Anesthesiology
1996; 85:889–900

Volatile General Anesthetics Produce Hyperpolarization of Aplysia *Neurons by Activation of a Discrete Population of Baseline Potassium Channels*

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Background: **The mechanism by which volatile anesthetics act on neuronal tissue to produce reversible depression is unknown. Previous studies have identified a potassium current in invertebrate neurons that is activated by volatile anesthetics. The molecular components generating this current are characterized here in greater detail.**

Methods: **The cellular and biophysical effects of halothane and isoflurane on neurons of *Aplysia californica* were studied. Isolated abdominal ganglia were perfused with anesthetic-containing solutions while membrane voltage changes were recorded. These effects were also studied at the single-channel level by patch clamping cultured neurons from the abdominal and pleural ganglia.**

Results: **Clinically relevant concentrations of halothane and isoflurane produced a slow hyperpolarization in abdominal ganglion neurons that was sufficient to block spontaneous spike firings. Single-channel studies revealed specific activation by volatile anesthetics of a previously described potassium channel. In pleural sensory neurons, halothane and isoflurane increased the open probability of the outwardly rectifying serotonin-sensitive channel (S channel). Halothane also inhibited a smaller noninactivating channel with a linear slope conductance of ~40 pS. S channels were activated by halothane with a median effective concentration of approximately 500 μM (0.013 atm), which increased channel activity about four times. The mechanism of channel activation involved shortening the closed-time durations between bursts and apparent recruitment of previously silent channels.**

Conclusions: **The results demonstrate a unique ability of halothane and isoflurane to activate a specific class of potassium channels. Because potassium channels are important regulators of neuronal excitability within the mammalian central nervous system, background channels such as the S channel may be responsible in part for mediating the action of volatile anesthetics. (Key words: Anesthetic mechanism, *Aplysia californica*, ion channel, potassium channel, volatile anesthetics, halothane, isoflurane.)**

VOLATILE general anesthetics are one of the most widely used groups of drugs. Their ability to produce a safe, reversible state of unconsciousness, concurrent amnesia, and analgesia makes them a remarkable class of agent. Although substantial progress has been made in understanding the molecular properties of anesthetic agents and their isomeric variants,[1] the mechanism by which they induce anesthesia remains obscure. The lipid bilayer alone appears to be an unlikely target site, given that clinical concentrations of volatile anesthetics produce relatively insignificant effects on lipid properties, such as membrane fluidity.[2,3] Recently, evidence has accumulated that volatile anesthetics act on ion channels. For example, several types of voltage-gated potassium (K^+) channels, as well as N-type calcium (Ca^{2+}) channels, may be inhibited by volatile anesthetics at clinical concentrations.[4,5] Volatile anesthetics also potentiate responses of the γ-amino butyric acid ($GABA_A$) receptor channel complex.[6–8] Yet although volatile anesthetic effects can be shown on several voltage-gated and ligand-gated ion channels, no comprehensive mechanism has been elucidated that can explain the anesthetic state.

Remarkably, sensitivity to volatile anesthetics has been highly conserved throughout evolution. A span

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Received from the Departments of Anesthesia and Physiology, School of Medicine, University of California, San Francisco, California. Supported by a Zeneca Pharmaceuticals FAER Young Investigator Award (C. Spencer Yost) and National Institute of Health research grants GM51372 (C. Spencer Yost) and DA08373 (John R. Forsayeth). Received for publication February 22, 1996. Accepted for publication June 17, 1996.

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of about 300 million years separates *Drosophila* from humans, yet both are about equally sensitive to the effects of volatile anesthetics.[9,10] The broadly consistent actions of volatile anesthetics have enabled investigators to study their actions in a variety of experimental models. Nicoll and Madison[11] reported that anesthetics hyperpolarize both frog motorneurons and rat hippocampal neurons at concentrations in the clinical range, possibly by an increase in K^+ conductance. Reports of the hyperpolarizing actions of anesthetics in mammalian neurons[6,12,13] and the finding that low concentrations of anesthetics hyperpolarize and inhibit spontaneous activity of neurons in the pond snail *Lymnaea stagnalis*[14] suggest that one target of volatile anesthetics may be a conserved class of K^+ channels.

We have studied volatile anesthetic actions on spike firings and on single noninactivating ion channels in the nervous system of the marine mollusk, *Aplysia californica*, in which sensory processing and simple forms of learning have been extensively investigated at the molecular level.[15] These studies revealed a direct effect of the volatile agents to activate the serotonin-sensitive K^+ channel (S channel), thereby contributing to a reversible inhibition of neuronal activity.

Materials and Methods

Intracellular Recordings

Adult *A. californica* (weighing 200 to 500 g) were obtained from Marinus, Inc. (Long Beach, CA). Abdominal ganglia were excised and pinned in a recording chamber filled with artificial sea water (ASW) containing 1 g/l glucose and 10 mM HEPES at pH 7.6. The feeding artery was cannulated and continuously perfused at a rate of 5 μl/min with ASW.[16] The stainless steel cannula ran from a Rheodyne high performance liquid chromatography sample injector valve (Rheodyne, Berkeley, CA) connected to an infusion pump. Injection of experimental solutions into the sample port prevented diffusion of volatile anesthetics into the air.

Intracellular recordings were made from as many as four neurons at a time with glass microelectrodes filled with 2 M potassium citrate and having resistances of 10 to 30 MΩ. Saturated stock solutions of halothane and isoflurane (Ohmeda, Murray Hill, NJ) were dissolved in ASW and stored in gas-tight bottles. The anesthetic concentrations of experimental solutions were measured using gas chromatography. Anesthetics were delivered to the HPLC injector with gas-tight syringes. Data were recorded with a chart recorder (Astro-Med MT8800, Astro-Med, West Warwick, RI) and also digitally recorded (Vetter 3000A, A. R. Vetter, Rebersburg, PA) for later analysis.

