Anesthesiology
74:83–88, 1991

Anesthetic Effects on Resting Membrane Potential Are Voltage-dependent and Agent-specific

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Membrane hyperpolarization (increase in resting potential) together with a conductance increase has been suggested as a common mechanism of anesthetic action. The current study compared the effects of halothane, enflurane, and isoflurane on resting membrane potential and conductance of hippocampal CA1 neurons *in vitro*. At 1 MAC, halothane produced significant ($P < 0.01$) hyperpolarization (-2.8 ± 1.3 mV, mean ± SD) accompanied by a conductance increase (6.2 ± 2.7%). Enflurane also produced a significant ($P < 0.001$) hyperpolarization (-3.15 ± 1.2 mV); however, this was accompanied by a conductance decrease (-4.5 ± 1.5%). Isoflurane produced variable effects. Anesthetic-induced hyperpolarization was maximal in neurons with more negative initial resting potentials and was reduced by depolarization. Across agents, these relatively small changes in resting potential were not correlated with decreases in excitability as measured by synaptically evoked population spike depression. The results are not consistent with a common action of the three agents on a single ionic channel. (Key words: Anesthetic action: theories. Anesthetics, volatile: halothane, isoflurane, enflurane. Brain: hippocampal slice, hyperpolarization, resting potential.)

UNITARY HYPOTHESES of anesthetic action are attractive for their elegance and simplicity. In their usual form, they postulate that all general anesthetic agents act in fundamentally similar ways to bring about anesthesia. Several mechanisms have been proposed at the level of the nerve cell membrane.[1–6] One possibility raised by several investigators is that many anesthetic agents hyperpolarize central neurons by increasing conductance through a potassium[7–9] or chloride channel.[10,11] The functional effect of this hyperpolarization would be to raise the threshold for impulse initiation and thus decrease the probability that a cell will discharge in response to a given excitatory synaptic input.

This hypothesis has been proposed for a large variety of agents, including volatile anesthetics, barbiturates, ethanol, and opioids.[8,9] We have examined its validity for a more uniform class, that of volatile anesthetic agents. These agents have been reported to hyperpolarize a variety of neurons,[8,12,13] including hippocampal and cortical pyramidal neurons. There are, however, conflicting reports that volatile anesthetics do not affect resting membrane potential in these neurons. The hippocampal cortex is an important structure for the study of anesthetic effects, which have been extensively described both *in vivo* and *in vitro*. The hippocampus, particularly CA1 pyramidal neurons, is among the best characterized structures in the central nervous system.[14–16] In the current study, two halogenated ethers, isoflurane and enflurane, and a halogenated hydrocarbon, halothane, were examined for their effects on resting membrane potential and conductance in CA1 pyramidal neurons of the *in vitro* hippocampal brain slice preparation. The results show that at equipotent anesthetic concentrations, even structurally similar agents do not exert common hyperpolarizing effects on resting potential or common changes in conductance.

Materials and Methods

PREPARATION

Experiments were conducted on 31 hippocampal slices from male Sprague-Dawley rats (150–250 g). The protocol was approved by Stanford University's Institutional Animal Care Committee. Rats were anesthetized with diethyl ether, the heart stopped by a blow to the back of the thorax, and the brain rapidly removed and placed in precooled (10° C) artificial cerebrospinal fluid (ACSF). The ACSF had the following composition (millimolar concentrations): NaCl 134; KCl 3.25; $CaCl_2$ 1.6; KH_2PO_4 1.25; $MgSO_4$ 2; $NaHCO_3$ 16; and dextrose 10. After dissection of the hippocampal formation, transverse slices of hippocampus (400 μm) were cut with a McIlwain tissue chopper (Mickel Laboratory Engineering Co., UK). Slices were maintained on a nylon mesh screen at the gas–liquid interface in a chamber based on the design of Richards and Tegg.[17] Oxygenated (95% oxygen/5% carbon dioxide) and prewarmed (35° C) ACSF was continuously perfused through the chamber at a rate of 0.25–0.5 ml/min.

