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Inhalational Anesthetic Actions on Voltage-Gated Ion Currents of Bovine Adrenal Chromaffin Cells

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SUMMARY

The effects of the inhalational anesthetics halothane and isoflurane on voltage-gated ionic currents in bovine adrenal chromaffin cells were examined using patch-clamp techniques. Halothane (1.5% atmospheres, 0.90 mM in solution) and isoflurane (2.5% atmospheres, 0.78 mM in solution) diminished the Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$) "hump" by 52 ± 3% (n = 16 cells) and 40 ± 4% (n = 6), respectively. These concentrations of halothane and isoflurane had virtually no effect on the rapid inward Na^+ current and exerted only minor effects on outward K^+ currents in the absence of external Ca^{2+}. The effectiveness of halothane (0.90 mM) was reduced by increasing the external Ca^{2+} concentration ($[Ca^{2+}]_o$); $I_{K(Ca)}$ was decreased by 64 ± 4% (n = 4) with 1 mM $[Ca^{2+}]_o$ but by only 37 ± 4% (n = 4) with 10 mM $[Ca^{2+}]_o$. Voltage-activated Ca^{2+} current (I_{Ca}), isolated by intracellular perfusion with Cs^+ and tetraethylammonium in the presence of 15–30 μM external tetrodotoxin, was insensitive to 0.90 mM halothane and partially reduced by 0.78 mM isoflurane (24 ± 7%, n = 9). Halothane at 1.4 mM decreased I_{Ca} by approximately 30 ± 5% (n = 7), with a slight enhancement of the rate of activation and no change in the voltage dependence of activation. Higher levels of halothane produced a clear enhancement of the rates of both activation and deactivation of I_{Ca}. Detailed examination of the Na^+ current showed that excessive levels of halothane (~3 mM) decreased the peak amplitude by 26 ± 7% (n = 6) and enhanced the rates of activation and inactivation, while shifting the voltage dependence of activation by 8.3 ± 2.3 mV (n = 3) and the steady state inactivation by 6 mV towards hyperpolarized levels. Excised membrane patch-clamp measurements were performed to assess the direct effect of halothane on the large conductance "BK" channels underlying $I_{K(Ca)}$. Halothane (0.90 mM) with ~900 nM free Ca^{2+} at the cytoplasmic side of the patch decreased the probability of opening (p_o) by 42 ± 10% (n = 8), with no alteration in the open channel amplitude. With the cytoplasmic $[Ca^{2+}]$ reduced to ~100 nM, halothane diminished p_o by 65 ± 8% (n = 8), suggesting that Ca^{2+} can antagonize the effect of halothane on BK channels, consistent with whole-cell experiments. Although these results show that several channel types in chromaffin cells are affected by inhalational agents, BK channels appear to be more sensitive than other voltage-dependent channels. Our results may explain, at least in part, the increased excitability occasionally observed in neuronal tissue with anesthetic application.

Clinical anesthesia is thought to be the result of a depression of synaptic transmission and/or excitability within the central nervous system. It is well known that volatile anesthetics, at sufficiently large concentrations, can perturb the structure of the lipid bilayer by enhancing the fluidity, or more exactly, decreasing the microviscosity of the cellular membrane. As a result, the mobilities of intramembranous molecules are known to be augmented (1), perhaps facilitating membrane channel state transitions. For instance, halothane diminishes neuronal I_{Na} (2, 3) by, at least in part, increasing the rate of inactivation (2). Although the anesthetic-induced reductions in I_{Na} are consistent with an overall decrease in excitability, such effects were observed outside the clinically useful concentration range. The long-held view that general anesthetics affect cellular function exclusively by nonspecific alterations in lipid character has been questioned by recent findings showing that the inhalational agents affect voltage-gated ion channels at lower concentrations. At more clinically relevant anesthetic levels, voltage-activated Ca^{2+} channels in both cardiac (4–7) and neuronal cells (8–10) have been shown to be depressed. In excitable cells exhibiting stimulus-secretion coupling, decreased Ca^{2+} entry through voltage-activated Ca^{2+} channels would depress evoked transmitter release to diminish synaptic transmission. Volatile agents have been postulated to enhance as well as depress different classes of channels to contribute to the overall anesthetic state. Franks and Lieb (11) reported that halothane

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ABBREVIATIONS: BK, large conductance voltage and calcium activated K^+ channel; I_{Na}, Na^+ current; $I_{K(Ca)}$, Ca^{2+}-dependent K^+ current; I_{Ca}, Ca^{2+} current; I_K, K^+ current; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $[Ca^{2+}]_o$, external Ca^{2+} concentration; $[Ca^{2+}]_i$, internal Ca^{2+} concentration; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HVA, high voltage-activated; AHP, afterhyperpolarization; SK, intermediate conductance calcium activated K^+ channel; TEA, tetraethylammonium.

activates a novel K^+ conductance in snail neurons, resulting in a hyperpolarization. Furthermore, several investigators have proposed that anesthetics activate neuronal Ca^{2+}-dependent K^+ conductance by increasing the intracellular Ca^{2+} concentration (12, 13), leading to reduced neuronal excitability.

The purpose of the present study was to investigate the effects of the inhalational anesthetics halothane and isoflurane on the membrane currents in isolated bovine adrenal chromaffin cells. Chromaffin cells have been used extensively as models for neuronal electrophysiology and exocytotic secretion. Previous investigators have described chromaffin cell electrophysiology in detail (14–16). A preliminary account of this work has been presented to the American Society of Anesthesiology (17).

Materials and Methods

Cell culture and preparation. Bovine adrenal chromaffin cells were generously supplied by Dr. Y. I. Kim from the Department of Biomedical Engineering of the University of Virginia and were isolated according to a previously reported method (18), with modifications (19). Cells were immobilized for patch-clamp experiments by adhering the cells to poly-L-lysine-coated coverslips. Cells were maintained in an incubator at 37° in 5% CO_2/95% air. Best results were obtained from cells used within 2 days of isolation.

Solutions. Table 1 lists the contents of each of the solutions used in this study. Solutions A, B, and C were adjusted to pH 7.4 with 2.5 N NaOH and were used as the external bathing solutions. Solution D, adjusted to pH 7.3 with 2.5 N HCl, was used within the patch pipette to measure outward K^+ currents. Solution E, adjusted to pH 7.3 with TEA-OH, was also used as an intracellular solution to suppress outward currents and reveal inward currents. To counteract the well documented Ca^{2+} channel "run-down" phenomenon (14), 5 mM ATP (equine Mg^{2+} salt) was included in the patch pipette solutions (solutions D and E), which significantly enhanced the longevity of I_{Ca} and $I_{K(Ca)}$. To block Na^+ channels and isolate I_{Ca}, 15–30 μM tetrodotoxin was applied extracellularly. Solutions F and G, adjusted to pH 7.4 with KOH, were used to record unitary K^+ channels in excised membrane patches. Solutions F and G were prepared according to a custom program based on the method of Stockbridge (20), to have free Ca^{2+} concentrations of approximately 100 nM and 900 nM, respectively. This program, which predicts free Ca^{2+} concentrations as a function of the total calcium and EGTA concentrations, yields values in close agreement with a previous study (21).

Inhalational anesthetics were equilibrated in the bathing solution at room temperature by bubbling for at least 20 min with filtered air passed through a calibrated vaporizer. We chose to emphasize equi-anesthetic levels, which were multiples of the anesthetic ED_{50} or minimum alveolar (gas) concentration, i.e., 0.75% and 1.25% atmospheres for halothane and isoflurane, respectively. These concentrations were set with the vaporizer in terms of volume percent and were verified by gas chromatography with sampling directly from the recording chamber. Values in solution are reported in Table 2 and represent 90–100% of the anticipated value calculated from the gaseous/aqueous partition coefficient (22) at 20°. Control solutions were bubbled with filtered air only. Control, anesthetic treatment, and recovery phases were evaluated in each cell tested. Complete solution exchange was accomplished within 20 sec and measurements were made after approximately 90 sec of solution application. Alterations in membrane currents after treatment with anesthetics were persistent for several minutes regardless of whether or not the perfusion was constant.

Whole-cell patch-clamp recording. A coverslip with adherent cells was transferred to a chamber mounted on a Zeiss Axiovert inverted microscope where bathing solutions could be exchanged. Standard whole-cell voltage-clamp methods were used, as described by Hamill *et al.* (23). It was usually necessary to wait 4–6 min after initiating the whole-cell recording configuration, to establish a stable base line. Voltage-clamp measurements were performed using either a model 8900 (Dagan Corp., Minneapolis, MN) or an Axopatch 200 (Axon Instruments, Foster City, CA) patch-clamp amplifier. The Axopatch 200 offered the advantage of cancellation of the capacitive transient by estimation of the series resistance and cell membrane capacitance in the whole-cell recording mode. When the Axopatch 200 was used series resistance compensation was typically set at 85% or greater, whereas when the Dagan 8900 was used resistance compensation was increased to a level immediately below the threshold for ringing, which yields 80–90% compensation. Patch electrodes were prepared from borosilicate glass (1B150F-4; World Precision Instruments, Inc., Sarasota, FL). After the fabrication of pipettes with a two-stage micropipette puller (Narishige Co., Tokyo, Japan), pipette tips were heat-polished with a microforge (Narishige Co.). All experiments were conducted at room temperature (20–22°).

