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Halothane and Isoflurane Decrease the Open State Probability of K^+ Channels in Dog Cerebral Arterial Muscle Cells

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Background: **Both halothane and isoflurane evoke cerebral vasodilation. One of the potential mechanisms for arterial vasodilation is enhanced K^+ efflux resulting from an increased opening frequency of membrane K^+ channels. The current study was designed to determine the effects of volatile anesthetics on K^+ channel current in single vascular smooth muscle cells isolated from dog cerebral arteries.**

Methods: **Patch clamp recording techniques were used to investigate the effects of volatile anesthetics on macroscopic and microscopic K^+ channel currents.**

Results: **In the whole-cell patch-clamp mode, in cells dialyzed with pipette solution containing 2.5 mM EGTA and 1.8 mM $CaCl_2$, depolarizing pulses from −60 to +60 mV elicited an outward K^+ current that was blocked 65 ± 5% by 3 mM tetraethylammonium (TEA). Halothane (0.4 and 0.9 mM) depressed the amplitude of this current by 18 ± 4% and 34 ± 6%, respectively. When 10 mM EGTA was used in the pipette solution to strongly buffer intracellular free Ca^{2+}, an outward K^+ current insensitive to 3 mM TEA was elicited. This K^+ current, which was reduced 51 ± 4% by 1 mM 4-aminopyridine, was also depressed by 17 ± 5% and 29 ± 7% with application of 0.4 and 0.9 mM halothane, respectively. In cell-attached patches using 145 mM KCl in the pipette solution and 5.2 mM KCl in the bath, the unitary conductance of the predominant channel type detected was 99 pS. External application of TEA (0.1 to 3 mM) reduced the unitary current amplitude of the 99 pS K^+ channel in a concentration-dependent manner. The open state probability of this 99 pS K^+ channel was increased by 1 μM Ca^{2+} ionophore (A23187). These findings indicate that the 99 pS channel measured in cell-attached patches was a TEA-sensitive Ca^{2+}-activated K^+ channel. Halothane and isoflurane reversibly decreased the open state probability (NPo), mean open time, and frequency of opening of this 99 pS K^+ channel without affecting single channel amplitude or the slope of the current-voltage relationship.**

Conclusions: **Halothane and isoflurane suppress the activity of K^+ channels in canine cerebral arterial cells. These results suggest that mechanisms other than K^+ channel opening likely mediate volatile anesthetic-induced vasodilation. (Key words: Anesthetics, volatile: halothane; isoflurane. Circulation, cerebral: middle cerebral artery. Current: K^+. Patch-clamp: single channel.)**

THE volatile anesthetic halothane is a potent cerebral vasodilator. It increases cerebral blood flow in a concentration-dependent manner both in animals[1] and humans.[2] Because isoflurane causes a relatively less cerebral vasodilation than does halothane,[3] it is the anesthetic of choice for neurosurgical procedures. Both isoflurane and halothane also relax other vascular beds including isolated rat[4] and rabbit aorta,[5] canine carotid artery,[6] and porcine coronary artery.[7] Although volatile anesthetics are potent vasodilators, the mechanisms by which they relax vascular smooth muscle are not well understood. Recent studies from our laboratory have shown that volatile anesthetics suppress voltage-dependent Ca^{2+} channel current in both cardiac[8,9] and vascular smooth muscle cells.[10] These findings indicate that one possible mechanism by which volatile anesthetics dilate vascular smooth muscle might be *via* a direct effect on Ca^{2+} channel currents.

However, one other potential mechanism for arterial vasodilation is enhanced K^+ efflux, enabled by an increased open state probability of membrane K^+ channels. Based on pharmacologic and biophysical properties, a number of K^+ channel types have been identified in vascular smooth muscle membranes.[11–18] The

