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Effects of isoflurane on hippocampal seizures at immature rats in vivo

Досліджували ефект ізофлюрану на епілептичну активність у СА3-зоні гіпокампа молодих щурів in vivo. Було встановлено, що ізофлюран, у концентрації яка використовується у клінічній практиці, усуває епілептичну активність у гіпокампі. У щурів, які знаходились під ізофлюрановою анестезією, епілептична активність може бути викликана аплікацією проконвульсантів, але ця активність якісно відрізняється від тої, що отримана від неанестезованих щурів. Це спостереження свідчить про те, що механізми задіяні у припиненні та запобіганні епілептичної активності відрізняються.

INTRODUCTION

Isoflurane is a one of the widely used volatile anesthetic agents which has no reported organ toxicity and produces electrographic suppression at clinically useful concentrations in pediatric and adult patients. The information about cellular mechanism of action of isoflurane bases commonly on the *in vitro* studies. It was shown, that isoflurane increases inhibitory synaptic transmission and decreases excitatory synaptic transmission by both pre- and postsynaptic mechanism in hippocampal and cortical neurons [1, 2, 6, 11, 12, 17, 22]. A different sensitivity of adult and young rats to isoflurane has been demonstrated [7]. One of the possible mechanisms of age-related vulnerability to anesthesia is the increasing susceptibility of excitatory synaptic neurotransmission to anesthetic action [20]. In the first postnatal weeks a number of major developmentally regulated changes in synaptic transmission properties take place, among them: establishment of synaptic contacts, increasing of glutamate receptor expression [5, 23], and a switch from excitatory to inhibitory effects of $GABA_A$ receptor-mediated signals [4, 15]. However, the number of binding sites of the excitatory amino acid, N-methyl-D-aspartate (NMDA), is reduced in the aged rat hippocampus and neocortex [3.8]. In addition, GABA-evoked currents in aged neurons displayed significantly greater maximal response and lesser degree of use-dependent receptor desensitization compared to young neurons [9]. Therefore, age-induced augmentation of anesthetic actions could be due to synergism between neuronal maturation and anesthesia in depressing synaptic excitation and/or enhancing synaptic inhibition. Isoflurane anesthesia for management status epilepticus has been recommended by many practitioners [10, 21]. Most of the in vivo studies of anticonvulsant effect of isoflurane on epilepsy models were performed on adult animals. However, only a few investigations were made in vivo on the hippocampus of the animals at the early postnatal stages [14, 16]. That can be explained by deep "bedding" of the hippocampus (especially CA3 zone) and by lack of the detail information about the stereotaxic coordinates of the young animal's brain.

The changing physiology during development raises important questions about the effectiveness of isoflurane in seizures management. The goal of the present study was to examine effect of isoflurane on seizure activity at CA3 pyramidal region of hippocampus on immature rats.

METHODS

To determine the anticonvulsant action of isoflurane, we used the high potassium-low magnesium model of epilepsy [13]. Sprague-Dawley rats at the postnatal (P) age 10-12 were used throughout experiments. Briefly, on the day of testing, a pup was removed from the litter, weighed, and anesthetized. Buprenex was used for general analgesia. The cannula for local drugs application with a wire electrode for recording extracellular field potential changes was implanted into the hippocampus, and the tip of the electrode and cannula was aimed at the pyramidal cell layer in the CA3 region under stereotaxic and electrophysiological guidance (2.0–2.5 mm caudal to bregma; 2.0–2.5 mm from midline; depth, 2700-3100 mm) using a micromanipulator. A silver reference electrode was implanted into the cerebellum. The cannula and recording and reference electrodes were fixed with dental acrylic. After surgery the isoflurane anesthesia was stopped and pups were left to recover from anesthesia for 10-15 minutes, and then electrophysiological data were recorded uninterrupted for 60-120 min. The electrode was connected to differential amplifier (A-M Systems, Carlsborg, WA). The signals from the recording electrode were amplified (x1000) with filter settings of 0.1–5000 Hz. After the recordings, the rat was anesthetized, and the brain was dissected out. Sagital slices were cut 300 mm thick using a vibroslicer Leica VT 1000S (Leica Microsystems, Nussloch GmbH, Germany). Electrode position verification was done under light microscopical evaluation. Drugs were applied locally through the stainless-steel cannula (inner diameter 100 mm) using microsyringes. The proconvulsant solution – artificial cerebrospinal fluid (ACSF) with high potassium/low magnesium- was following composition: 115 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 24 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 11 mM glucose. In these conditions, the extracellular concentration of Mg^{2+} ions does not drop to zero because the contamination by Mg^{2+} of the other constituents of the application solution and could reach 0.08 mM [19]. Solutions at amount 5 ml were applied every 5 minutes up to 10 times.