Voltage-clamp data acquisition and analysis. Data acquisition was performed using the pCLAMP system, version 5.5.1 (Axon Instruments), coupled to an IBM-compatible, 386-based microcomputer. The CLAMPEX routine was used to clamp cells at −80 mV and to apply step depolarizations, with standard P/n analysis to estimate leakage and capacitive current (24). By this method, the ensemble of the currents elicited by $n = 4$ hyperpolarizing subpulses of amplitude $P/4$ were added to the current evoked by a depolarizing test voltage (V_T) from the holding potential (HP), where $P = V_T - HP$. Unless otherwise noted, all whole-cell currents were filtered at 2 kHz with a four-pole Bessel low-pass filter and were digitized at 10 kHz. Current records were analyzed off-line using a program optimized for the evaluation of the peak $I_{K(Ca)}$. Fig. 1A illustrates whole-cell current evoked by a voltage step from a holding potential of −80 mV to +30 mV in the presence and the absence of external Ca^{2+}. As originally described by Marty and Neher (16), the current-voltage (I-V) curve (Fig. 1B) for the outward current exhibits a distinct N-shape or "hump" in the presence of extracellular Ca^{2+}, due to the activation of a Ca^{2+}-dependent K^+ conductance. The magnitude of $I_{K(Ca)}$, which is represented by the hump, can be assessed by drawing a tangent between the inflection points on each side of the hump and measuring the distance from the peak of the hump to a point along the tangent at the corresponding potential (16).

TABLE 1
Composition of external and internal recording solutions
This table lists the contents of the internal pipette and external bath patch-clamp solutions used in the present study.

Solution	Composition (mM)													
	NaCl	$CaCl_2$	KCl	KOH	$MgCl_2$	Mg-ATP	$CoCl_2$	CsCl	CsOH	TEA-Cl	HEPES	EGTA*
A	140	2	5		1						10	
B	142		5		1						10	0.5
C	141		5		1		1				10	
D		1	100	42		5					5	10
E			100	42		5		120	30	20	10	11
F		7.32	100	42							10	10
G		9.32	100	42							10	10

*97% purity.

TABLE 2
Vaporizer settings and the associated anesthetic concentration in solution
The concentrations of both anesthetics were sampled directly from the recording chamber and determined by gas chromatography at room temperature (20–22°). Values are reported as mean ± standard deviation (four experiments).

Halothane		Isoflurane	
Volume	Concentration	Volume	Concentration
%	mM	%	mM
0.75	0.42 ± 0.03	1.25	0.38 ± 0.02
1.5	0.90 ± 0.03	2.5	0.78 ± 0.01
2.25	1.42 ± 0.05	3.75	1.22 ± 0.04

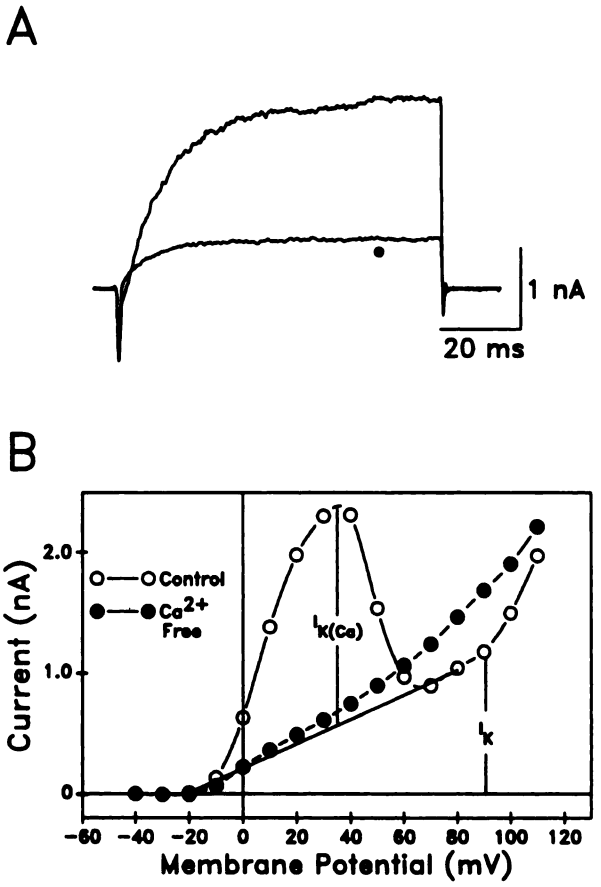

Fig. 1. Voltage-sensitive membrane currents from bovine adrenal chromaffin cells. Under control conditions the external and internal pipette solutions were solutions A and D (see Table 1), respectively; the external solution was changed to solution B (see Table 1) under Ca^{2+}-free conditions. A, Whole-cell current evoked by a test potential to +30 mV from a holding potential of −80 mV with normal $[Ca^{2+}]_o$ (2 mM) and after treatment with Ca^{2+}-free solution (extracellular Ca^{2+} replaced with 0.5 mM EGTA). B, The outward current versus voltage (I-V) relation for the cell under normal and Ca^{2+}-free conditions shows that the $I_{K(Ca)}$ hump is abolished in the absence of Ca^{2+} entry. $I_{K(Ca)}$ was quantified by drawing a tangent between the inflection points on each side of the hump and measuring the distance from the peak outward current to a point along the tangent at the corresponding voltage. The Ca^{2+}-independent I_K was estimated as the outward current evoked by a test pulse to +90 mV, well beyond the hump.

In addition, the magnitude of the outward current evoked by a step pulse to +90 mV, which was beyond the I-V curve hump, was used to estimate the Ca^{2+}-independent I_K. As described earlier, a possible outcome of decreased microviscosity produced by administration of general anesthetics is altered rates of channel activation and inactivation. For kinetic analysis of inward currents, nonlinear curve fitting of digitized traces was performed using the SYSTAT NONLIN software module. Generally, 30 or fewer iterations based on the SIMPLEX algorithm were required for convergence. The voltage dependence of activation, measured in terms of permeability or conductance as a function of voltage, was quantitatively described using the Boltzmann function:

$$f(V) = \frac{1}{1 + \exp\left(\frac{-(V - V')}{k'}\right)}$$

where V is the command voltage, V' is the potential where $f(V)$ is half-maximal, and k' is the slope factor.

Ca^{2+}-dependent K^+ channel data acquisition and analysis. Gigaseals were first attained with normal Ca^{2+} concentrations (solution A; see Table 1) in the external bath. Upon patch excision to achieve the inside-out variation, the membrane patches were voltage-clamped to +30 mV and the cytoplasmic side of the membrane was perfused with isotonic K^+ solutions F and G (see Table 1). Single K^+ channel currents were filtered at 1 kHz with a four-pole low-pass Bessel filter and were recorded at room temperature (20–22°) using the FETCHEX routine (Axon Instruments). Current segments were saved to the computer hard disk and were analyzed off-line with a custom program designed to assess open channel amplitude and kinetics. The probability of a channel being open (p_o) was estimated according to the method of Aldrich and Yellen (25). For kinetic analysis, event detection was achieved using half-amplitude thresholding, as described by Colquhoun and Sigworth (26). Complete kinetic analyses require the presence of a single channel in the membrane patch, which proved difficult to attain, perhaps due to the high density of Ca^{2+}-dependent K^+ channels in bovine adrenal chromaffin cells (16). The use of high resistance ($R >$ 15 MΩ), small-tipped micropipettes merely reduced the likelihood of observing any channel activity, rather than isolating a single BK channel. Although the existence of multiple homogeneous channels clearly obscures estimates of closed times because particular channel closures cannot be distinguished, open time kinetics may be adequately assessed if the number of simultaneous openings, which biases the data against long openings, is small (27). Therefore, under conditions of low p_o single-channel openings were binned and an exponential function, $f(t)$, of the form:

$$f(t) = \sum_{i=1}^{3} A_i \exp(-t/\tau_i)$$

was fitted to the open time distributions using the SYSTAT NONLIN software routine.

Statistics. Where appropriate, data are presented as mean ± standard error of percentage of control for the number of cells tested (n). Statistical significance of a drug effect was determined using Student's t test, with $p < 0.05$ considered as significant.

Results

Anesthetic action on outward currents. As shown in Fig. 2A, administration of 0.90 mM halothane resulted in a large reduction in outward current with little or no apparent change in I_{Na}. The I-V plot, shown in Fig. 2B, reveals that the $I_{K(Ca)}$ hump was reversibly affected by the anesthetic; 0.90 mM halothane diminished $I_{K(Ca)}$ by 52 ± 3% (mean ± standard error, n = 16 cells). Both halothane and isoflurane reduced $I_{K(Ca)}$ in a concentration-dependent manner (Fig. 3); 0.78 mM isoflurane decreased $I_{K(Ca)}$ by 40 ± 4% (n = 6). Application of each anesthetic was accomplished by steady perfusion of the treatment solution for 90 sec; the peak outward current steadily decreased to a diminished level, often within 30 sec of exposure, without showing any time-dependent effects that might suggest a different action at lower anesthetic concentrations. The outward current elicited at very depolarized potentials, well beyond the $I_{K(Ca)}$ hump, was modestly reduced by both of the inhalational anesthetics. The magnitude of the outward current evoked by a command potential of +90 mV fell by 14 ± 2% (n = 11) and 16 ± 4% (n = 6) with application of 0.90 mM halothane and 0.78 mM isoflurane, respectively. To further delineate the depression of Ca^{2+}-independent I_K, we replaced extracellular Ca^{2+} with 0.5 mM EGTA to eliminate the contribution of $I_{K(Ca)}$ to the whole-cell current, as shown in Fig. 1. Marty and Neher (16) previously reported that the absence of extracellular Ca^{2+} not only abolishes the $I_{K(Ca)}$ hump but also alters the permeability of the voltage-gated Ca^{2+} channels to monovalent cation flux. They reported that test potentials in