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most common K^+ channel in vascular smooth muscle is the Ca^{2+}-activated K^+ channel. The open state probability of this channel is low at resting membrane potential but increases in proportion to membrane depolarization and elevation of intracellular Ca^{2+} concentration.[11–13] The resulting enhanced K^+ efflux will induce membrane repolarization or hyperpolarization,[14–15] reduce the open state probability of voltage-dependent Ca^{2+} channels, and in turn cause vascular relaxation. In addition to this K^+ channel type, an outwardly rectifying (delayed rectifier) K^+ current that is voltage- but not Ca^{2+}-dependent has been observed in vascular muscle membranes from cat cerebral artery,[16] rabbit pulmonary artery,[17] and rabbit portal vein.[18] An inward rectifier K^+ current activated at potentials close to the K^+ equilibrium potential also has been reported in vascular smooth muscle.[19] This K^+ channel type may play a role in setting the level of the resting membrane potential.[19] Another distinct K^+ channel in vascular smooth muscle cells is the ATP-sensitive (K_{ATP}) channel that is activated by low cytosolic ATP levels.[20] The K_{ATP} channel is only weakly voltage-sensitive and is not activated by changes in intracellular Ca^{2+} concentrations.

Even though a substantial number of K^+ channel types are found in vascular smooth muscle cells, the direct effect of volatile anesthetics on these K^+ channels in arterial membranes is not well understood. Therefore, in the current study we applied the patch-clamp technique to examine the effect of halothane and isoflurane on K^+ channel currents in isolated dog cerebral arterial muscle cell membranes.

Methods

These experiments were approved by the Medical College of Wisconsin Animal Care and Use Committee.

Cell Isolation

Adult mongrel dogs of either sex weighing 15–25 kg were placed in a plexiglass box and anesthetized with halothane. After attainment of surgical anesthesia, brains were removed. The middle cerebral arteries were dissected free of arachnoid and placed in cold Krebs' solution. Vessels were cut into small pieces and placed in 2-ml vials containing enzyme solution of the following composition (mM): NaCl 135, KCl 5.2, $MgCl_2$ 1.0, HEPES 10, $CaCl_2$ 0.04, glucose 10, and collagenase CLS II 500 U/ml (Worthington Biochemical, Freehold, NJ), dithiothreitol 4 mM (Sigma Chemical, St. Louis, MO) and papain 2 U/ml (Worthington Biochemical, Freehold, NJ). The enzyme solution was maintained at 37°C and stirred at 10 rpm by a microstirrer for 1–1.5 h. The supernatant was then removed and diluted with Tyrode's solution of the following composition (mM): NaCl 135, KCl 5.2, $MgCl_2$ 1.0, HEPES 10, $CaCl_2$ 1.8, and glucose 10. The dispersed cells were kept at 4°C until used.

Voltage-Clamp Recording

Dispersed cells were placed in a perfusion chamber (22°C) on the stage of an inverted microscope (IMT-2, Olympus Optical, Tokyo, Japan) equipped with modulation contrast. At 500× magnification, a hydraulic micromanipulator (MO-203, Narishige, Tokyo, Japan) was used to position heat-polished borosilicate patch pipettes with tip resistance of 1–5 MΩ on the membranes of single cerebral arterial muscle cells. Once high resistance seals (2–10 GΩ) were formed, the pipette patch was removed by negative pressure to give access to the whole cell. Whole-cell K^+ currents were elicited every 5–10 s by 200 ms depolarizing pulses generated by a computerized system (PClamp Software, Axon Instruments, Burlingame, CA). The currents were amplified by a List EPC-7 patch-clamp amplifier (List-Electronics, Darmstadt-Eberstadt, Germany), and the amplifier output was low-pass filtered at 1 KHz. All data were digitized (sampling rate = 10,000/s) and stored on a hard disk to permit analysis at a later time. Leak and capacitive currents in whole-cell recording were subtracted from each record by linearly summating scaled currents obtained during 10 mV hyperpolarizing pulses.

The composition of the pipette solution used for whole-cell K^+ current recording was (in mM): KCl 70, K^+-glutamate 60, K_2ATP 5, EGTA 2.5, HEPES 10, $MgCl_2$ 1, and $CaCl_2$ 1.8; the computer-calculated free intracellular $[Ca^{2+}]$, using the program Chelator by Theo J. M. Shoenmakers, was 10^{-6} M (pH = 7.2). A variant of this solution contained 10 mM EGTA and zero $CaCl_2$, giving a free $[Ca^{2+}]_i$ of $\leq 10^{-9}$ M. The external solution contained (in mM): $CaCl_2$ 1.8, NaCl 135, KCl 5.2, $MgCl_2$ 1.0, HEPES 10, and glucose 10 [pH = 7.4].