ELECTROPHYSIOLOGY

The arrangement of stimulating and recording electrodes is shown diagrammatically in figure 1*A*. A bipolar tungsten stimulating microelectrode was placed on Schaffer-collateral fibers to electrically activate excitatory synaptic inputs to CA1 pyramidal neurons. Extracellular field potentials were recorded *via* glass fiberfill recording electrodes (filled with ACSF, resistance 0.5–1.0 Mohm) placed at the border between stratum pyramidale and stratum

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Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California. Accepted for publication August 10, 1990. Supported by National Institutes of Health grant NS13108 to JJK. Presented in part at the annual meeting of The Society for Neuroscience, Toronto, Canada, November 12–16, 1988.

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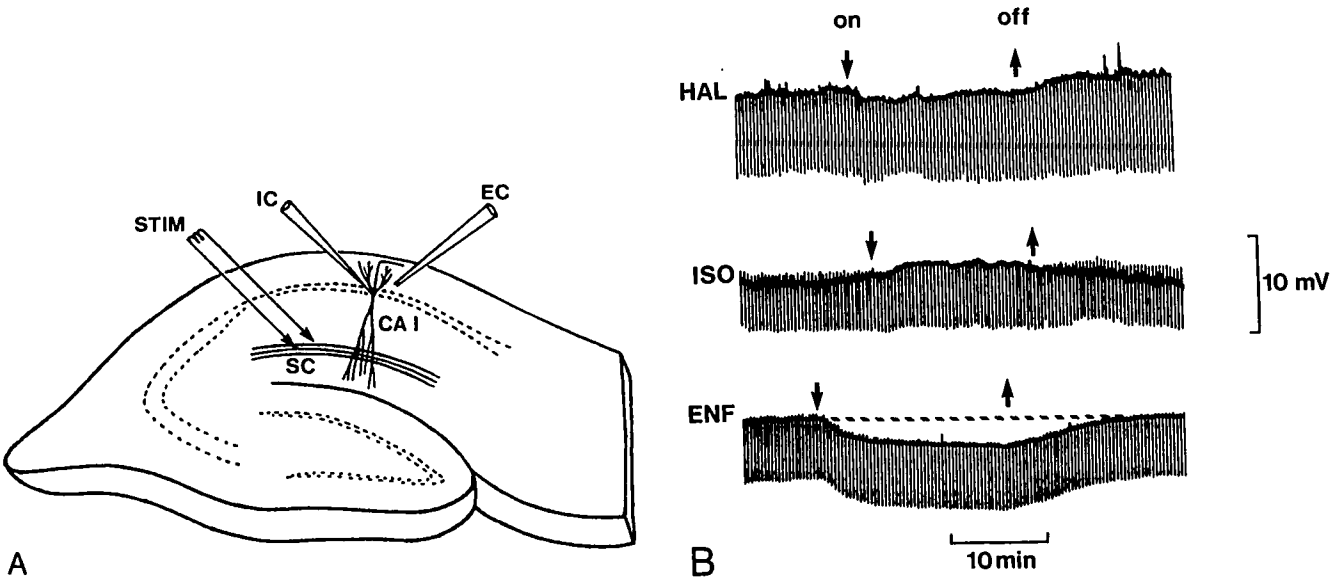

FIG. 1. (*A*) Hippocampal slice preparation. Diagram shows placement of stimulating (STIM) and recording electrodes (IC = intracellular; EC = extracellular). (*B*) Records of resting potential showing the effects produced by 1 MAC of halothane (HAL; 1.2 vol%), isoflurane (IS0; 1.4 vol%), and enflurane (ENF; 1.8 vol%) applied for the indicated times (arrows). Negative potential deflections are responses to hyperpolarizing constant current pulses (150 ms, 0.2–0.3 nA, 0.04 Hz) used to measure membrane conductance.

oriens to measure synaptically evoked field potentials at the somatic level. Recorded signals were amplified, filtered (bandpass 1–10 KHz), and digitally stored for later analysis. A wide bandpass filtering was used to avoid any distortion of the field potential signal (component frequencies 400 Hz–2 KHz). Records of evoked responses are averages of ten individual field potentials recorded at a stimulus frequency of 0.08 Hz. This stimulus frequency does not produce facilitation or disinhibition of synaptic responses, as can occur with higher-stimulus frequencies,[18] and was chosen in order not to bias the results. Population excitatory postsynaptic potential (EPSP) amplitude was measured as the peak positivity, and population spike (PS) amplitude was measured from threshold to peak negativity. Criteria for accepting a slice included stable PSs of at least 4 mV amplitude evoked by stimuli of low intensity (usually <10 V; 20 μA constant current).

