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Mechanism of antiseizure effect of isoflurane in the immature rat hippocampus

Леткий анестетик ізофлюран часто використовується для припинення довготривалого епілептичного нападу у дітей. Проте механізм антиконвульсивної дії цього препарату на ранніх стадіях розвитку мозку не відомий. Ми досліджували збудливу та гальмівну провідності у антиепілептичному ефекті ізофлюрану, використовуючи комбінацію методу patch-clamp у конфігурації "ціла клітина" та реєстрації зовнішньоклітинного потенціалу на двох моделях епілепсії у гіпокампальних зрізах мозку молодих щурів. Наші результати свідчать про те, що пригнічення збудливої синаптичної системи не відіграє суттєвої ролі у антиконвульсивній дії ізофлюрану. Останній зменшує синхронізацію нейрональной активності переважно через підвищення ГАМК-зумовленого гальмування як фазової, так і тонічної хлорної провідності.

INTRODUCTION

Status epilepticus is a common neurological emergency estimated to have an incidence of about 100,000 cases per year and the highest rate of occurrence in children is less than one year old. Prolonged seizure activity itself produces irreversible cerebral damage, independent of accompanying hypoxia, acidosis, and consequent biochemical derangements. Although the majority of children who suffer continuous seizures respond to intravenously administered drugs, some require other modalities of treatment including general anesthesia [9]. The volatile anesthetic agent isoflurane is one of the primary choices in clinical practice for management of refractory status epilepticus in children [3, 6, 10]. In our recent study we showed that isoflurane effectively stops hippocampal seizures in high-potassium/ low magnesium model of epilepsy in immature rat [4]. However the mechanism by which isoflurane produces its antiseizure effect remains to be clarified. It has been well documented that potentiation of GABA-induced Cl^- conductance and decreasing excitatory synaptic transmission are a primary targets for isoflurane anesthesia [1, 2, 7]. In the hippocampus, two distinct forms of GABAergic inhibition have been identified, phasic inhibitory postsynaptic currents that are the consequence of the vesicular release of GABA and tonic conductance that is activated by low ambient concentrations of extracellular GABA [8, 11]. The receptors underlying tonic and phasic inhibitory conductances in hippocampal neurons are pharmacologically and biophysically distinct, suggesting that they serve different physiological roles. In our previous study isoflurane had substantial effect on tonic and phasic inhibitory conductances as well as on excitatory postsynaptic transmission [5]. In this study we aimed to clarify participation of these systems in antiseizure effect of isoflurane.

METHODS

Slice preparation

Sprague-Dawley rats were deeply anaesthetized using isoflurane and decapitated. The brain was removed and placed into ice-cold 'cutting solution' of the following composition (mM): sucrose – 250, KCl – 2, $CaCl_2$ – 0.5,

$MgCl_2$ – 7, $NaHCO_3$ – 26, NaH_2PO_4 – 1.2 and glucose 11 (pH=7.4). Transverse hippocampal slices (500 μm) were cut using specific vibroslicer (Leica 1000S). After dissection, slices were kept in an oxygenated (95 % O_2-5% CO2) artificial cerebrospinal fluid (ACSF) solution of the following composition (mM): NaCl 126, KCl 3.5, $CaCl_2$ 2.0, $MgCl_2$ 1.3, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11 (pH 7.3) at 30-32 °C for at least 1.5 h before use.

Electrophysiological recordings

For recordings, slices were transferred to a recording chamber and superfused at 30-32 °C at a rate of 1.3 mL/min with the oxygenated ACSF. Patch electrodes were filled with a solution of the following composition (in mM): K-gluconate 117.5, KCl 17.5, NaCl 8, HEPES 10, EGTA 10, Na_3GTP 0.2, and MgATP 2 (pH 7.3). Patch-clamp recordings in whole-cell configuration were made from the visually identified pyramidal cells. The recordings were performed using an Axopatch 200B amplifier (Axon Instruments). Extracellular field potential recordings were obtained from CA3 pyramidal cell layer using metal electrodes of 50 μm diameter and the signal was amplified using a custom-made amplifier with enhanced electromagnetic interference noise suppression (bandpass 0.1 Hz – 4 kHz; amplification x1000). Peak-to-peak noise was in the range of 20 μV. All recordings were digitized (10 kHz) online with an analogue-to-digital converter Digidata 1322A (Axon Instruments) and analyzed using Clampfit and Origin softwares.