Single channel currents were recorded in a cell-attached configuration using the standard patch-clamp technique.[13] The bath solution was the same as that used for whole-cell recordings. The pipette solution contained (mM): KCl 145, $CaCl_2$ 1.8, $MgCl_2$ 1.1, and HEPES 5 (*p*H = 7.4).

Statistical analysis of single channel activity was performed using Pstat software (Axon, pClamp version 5.5). The mean open time, channel open probability (Po), and amplitude were analyzed for each cell and stored. Transitions from closed to open states were defined as a change in 50% below or above baseline relative to the predominant channel amplitude. The open state probability (NPo) was defined by the relation, NPo = I/i, where I is the time-averaged mean current, N is number of channels, i is single-channel current amplitude, and Po is channel open probability. Current-voltage relationships were fitted by least-square linear regression for determination of slope conductance.

After recording of K^+ currents in control solutions, the inflow perfusate was changed to one in which a given concentration of anesthetic agent had been equilibrated at room temperature. Halothane was prepared in final bath concentrations of 0.4 and 0.9 mM, which was equivalent to 0.7 and 1.5%, respectively.[21] Isoflurane was prepared in a final bath concentration of 1.2 mM, which was equivalent to 2.7%. Anesthetic content of the perfusate from the perfusion chamber was sampled and verified by gas chromatography. Drug effects were completed within 2–3 min, and measurements were made after stabilization of drug-induced changes. The inflow perfusate containing the anesthetics was then changed to the control solution, and K^+ channel currents were measured again.

Isometric Tension Recording

Rings of middle cerebral arteries were mounted between two Tungsten wire triangles in 15 ml water-jacketed organ bath. Isometric tension was recorded on a model 7 Grass polygraph (Quincy, MA). The temperature was maintained at 37°C. The rings were stretched progressively to a final optimal tension of approximately 750 mg. The optimal tension was previously determined by length-tension studies using 40 mM KCl (unpublished observation). The integrity of each ring was examined by the contractile response to 40 mM KCl added to the bathing media. Isometric contractions were determined in middle cerebral arterial rings that were equilibrated in physiologic salt solution (*p*H 7.4) of the following composition (in mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.6, $MgSO_4$ 1.17, $NaHCO_3$ 27.8, NaH_2PO_4 1.18, EDTA 0.026, glucose 5.5, and HEPES (4(2-)2-hydroxyethyl)(-1-piperazineethane-sulfonic acid) 5. The solution was aerated with 93.5% O_2 and 6.5% CO_2.

The arterial rings were washed repeatedly, and after a 60-min equilibration period, they were contracted with $PGF_{2\alpha}$ (3 μM) before and after 15 min exposure to tetraethylammonium. During a stable $PGF_{2\alpha}$-induced contraction, different concentrations of halothane or isoflurane were bubbled into the tissue bath using a vaporizer (Dragerwerk, Lubeck, Germany) to determine a dose-response relation. The preparations were exposed to volatile anesthetics for 15 min. Arterial rings were rinsed and rested for 30 min between exposures to different anesthetics. The concentrations of halothane (0.29 mM and 0.43 mM equivalent to 0.98% and 1.5%, respectively, at 37°C) and isoflurane (0.21 mM and 0.43 mM equivalent to 1.1% and 2.1%, respectively, at 37°C) in the bath were determined by gas chromatography.

Statistics

Results are expressed as mean ± SEM. Where appropriate, data were analyzed by Student's *t* test ($P < 0.05$).

Results

Effect of Halothane on Macroscopic K^+ Current

Figure 1A depicts a macroscopic outward current obtained from single canine middle cerebral arterial smooth muscle cell dialyzed with a pipette solution containing a free intracellular calcium concentration of 1 μM to enhance Ca^{2+}-dependent K^+ current. The current was activated progressively by 200 ms depolarizing pulses from a holding potential of −60 mV (fig. 1A, *top*) to consecutive more positive membrane potentials. Stepwise depolarization from a holding potential of −60 mV to beyond −30 mV elicited an outward current, which showed a mean peak amplitude of 1041 ± 140 pA at +60 mV (n = 10). We examined the effect of 3 mM tetraethylammonium (TEA), a Ca^{2+}-activated K^+ channel blocker,[11,13] on this outward current. As illustrated in figure 1B, addition of TEA significantly decreased peak current amplitude at +60 mV by 65 ± 5%.