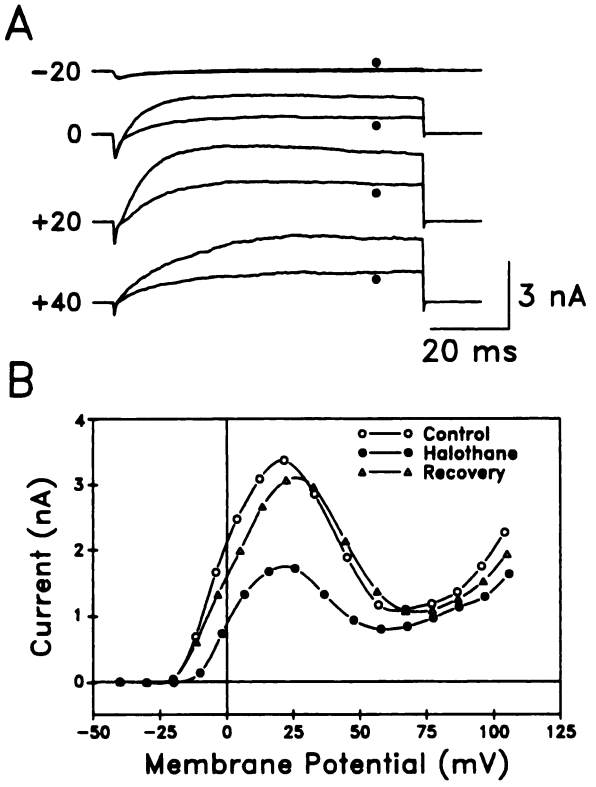

Fig. 2. Voltage-activated membrane currents from bovine adrenal chromaffin cells in the absence and presence of the inhalational anesthetic halothane. External and internal pipette solutions were solutions A and D (see Table 1), respectively. A, The current traces illustrated were elicited by 80-msec depolarizing pulses ranging from −20 to +40 mV, in 20-mV increments, from a holding potential of −80 mV. Records are from the same cell before and during exposure to 0.90 mM halothane (●). B, Outward current-voltage (I-V) relations for the cell identified in A. Halothane primarily depresses $I_{K(Ca)}$.

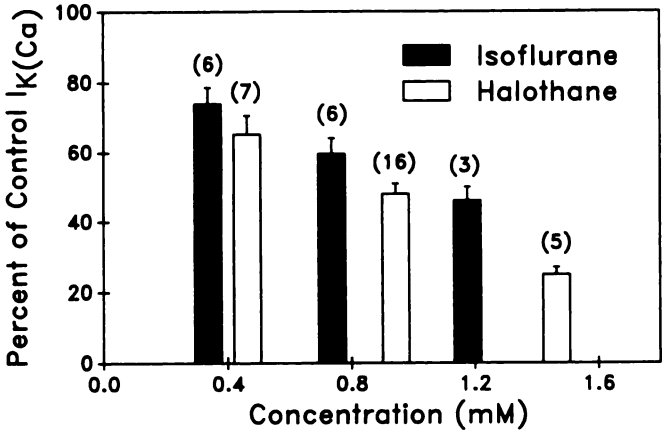

Fig. 3. Dose-dependent depression of $I_{K(Ca)}$ by the inhalational anesthetics halothane and isoflurane. Data are presented as the mean ± standard error of percentage of control $I_{K(Ca)}$ after anesthetic treatment. The number of cells used at each anesthetic concentration is given in *parentheses immediately above each bar*. All anesthetic effects were significantly different ($p < 0.01$–0.05) from control values. External and internal pipette solutions were the same as in Fig. 2. The magnitude of $I_{K(Ca)}$ was estimated graphically from the I-V plot (see Materials and Methods and Fig. 1).

excess of +20 mV resulted in an outward current consisting of substantial components of I_K and K^+ efflux through Ca^{2+} channels (16) (Fig. 2B). Anesthetic treatment with 0.90 mM halothane and 0.78 mM isoflurane in Ca^{2+}-free bathing solution produced only modest reductions in outward current (Fig. 4). In response to a command pulse to +90 mV, halothane and isoflurane diminished the outward current by 9 ± 1% ($n = 3$) and 14 ± 2% ($n = 3$), respectively. At a test voltage of +10 mV, where the cation flux through Ca^{2+} channels is small and I_K predominates, halothane decreased the outward current by 15 ± 4%, whereas isoflurane had no significant effect. Clearly, the effect of these two anesthetics on Ca^{2+}-independent outward current is relatively small.

Given the importance of Ca^{2+} to the activation of $I_{K(Ca)}$, we examined the efficacy of halothane at different $[Ca^{2+}]_o$ values. Control, treatment, and recovery phases were studied with each cell at two different $[Ca^{2+}]_o$ values. In the absence of anesthetics, elevation of $[Ca^{2+}]_o$ from 2 to 10 mM resulted in a 91 ± 6% ($n = 4$) increase in $I_{K(Ca)}$, whereas a reduction of $[Ca^{2+}]_o$ from 2 to 1 mM produced a 30 ± 6% ($n = 4$) decrease in $I_{K(Ca)}$. Fig. 5A summarizes the comparative effects of halothane on

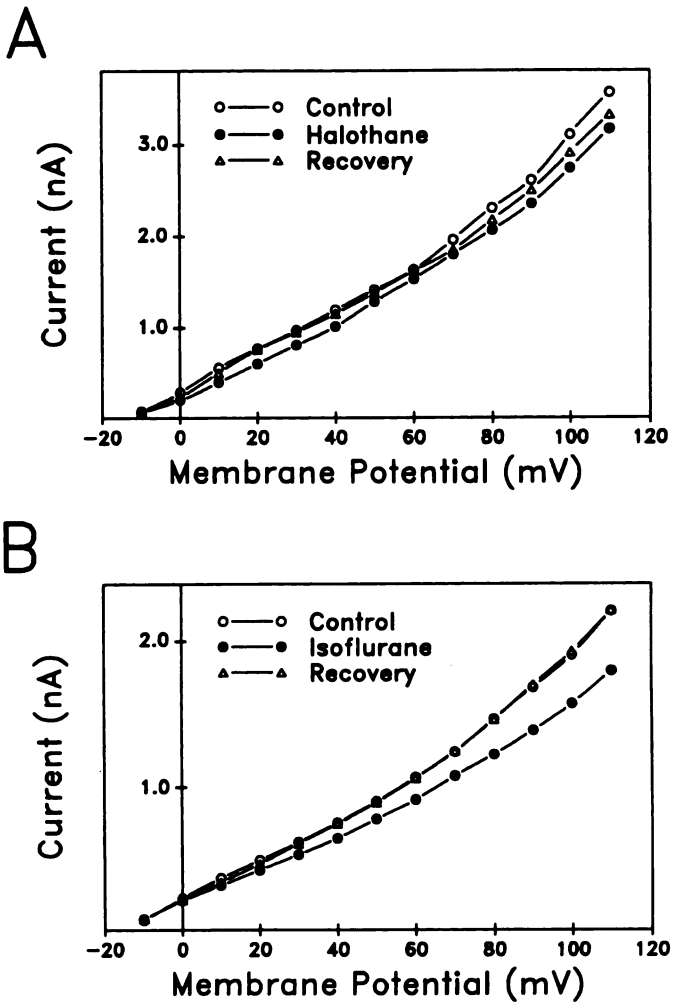

Fig. 4. Limited effect of the volatile anesthetics on outward currents in the absence of external Ca^{2+}. External and internal solutions were solutions B and D (see Table 1), respectively. A, I-V relations for a typical cell under control, 0.90 mM halothane, and recovery conditions. B, I-V curves for a typical cell exposed to control, 0.78 mM isoflurane, and recovery conditions.

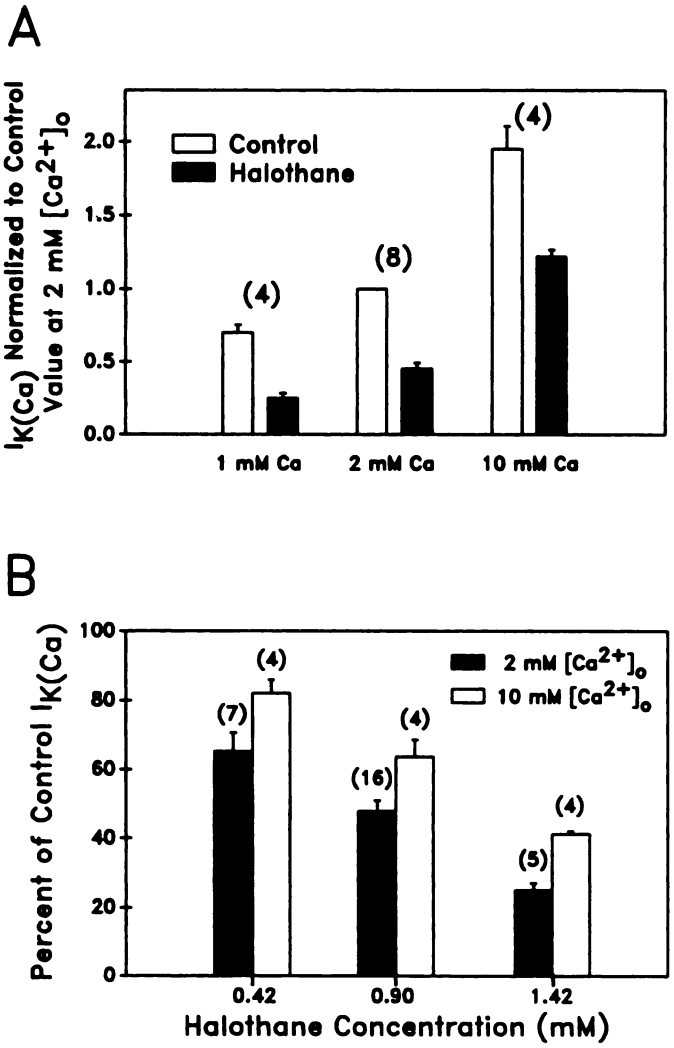

Fig. 5. Comparative effectiveness of halothane on $I_{K(Ca)}$ at different $[Ca^{2+}]_o$ values. Data are presented as mean ± standard error, with the number of cells used given in *parentheses*. A, Data from each cell were normalized to the $I_{K(Ca)}$ magnitude at 2 mM $[Ca^{2+}]_o$ measured in the same cell. Comparison of anesthetic action at different $[Ca^{2+}]_o$ values reveals that anesthetic potency is inversely related to $[Ca^{2+}]_o$. B, The presence of elevated $[Ca^{2+}]_o$ rendered the inhalational anesthetic at each concentration examined significantly less effective ($p < 0.05$) in the blockade of $I_{K(Ca)}$.