Patch-Clamp Methods

Isolation and Culture of Neurons. Abdominal ganglion neurons or sensory neurons of the pleural ganglion ventrocaudal cluster were separately dissociated and cultured according to a procedure that was modified from Schacher and Proshansky.[17] The ganglia were dissected from adult *A. californica* (weighing 200 to 300 g) and suspended for 4 hr at room temperature in Leibovitz's L-15 medium (Gibco BRL, Grand Island, NY) with ASW (L-15/ASW) containing 1% dispase, grade II (Boehringer Mannheim, Indianapolis, IN). The tissue was rinsed with L-15/ASW medium containing 1% horse serum, and individual neurons from the abdominal ganglion or the isolated ventrocaudal cluster[18] were dissociated by drawing the tissue through the fire-polished tip of a Pasteur pipette. Isolated neurons were plated into uncoated 35-mm plastic Petri dishes and maintained at 18°C in L-15/ASW media (Gibco BRL) with 50 μg/ml gentamicin. Neurite outgrowth was observed within 24 to 48 hours of plating, and the cells appeared healthy for as long as a week.

Electrophysiologic Methods. Single-channel activity was recorded from cell-attached patches or excised, inside-out patches according to the technique described by Hamill and colleagues.[19] Patch electrodes were pulled from borosilicate capillary tubes (Dynalab, Rochester, NY), the shanks were coated with Sylgard (Dow Corning, Midland, MI), and the tips were heat polished. Currents were measured with a List EPC-7 amplifier (List Electronic, Darmstadt, Germany) and digitally recorded on videotape at a sample rate of 44.1 kHz. The current records were analyzed with an LSI 11/73 computer (Indec Systems, Capitola, CA) after being filtered with an eight-pole Bessel filter (−3 dB at 2 kHz, Frequency Devices, Haverhill, MA) and digitized at 5 kHz. All experiments were performed at room temperature (21 to 23°C).

Solutions. The patch electrode filling solution contained 460 mM NaCl, 55 mM $MgCl_2$, 11 mM $CaCl_2$, 10 mM KCl, 10 mM HEPES. The pH was adjusted to 7.6 with NaOH. In some experiments the recording pipette solution also contained 10 mM tetraethylammo-

nium (TEA), which blocks Ca^{2+}-activated K^+ channels ($K_{[Ca]}$ channels) at an extracellular site with a Kd of ~0.3 mM.[20] The bathing solution contained 360 mM potassium methanesulfonate, 277 mM sucrose (to adjust osmolarity), 10 mM HEPES, 10 mM TEA chloride (TEA-Cl), 5 mM $MgCl_2$, 1.5 mM EGTA, and 1 mM $CaCl_2$. The pH was adjusted to 7.6 with methanesulfonic acid. The free [Ca^{2+}] was estimated to be ~80 nM.[21] The isotonic K^+ bathing solution was used to zero the cell's resting potential so that the patch potential was the same as the voltage applied to the electrode. The voltage error introduced by this procedure was generally less than 5 mV. An error as large as 10 mV was measured in a few experiments as a shift in the single-channel *i*-V relation after patch excision. The contribution of this source of error was minimized by rejecting experiments in which there was a change in the single-channel current after excising the patch at the end of an experiment. Before seal formation, the voltage offset between the patch electrode and the bath solution was adjusted to produce zero current. Seal resistances ranged from 20 to 40 GΩ.

Delivery of Volatile Anesthetics. Saturated stock solutions of halothane or isoflurane were created in gas-tight bottles by dissolving excess volatile anesthetic into the bath solution. The stock solutions were diluted to the final experimental concentrations with a calibrated gas-tight syringe (Hamilton, Reno, NV) and stored in gas-tight bottles. Experimental solutions were perfused onto the cell or excised patch from a narrow Teflon pipette that was placed in the bath. All solutions were delivered at a rate of 25 μl/min from a Rheodyne HPLC injector that was connected to an infusion pump. Perfusion with the bathing solution alone had no effect on the mean open probability. The volatile anesthetic concentrations in both the stock solutions and the experimental solutions were measured using gas chromatography. Partial pressures were converted to aqueous concentrations using an ASW-gas partition coefficient (37°C) of 0.76.

Analysis of Single-Channel Properties. Multiple channel recordings were obtained from most of the patches, whereas single-channel activity was only observed in a few experiments. To estimate the number of channels, we used the "MAX" estimator method,[22] which consisted of a visual review of each entire experiment (~8 min of data or more per experiment) to determine the maximum number of simultaneously open channels. Although the MAX estimator can underestimate channel number, it works well for patches with four or fewer channels even when the open probability is small.[22] The single-channel current amplitudes were measured by fitting cursors to the closed-channel level and to the average level of open-channel noise. In some experiments with multiple active channels, amplitude histograms were constructed from several minutes of data to verify the channel current amplitudes and the number of channels. When TEA was present in the recording pipette, there were usually two or three active channels in each patch, and in most cases they exhibited the same *i*-V relation. Patches with a heterogeneous population of channels were excluded from analysis.

Measurements of mean open probability (NP_o) for anesthetic-sensitive channels were made using custom software that sets the open-channel level to unity and integrates the data records while correcting for small shifts in the closed-channel level from sweep to sweep. Because S channels can spontaneously enter a high-activity mode,[23] we averaged the Np_o for at least a 2-min period immediately after drug delivery. We tested for the homogeneity of single-channel current amplitudes by constructing amplitude histograms from several minutes of data or more and looking for amplitude peaks that were not integer multiples of the lowest-amplitude open-channel peak.

