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THE DIFFERENTIAL EFFECTS OF VOLATILE ANESTHETICS ON ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING AND RECOVERY AFTER HYPOXIA IN RAT HIPPOCAMPAL SLICE CA1 PYRAMIDAL CELLS

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Abstract—Two volatile agents, isoflurane and sevoflurane have similar anesthetic properties but different potencies; this allows the discrimination between anesthetic potency and other properties on the protective mechanisms of volatile anesthesia. Two times the minimal alveolar concentration of an anesthetic is approximately the maximally used clinical concentration of that agent; this concentration is 2% for isoflurane and 4% for sevoflurane. We measured the effects of isoflurane and sevoflurane on cornus ammonis 1 (CA1) pyramidal cells in rat hippocampal slices subjected to 10 min of hypoxia (95% nitrogen 5% carbon dioxide) and 60 min of recovery. Anesthetic was delivered to the gas phase using a calibrated vaporizer for each agent. At equipotent anesthetic concentrations, sevoflurane (4%) but not isoflurane (2%), enhanced the initial hyperpolarization (6.7 vs. 3.4 mV), delayed the hypoxic rapid depolarization (521 vs. 294 s) and reduced peak hypoxic cytosolic calcium concentration (203 vs. 278 nM). While both agents reduced the final membrane potential at 10 min of hypoxia compared with controls, 4% sevoflurane had a significantly greater effect than 2% isoflurane (−24.4 vs. −3.5 mV). The effect of these concentrations of isoflurane and sevoflurane was not different for sodium, potassium or ATP concentrations at 10 min of hypoxia, the only difference at 5 min of hypoxia was that ATP was better maintained with 4% sevoflurane (2.2 vs. 1.3 nmol/mg). If the same absolute concentration (4%) of isoflurane and sevoflurane is compared then the cellular changes during hypoxia are similar for both agents and they both improve recovery. We conclude that an anesthetic's absolute concentration and not its anesthetic potency correlates with improved recovery of CA1 pyramidal neurons. The mechanisms of sevoflurane-induced protection include delaying and attenuating the depolarization and the increase of cytosolic calcium and delaying the fall in ATP during hypoxia. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: isoflurane, sevoflurane, ischemia, anoxia, calcium, ATP.

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Abbreviations: aCSF, artificial cerebrospinal fluid; CA1, cornus ammonis 1; MAC, minimum alveolar concentration; S.E.M., standard error of the mean.

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There is an increased risk of stroke and/or neurologic deficit for patients undergoing surgical procedures. Isoflurane and sevoflurane are two volatile anesthetics that are commonly used in neurologic and cardiac surgery. Anesthetics, which are required for surgical procedures, may act to reduce neurological deficits. Since anesthetics do not have the same mechanisms of action, they can differentially affect anesthesia, cellular properties and neuronal damage. Indeed certain anesthetics have been shown to improve recovery from neurologic insults while others appear to have little efficacy. A number of investigations have shown that volatile anesthetics can reduce ischemic cerebral injury in animals (Bendo et al., 1987; Werner et al., 1995; Engelhard et al., 1999; Toner et al., 2002). Isoflurane has been shown to alter outcome after either focal or incomplete global ischemic brain injury (Warner et al., 1993; Toner et al., 2002; Tsai et al., 2004; Zheng and Zuo, 2004). Halothane and sevoflurane both substantially reduced the volume of infarction after focal ischemia compared with the awake state (Warner et al., 1993). Recent studies demonstrate that isoflurane may only delay but not prevent cerebral infarction in rats subjected to ischemia; thus the true extent of long term anesthetic protection is unknown (Kawaguchi et al., 2000, 2004; Elsersy et al., 2004). The protective efficiency of volatile anesthetics, the relationship between an anesthetic's potency and its protective efficacy, and the mechanisms by which volatile anesthetics protect against hypoxic and ischemic brain injury remain unclear. Volatile anesthetic agents can have different potencies even when their physical structures are similar. This allows a differentiation between anesthetic potency and anesthetic concentration in determining the mechanism of anesthetic protection against hypoxic and ischemic neuronal injury. We have examined isoflurane and sevoflurane, two similar volatile anesthetics, which have a two-fold difference in their anesthetic potency at the same absolute concentration.

Ischemia and hypoxia trigger and activate events during and immediately after the insult that leads to the long-term damage. Acute brain slices from adult animals cannot be used to examine long-term recovery and damage; however, they are useful for examining the effects of anesthetics on the triggers and mechanisms, which result in the long term damage after hypoxia. Hippocampal cornus ammonis 1 (CA1) pyramidal neurons are known to be extremely vulnerable to hypoxia and ischemia and they demonstrate a stereotyped response to the insults that is char-

acterized by an initial hyperpolarization followed by a depolarization. When the membrane completely depolarizes, the damage becomes irreversible, even if oxygen is reintroduced (Tanaka et al., 1997; Taylor and Narasimhan, 1997; Wang et al., 1999). The aim of the present study is to compare the effective concentrations of isoflurane and sevoflurane for protection against hypoxia-induced damage and to examine the mechanisms by which these volatile anesthetics may protect against neuronal damage.