$I_{K(Ca)}$ at each of the $[Ca^{2+}]_o$ values. To reduce experimental variance, data were normalized to the control $I_{K(Ca)}$ magnitude at a $[Ca^{2+}]_o$ of 2 mM in the same cell. Note that comparison of control and halothane treatment phases at the same $[Ca^{2+}]_o$ shows proportionally reduced anesthetic effectiveness with higher $[Ca^{2+}]_o$ values. Halothane at 0.90 mM diminished $I_{K(Ca)}$ by 37 ± 4% ($n = 4$) and 64 ± 3% ($n = 4$) at $[Ca^{2+}]_o$ values of 10 and 1 mM, respectively. Fig. 5B shows the dose-dependent suppression of $I_{K(Ca)}$ by halothane at 2 mM and 10 mM $[Ca^{2+}]_o$, indicating a shift toward higher anesthetic concentrations for $I_{K(Ca)}$ blockade with elevated $[Ca^{2+}]_o$. Presumably, increased $[Ca^{2+}]_o$ permits greater Ca^{2+} entry and an increased $[Ca^{2+}]_i$ (28). Therefore, it is possible that the anesthetic might interfere with the Ca^{2+}-dependent K^+ channel at a Ca^{2+} activation step, although we cannot exclude the possibility of activation of a less anesthetic-sensitive population of K^+ channels at higher $[Ca^{2+}]_o$ levels.

Effect of anesthetics on inward currents. To examine whether anesthetic-induced reduction of voltage-gated Ca^{2+} entry could account for the observed suppression of $I_{K(Ca)}$, pipettes were filled with a solution containing both Cs^+ and TEA to block outward currents. For the evaluation of I_{Ca}, cells were voltage-clamped to the same range of potentials as for the outward currents. Under control conditions peak I_{Ca}, evoked by potentials from −10 to +10 mV, ranged from −60 to −330 pA. To quantify alterations in I_{Ca} gating with higher concentrations of halothane, currents were fitted with equations similar to those used by Doupnik and Pun (29). I_{Ca} activation exhibited a brief delay (~1 msec) in onset and a variable pattern of inactivation under both control and anesthetic-treated conditions; therefore, the currents were described by a model with m^2 activation kinetics and an inactivation term, h:

$$I_{Ca} = Am^2h = A(1 - e^{-t/\tau_m})^2 e^{-t/\tau_h}$$

Using a nonlinear curve-fitting routine, the current amplitude A and the time constants τ_m and τ_h were allowed to converge independently for current records evoked by potentials ranging from −20 to +30 mV. Halothane at 0.90 mM, which reduced $I_{K(Ca)}$ by >50%, had no apparent effect on I_{Ca} ($n = 7$) (Fig. 6A). Significant depression of I_{Ca} was observed only in the presence of ≥1.42 mM halothane (Fig. 6B). Halothane at 1.42 mM reduced both peak and plateau components of I_{Ca} to the same extent, by 29 ± 2% ($n = 7$) and 30 ± 5% ($n = 7$), respectively. Although the I-V curve shown in Fig. 6C revealed no obvious shift in the voltage dependence of I_{Ca} after anesthetic treatment, steady state activation was evaluated by scaling peak I_{Ca} evoked by potentials ranging from −30 to +40 mV, according to the constant field equation (30):

$$P_{Ca} = I_{Ca} \frac{RT}{4VF^2} \frac{1 - \exp(-2FV/RT)}{[Ca^{2+}]_i - [Ca^{2+}]_o \exp(-2FV/RT)}$$

where $[Ca^{2+}]_i$ is estimated to be 100 nM, V is the command potential, and the constants R, T, and F have the standard meanings. With P_{Ca} at each potential normalized to the peak value and assuming m^2 kinetics, Ca^{2+} channel activation could be described by the Boltzmann function. Under control conditions V' and k' were −16 ± 2 mV ($n = 6$) and 10 ± 1 mV ($n = 6$), respectively. No significant changes were observed in V' or k', verifying no shift in the voltage dependence of activation in the presence of 1.42 mM halothane (Fig. 6D). To determine whether the halothane-induced reduction of I_{Ca} is use or frequency dependent, that is, requiring channel opening leading to subsequent blockade, as is the case for open channel blocking schemes, a train of six pulses 80 msec in duration were applied at a rate of up to 1 Hz. The anesthetic at 1.42 mM did not alter the use-dependent decline in the magnitude of I_{Ca}, suggesting that channel opening is not required for anesthetic blockade. Paired comparison of control and halothane-treated currents did reveal a slight increase in the rate of activation, with no apparent change in the rate of inactivation, in the presence of the anesthetic. Statistically significant reductions in τ_m ranging from 0.8 to 0.4 msec were observed for potentials ranging from −10 to +30 mV. Application of halothane at 3% (2 mM, estimated), which decreased peak I_{Ca} by 58 ± 2% ($n = 5$), showed the enhanced rate of activation and deactivation much more clearly, with no effect on the voltage dependence of I_{Ca} activation. As illustrated in Fig. 7A for a test pulse to −10 mV, ~2 mM halothane decreased τ_m from 2.9 ± 0.2 msec ($n = 5$) to 1.4

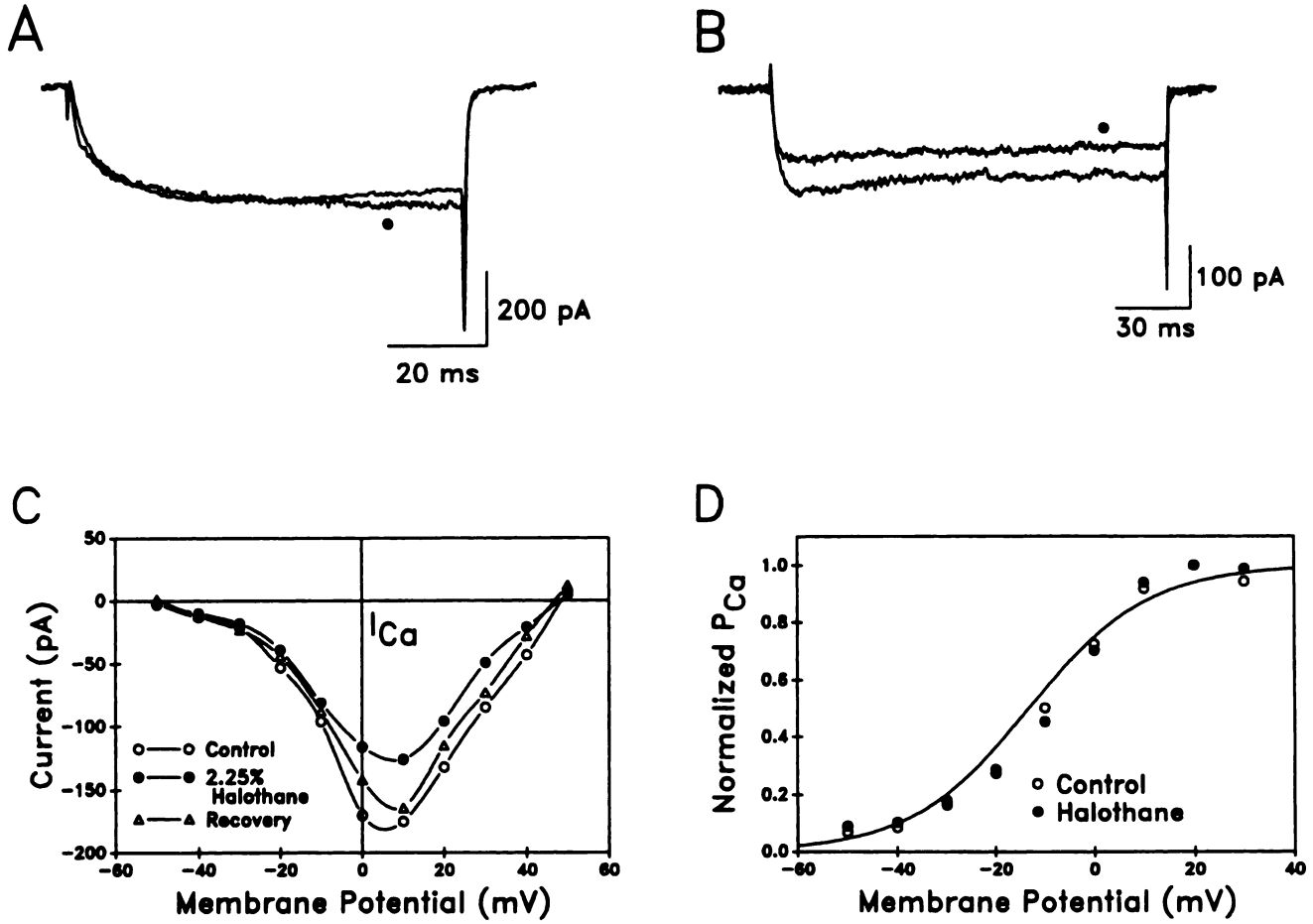

Fig. 6. Effect of moderate and high doses of halothane on voltage-gated I_{Ca} in adrenal chromaffin cells. External and internal pipette solutions were solutions A and E (see Table 1), respectively, with 15–30 μM tetrodotoxin added to suppress voltage-dependent I_{Na}. The current traces shown in A and B were elicited by a command pulse from a holding potential of −80 mV to 0 mV. ●, Halothane treatment. A, Halothane at 0.90 mM had virtually no observable effect on I_{Ca}. B, Halothane at 1.42 mM partially decreased both peak and plateau I_{Ca} by approximately 30%. C, The I-V relations for the same cell under control and 1.42 mM halothane-treated conditions. The effect of the anesthetic was fully reversible. D, The voltage dependence of activation, as calculated using the constant field equation, revealed virtually no change with 1.42 mM halothane. *Solid line*, best fit to the Boltzmann function, with $V' = -13.3$ mV and $k' = 12.2$ mV.