To analyze the kinetics of single-channel current fluctuations, we used software designed to detect channel transitions in multiple channel recordings[24] and other software to fit exponential components by a maximum-likelihood fitting routine.[25] Open- and closed-state transitions were measured using the half-threshold detection method.[26] We restricted our analysis of channel kinetics to experiments with no more than two simultaneously open channels that both exhibited the same *i*-V relation. Simultaneous openings were discarded from analysis. Because the presence of an additional channel in the patch will shorten the closed-time measurements, we simulated single-channel recordings with two channels using a wide range of rate constants.[25] The closed times obtained from analysis of the simulated records were shorted by as much as one half the duration of their known value. Thus our closed-time measurements could be inaccurate by as much as a factor of two. We report the kinetic analysis results as apparent rates without applying any of the different correction procedures because our conclusions do not depend heavily on the frequency response of our re-

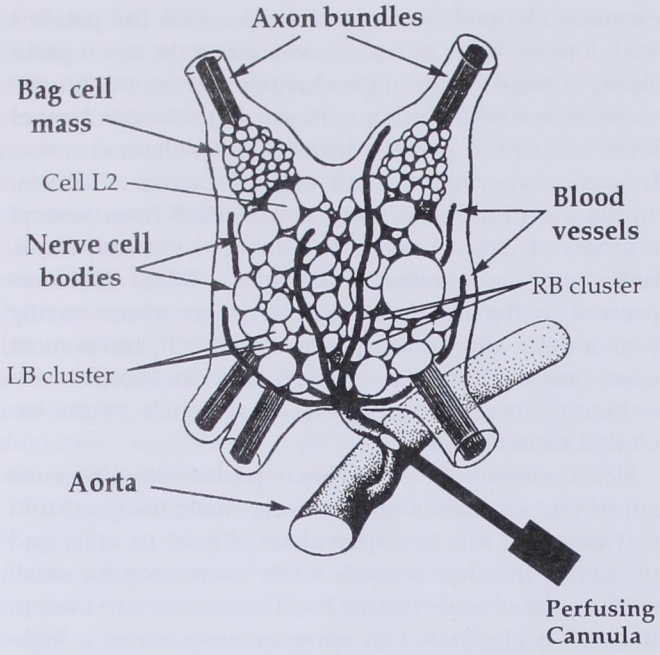

Fig. 1. Abdominal ganglion of *Aplysia californica*.

cording system. Although the undetected brief openings and closures will affect the durations of slower kinetic components,[26] most of the fast transitions described by τ_{o1} and τ_{c1} were detected. The experimentally measured dead time of the recording system was ~90 μs.[26] The estimated number of missed events was based on the algorithm

$$1 - \exp(-D/\mu m) \quad (1)$$

where D is the dead time of the recording system and μ is the mean open time or closed time obtained from the exponential fit.[27] Although we could not measure the true durations of events briefer than D, we obtained accurate measurements of the mean durations of the rapidly declining exponential distributions by not including the first bin (0 to 0.2 ms) in the fitting procedure. This method required a sufficient number of events with durations longer than D to define the curve. The briefest exponential component that we fit by this method had a time constant of 0.2 ms, for which we estimated that ~36% of the events were missed.

Results

Membrane Potential Responses to Anesthetics

We first studied the membrane potential responses to anesthetics in the nerve cell bodies of the excised abdominal ganglion from *A. californica*, a preparation that was used previously to study the neuromodulatory effects of neuropeptides.[16] As shown in figure 1, the abdominal ganglion of *Aplysia* is composed of about 1,000 neurons. It is perfused by a vessel that branches from the animal's central aorta. The neuronal cell bodies are large and invested with a connective tissue sheath. A specialized mass of neurons, called the bag cell mass, occupies a cephalad position within the ganglion and has important neuroendocrine functions. Axonal connections are made to other ganglia and to peripheral sites through major bundles exiting the ganglion.

This preparation permits continuous infusion and drug delivery through the cannulated feeding artery. Spontaneous neuronal firing was monitored simultaneously in four different neurons. Halothane ($CF_3CHClBr$) potently and reversibly hyperpolarized abdominal ganglion neurons and depressed action potential firing rates. Halothane was active at concentrations within or less than the minimum alveolar concentration that produces anesthesia in mammals.

Figure 2A shows the effects of halothane on two different cells, and their membrane potential responses were recorded simultaneously. These cells, which both displayed spontaneous action potentials, were identified as either from within the LB cluster (top) or as an L2 cell (bottom). Arterial perfusion with 400 μM halothane (0.011 atm) halted spike firings in both cells and produced a slow hyperpolarization of as much as −11 mV that lasted several minutes after halothane was cleared from the perfusion system. Perfusion with 400 μM isoflurane ($CF_3CHClOCHF_2$) produced a comparable level of hyperpolarization and also inhibited spike firing in a similar manner (data not shown). Perfusion with anesthetic-free ASW did not affect neuronal activity.

The spontaneous firing of cells in three well-characterized clusters within the ganglion[28] showed differing sensitivity to inhibition by halothane. During several experiments, perfusion of 400 to 500 μM halothane (0.011 to 0.013 atm) inhibited 11 of 12 LB and LC neurons of the left lower quadrant, 5 of 7 L2-L6 neurons of the left upper quadrant, but only 1 of 5 RB neurons. The membrane hyperpolarization of an L2 neuron depended on the concentration of halothane (fig. 2B), with a half-maximal effective concentration (EC_{50}) of 133 μM (0.003 atm). Although all the neurons we studied were inhibited by halothane, much higher concen-