The effects of halothane on this TEA-sensitive K^+ current was examined in eight cells. Representative tracings showing the effects of low and high doses of halothane on K^+ currents elicited by depolarizing pulses (200 ms duration) from −60 mV to +60 mV are presented in figure 2 (*top*). Halothane reversibly and dose-dependently suppressed macroscopic K^+ channel current. The effects of halothane on the current-voltage (I–V) relationship for K^+ channel activation are shown

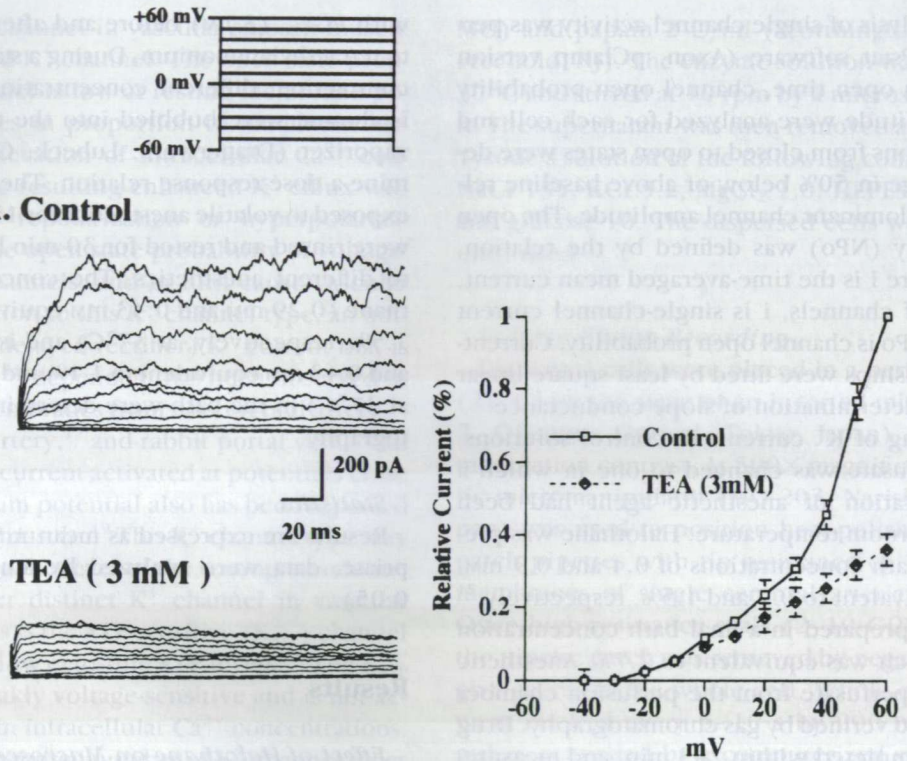

Fig. 1. Whole-cell outward K^+ current in a single canine cerebral arterial muscle cell dialized with 1.8 mM $CaCl_2$ and 2.5 mM EGTA before (*A*) and after (*B*) addition of 3 mM tetraethylammonium (TEA). Cells were progressively depolarized from a holding potential of −60 mV to the corresponding potentials indicated on the pulse protocol. An outward current was elicited whose amplitude was markedly depressed by TEA. (*C*) Peak current-voltage relations obtained before and after exposure to 3 mM TEA in two canine cerebral arterial muscle cells.

in figure 2 (*bottom*). This anesthetic agent produced concentration-dependent suppression of the K^+ channel current amplitude over the entire voltage range studied, without shifting the voltage-dependency of the I–V relationship. Low and high concentrations of halothane depressed peak K^+ current at +60 mV by 18 ± 4% and 34 ± 6%, respectively.

