augmented the amplitude of the response to submaximal doses of exogenous glycine (Fig. 1). These findings suggest that the local concentration of released glycine in the synaptic cleft is comparatively high and sufficient to saturate postsynaptic receptors even for the miniature currents. The amplitude of miniature currents may depend on the local density of the receptor on the postsynaptic membrane. The decay time constant of the miniature IPSC became significantly longer with an unchanged amplitude (Fig. 4), suggesting that isoflurane prolonged the mean open time of the glycine-activated channel and/or that effective concentrations of glycine remained longer in the cleft due to the leftward shift of the concentration–response curve. In some cells, 1 mM isoflurane evoked a small inward current by itself (Fig. 1, inset), which might be due to a GABA-mimetic action as described for other volatile anesthetics (Wu et al., 1994; Kira et al., 1998).

4.2. Presynaptic actions of isoflurane

In addition to the postsynaptic actions, we describe a presynaptic action of low (0.1 mM) and high (1 mM) concentrations of isoflurane. The minimum alveolar concentration of isoflurane for rats has been reported to correspond to 0.31 mM aqueous concentration at room temperature (Franks and Lieb, 1996). The concentrations used in the present study are, therefore, 0.3 and 3 times the minimum alveolar concentration. Both concentrations increased the frequency of miniature IPSCs without changing their peak amplitude and the effect was dose dependent (Fig. 3). This finding is consistent with the results of Gomez et al. (2000) showing that isoflurane increased the basal release of acetylcholine in cortical slices of the rat brain. The enhancement of the miniature IPSC frequency may be ascribed to an increase in $[Ca^{2+}]_i$ in the presynaptic terminal. Actually, there have been several reports demonstrating that volatile anesthetics increase the cytoplasmic Ca^{2+} concentration in muscle (Kunst et al., 1999;

Blanck et al., 1992) and in neurons (Hawa et al., 1999). The regulation of $[Ca^{2+}]_i$ is a complex matter and it is not known which processes are responsible for the postulated increase in Ca^{2+} concentration.

$[Ca^{2+}]_i$ may be increased when Ca^{2+} flows into the cell through voltage- or receptor-operated Ca^{2+} channels. If K^+ channels are blocked by isoflurane, the presynaptic membrane will be depolarized, resulting in Ca^{2+} influx into the presynaptic terminal through voltage-gated Ca^{2+} channels. Clinically relevant concentrations of general anesthetics have been reported not to affect voltage-dependent K^+ channels (Haydon and Urban, 1986; Saint, 1992; Franks and Lieb, 1994). Recently, a cloned human intermediate conductance Ca^{2+}-activated K^+ channel was shown to be rapidly and reversibly inhibited by volatile anesthetics including isoflurane (Namba et al., 2000). This inhibition may result in an additional increase in $[Ca^{2+}]_i$ of presynaptic nerve terminals under physiological conditions.

4.3. Release from intracellular stores is responsible for the $[Ca^{2+}]_i$ increase

Another mechanism to increase $[Ca^{2+}]_i$ is Ca^{2+} release from stores in the presynaptic terminal. This idea was supported by the finding that the frequency of the miniature IPSC was also increased in Ca^{2+}-free external solution (Fig. 5). Thapsigargin is an inhibitor of Ca^{2+}-ATPases, which are responsible for the uptake of Ca^{2+} into stores (Treiman et al., 1998). Thapsigargin itself enhanced the frequency but not the amplitude of miniature IPSCs (Fig. 6), indicating that presynaptic terminals have thapsigargin-sensitive Ca^{2+} stores. The constancy of the amplitude is consistent with the finding of Tapia et al. (1997) that the glycine response was not affected by an increase in Ca^{2+} concentration in the postsynaptic neuron, since $[Ca^{2+}]_i$ of the postsynaptic neuron may have also been increased by thapsigargin. Isoflurane did not affect the IPSC frequency in the presence of thapsigargin, suggesting that the increase in $[Ca^{2+}]_i$ is caused predominantly by release from intracellular store sites and/or inhibition of Ca^{2+} uptake. Actually, inhibition of sarcoplasmic membrane Ca^{2+}-ATPase by volatile anesthetics (halothane, isoflurane and sevoflurane) has been reported, which results in Ca^{2+} accumulation in the cytoplasm (Kosk-Kosicka and Roszczynska, 1993; Franks et al., 1995a,b; Franks et al., 1998). Kindler et al. (1999) reported that isoflurane in concentrations 0.5, 1 and 2 times the minimum alveolar concentration increased the basal $[Ca^{2+}]_i$ of neurons in cortical slices in a dose-dependent manner.

The results suggest that isoflurane increased $[Ca^{2+}]_i$ in the presynaptic nerve terminal by enhancing release from and/or inhibiting Ca^{2+} uptake into stores. Inhibition of a K^+ channel may also be responsible. This $[Ca^{2+}]_i$ increase augments the amount of glycine released in the resting state of the presynaptic nerve terminal and may influence the background conductance of postsynaptic neurons. Whether this action is specific for glycinergic nerve terminals and how it relates to clinical anesthesia are important issues yet to be resolved.

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