For intracellular recording, glass fiberfill recording electrodes (1.0 mm OD, thin-wall; Sutter Instruments, Novato, CA) were pulled on a P-80/PC Flaming Brown micropipette puller. Electrodes were filled with 4 M potassium acetate and beveled to produce tip resistances of 80–140 Mohms. A Getting microelectrode bridge amplifier (model 5A) was used to inject current and to measure potential changes across CA1 neuron somatic membrane. Recorded signals were amplified and digitally stored for later analysis. Membrane conductance was measured by injecting hyperpolarizing current pulses through the intracellular recording electrodes. Resting membrane potential was continuously monitored and recorded on a strip chart recorder, and absolute values were determined by withdrawing the electrode from impaled neurons after each experiment. Experiments were done only on cells with an initial stable resting potential of at least 60 mV for a 10–15-min period.

Anesthetic Administration and Analysis

Volatile anesthetic agents were applied as a vapor *via* the prewarmed and humidified oxygen–carbon dioxide gas stream above the slices with the use of calibrated commercial vaporizers (Ohio Medical Products). Anesthetic agents were administered over a concentration range of 0.5 to 2 MAC. Each preparation was exposed to two or three concentrations of a single anesthetic, usually beginning with a low concentration. Concentrations were randomized in several preparations; however, no differences were noted when a low concentration was tested on a neuron that previously had been exposed to higher concentrations, as opposed to a naive neuron. Concentration-dependent effects were observed and increased monotonically with increasing concentrations of each agent. Agents were compared at the equipotent anesthetic level for rats (1 MAC): halothane 1.1–1.2, isoflurane 1.4, and enflurane 1.8 vol%.[19] Vapor concentrations were applied for up to 30 min to ensure that equilibrium was achieved. Accurate measurements of volatile anesthetic concentrations in the vapor phase were obtained with an infrared absorption monitor (model AAM222; Puritan-Bennett Co.). Sample gas was drawn continuously from the tissue

chamber at a site <1.0 mm above the brain slice. Chamber concentrations reached steady state within 5 min of adding the anesthetic to the gas stream. An approximation of the corresponding cerebrospinal fluid concentrations (millimolar) of the anesthetics can be obtained from MacIver and Roth.[20] Enflurane and isoflurane were obtained from Anaquest, Inc.; halothane was obtained from Ayerst Laboratories, Inc., Philadelphia, PA. All chemicals used in the ACSF were reagent grade or better and were obtained from J. T. Baker Chemical Co.; water for solutions was high-performance liquid chromatographic and spectrophotometry grade and was obtained from EM Science, Inc. (Gibbstown, NJ).

Data Analysis

Effects on resting membrane potential and conductance were determined by comparing the mean resting potential during a 5-min period immediately before anesthetic administration with the mean potential during the last 5 min of a 20-min exposure period. Steady-state effects of anesthetics were observed within 10–15 min after anesthetic administration. Time-matched control and anesthetic measures were compared, and statistical significance was determined with a Wilcoxon paired-sample test. Only neurons that exhibited recovery from the anesthetic effect were included in the analysis. Data from field potential measurements were treated in a similar manner; statistical comparisons were made to time-matched control data. Analysis of variance (ANOVA) was used to compare effects among anesthetic agents.