In a separate series of experiments, we used a whole-cell mode in which $[Ca^{2+}]_i$ was strongly buffered with 10 mM-EGTA in the pipette solution[11] to minimize opening of the Ca^{2+}-dependent K^+ channel current and examined the effect of halothane on Ca^{2+}-independent K^+ channel current. Figure 3A depicts a macroscopic outward current recorded from canine middle cerebral arterial muscle cell and represents the most commonly encountered current pattern. The current was activated progressively by 200 ms depolarizing pulses from a holding potential of −60 mV (fig. 3A, *top*) to consecutive more positive membrane potentials. The mean peak current in these cells was 423 ± 58 pA at +60 mV (n = 7). This outward current displayed properties of a delayed rectifier K^+ current, that is the rate of activation became faster at more positive voltages and exhibited very little inactivation during 200 ms command pulses. Application of 3 mM TEA had little effect on this outward current (n = 4). Addition of 1 μM Ca^{2+} ionophore (A23187) also did not affect the amplitude of this outward K^+ current (n = 3), indicating that the high EGTA concentration (10 mM) in the pipette solution strongly buffered changes in $[Ca^{2+}]_i$ to minimize the Ca^{2+}-activated K^+ current in these cells. We then examined the effects of 4-aminopyridine (1 mM, 4-AP), a K^+ channel blocker, on this outward current. Recordings in figure 3B show that 4-AP significantly decreased the peak amplitude of this current. Figure 3C summarizes the current-voltage (I–V) relationship plotted as percent of maximum current before and after the addition of 4-AP to the external solution in four paired experiments. Application of 1 mM 4-AP reduced the peak current at +60 mV by 51 ± 4%. When K^+

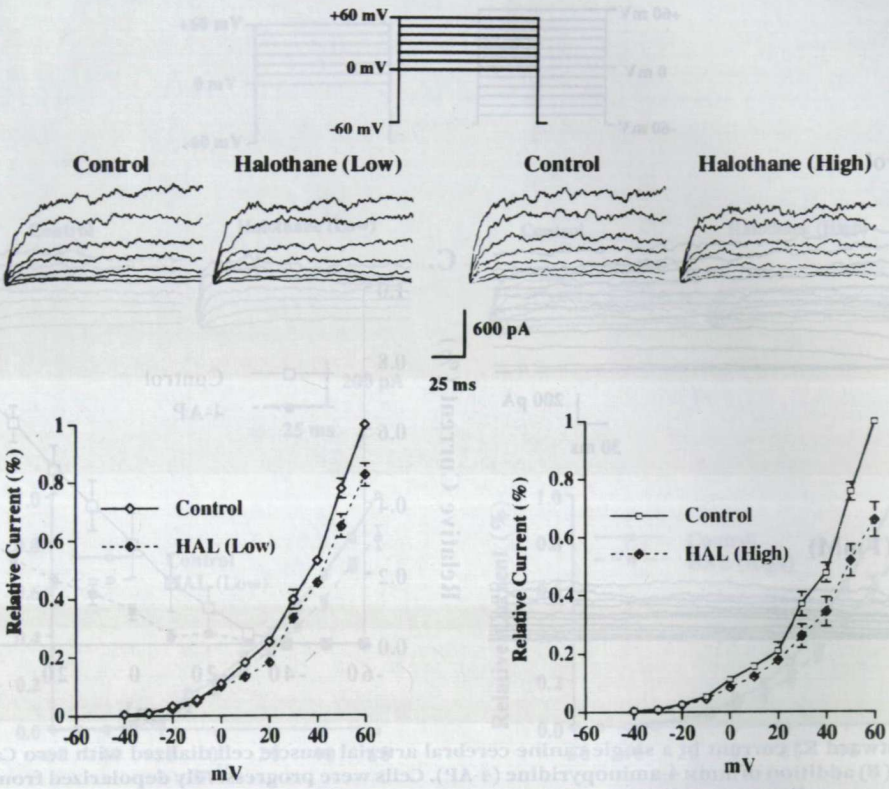

Fig. 2. Modulation of macroscopic K^+ channel current by low (0.4 mM equivalent to 0.7%) and high (0.9 mM, equivalent to 1.5%) concentrations of halothane in canine cerebral arterial muscle cells dialized with 1.8 mM CaCl and 2.5 mM EGTA. (*Top*) The pulse protocol and actual recordings of macroscopic K^+ current traces in control solution and after exposure to low or high doses of halothane. Voltage steps from a holding potential (HP) of −60 mV to the corresponding potentials elicited an outward current whose amplitude was depressed markedly and reversibly by halothane. (*Bottom*) The peak current-voltage relationship in control solution and after exposure to low and high doses of halothane, 0.4 (n = 4) and 0.9 mM (n = 5), respectively. Cells were depolarized progressively from a HP of −60 mV to the corresponding potentials indicated on the abscissa. Points with error bars represent the mean ± SEM.