To study the effects of isoflurane on neuronal activity the anesthetic mask connected to isoflurane vaporizer (Isotec 3, Ohmeda Medical System) was put on the pup at all times during the experiment. The concentration of isoflurane was regulated by vaporizer. The ventilator was connected to a scavenger system; a surge tank was used and a negative pressure of a 10-cm water column applied to prevent the loss of isoflurane into the room.

Recordings were digitized (10 kHz) online with an analogue-to-digital converter (Digidata 1322A; Axon Instruments) and analyzed off-line with the Axon package MiniAnalysis program (Jaejin Software, Leonia, NJ0 and Origin 5.0 (Microcal Software, Northampton, MA).

Tonic was arbitrary used to describe sustained rhythmic spikes whereas clonic was defined as bursts of rhythmic spikes interspersed with lower amplitude, non-rhythmic activity [18]. The tonic and clonic rhythms refer to the EEG correlates to the tonic and clonic behaviors observed during seizures. In this study we used these terms arbitrarily to describe the EEG patterns that typically occur in conjunction with behavioral seizures. The term seizure-like activity (SLA) was used to describe either ictal or interictal activity.

RESULTS AND DISSCUSION

To explore the effect of isoflurane on seizures induced during early postnatal life, we used high potassium/low magnesium model of epilepsy at immature rats *in vivo* [13]. We focus our attention on CA3 pyramidal region of the hippocampus as this area is one of the most seizure prone areas of the brain and is most commonly found as the seizure focus in epilepsy surgery. Seizures occurred after the 5-9th microinjection of 10mM $[K]_o$ / low $[Mg]_o$ in ACSF into the CA3 region of hippocampus.

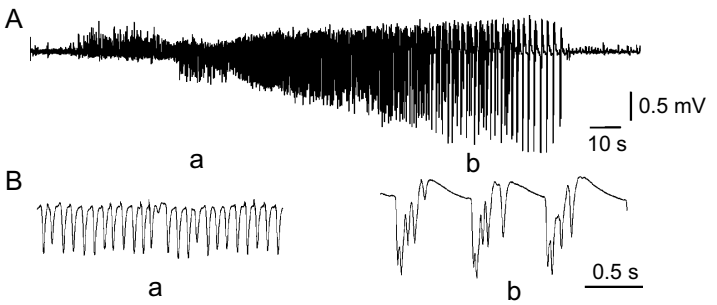

Fig.1. Seizure like-activity induced by high potassium/low magnesium in P10 rat. (A) Extracellular field potential recordings from CA3 pyramidal cell layer in the presence of 10mM $[K]_o$ / low $[Mg]_o$ in vivo. (B) Example of ictal-like event and its phases from A shown on expanded time scale: (a) tonic- and (b) clonic-like discharges.

Electrographic seizures had typical ictal morphology with tonic- and clonic-like discharges (Fig. 1 *a, b*). Once induced, seizures were generated in response to each additional microinjection.