Since CA1 neurons exhibited considerable variation in both resting membrane potential (−60–−73 mV) and conductance (32–58 Mohm; 17–31 ns), data were normalized and anesthetic effects expressed as percent change ([drug − control] ÷ control × 100). Thus, a conductance increase of 6.2% would result from a 1.43-ns change in conductance in a neuron with a control measure of 23.21 ns and drug-measure of 24.64 ns. Normalization reduced the variability within data sets to <15% (*e.g.*, range and conductance change produced by 1 MAC of halothane = 5.8–6.6%), well within the restriction limits for propagated errors of percentages. Data for EPSP, PS, and threshold were treated in the same manner.

Results

Resting Membrane Potential

Halothane at 1 MAC produced a significant ($P < 0.01$) mean hyperpolarization of −2.8 ± 1.3 mV (SD) in 11 neurons compared to control (fig. 1*B*). Enflurane produced a mean hyperpolarization of −3.15 ± 1.2 mV in 10 neurons ($P < 0.001$ compared to control; $P < 0.05$ compared to halothane by ANOVA). Isoflurane, on the other hand, produced variable effects: in the presence of this anesthetic, 4 of 7 neurons were hyperpolarized by a mean value of −1.35 ± 0.5 mV, and 3 neurons were depolarized by a mean value of 1.6 ± 0.7 mV. In pooled data, the effects of isoflurane on CA1 neuron resting potential were not significant at the $P < 0.05$ level (−0.09 ± 1.7 mV, n = 7 compared with control). Anesthetic effects on resting membrane potential are shown in figure 2.

Conductance

Anesthetic effects on resting membrane potential were not accompanied by consistent effects on membrane conductance (fig. 2). Halothane at 1 MAC produced a small mean conductance increase of 6.2 ± 2.7% (SD), whereas

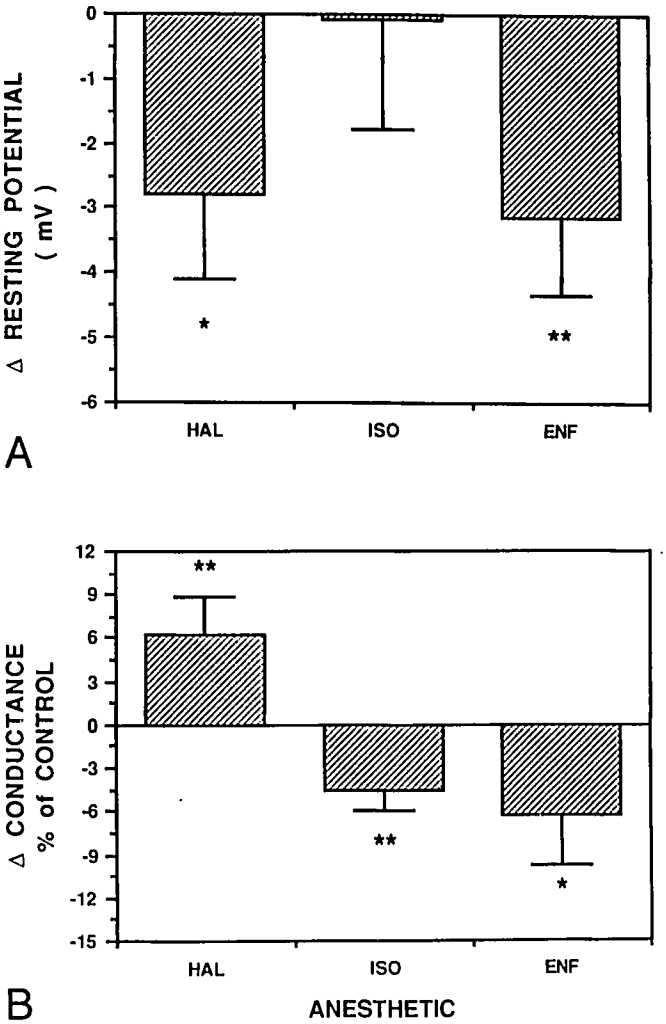

FIG. 2. Anesthetic effects on CA1 neuron (*A*) resting membrane potential (change in millivolts) and (*B*) conductance (percent change from control). Results are mean ± SD from 28 CA1 neurons exposed to the anesthetic agent at 1 MAC. *$P < 0.01$; **$P < 0.001$.