glutamate and KCl were replaced with CsCl in the pipette solution, we were unable to measure an outward current (n = 4), suggesting that K^+ was the charge carrier.

We studied the effects of halothane on this Ca^{2+}-independent K^+ current in 12 cells. Representative tracings showing the effects of low and high doses of halothane on the Ca^{2+}-independent K^+ channel currents, elicited by depolarizing pulses (200 ms duration) from −60 mV to +60 mV, are presented in figure 4 (*top*). Halothane reversibly and dose-dependently suppressed macroscopic K^+ channel current. The effect of halothane on the current-voltage (I–V) relationship for K^+ channel activation is shown in figure 4 (*bottom*). Low and high doses of this anesthetic agent produced concentration-dependent suppression of K^+ channel current amplitude over the entire voltage range studied, without shifting the voltage dependency of the I–V relationship. Low and high concentrations of halothane depressed peak K^+ current at +60 mV by 17 ± 5% and 29 ± 7%, respectively. However, there was no significant difference in the sensitivity of Ca^{2+}-dependent and -independent K^+ currents to halothane.

Effect of Halothane and Isoflurane on Single-channel K^+ Current

After macroscopic current measurements, the effects of halothane also were examined on single-channel K^+ currents recorded from cell-attached patches of dog middle cerebral arterial muscle cells. We also analyzed the effect of isoflurane on single-channel K^+ currents, because our laboratories have shown earlier that this anesthetic agent also decreases whole-cell K^+ current in cerebral arterial muscle cells.[22] Figure 5A shows