EXPERIMENTAL PROCEDURES

Slice preparation

All procedures involving animals were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the State University of New York Downstate Medical Center. The minimal number of animals needed to carry out the experiments was used, and any potential pain or suffering was alleviated by anaesthetic application before the procedure. Sprague–Dawley rats (100–120 days old) were anesthetized with 2% isoflurane for 2 min, decapitated, and their brain quickly removed and placed into chilled (2–4 °C) artificial cerebrospinal fluid (aCSF) (Zhu et al., 1997). Hippocampal slices of 400 μm thickness for intracellular electrophysiological recording, sodium, potassium and ATP measurements and 300 μm for calcium imaging were sectioned in chilled aCSF (4–6 °C) using a vibratome (Wang et al., 1999). The thinner sections improved visualization for the imaging experiments. The slices were stored in a beaker containing aCSF saturated with 95% O_2 and 5% CO_2, and remained there for approximately two hours at 25 °C. The composition of the aCSF was, in mmol/L NaCl, 126; KCl, 3; KH_2PO_4, 1.4; $NaHCO_3$, 26; $MgSO_4$, 1.3; $CaCl_2$, 1.4 glucose, 4; at pH, 7.4, and was equilibrated with 95% O_2–5% CO_2. Slices were transferred to a tissue chamber and maintained at 37 °C in this chamber. Hypoxia was generated by switching the gas to 95% N_2–5% CO_2.

Electrophysiology

The hippocampal slice was submerged in the recording chamber and perfused with aCSF at a rate of 3.0 ml/min. The temperature in the recording chamber was maintained at 37 °C.

A bipolar stimulating electrode was placed in the Schaffer collateral pathway and then a CA1 pyramidal neuron was impaled with a glass-micropipette filled with 4 mol/l K acetate (70–120 MΩ). Only neurons with stable resting potentials of at least −60 mV for 15 min with high amplitude, short duration action potentials that showed spike frequency accommodation and were activated by short latency Schaffer collateral stimulation were examined (Wang et al., 1999).

The electrophysiological parameters were measured as previously described by Tanaka et al. (1997). The latency of the rapid depolarization was measured from the onset of hypoxia to the onset of the rapid depolarization, which was estimated by extrapolating the slope of the rapid depolarization to the slope of the slow depolarization. The onset of the rapid depolarization was measured as the membrane potential crossing the extrapolated slope of the slow depolarization and the rapid depolarization.

The untreated group received 20 min of perfusion, then 10 min of hypoxia followed by 60 min of reperfusion. The anesthetic-treated groups received 10 min of perfusion, 10 min of anesthetic treatment, then 10 min of hypoxia with anesthetic followed by 60 min of reperfusion.

ATP measurement

ATP was measured in the CA1 region of individual slices. Slices were incubated in beakers under conditions similar to those described for the electrophysiological experiments. For these experiments a period of 5 or 10 min of hypoxia was examined. After hypoxia, or an equivalent time period, the slices were removed from the beaker, rapidly frozen in liquid nitrogen, lyophilized and the CA1 regions dissected and weighed. The ATP was then extracted by homogenizing the tissue in 3 N ice-cold perchloric acid and measured, after neutralization, using the firefly luciferin–luciferase assay (Kass et al., 1992).

Sodium and potassium measurements

To measure sodium and potassium concentrations, slices were placed in agitated ice-cold (4 °C) isotonic sucrose for 10 min after the experiment. This procedure was used to wash ions from the extracellular space. The CA1 regions of the slices were microdissected; CA1 regions from different slices from the same animal were pooled so that there was enough tissue to measure sodium and potassium. The pooled tissue was dried at 85 °C for 48 h and weighed. Diluted nitric acid (0.1 N) was added to the tissue. The extract was assayed in a flame photometer (Kass et al., 1992; Amorim et al., 1999).

Calcium imaging

Slices were placed in a small beaker containing 6 ml aCSF, 9 μmol/l Fura-2 AM (Molecular Probes; Eugene, OR, USA), 0.01% pluronic acid and 50 μl of DMSO for 45 min. They were then washed in fresh aCSF and incubated for an additional 45 min to remove extracellular Fura-2 and allow the AM moiety of intracellular Fura-2 AM to hydrolyze. The slices were maintained at 33 °C from the initial incubation until they were placed in a tissue chamber on the microscope stage; maintenance at this temperature improved dye loading. The slices were maintained at 37 °C in the tissue chamber on the microscope stage throughout the experiment. An Incyt Im2 dual wavelength imaging system and its associated software were used for measuring Ca^{2+} (Intracellular Imaging Inc., Cincinnati, OH, USA). A long working distance Nikon Plan Fluor 20× UV objective (n.a. 0.5) and a Nikon TMS inverted microscope were attached to a filter changer (340 nm and 380 nm filter), a 300 W Xenon light source and a low light level CCD camera for image acquisition (Wang et al., 1999, 2000).

We used Ca buffers in solution for calibration, all values represent Ca^{2+} concentrations corrected for background fluorescence in time matched, unlabeled (no Fura-2) slices subjected to hypoxia. This was done to account for the increase in background fluorescence due to the increase in NADH levels during hypoxia (Wang et al., 1999, 2000).

Statistics

Either chi-square, ANOVA followed by Newman-Keuls multiple comparison test or Student's *t*-test was used to test significance (Prism, GraphPad Software, San Diego, CA, USA). Data are expressed as mean±standard error of the mean (S.E.M.); $P<0.05$ was considered significant. Percentage values are used in the text to enhance readability and facilitate comparison, however, all statistics are done on the absolute numbers, which are shown in the figures and tables. Any stated difference in the results section is a statistically significant difference.

RESULTS

Electrophysiological recovery

Intracellular recordings were made from CA1 pyramidal neurons in rat hippocampal slices before, during and after