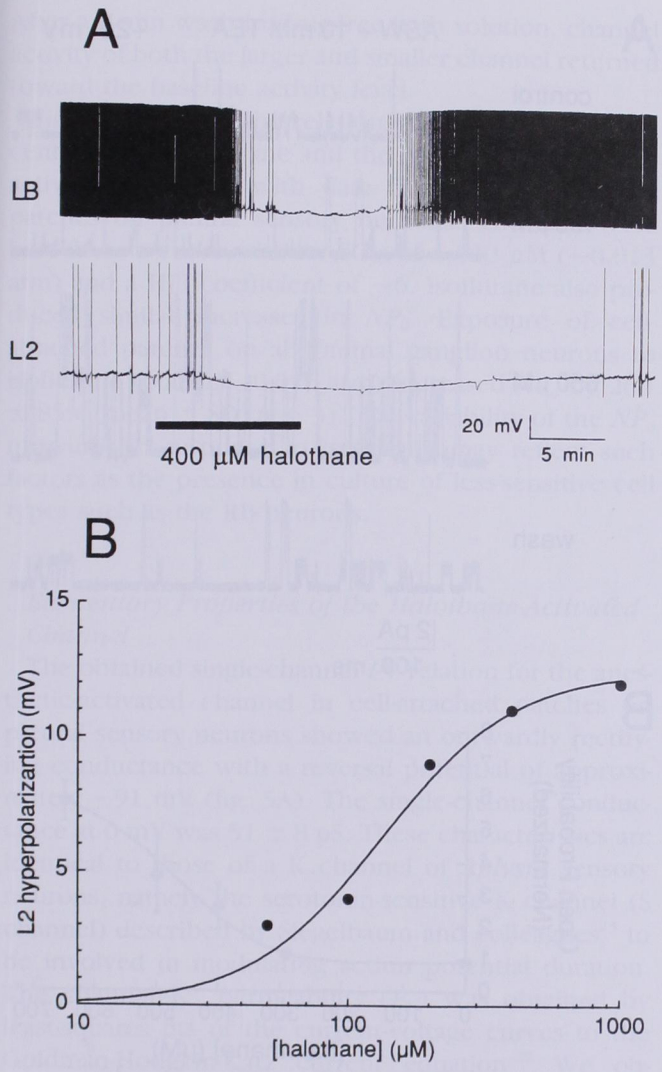

Fig. 2. Halothane inhibits spike firings and hyperpolarizes neurons. (*A*) Simultaneous intracellular recordings from intact abdominal ganglion neurons: L2 and an unidentified LB cluster neuron. Application of 400 μM (0.013 atm) halothane (bar across bottom) inhibited spike firings and slowly hyperpolarized both neurons for many minutes. The delayed response is due to a 3-min dead time in the perfusion system, as measured with the dye fast green. The effects of halothane on the membrane potential were smoothly graded, with no apparent spontaneously occurring synaptic inputs that could account for the hyperpolarization. (*B*) Dependence of membrane hyperpolarization on the concentration of halothane. All points are from a single experiment with neuron L2. The resting potential was approximately −40 mV. The smooth curve has the form

$$V_m = V_{m(max)}/[1 + (K/[An])^N] \quad (2)$$

where V_m is the membrane potential and N is the Hill coefficient of 1.75. The fit was obtained with K = 133 μM.

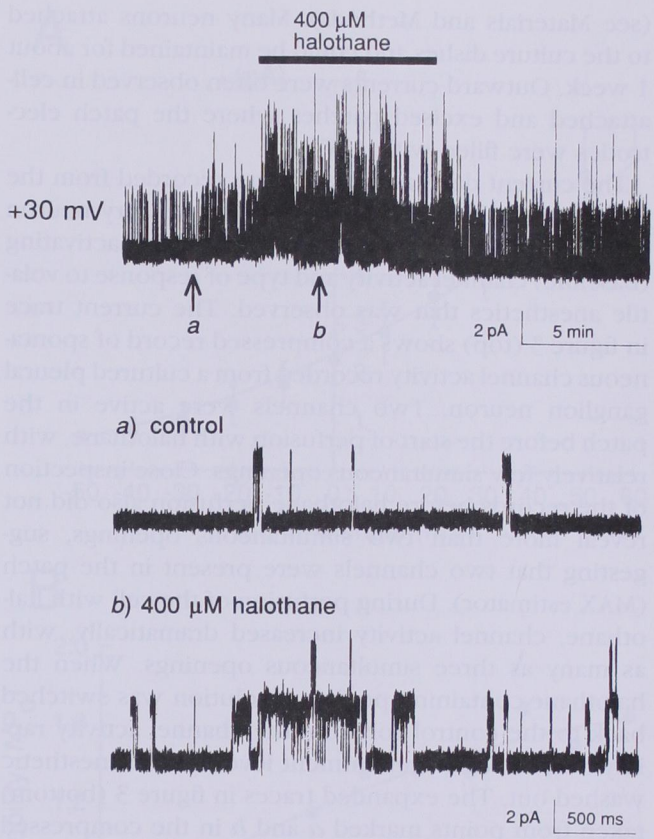

Fig. 3. Effect of halothane on single K^+ channel activity. Unitary outward currents were recorded with ASW and 10 mM tetraethylammonium in the recording pipette at a holding potential of +30 mV. The top trace is a continuous segment of data recorded during a 20-min period from a cell-attached patch on a pleural sensory neuron. Bar indicates a 7-min period when the cell was perfused with bath solution containing 400 μM halothane. Traces (*a*) and (*b*) were taken from the indicated points on the upper trace, showing single-channel activity on an expanded time scale before (*a*) and during (*b*) perfusion of the cell with halothane-containing solution.

trations (greater than 1 mM) were required to inhibit cells in the RB cluster. Based on a constant EC_{50} in the other neurons studied, we discounted the effect of anesthetics on the RB cluster because the concentration of halothane required was outside the clinically relevant range.

A Serotonin-Sensitive Baseline Channel Activated by Halothane

To identify the ionic nature of the currents responsible for the hyperpolarization induced by halothane, we isolated neurons from abdominal and pleural ganglia

(see Materials and Methods). Many neurons attached to the culture dishes and could be maintained for about 1 week. Outward currents were often observed in cell-attached and excised patches where the patch electrodes were filled with ASW.

The current data in figure 3 were recorded from the surface of an isolated *Aplysia* pleural sensory neuron and are representative of the pattern of noninactivating (baseline) channel activity and type of response to volatile anesthetics that was observed. The current trace in figure 3 (top) shows a compressed record of spontaneous channel activity recorded from a cultured pleural ganglion neuron. Two channels were active in the patch before the start of perfusion with halothane, with relatively few simultaneous openings. Close inspection of the records before halothane perfusion also did not reveal more than two simultaneous openings, suggesting that two channels were present in the patch (MAX estimator). During perfusion of the cell with halothane, channel activity increased dramatically, with as many as three simultaneous openings. When the halothane-containing perfusion solution was switched back to the control solution, the channel activity rapidly decreased to pretreatment levels as the anesthetic washed out. The expanded traces in figure 3 (bottom) taken from points marked *a* and *b* in the compressed record indicate that the single-channel currents in the presence of halothane had the same current amplitude as found in the baseline condition (i = ~3.4 pA). Control experiments in which solutions containing no anesthetic were perfused onto patched neurons consistently found no change in activity of these outward currents (n = 8).