Experiments with isoflurane were performed at P10-P12. The suppression of epileptiform activity to a given proconvulsant stimulus by an anesthetic can be considered as a summation of the suppression of basal (pre-stimulus) activity and response capability (increased by proconvulsant). We therefore explored the action of isoflurane in control conditions and during SLA. Exposure of the rat pups to 2.5% isoflurane evoked a sequential process of neuronal activity pattern changes in the CA3 hippocampal field (Fig. 2). During the first two minutes of isoflurane

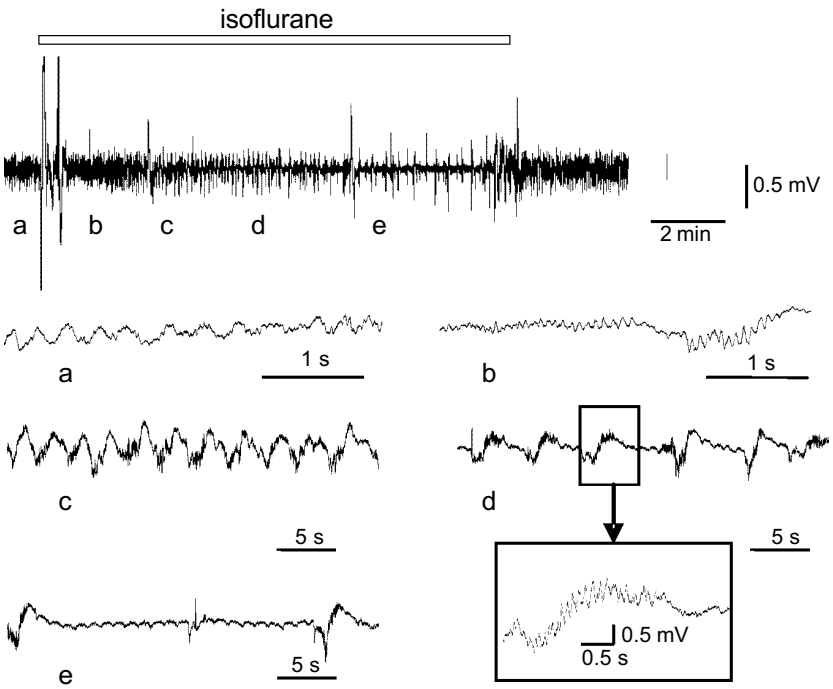

Fig.2. Effects of isoflurane on neuronal activity pattern recorded from CA3 cell layer in P11 animal. (A) Field potential recording during control conditions and following exposure of 2.5% isoflurane. (B) Parts of the traces from A are shown on an expanded time scale: (a) neuronal activity in control; (b) increasing beta activity during 2 min of exposure to isoflurane; (c) appearance high-amplitude, low-frequency waves after 2 min of general anesthesia; (d, e) decreasing of frequency of waves after 5 min of exposure to isoflurane. The enlarged framed portion of d shows the 16 Hz brushes on the high-amplitude waves.

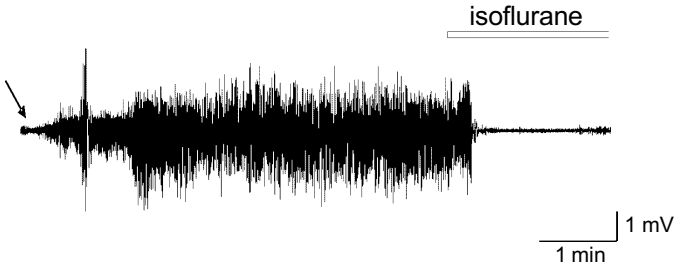

Fig. 3. Effect of isoflurane on high potassium/low magnesium induced SLA in P11 pups. Application of ACSF with 10mM $[K]_o$ / low $[Mg]_o$ is indicated by the arrow. Isoflurane at 2.5 % completely suppressed the seizure-like activity

anesthesia increasing amounts of beta-range (14–16 Hz) frequencies were recorded (14–16 Hz). High-amplitude, low-frequency waves (0.4–0.2 Hz) were evoked after 2–5 minutes of isoflurane exposure. The frequency of these waves gradually decreased with continuous exposure to isoflurane.