± 0.1 msec ($n = 5$). Assessment of Ca^{2+} tail currents, low-pass filtered at 5 kHz and digitized at a rate of 50 kHz, also revealed an augmented rate of deactivation in the presence of ~2 mM halothane. Tail currents were evoked by first maximally activating Ca^{2+} channels by a step to +40 mV and then rapidly repolarizing the membrane to potentials ranging from −70 to −40 mV. In Fig. 7B, the digitized tail current was plotted on a logarithmic scale over a range of 90% to 10% of the maximum inward current magnitude. The tail current decays were well fitted by a single-exponential time constant, τ', which yields a τ_m of $2\tau'$, assuming m^2 kinetics. For a repolarization to −50 mV, τ_m was decreased significantly ($p < 0.001$), from a control level of 1.06 ± 0.10 msec ($n = 5$) to 0.57 ± 0.05 msec. According to the method of Hodgkin and Huxley (31), both τ_m and the steady state activation (m) are functions of the m gate forward and backward rate constants, α_m and β_m, respectively:

$$\tau_m = \frac{1}{\alpha_m + \beta_m}$$

$$m_\infty = \frac{\alpha_m}{\alpha_m + \beta_m}$$

In the presence of high levels of halothane, the absence of a shift in m with proportionally equivalent decreases in τ_m over a wide range of potentials suggests that the inhalational anesthetic may act on I_{Ca} by increasing α_m and β_m to the same extent, possibly by altering membrane fluidity or microviscosity.

Administration of isoflurane yielded effects on I_{Ca} qualitatively similar to those seen with halothane. Fig. 8 illustrates the dose-dependent reduction of I_{Ca} with both of the inhalational agents. We did observe a greater sensitivity of I_{Ca} to isoflurane, compared with halothane. Isoflurane at 0.78 mM depressed peak I_{Ca} by 24 ± 7% ($n = 9$), although 0.38 mM isoflurane appeared without effect ($n = 5$).

Application of halothane concentrations as high as 1.42 mM had no effect on chromaffin cell I_{Na}, although reductions of I_{Na} became apparent at higher levels, well beyond the clinically relevant range. Peak I_{Na} was diminished by 26 ± 7% ($n = 6$) with application of 5% (3 mM, estimated) halothane, as depicted in Fig. 9A. Fig. 9B summarizes the effect of ~3 mM halothane on peak I_{Na} evoked by potentials ranging from −50 to +50 mV, depicting a subtle shift in voltage dependence. $G_{Na}(V)$ was calculated based on Ohm's law and was well fit by the Boltzmann function. Under control conditions, V' and k' were −15.4

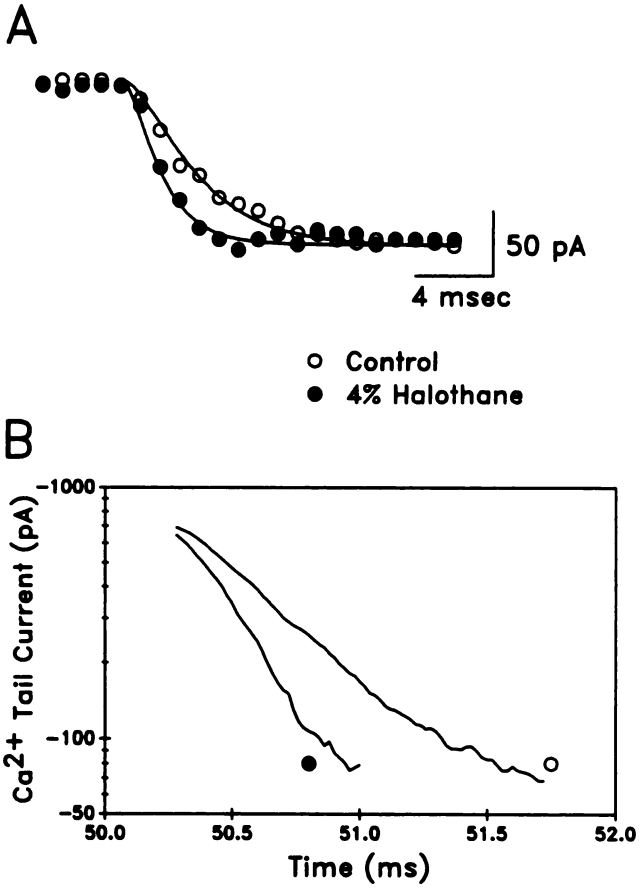

Fig. 7. Effect of a high concentration of halothane on chromaffin cell voltage-activated I_{Ca} activation and deactivation kinetics. External and internal pipette solutions were the same as in Fig. 6. A, Halothane at 3% (~2 mM) clearly produces an enhancement of the rate of activation of I_{Ca}. The first 15 msec of each of the currents elicited by a test pulse to −10 mV from a holding potential of −80 mV are shown. The halothane-treated I_{Ca} was scaled by a factor of 1.72 to readily distinguish changes in activation. One of every five samples from the digitized current records is shown (○, control; ●, halothane). *Solid lines*, result of fitting the current record using m^2h Hodgkin-Huxley kinetics; control τ_m, 2.8 msec; halothane τ_m, 1.5 msec. B, Halothane (~2 mM) enhances the rate of Ca^{2+} channel deactivation. After a test pulse to +40 mV, the cell was repolarized to −50 mV to elicit an inward Ca^{2+} tail current, which was low-pass filtered with a four-pole Bessel filter with a corner frequency of 5 kHz and digitized at a rate of 50 kHz. The halothane-treated tail current (●), which was scaled by a factor of 1.81 for comparison with the control tail current (○), was plotted on a logarithmic scale to clearly identify alterations in the time course of decay. Fitting the current records to single-exponential decays assuming m^2 Hodgkin-Huxley kinetics yielded control τ_m of 1.16 msec and halothane τ_m of 0.61 msec.

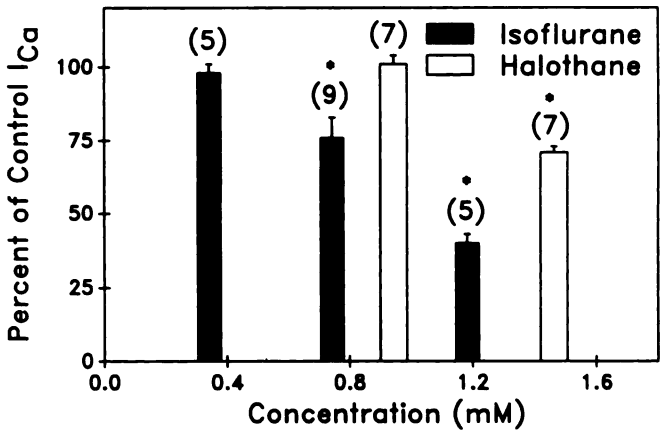

Fig. 8. Dose-dependent depression of peak inward I_{Ca} by the inhalational anesthetics halothane and isoflurane. Peak I_{Ca} was evoked by test pulses to −10 to +10 mV from a holding potential of −80 mV. Data are presented as the mean ± standard error of percentage of control I_{Ca} remaining after anesthetic treatment. The number of cells used at each anesthetic concentration is given in *parentheses immediately above each bar*. *, Statistically significantly different from control ($p < 0.05$). External and internal pipette solutions were the same as in Fig. 7.

± 1.4 mV ($n = 3$) and 6.6 ± 0.2 mV ($n = 3$), respectively. The voltage dependence of activation was shifted toward hyperpolarized potentials by ~3 mM halothane. V' was reversibly decreased by 8 ± 2 mV ($n = 3$) in the presence of the anesthetic; however, no change in k' was observed. To assess halothane-induced alterations in kinetics, I_{Na} was low-pass filtered at 5 kHz and digitized at 40 kHz. The rate of Na^+ channel activation, estimated by measuring the time to peak (t_p) of I_{Na}, was increased from 3.9 ± 0.6 msec ($n = 3$) to 2.0 ± 0.2 msec ($n = 3$) for a test pulse to −30 mV. The rapid inactivation phase of I_{Na} was well fitted by a single-exponential function in the absence and presence of halothane. For command voltages to −20 mV, the time constant of inactivation, τ_h, fell from a control level of 5.8 ± 0.4 msec ($n = 3$) to 2.7 ± 0.1 msec ($n = 3$) after halothane treatment (Fig. 9C). Because the inhalational anesthetic enhanced the rate of I_{Na} inactivation, possible changes in steady state inactivation by halothane were assessed. Each cell was voltage-clamped for 5 sec at a prepulse potential ranging from −90 to −40 mV and I_{Na} was evoked by a voltage step to 0 mV. Peak I_{Na} for each prepulse voltage was normalized to the maximum I_{Na} for each trial. Under both control and halothane-treated conditions, steady state inactivation was well described by the Boltzmann function (Fig. 9D). Application of ~3 mM halothane shifted V' from −61 to −66 mV, with little effect on the slope factor, k'. These observations suggest that at supraclinical inhalational anesthetic levels the forward inactivation rate constant (α_h) may be accelerated to account for the reduction in chromaffin cell I_{Na}.