Application of isoflurane

To study the effects of isoflurane we used specific isoflurane vaporizer (Isotec 3, Ohmeda Medical System). The concentration of isoflurane was regulated by vaporizer and set on 2.5 % which was shown to be an effective concentration to stop hippocampal seizures in vivo [4]. Application was made using polytetrafluoroethylene (Teflon) tubes to minimize loss and binding of isoflurane.

RESULTS AND DISSCUSION

To evaluate the role of excitatory and inhibitory conductances in antiseizure effect of isoflurane we used combination of whole-cell patch-clamp and extracellular field potential recording techniques on two models of epilepsy in a hippocampal slice preparation. Recordings were performed from CA3 pyramidal layer of hippocampus from rats at postnatal (P) day 10 to 12. Application of 15 μM bicuculline led to blockade phasic and tonic chloride conductances and induced ictal-like discharges in CA3 pyramidal layer of hippocampus (Fig.1). In the extracellular field potential recordings the ictal-like events associated with rhythmic burst of population spikes. In whole-cell recordings at holding potential -20mV inward currents were synchronized with ictal-like activity. These currents reversed near the reversal potential for glutamatergic postsynaptic currents (0mV) and were completely blocked by AMPA and NMDA receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 mM) and D-2-amino-5-phosphonovalerate (D-APV, 50 mM) suggesting a contribution of AMPA and NMDA receptors. Application of isoflurane in concentrations effective to stop seizure in vivo [4] did not affect of bicuculline-induced ictal-like activity. These data demonstrated that the primary effect of isoflurane is through GABAergic mechanism.

It has been shown that tonic chloride conductance in hippocampal neurons is insensitive to the inhibitory effects of $GABA_A$ receptor antagonist, gabazine in small concentrations (up to 10 μM) [11]. In our previous study application of 1 μM gabazine abolished spontaneous inhibitory postsynaptic currents but did not affect tonic current [5]. When extracellular potassium concentration was increased to 6mM application 1 μM of gabazine evoked ictal-like discharges in CA3 pyramidal layer of hippocampus (Fig.2). Application of isoflurane reduced frequency of gabazine-in-

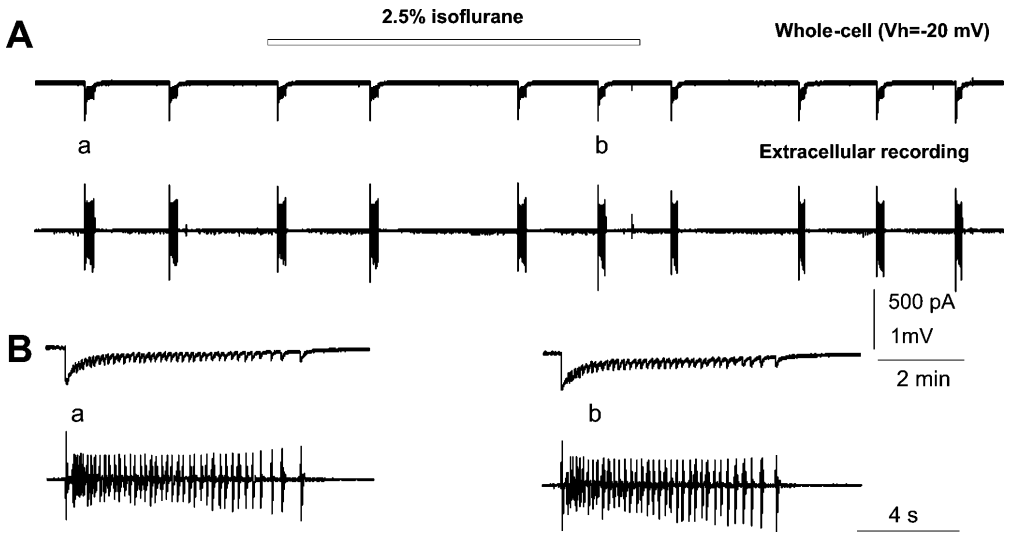

Figure 1. Effect of isoflurane on bicuculline-induced seizure–like activity. (A) Example of whole-cell voltage-clamp and extracellular recordings from CA3 pyramidal layer of hippocampus from P10 rats in the presence of 15 μM bicuculline before, during and after application of 2.5% isoflurane. (B) Parts of recording presented in A in the expended scales

duced interictal-like events from 0.30 Hz to 0.025 Hz (Fig.2). In whole-cell recordings outward, bicuculline sensitive currents appeared in the presence of isoflurane simultaneously with interictal discharges. These currents were mediated by extracellular $GABA_A$ receptors