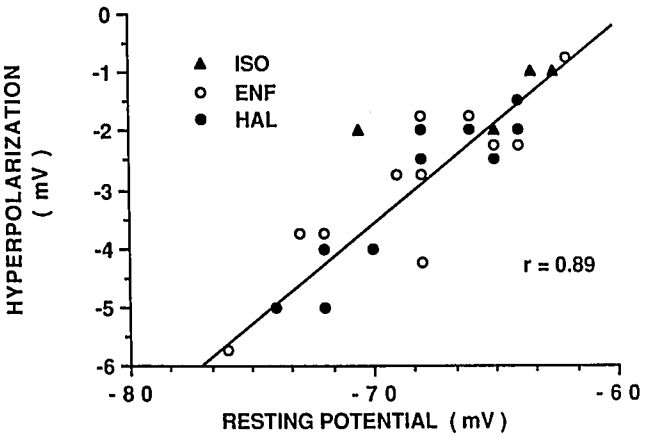

FIG. 3. The correlation between preanesthetic control resting membrane potential and the degree of hyperpolarization produced by 1 MAC of each anesthetic. Only the CA1 neurons that were hyperpolarized by isoflurane were included in the data set. Line-fit and correlation coefficient (r) are from a linear regression analysis. All cells were in control ACSF (4.5 mM K^+) and 0.0 nA of clamp current.

isoflurane and enflurane decreased conductance by −4.5 ± 1.5% and −6.2 ± 3.5%, respectively. The hyperpolarization produced by halothane was usually (8 of 11 neurons) accompanied by a conductance increase. Two neurons exposed to halothane did not exhibit a conductance change within the limits of experimental measurement error (±1.5%). One neuron exhibited a conductance decrease (−2.2%). In contrast, the hyperpolarization produced by enflurane always (10 of 10 neurons) occurred together with a conductance decrease. Isoflurane either did not alter conductance (2 of 7 neurons) or produced a conductance decrease, regardless of whether the membrane was hyperpolarized or depolarized by the anesthetic.

VOLTAGE-DEPENDENT EFFECTS

Anesthetic effects on resting membrane potential were dependent on the preanesthetic control resting potential. Higher (more negative) initial resting potentials were correlated with larger anesthetic-induced hyperpolarizations for all three agents at 1 MAC (r = 0.89) (fig. 3). In an experimental test of the relationship, membrane potential was manipulated either by changing extracellular potassium concentration, which affected all the cells in the slice, or by direct constant current injection through the microelectrode, which affected only the impaled cell.

Increasing extracellular potassium from 4.50 to 5.75 mM resulted in a depolarization of 9.8 ± 1.2 mV and reduced the hyperpolarization produced by 1 MAC of enflurane (fig. 4*A*). Depolarizing CA1 neurons 10 mV with injected current also abolished the hyperpolarization (−1.2 ± 2.5 mV [SD]; n = 4). The relatively small hyperpolarization produced by 1 MAC of isoflurane could be markedly increased by imposing a −10-mV hyperpolarization through the intracellular microelectrode (fig. 4*B*). In 4 of 5 neurons studied, hyperpolarizing current injection revealed a possible additional effect of isoflurane; a further hyperpolarization was observed after termination of the anesthetic exposure (fig. 4*B*).