Direct effect of halothane on Ca^{2+}-activated K^+ channels. The lower anesthetic concentrations used in this study were most effective in reducing $I_{K(Ca)}$, whereas I_{Ca}, I_{Na}, and I_K were comparatively less sensitive. To determine whether decreased $I_{K(Ca)}$ can be attributed to anesthetic-induced reductions in single-channel current amplitude and/or probability of channel opening (p_o), unitary Ca^{2+}-activated BK channel activity was measured in excised inside-out membrane patches. Because the effect of halothane on the whole-cell current appeared to be antagonized by elevated Ca^{2+} entry, channel activity was examined under conditions of physiologically relevant Ca^{2+} concentrations, approximately 100 nM and 900 nM free Ca^{2+} (solutions F and G, respectively). BK channel openings were easily distinguished by 1) the extremely large conductance of the open state (140–290 pS) (32) and 2) enhanced p_o with increased cytoplasmic Ca^{2+}. Despite using extremely small-diameter micropipettes to isolate a single BK channel, only patches with multiple BK channels were attained (eight of 52 patches attempted). Fig. 10, A and B, illustrates portions of current records from the same membrane patch, exhibiting three BK channels, before and during application of 0.90 mM halothane with ~900 nM Ca^{2+}. Among the patches investigated with ~900 nM Ca^{2+} at the inner surface of the membrane, p_o ranged from 0.008 to 0.3493, averaging 0.1401 ± 0.0507 ($n = 8$ patches). The current amplitude of channel openings evoked at

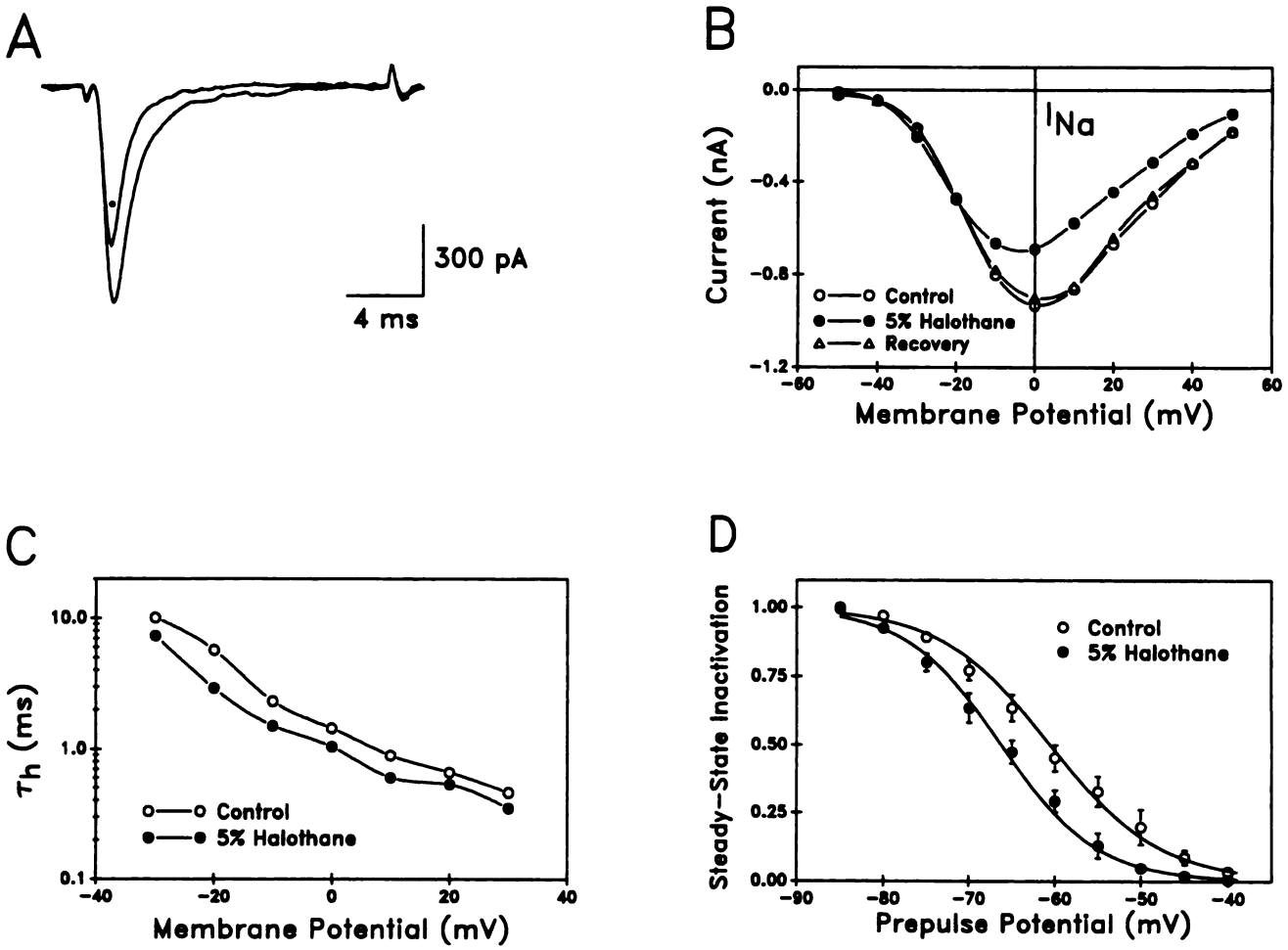

Fig. 9. Inward I_{Na} in the absence and the presence of 5% (~3 mM) halothane. External and internal pipette solutions were solutions C and D (see Table 1), respectively. A, The current traces were evoked by a 16-msec step potential to 0 mV from a holding potential of −80 mV. I_{Na} was digitized at 40 kHz with the four-pole Bessel low-pass filter set at a corner frequency of 5 kHz. Records are from the same cell before and during (●) exposure to halothane. B, Comparison of I-V curves for the cell shown in A suggests a subtle shift in the voltage dependence of activation. C, Halothane enhances the rate of I_{Na} inactivation, as shown by the decrease in the time constant of inactivation, τ_h, with halothane administration. Same cell as depicted in A. D, Application of halothane (~3 mM) reversibly shifts the steady state inactivation towards more hyperpolarized potentials. Steady state inactivation in the presence and the absence of the anesthetic was well fitted by the Boltzmann function: control, $V' = -60.6$ mV, $k' = -6.4$ mV; halothane, $V' = -66.3$ mV, $k' = -5.8$ mV. Data shown as mean ± standard error ($n = 5$).

+30 mV, 6.69 ± 0.41 pA ($n = 8$) under control conditions, was unchanged with application of anesthetic. The all-points histograms in Fig. 10, C and D, from control and halothane-treated current records, respectively, clearly reflect the reduced p_o observed in the presence of the anesthetic. We did occasionally observe a Ca^{2+}-dependent low-current amplitude event in our membrane patch recordings, shown by the cluster of points with a mean of 2.6–2.8 pA in Fig. 10, C and D, which may represent a subconductance state of the BK channel consistent with previous studies (33, 34) or a separate lower conductance Ca^{2+}-dependent K^+ channel type (32). The appearance of the lower conductance was rare and could be easily distinguished from the large conductance openings. Exposure to halothane (0.90 mM) and isoflurane (0.78 mM) diminished p_o by 42 ± 10% ($n = 8$) and 31 ± 5% ($n = 2$), respectively, with no change in BK channel amplitude. As expected, exposure of membrane patches to ~100 nM Ca^{2+} markedly decreased BK channel p_o; however, the degree of reduction seemed to vary from patch to patch, with factors ranging from 5 to 300. This reduced level of cytoplasmic Ca^{2+} appeared to potentiate significantly ($p < 0.05$) the effect of halothane (0.90 mM) on channel activity; p_o decreased by 65 ± 8% ($n = 8$) in the presence of ~100 nM Ca^{2+}. This observation at the level of excised BK channels was consistent with earlier, less direct, whole-cell recordings that suggested Ca^{2+} antagonism of the halothane-induced blockade of $I_{K(Ca)}$.

Under control conditions with ~100 nM Ca^{2+}, with p_o sufficiently low that simultaneous openings were unlikely (<0.5% of the data record), BK channel open lifetimes were assessed. Channel openings were either brief ($\tau_f = 0.7$ msec) or longer ($\tau_s = 3.5$ msec) in duration; longer events constituted 16% of the observed events, based on multiexponential curve fitting (Fig. 11A). Exposure to 0.90 mM halothane produced little effect on the time constants themselves, although the contribution of long duration events was substantially reduced, to only 6% (Fig. 11B). Furthermore, the frequency of channel openings, averaging 1.2 ± 0.5 Hz ($n = 8$) under control conditions, was diminished by 44 ± 13% ($n = 8$).