The isoflurane at a concentration of 2.5 % stopped SLA induced by 10mM $[K]_o$ / low $[Mg]_o$ ACSF during 10–20 s after exposure (Fig. 3). The neuronal activity observed after seizure disappearance was similar to activity under isoflurane anesthesia as shown in figure 2.

While isoflurane in clinically relevant concentration stops hippocampal seizures, we have revealed that seizures can be evoked by proconvulsant agents while the animal is under isoflurane anesthesia. The figure 4 shows that SLA induced during anesthesia was qualitatively different from that obtained in nonanesthetized conditions. No clonic phase was observed in this condition. The decrease in amplitude and frequency of SLA and the changing qualitative parameters of seizures

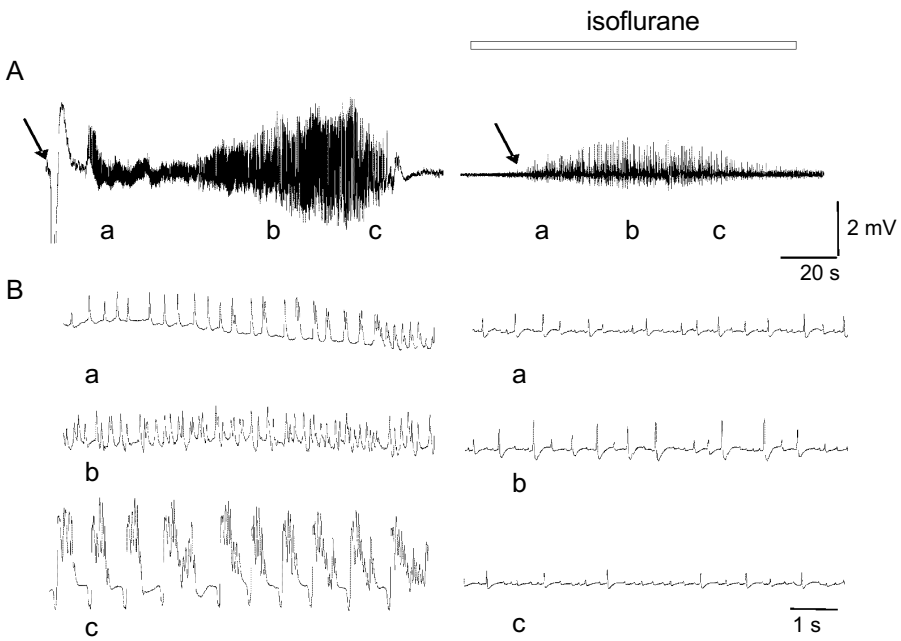

Fig 4. Seizure-like activity induced by high potassium/ low magnesium ACSF recorded from CA3 cell layer of nonanesthetized rats and animals under general anesthesia. (A) SLA in the presence of 10mM $[K]_o$/ low $[Mg]_o$ were detected on the same P12 animal in awake condition (left) and following isoflurane anesthesia (2.5%) (right). (B) Parts of the traces form A illustrating the phases of SLA. Note: the seizure-like activity evoked when the animal was under isoflurane anesthesia was smaller in amplitudes and lower in frequency.

demonstrate that isoflurane only partially blocks the systems which involved in generation seizures. This observation suggests that the mechanism by which seizures are terminated differ from the mechanisms responsible for preventing of the initiation of seizures. Our results show that general anesthesia with isoflurane can be effectively used in seizure treatment in developing brain. However prolongation of isoflurane exposure does not prevent the seizure reappearance.

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EFFECTS OF ISOFLURANE ON HIPPOCAMPAL SEIZURES AT IMMATURE RATS IN VIVO

In the present study we examined the effect of isoflurane on seizure-like activity at hippocampal CA3 pyramidal region of immature rats in vivo. We found that isoflurane in clinically relevant concentrations effectively stops hippocampal seizures. When animal was under isoflurane anesthesia seizure-like activity still can be evoked by application of proconvulstant agents, but this activity was qualitatively different from that obtained in nonanesthetized rats. This observation suggests that the mechanism by which seizures are terminated differ from the mechanism responsible for preventing of the initiation of seizures.

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