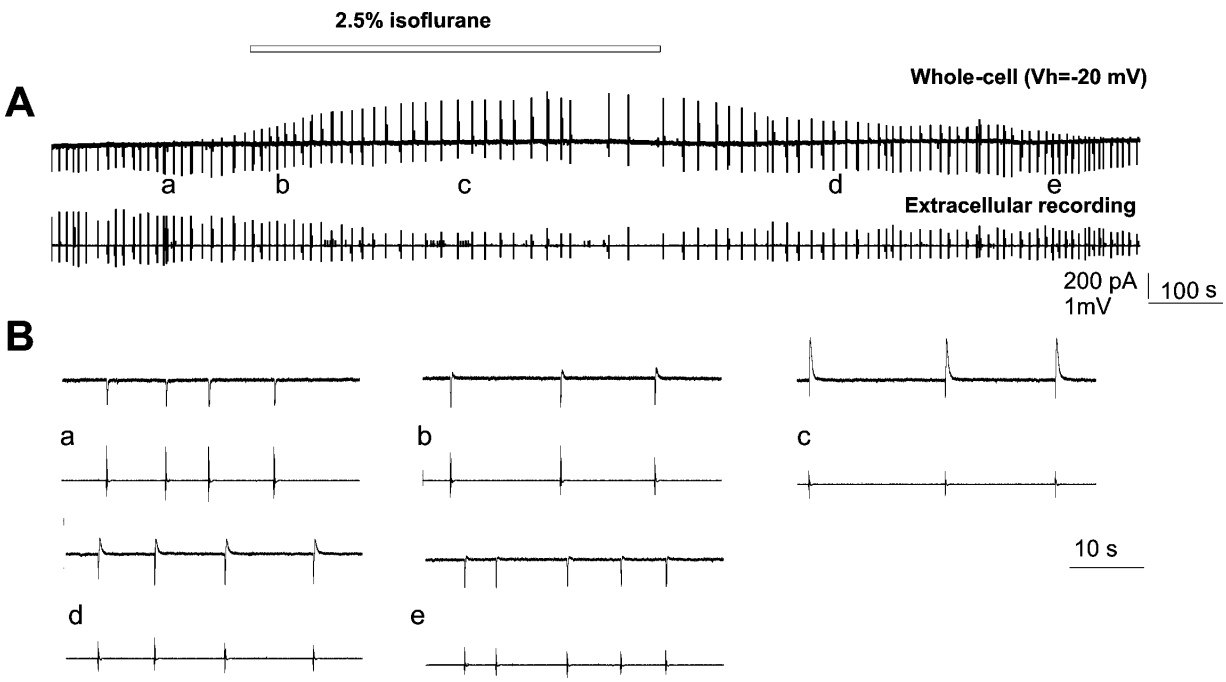

Figure 2. Effect of isoflurane on gabazine-induced seizure–like activity (A) Example of recording before, during and after application of 2.5% isoflurane. Whole-cell voltage-clamp and extracellular recordings were simultaneously made from CA3 pyramidal layer of hippocampus from P12 rats in the presence of 1μM gabazine. Application of isoflurane led to decreasing of frequency of interictal discharges induced by application of gabazine. (B) Parts of recording presented in A in the expended scales

because the current reversed polarity at the equilibrium potential for Cl^- and never observed in the presence of bicuculline, $GABA_A$ receptor antagonist.

Our results demonstrate that although isoflurane affects different neuronal targets of the immature brain anticonvulsive action of this volatile anesthetic mainly account for the enhancing of GABAergic inhibition.

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MECHANISM OF ANTISEIZURE EFFECT OF ISOFLURANE IN THE IMMATURE RAT HIPPOCAMPUS

The volatile anesthetic isoflurane is often used in children in the management of refractory status epilepticus. However the mechanism of anticonvulsant action of isoflurane during early brain development is not clear. In this study we explore the role of excitatory and inhibitory conductances in antiseizure effect of isoflurane using combination of whole-cell patch-clamp and extracellular field potential recording techniques on two models of epilepsy in a hippocampal slice preparation from immature rat. Our data demonstrated that decreasing of excitatory synaptic transmission does not account for antiseizure effect of this volatile anesthetic agent. Isoflurane decreases the synchronization of neuronal activity mainly through the enhancing of GABAergic inhibition by influencing both phasic and tonic chloride conductances.

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