Concentration Dependence of Halothane Action

Figure 4A shows that gating of the halothane-activated channel depends on the concentration of halothane. Besides the halothane-activated channel, this cell-attached patch on a pleural sensory neuron also included a smaller conductance channel that opened relatively more frequently in the control condition (fig. 4A, control). The activity of the larger-conductance channel increased with the perfusion of 400 μM halothane and increased even more so with perfusion of 650 μM halothane, with the simultaneous opening of two channels becoming apparent. Opposite effects were seen on the smaller-conductance channel whose activity decreased in the presence of 400 μM halothane and was completely inhibited by 650 μM halothane.

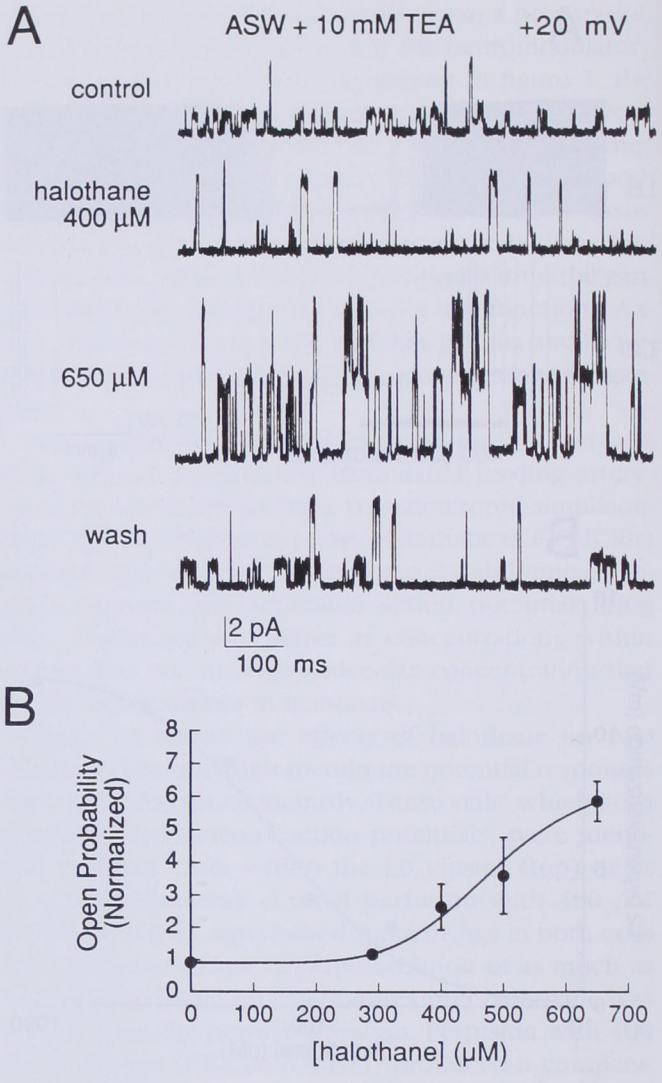

Fig. 4. Concentration-dependent effect of halothane on single-channel currents. (*A*) Representative records before and during perfusion of a cell-attached patch on a pleural sensory neuron with the indicated concentrations of halothane. The recording pipette contained ASW and 10 mM tetraethylammonium. V_h was +20 mV. Large-amplitude openings became more frequent in the presence of halothane, whereas small-amplitude openings were rare. Both types of channel activity returned toward control levels after wash. (*B*) Concentration-dependent actions of halothane are shown. Mean open probability (NP_o) measures were obtained from experiments in which halothane was delivered to cell-attached patches on pleural sensory neurons. The recording pipettes contained ASW and 10 mM tetraethylammonium. V_h was +20 mV. NP_o increased in proportion to the concentration of halothane. The smooth curve was drawn with equation 1 and was best fit with a Hill coefficient (N) of ~6 and K = ~500 μM. Error bars represent SEM. Mean open probability values were normalized to the average mean open probability for 2 or 3 min of continuous data immediately before halothane perfusion.

After a 2-min wash in drug-free bath solution, channel activity of both the larger and smaller channel returned toward the baseline activity level.

Figure 4B shows the relationship between the concentration of halothane and the NP_o of the halothane-activated channel, with data from 25 cell-attached patches on pleural sensory neurons. The data were best fit to a curve with an EC_{50} of ~500 μM (~0.013 atm) and a Hill coefficient of ~6. Isoflurane also produced similar increases in NP_o. Exposure of cell-attached patches on abdominal ganglion neurons to isoflurane (600 μM, 0.016 atm) increased NP_o by 260 ± 85% (mean ± SD, n = 3). The variability of the NP_o measures in response to isoflurane may reflect such factors as the presence in culture of less-sensitive cell types such as the RB neurons.

Elementary Properties of the Halothane-Activated Channel

The obtained single-channel *i*-V relation for the anesthetic-activated channel in cell-attached patches on pleural sensory neurons showed an outwardly rectifying conductance with a reversal potential of approximately −91 mV (fig. 5A). The single-channel conductance at 0 mV was 51 ± 8 pS. These characteristics are identical to those of a K channel of *Aplysia* sensory neurons, namely the serotonin-sensitive K channel (S channel) described by Siegelbaum and colleagues[23] to be involved in modulating action potential duration. The channel K^+ permeability (P_K) was obtained by least-squares fits of the current-voltage curves to the Goldman-Hodgkin-Katz current equation.[29] We obtained a value for P_K of 0.8×10^{-13} cm^3/s, which was close to the P_K value reported for the S channel of 1×10^{-13} cm^3/s.[20] The selectivity ratio of Na^+ to K^+ (P_{Na}/P_K) for the anesthetic-activated channel was ~0.02, indicating a high degree of K^+ selectivity.

The S channels we observed showed little or no dependence of the NP_o on the patch potential. In many experiments, halothane-activated channels exhibited about the same NP_o value across a range of patch potentials. Figure 5B illustrates three independent experiments in which halothane-activated channels showed low voltage dependence. The steepness of the voltage-dependent gating was within the range measured for the S channel.[20] The data for each experiment were best fit to a Boltzmann relation. The mean steepness was ~28 ± 7 mV (n = 3).