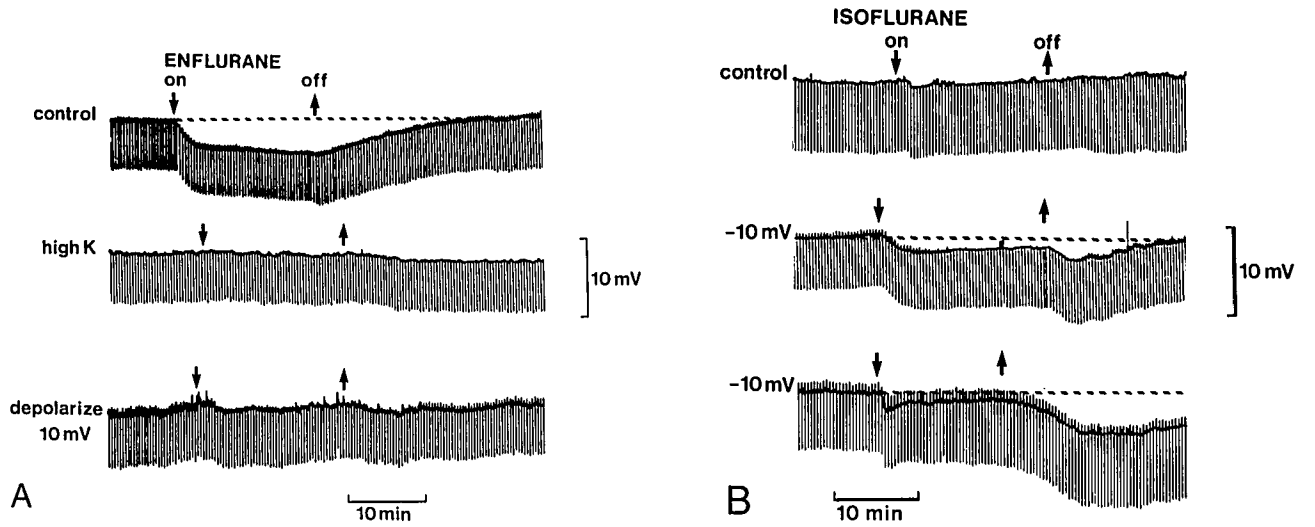

FIG. 4. (*A*) Enflurane-induced hyperpolarization was blocked by depolarization using either high K^+ levels (5.75 mM) in the ACSF, or by constant current injection to shift the resting potential +10 mV. (*B*) Isoflurane-induced hyperpolarization was increased by constant current injection to shift the resting potential −10 mV. Note the late hyperpolarizing response transiently observed after wash-out of isoflurane (4 of 5 neurons; data from two different CA1 neurons shown).

Effects on Evoked Synaptic Responses

A comparison of anesthetic effects on synaptically evoked field potentials is provided in figure 5. Halothane, isoflurane, and enflurane produced a marked (~100%) depression of evoked PS amplitudes in the presence of equianesthetic levels of each agent (1 MAC; see reference 18 for full concentration–response relationships). Depression of PS amplitudes was accompanied by depression of field EPSP amplitudes. As we have reported previously,[18,20,21] however, anesthetics differ with respect to the degree of EPSP depression associated with block of synaptically evoked PSs. Halothane was relatively ineffective at depressing EPSP amplitudes (<15% of control at 1 MAC), whereas the halogenated ethers produced more pronounced effects (isoflurane ~38% ≥ enflurane ~36%).

The anesthetics produced differential effects on the PS threshold, measured as the point of inflection on the rising phase of field EPSPs (fig. 5). Halothane increased the threshold such that greater (+14%) levels of synaptic input were required to produce PS discharge.[20,21] In contrast, both halogenated ethers decreased the PS threshold—enflurane by −42%, and isoflurane by −37%. We have previously reported that a third halogenated ether anesthetic, methoxyflurane, decreased threshold by ~22%.[18]