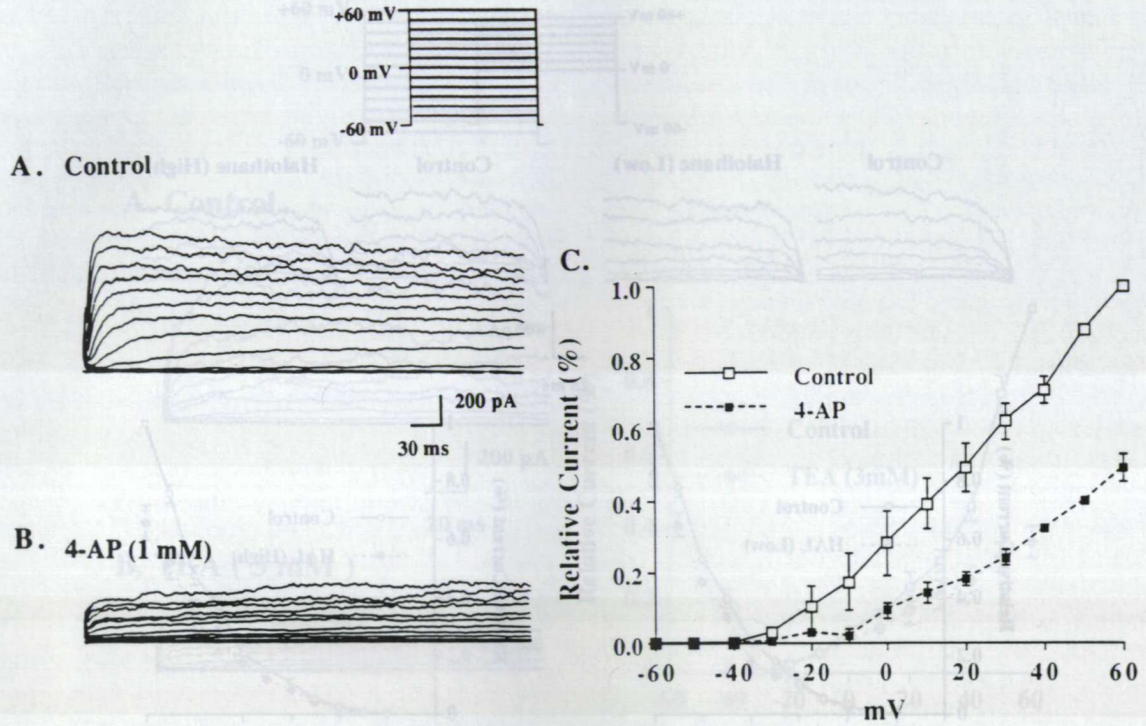

Fig. 3. Whole-cell outward K^+ current in a single canine cerebral arterial muscle cell dialized with zero Ca^{2+} and 10 mM EGTA before (*A*) and after (*B*) addition of 1 mM 4-aminopyridine (4-AP). Cells were progressively depolarized from a holding potential of −60 mV to the corresponding potentials indicated on the pulse protocol. An outward current was elicited whose amplitude was markedly depressed by 4-AP. (*C*) Peak current-voltage relations obtained before and after exposure to 1 mM 4-AP in four canine cerebral arterial muscle cells. Points with error bars represent the mean ± SEM.

representative tracings of single-channel K^+ currents recorded at various pipette potentials from cell-attached patches. The current-voltage relationship of this single-channel K^+ current recorded from six cells revealed a unitary slope conductance of 99 pS when determined at pipette potentials between +40 mV and −40 mV (fig. 5B). Assuming that intracellular K^+ concentration was comparable to that in the pipette solution, the absence of detectable K^+ currents at a pipette potential of −60 mV suggested that intracellular membrane potential may reflect this value. When incremental negative pipette potentials were applied, there was reversal of single-channel current at potentials negative to −60 mV (fig. 5). Application of tetraethylammonium (TEA, 0.1–3.0 mM) to the extracellular membrane surface *via* the pipette solution, produced a concentration-dependent reduction of the single-channel current amplitude (fig. 6). Figure 7 illustrates representative tracings showing the effect of the Ca^{2+} ionophore (A23187) on single channel K^+ currents. Addition of the Ca^{2+} ionophore (A23187, 1 μM) in the bathing solution increased the open state probability of the channel from 0.015 ± 0.001 to 0.040 ± 0.021 ($p < 0.05$) with out a significant reduction on unitary current amplitude. These findings suggest that the 99 pS K^+ channel is a Ca^{2+}-activated K^+ channel. In some patches, a smaller amplitude current also was observed, indicating the presence of second channel type or a subconductance state of the 99 pS K^+ channel (fig. 8). These smaller openings were observed in only 10% of patches. However, the detection of this channel may have been influenced by the predominance of the 99 pS channel type, which made single-channel analysis of the small amplitude current difficult.

The effects of halothane and isoflurane on these 99 pS K^+ currents were determined in cell-attached patches of dog middle cerebral arterial muscle cells. Figure 9 depicts the summary of statistical analysis of mean open time, open state probability, and event frequency obtained before, during, and after exposure to 0.9 mM halothane. Halothane decreased event fre-