Discussion

As described previously (14, 15), incremental step depolarizations larger than −50 mV elicit a rapid inward I_{Na}, whereas

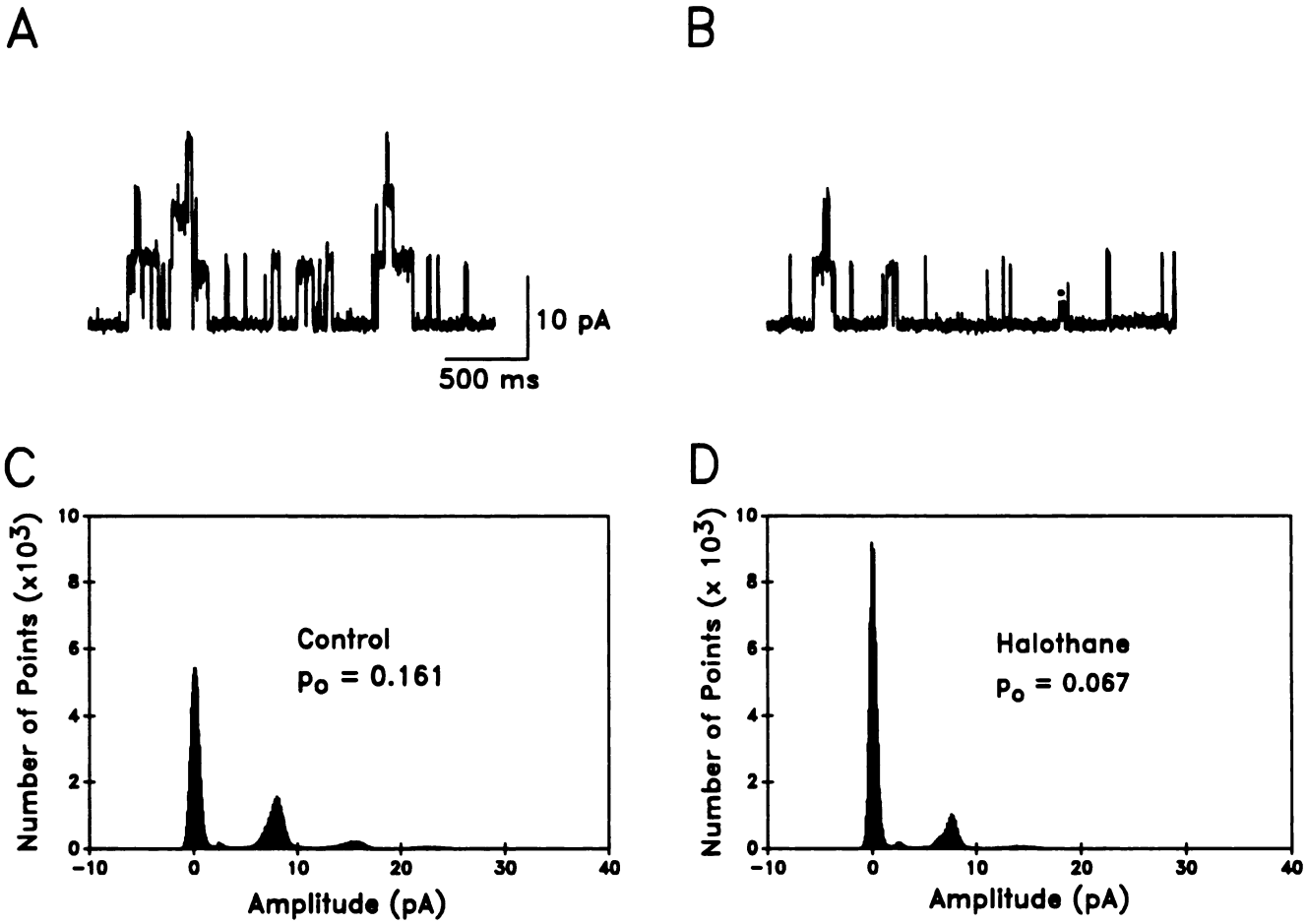

Fig. 10. Effect of 0.90 mM halothane on an excised inside-out membrane patch, containing three BK channels, in the presence of ~900 nM Ca^{2+} at the cytosolic side. Solution F was used in the patch pipette, whereas solution G was used in the external bath (see Table 1). The membrane patch was voltage-clamped to +30 mV. Channel openings (outward current) are shown as *upward deflections*. ●, A clear low conductance opening, possibly a subconductance state of the BK channel. These *traces* are representative of the data records of 90-sec duration under control (A) and halothane-treated (B) conditions. The all-points histograms (96,000 total points) for control (C) and halothane treatment (D) data clearly show a reduction in the probability of channel opening (p_o) with anesthetic application.

voltages more positive than −20 mV trigger slower activating outward current components carried by K^+ ions and a smaller inward I_{Ca}. The I-V curve shows an N-shape form with a hump corresponding to $I_{K(Ca)}$. A major determinant of the N-shape form of the I-V curve for the outward currents is the voltage dependence of I_{Ca} in these cells. Direct voltage dependence of $I_{K(Ca)}$, however, is well demonstrated by the observation that $I_{K(Ca)}$ evoked by potentials from +10 to +20 mV typically increases, although I_{Ca} decreases over the same voltage range. By using a pipette solution with K^+ replaced by Cs^+ and TEA, a sustained HVA I_{Ca} can be readily distinguished from I_{Na}. As reported in previous studies (15, 35), I_{Ca} inactivation often varied from cell to cell. The type of Ca^{2+} channels in chromaffin cells is still uncertain (35, 36); consequently, this study has considered anesthetic actions on the cumulative I_{Ca}.

Anesthetic action on inward currents. Our findings indicate that the inhalational anesthetics halothane and isoflurane have no apparent effect in I_{Na} even at concentrations as high as 1.4 mM and 1.2 mM, respectively. Halothane at an excessive concentration of 5% (~3 mM) depressed I_{Na} and increased the rate of inactivation. Haydon and Urban (37), using squid giant axons maintained at 6°, reported a halothane-induced reduction in I_{Na} with an ED_{50} of 2 mM. Given that decreasing temperature increases aqueous solubility and reduces partitioning into lipids by volatile anesthetics, anesthetic effects at such a reduced temperature predict more potent effects at higher temperatures. However, recent work at room temperature with mammalian GH_3 clonal pituitary cells (9), as well as the present work, indicates little sensitivity of I_{Na} to halothane, arguing against a role for neuronal Na^+ channel blockade by inhalational anesthetics under clinically relevant conditions. Consequently, the low sensitivity of mammalian Na^+ channels to general anesthetics may be attributed to a perturbation of the membrane lipid environment of the channel. Furthermore, in rat brain synaptosomes anesthetic-induced blockade of $^{24}Na^+$ flux occurs only with supraclinical anesthetic concentrations, which measurably disorder lipids (38).

In contrast, voltage-gated Ca^{2+} channels in various types of excitable cells have been shown to be targets of anesthetic action at lower concentrations. In cardiac cells, where a depression of I_{Ca} may contribute to the anesthetic-induced depression of myocardial function, halothane and isoflurane at clinically relevant dosages suppress Ca^{2+}-mediated slow action potentials (4, 5). More recent voltage-clamp studies have shown that the

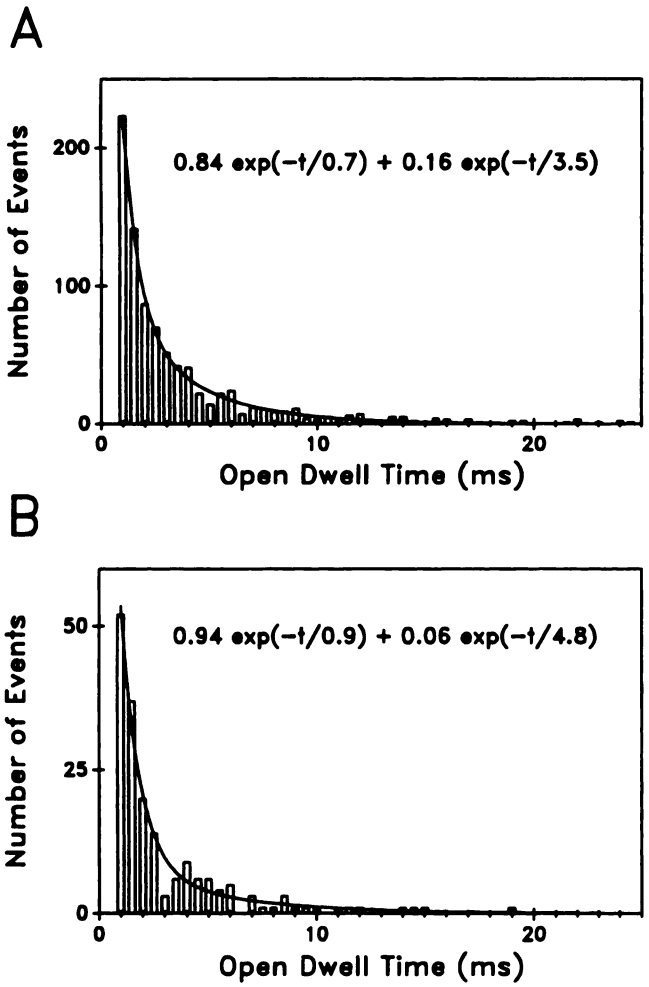

Fig. 11. Open-time histogram from a patch containing three BK channels, in the presence of ~100 nM Ca^{2+}. The *smooth curve* represents a best fit of the function *f(t)* (see Materials and Methods), achieved with a biexponential function for both control and treatment conditions. Addition of a third exponential term failed to identify an unique component. Also shown is *f(t)* after normalization of the amplitude of each exponential (A_i) to the sum of the amplitude terms ($\sum A_i$). A, Under control conditions; B, after application of halothane (0.90 mM), where long duration openings were less prevalent.

magnitude of I_{Ca} is reduced by halothane and isoflurane in ventricular myocytes (7, 39), an effect confirmed in our laboratory.[1]

The efficacy of inhalational anesthetics on voltage-gated Ca^{2+} channels in neuronal cells has been somewhat unclear. Single-microelectrode studies of CA1 neurons of hippocampal slices showed that halothane suppresses HVA I_{Ca} (40) as well as low voltage-activated I_{Ca} (8). Halothane-induced depression of peak HVA I_{Ca} has been reported for dorsal root ganglion neurons (10) and GH_3 cells (9), with ED_{50} values of 1.5 mM and 0.8 mM, respectively. High K^+-evoked intracellular Ca^{2+} transients of clonal pheochromocytoma PC12 cells can be inhibited by even lower levels of halothane and isoflurane, with ED_{50} values of 0.75 mM and 0.60 mM, respectively (41, 42). Although cells of the adrenal medulla are of neural origin and share common properties of sympathetic neurons, we did not observe any change in chromaffin cell I_{Ca} with 0.90 mM halothane and we measured only a slight reduction with 0.78 mM isoflurane. Consistent with our findings, earlier investigators using biochemical techniques have shown that potential-operated Ca^{2+} entry in adrenal chromaffin cells is largely unaffected by 1–2% halothane (43, 44). In fact, Pocock and Richards (45) reported that the IC_{50} for inhibition of $^{45}Ca^{2+}$ uptake, as well as catecholamine secretion, was greater than 2 mM.