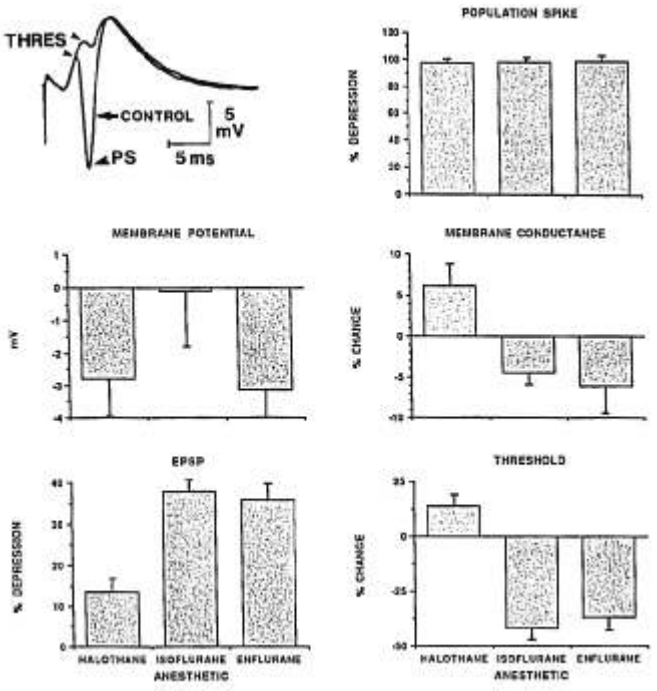

FIG. 5. All clinically used inhalation agents studied produced a marked depression of synaptically evoked population spike amplitudes (~100% at 1 MAC). Superimposed records show the effect of 1.2 vol% halothane on synaptically evoked field potential responses; note depression of population spike (PS) and increase in threshold (THRES; arrows). The bar graphs compare anesthetic actions on population spike output, resting membrane potential, membrane conductance, excitatory postsynaptic potential (EPSP), and threshold. The different anesthetics produce a block of population spike output accompanied by unique combinations of effects at synaptic and postsynaptic sites of action. Error bars show the standard deviation from the mean for n of at least five preparations for each response.

Discussion

The results do not support the hypothesis that all general anesthetics reduce output from central neurons by a common mechanism involving hyperpolarization. Even structurally similar volatile agents (such as isoflurane and enflurane, which are isomers) exerted clearly different effects on resting membrane potential and conductance. Only halothane fits the hypothesis that anesthetics may hyperpolarize neurons by increasing conductance through a potassium or chloride channel. It should be noted that even with halothane, effects on resting potential were small. Neither isoflurane nor enflurane increased conductance. Although enflurane consistently hyperpolarized CA1 neurons, this was accompanied by a decrease in conductance. An economical explanation for these different actions may involve effects on distinct ion channels for each anesthetic.

Effects on resting potential but not conductance were potential-dependent, suggesting that the current–voltage relationship of anesthetic actions on channels has a significant slope near the normal resting potential. Beyond this, the methodology of intracellular voltage recording does not permit speculation about mechanism. The general anesthetics used in the current study have been reported variously to hyperpolarize hippocampal CA1 neurons,[8] to exert no effect,[15,16] or, at high concentrations, to depolarize.[15] Isoflurane in particular also has been reported to produce inconsistent effects on resting membrane potential in normal untreated hippocampal slices, but to hyperpolarize neurons in slices treated with tetrodotoxin (TTX).[22]

Inasmuch as control membrane potentials in isolated preparations may be expected to vary among laboratories, the potential dependence of anesthetic effects on resting potential may account for some of the variability in previously reported results. Additional variability can be attributed to possible errors in anesthetic concentrations; this is the first study to actually measure applied concentrations of the volatile agents. Potential dependence may account also for isoflurane-induced hyperpolarization in the presence of TTX,[8,22] since TTX-treated cells may be expected to be somewhat hyperpolarized. Measurements of CA1 neuron resting potentials *in vivo* suggest that these cells are normally more depolarized than they are in the *in vitro* preparation. Thus, anesthetic-induced changes in resting potential are not likely to contribute greatly to neuronal depression associated with anesthesia *in vivo*.

At the equianesthetic levels (1 MAC) used for comparison, all of the agents depressed neuronal output, as seen in the near complete abolition of the PS (fig. 5). This effect was not correlated with changes in resting potential or conductance. The same is true of the relationship between depression of the synaptic potential and ability to generate impulses: they are not correlated across agents. The results are consistent with a multisite agent-specific mechanism of anesthesia, in which each agent acts by a unique spectrum of effects, including depression of excitatory synaptic transmission, depression of neuronal excitability, and enhanced inhibitory transmission.[1,20,23-26]

The authors thank Dr. J. Trudell, Dr. I. Mody, and Dr. D. V. Madison for helpful comments on the manuscript.

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