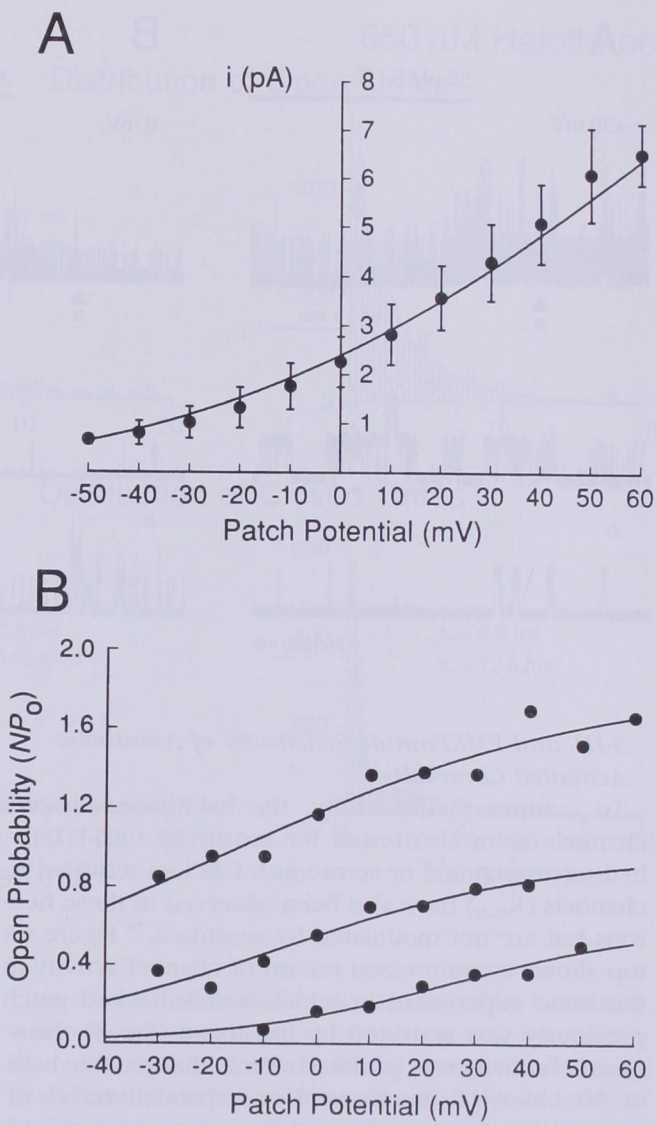

Fig. 5. Elementary properties of anesthetic-sensitive channels. (*A*) Single-channel amplitudes as a function of V_h. Single S-channel amplitudes with artificial seawater and 10 mM tetraethylammonium in the recording pipette (circles, n = 20). Single-channel amplitudes were unchanged in the presence of as much as 650 μM halothane. Error bars are standard deviations. (*B*) Dependence of the mean open probability (NP_o) of anesthetic-sensitive S channels on the patch potential. Three representative experiments are shown to indicate the range of variation in the voltage dependence and relative channel activity. The smooth curves drawn through the experimental points represent the best fit to a Boltzmann equation of the form

$$NP_{o(max)}/[1 + (\exp(V - V_{1/2})/k)] \quad (3)$$

The curve steepness (k) ranged from 20 to 40 mV.

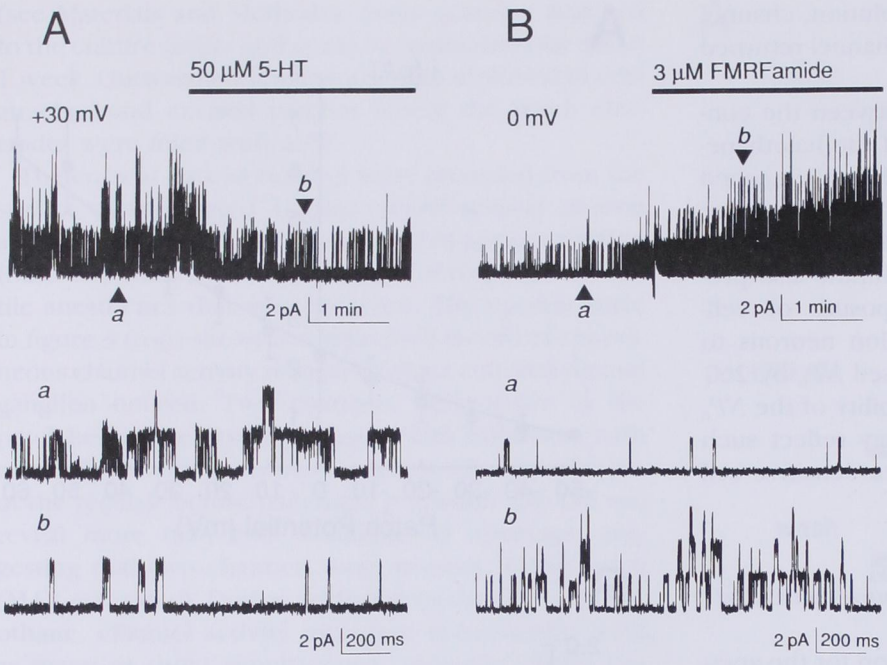

Fig. 6. Modulation of the halothane-activated channel is shown. (*A*) Five minutes of data recorded from the same cell-attached patch as in figure 2. Ten minutes elapsed from the time halothane perfusion was discontinued. Bar over the top tracing indicates a 3-min period when the cell was exposed to 50 μM 5-HT. Traces *a* and *b* shown below were taken from the indicated points on the upper trace, showing single-channel activity on an expanded time scale before (*a*) and during (*b*) perfusion of the cell with 5-HT. Vh = +30 mV. (*B*) Recordings of single-channel activity in a cell-attached patch on a pleural sensory neuron. Channel activity in this patch was previously found to be activated by halothane. The top trace shows channel activity before and after application of FRMFamide. Traces *a* and *b* show channel activity at indicated points of the upper trace. Vh = 0 mV.