With halothane at concentrations of 1.42 mM and greater, the rate of I_{Ca} activation and deactivation appeared enhanced, an effect consistent with an alteration of the lipid character surrounding the Ca^{2+} channel. Alternatively, halothane may unmask a component of I_{Ca} exhibiting faster kinetics. Clearly, unlike I_{Na}, the changes in I_{Ca} kinetics with exposure to halothane cannot account for the observed decrease in current magnitude.

Anesthetic action on outward currents. Bovine adrenal chromaffin cells express both $I_{K(Ca)}$ and I_K. Marty and Neher (16) have shown that the large conductance BK channel underlies $I_{K(Ca)}$, whereas two different K^+ channels comprise I_K. In the absence of external Ca^{2+}, which eliminates the contribution of $I_{K(Ca)}$ to the total outward current, the anesthetics exerted only minor effects. Halothane at 0.90 mM appeared to reduce I_K slightly, whereas the depressant effect of 0.78 mM isoflurane was most prominent at depolarized potentials. Currents evoked by potentials in excess of +20 mV in Ca^{2+}-free solution include a large component carried by K^+ ions through voltage-gated Ca^{2+} channels (16). Perhaps the effect of isoflurane on outward currents in the absence of external Ca^{2+} may in large part be attributable to blockade of Ca^{2+} channels, as described above. Previous studies have also reported minor effects of halothane on I_K. Halothane at concentrations of 6.4 mM or 3 mM is required to diminish by 50% the delayed outward I_K of the squid axon (37) and GH_3 clonal pituitary cells (9), respectively.

The interpretation of the effects on $I_{K(Ca)}$ of higher anesthetic concentrations is confounded by the concurrent blockade of I_{Ca}. For anesthetic levels above 0.90 mM halothane and 0.38 mM isoflurane, the reduction of $I_{K(Ca)}$ may be explained, to some extent, by diminished Ca^{2+} entry. The exact relationship between Ca^{2+} entry and Ca^{2+}-dependent K^+ channel activation is presently unclear, thus making it difficult to separate these two processes quantitatively. However, it is certain that the inhalational anesthetic-induced depression of $I_{K(Ca)}$ occurred at concentrations at which there was no demonstrable blockade of I_{Ca}.

Under physiological conditions intracellular Ca^{2+} plays a key role in the activation of Ca^{2+}-dependent K^+ channels. It is conceivable that our whole-cell observations can be attributed to decreased Ca^{2+} release from internal stores, although this is unlikely for three reasons. First, use of a high concentration of Ca^{2+} chelator, 10 mM EGTA, within the pipette solution theoretically precludes sizable changes in $[Ca^{2+}]_i$, except in a vicinity immediately proximal to the internal surface of the membrane (46). Second, resting $[Ca^{2+}]_i$ and basal secretion, a correlate of $[Ca^{2+}]_i$, were unaffected by halothane application to GH_3 clonal pituitary cells (47) and adrenal chromaffin cells (45), respectively. Indeed, increased synaptosomal $[Ca^{2+}]_i$, which would produce an effect in contrast to our observations, has been reported for halothane concentrations exceeding 3 mM (48). Third and most important, halothane reduced BK channel activity in excised membrane patches where the cytoplasmic Ca^{2+} concentration was fixed to a constant level. Thus, a direct

[1] J. J. Pancrazio and C. Lynch, unpublished observations.

anesthetic effect on BK channels accounts largely for the depression of whole-cell $I_{K(Ca)}$.

The effect of halothane on $I_{K(Ca)}$ was significantly antagonized by an elevation of $[Ca^{2+}]_o$. Although our whole-cell recordings cannot exclude the activation with elevated $[Ca^{2+}]_o$ of a separate class of Ca^{2+}-dependent K^+ channels that are less sensitive to halothane, a more likely hypothesis is that increased $[Ca^{2+}]_o$ may antagonize the effect of halothane on the same population of BK channels. In the absence of an external Ca^{2+} binding site, increased $[Ca^{2+}]_o$ presumably enhances Ca^{2+} entry, resulting in augmented transient $[Ca^{2+}]_i$, which then counteracts the depressant effect of halothane. Consistent with the whole-cell observations, elevated Ca^{2+} at the cytoplasmic side of the membrane patches significantly reduced the effectiveness of halothane on the p_o of the BK channels. These results suggest that the anesthetic acts, at least in part, by interfering with a Ca^{2+}-binding step of BK channel activation. Our single-channel results can be interpreted within the context of a BK channel gating model similar to that proposed by Moczydlowski and Latorre (33) for BK channels from rat plasma membranes:

$$\cdots C \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} O_B \underset{k_{-4}}{\overset{k_4[Ca^{2+}]}{\rightleftharpoons}} O_L$$

where C, O_B, and O_L represent a closed state, a brief open state, and a longer lasting open state, respectively. In the presence of ~100 nM Ca^{2+} and halothane, the second open state, O_L, was less prominent, perhaps due to anesthetic-induced reductions in k_4. Our data, however, cannot exclude possible anesthetic effects on closed state transitions, which may exhibit Ca^{2+} dependence (33). In fact, anesthetic effects on closed state transitions may well explain the marked reduction in the frequency of channel openings.

Several previous studies have shown that Ca^{2+}-dependent K^+ flux is depressed by anesthetics. Scharff and Foder (49) reported that the Ca^{2+}-dependent K^+ channel-mediated hyperpolarization in red blood cells is inhibited by halothane with an ED_{50} of 0.5 mM. Likewise, $^{86}Rb^+$ flux through Ca^{2+}-activated K^+ channels of rat glioma C6 cells is potently diminished by halothane (50). The local anesthetic procaine has been shown to affect BK channels of arterial and intestinal smooth muscle cells by depressing the open channel conductance (51, 52), a mechanism distinct from that presented in this study. Of particular interest, halothane has been reported to depress the fast and slow AHPs of hippocampal neurons with no impairment of voltage-activated Ca^{2+} entry (40, 53). Both AHP phases are carried by Ca^{2+}-dependent K^+ channels; specifically, the fast AHP is mediated by BK channels, whereas the slow AHP is due to intermediate conductance "SK" Ca^{2+}-activated K^+ channels (54). During the hippocampal epileptiform burst potential the fast AHP is triggered, which acts to repolarize the membrane. Suppression of this AHP phase increases the epileptiform burst duration (55). Accordingly, the incidence and duration of spontaneous firing of hippocampal neurons are escalated with low concentrations of halothane or isoflurane (40). We speculate that the inhalational agents alter hippocampal BK, and possibly SK, channel activity in a manner similar to that observed in chromaffin cells.

Excitatory effects of inhalational anesthetics on neuronal tissue are not uncommon. Transient excitation of the squid axon by general anesthetics, including halothane, appears to be due to reduction of a resting K^+ conductance component (56). Using isolated crayfish stretch receptors, MacIver and Roth (57) showed that a large number of anesthetic agents, including halothane, produce biphasic responses, that is, excitation at low concentrations and depression at higher levels. More recently, volatile agents have been shown to excite nociceptor fiber afferents in a manner mimicked by K^+ channel blockade (58). Therefore, the view that inhalational agents exclusively exert depressant effects in neuronal systems is not supported by electrophysiological studies.

Volatile anesthetics appear to produce a diverse number of effects on voltage-gated channels. The cellular bioelectrical response, either excitation or depression, to an anesthetic most likely depends on the individual anesthetic sensitivities of the channel types and other signaling processes expressed by that particular cell. A major point of interest of this study is that only some membrane targets are functionally altered by anesthetics at concentrations close to the clinically relevant range. Our results indicate that Ca^{2+}-activated BK K^+ channels, which are expressed in a diverse array of cell types including smooth muscle and pancreatic β cells (for review, see Ref. 32), may represent an anesthetic site of action. Several cell types, including endothelial cells, rely on Ca^{2+}-dependent K^+ channels to maintain a strong driving force ($V-E_{Ca}$) for Ca^{2+} during ligand-gated Ca^{2+} entry (59). Although the extent to which this mechanism exists in neuronal cells is not clear, it is conceivable that blockade of Ca^{2+}-activated K^+ channels may, under certain conditions, decrease Ca^{2+} influx to modulate Ca^{2+}-dependent cell function.

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