5-HT and FMRFamide Sensitivity of Anesthetic-Activated Channels

To confirm their identity, the halothane-activated channels were also tested for sensitivity to 5-HT (5-hydroxytryptamine or serotonin). Calcium-activated K channels ($K_{[Ca]}$) have also been observed in these neurons but are not modulated by serotonin.[20] Figure 6A top shows a compressed record of channel activity in the same experiment in which a cell-attached patch previously was activated by halothane (fig. 3). Now channel activity was inhibited after addition to the bath of 50 μM 5-HT. Representative expanded traces of channel activity marked *a* and *b* are shown below and reveal no apparent differences in the single-channel current amplitudes. In addition, the presence of 10 mM TEA in the recording pipette, a concentration that blocks $K_{(Ca)}$ currents[30] but not S channels[20] corroborates that the anesthetic-activated channel is the S channel.

Further evidence that the anesthetic-activated channel is the S channel was obtained in experiments in which pleural sensory neurons were perfused with the neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2), an endogenous activator of the S channel.[31–33] Figure 6B shows representative records of channel activity in a cell-attached patch on a pleural sensory neuron before and during exposure of the cell to 3 μM FMRFamide. The cell was previously exposed to 650 μM halothane, which increased channel activity about four times. The control trace in figure 6B shows the pattern of channel activity after a 12-min period to allow the channel to return to baseline after halothane was removed from the bath. The patch appeared to contain two S channels and a single 40 pS baseline channel, like the halothane-inhibited channel shown in figure 4A. After addition to the bath of 3 μM FMRFamide, additional S channels appeared in the recordings (fig. 6B, *b*).

Halothane Markedly Decreases Mean S-Channel Closed Times

To determine how volatile anesthetics affected the kinetics of S-channel activity, open- and closed-time histograms were constructed from records of channel activity before and during application of halothane. Figure 7 shows histograms from an experiment in which two S channels were active in a cell-attached patch. A high concentration of halothane (650 μM) was used to exaggerate the effects and identify changes in channel kinetics. The distribution of open times (fig. 7, upper panels), both in the presence and absence of anesthetic, were best fit with two exponential components. In this experiment, both the fast and slow exponential components of the open-time distributions decreased slightly in the presence of anesthetic. However, the

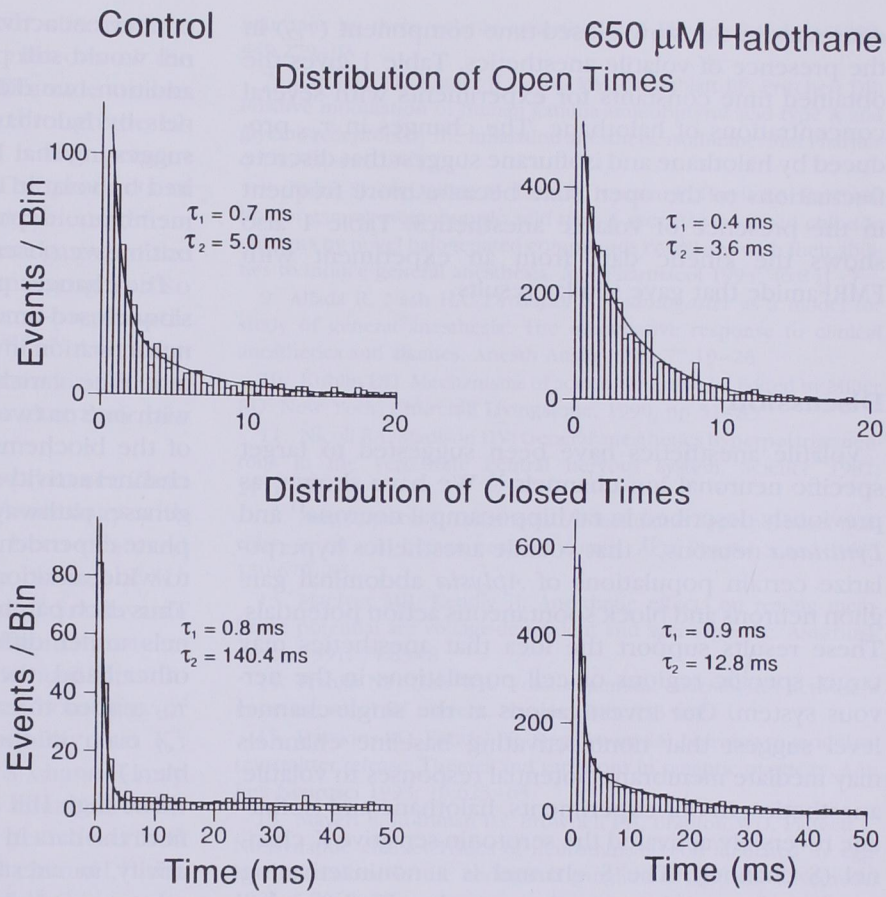

Fig. 7. Halothane primarily reduces the slow exponential component of S-channel closed times. Frequency histograms of open times (upper panels) and closed times (lower panels) in a cell-attached patch on a pleural neuron. The recording pipette contained artificial seawater and 10 mM tetraethylammonium. V_h was +20 mV. Histograms show open- or closed-time durations either before (*left*) and during (*right*) perfusion of the cell with 650 μM (0.017 atm) halothane. The smooth curve drawn through each histogram represents the maximum likelihood exponential fit to the data. The open and closed times were best fit by two exponential components with the indicated time constants.

more significant change in channel kinetics and the one that more likely explains the change in open probability appeared in the closed times. The lower panels show the closed-time distributions from the same experiment. The fast exponential component of the closed-time distribution was unchanged by halothane, whereas the slow component shortened dramatically from 140.4 ms to 12.8 ms in the presence of 650 μM (0.017 atm) halothane. These data are representative of kinetic analyses of several experiments that all showed

Table 1. Effect of Volatile Anesthetics on Single S-K Channel Kinetics

Drug (μM)		Channels per Patch	τ_o1 (ms)		τ_o2 (ms)		τ_c1 (ms)		τ_c2 (ms)	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post
Halothane	400	2	0.3	0.2	3.8	2.0	0.6	0.7	43.6	19.7
Halothane	400	2	0.2	0.3	7.3	9.9	0.7	0.5	176.3	139.5
Halothane	500	1	0.3	0.3	4.4	4.4	0.5	0.6	204.2	52.7
Halothane	650	2	0.4	0.4	4.8	4.0	0.5	0.5	186.8	16.5
Isoflurane	1000	2	0.7	0.8	3.9	3.8	0.7	0.7	117.4	55.4
FMRFa	3	4	0.3	0.5	6.1	6.2	0.5	0.5	274.2	22.4

Data from cell-attached patches on pleural sensory neurons held at a test potential of +20 mV. The number of active channels was determined for each experiment by the MAX estimator method.[22]

decreases in the slow closed-time component (τ_{c2}) in the presence of volatile anesthetics. Table 1 gives the obtained time constants for experiments with several concentrations of halothane. The changes in τ_{c2} produced by halothane and isoflurane suggest that discrete fluctuations to the open state became more frequent in the presence of volatile anesthetics. Table 1 also shows the kinetic data from an experiment with FMRFamide that gave similar results.

Discussion

Volatile anesthetics have been suggested to target specific neuronal ion channels.[34] We have shown, as previously described in rat hippocampal neurons[11] and *Lymnaea* neurons,[35] that volatile anesthetics hyperpolarize certain populations of *Aplysia* abdominal ganglion neurons and block spontaneous action potentials. These results support the idea that anesthetics may target specific regions or cell populations in the nervous system. Our investigations at the single-channel level suggest that noninactivating baseline channels may mediate membrane potential responses to volatile anesthetics. In our experiments, halothane and isoflurane reversibly activated the serotonin-sensitive K channel (S channel). The S channel is a noninactivating baseline channel that contributes to the membrane potential. S-channel activity can hyperpolarize neurons, leading to inhibition of spike firing and presynaptic inhibition of transmitter release.[31,36] The anesthetic-activated channel exhibited all the major properties reported for the S channel,[20,23] including inhibition by 5-HT (fig. 6A), outward rectification (fig. 5A), noninactivation, weak voltage dependence (fig. 5B), high selectivity for K^+ over Na^+, TEA-insensitivity, and activation by FMRFamide (fig. 6B). We conclude that the halothane-activated channel is the S channel.

Does the S channel mediate the hyperpolarizing actions of volatile anesthetics in the abdominal ganglion? S channels are present in abdominal ganglion sensory cells.[23] In addition, a TEA-resistant K^+ current with the *i*-V characteristics of an S current was previously observed in abdominal ganglion LB neurons (E. Mayeri and R. Jansen, personal communication). Our data indicate that S channels open more frequently in the presence of volatile anesthetics, which would contribute to membrane hyperpolarization. Although the small baseline channel was inhibited by halothane (fig. 4A), concurrent activation of the larger conductance S channel would still produce a net outward K^+ current. In addition, we did not observe activation of $K_{(Ca)}$ channels by halothane (fig. 6A). There is some evidence suggesting that $K_{(Ca)}$ channels in vertebrates are inhibited by volatile anesthetics,[37,38] which would produce membrane depolarization rather than the hyperpolarization we observed in intact ganglia.

The changes produced by volatile anesthetics in the slow closed-time component τ_{c2} suggests that S-channel activation involves a simple increase in the opening rate. The variability of τ_{c2} under control conditions with one or two channels in the patch may be a result of the biochemical pathways that act to modulate S-channel activity. The opposing actions of the 12-lipoxygenase pathway[39] and cyclic adenosine monophosphate-dependent protein kinase[30,31] could contribute to wide variations in baseline levels of channel activity. Thus each patch clamp experiment could involve channels under different modulatory conditions. On the other hand, the S channel mean open times τ_{o1} and τ_{o2} tended to exhibit relatively little variability, as did τ_{c1} even in the presence of 5 μM FMRFamide (table 1).

The high Hill coefficient that was obtained from the fit to the data in figure 4B may indicate positive cooperativity in anesthetic-receptor binding. However, we cannot preclude the possibility that the steepness of the fit may instead reflect a threshold at which the concentration of halothane is sufficient to recruit previously silent channels in the patch into an active mode. In the latter case, the high Hill coefficient we obtained may reflect two independent channel properties that are affected by halothane: (1) the opening kinetics as a function of τ_{c2}, which vary within a range of ~40 to 300 ms, and (2) the mode of the channel (active or inactive), which may change on a time scale lasting minutes.[20] Further experiments are necessary to test these hypotheses.

An altered pattern of gating or recruitment by halothane of previously silent channels into an active mode could result from direct actions of the drug on the channel or alternatively on components of the second messenger pathways that regulate channel activity. The actions of halothane and isoflurane are qualitatively similar to FMRFamide, a neuropeptide that activates the S channel but does not affect $K_{(Ca)}$ currents.[32,33] FMRFamide also produces a transient hyperpolarization of some abdominal ganglion neurons and cessation

of spike activity[33] that are similar to our observations of the actions of halothane in intact neurons of the abdominal ganglion. The actions of FMRFamide are mediated by arachidonic acid metabolism through the 12-lipoxygenase pathway.[40] This enzyme is also present in the mammalian nervous system.[41] The products of this pathway, such as (12s)-hydroperoxyeicosatetraenoic acid (12-HPETE), appear to modulate gating of the S channel directly.[39,42] These second messengers also produce membrane hyperpolarization and presynaptic inhibition of neurotransmitter release.[32,33,40] An attractive hypothesis is that volatile anesthetics mimic endogenous lipid-based second messengers or alter their rate of turnover to activate the S channel.

The S channel has not yet been cloned (but see Zhao and associates[43]). However, extensive homology with rodent brain K^+ channel proteins has been observed for homologs of the four *Drosophila* classes of K^+ channels: *Shaker*, *Shab*, *Shaw*, and *Shal*.[44] Small variations in the amino acid sequences of channel proteins may be responsible for the diversity of mammalian voltage-gated K^+ channels.[45] The widespread homology of K^+ channel proteins suggests the possibility that K^+ channels homologous to the *Aplysia* S channel may exist in mammalian neurons. Several channels with properties similar to the S channel have been described in vertebrate neurons, including the M channel,[46] the flicker channel,[47] and the RCK5 channel.[45] Our results raise the question of whether anesthetic activation of the S channel in *Aplysia* is unique or whether anesthetic-sensitive baseline channels are widespread and targeted by volatile anesthetics in mammalian neurons.

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