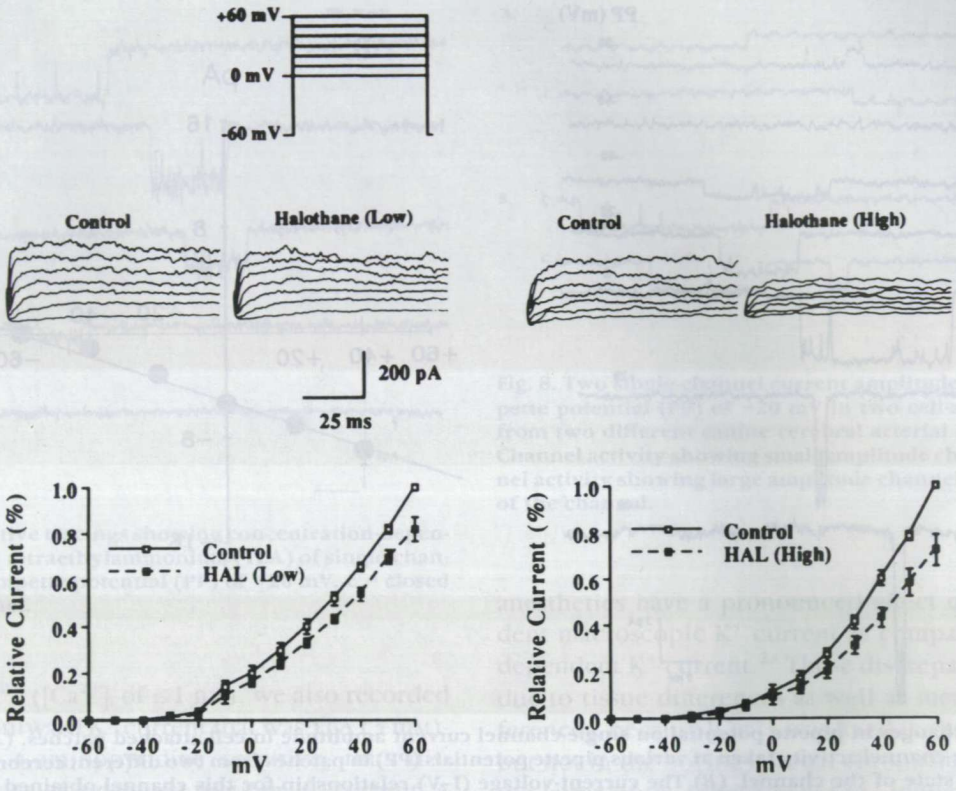

Fig. 4. Modulation of macroscopic K^+ channel current by low (0.4 mM equivalent to 0.7%) and high (0.9 mM, equivalent to 1.5%) concentrations of halothane in canine cerebral arterial muscle cells dialized with zero Ca^{2+} and 10 mM EGTA. (*Top*) The pulse protocol and actual recordings of macroscopic K^+ current traces in control solution and after exposure to low or high doses of halothane. Voltage steps from a holding potential (HP) of −60 mV to the corresponding potentials elicited an outward current whose amplitude was depressed markedly and reversibly by halothane. (*Bottom*) The peak current-voltage relationship in control solution and after exposure to low and high doses of halothane, 0.4 (n = 6) and 0.9 mM (n = 6), respectively. Cells were depolarized progressively from a HP of −60 mV to the corresponding potentials indicated on the abscissa. Points with error bars represent the mean ± SEM.

quency from 28.1 ± 6.9 to 14.9 ± 3.4 per 2-min recording interval, the mean open time from 14.1 ± 1.3 ms to 9.4 ± 1.5 ms (fig. 9; n = 9, $P < 0.05$), and the open state probability (NP_0) of the 99 pS K^+ channels from 0.011 ± 0.005 to 0.004 ± 0.002. Figure 10 shows representative tracings obtained before, during, and after exposure to 0.9 mM halothane at a pipette potential of −20 mV. The number of openings was greatly reduced during exposure to halothane. This effect of halothane on single-channel was reversed when the inflow perfusate was changed to the control solution. Halothane (0.9 mM) did not affect single channel amplitude, which was −3.0 ± 0.2 pA and −2.9 ± 0.2 pA at a pipette potential of −20 mV in the absence and presence of halothane, respectively (n = 9). This anesthetic had no significant effect on the slope of the I–V relationship, which was 99 pS in the absence and 93 pS in its presence. Similarly, the distribution of the unitary current amplitude was not altered, suggesting that halothane did not influence a separate conductance pathway but rather decreased the activity of the same channel type.

Similar experiments were performed at a single-channel level to examine the effects of isoflurane (1.2 mM) on the 99 pS K^+ channel. Figure 11 depicts single-channel recordings obtained before, during, and after exposure to 1.2 mM isoflurane. Single channel currents were recorded at a pipette potential of −20 mV. Similar to halothane, the number of single channel events seen during exposure to isoflurane was reversibly reduced; also like halothane, isoflurane did not affect single channel amplitude which was −3.4 ± 0.2 pA and −3.3 ± 0.2 pA at a pipette potential of −20 mV in the absence and in the presence of isoflurane, respectively (n = 5).

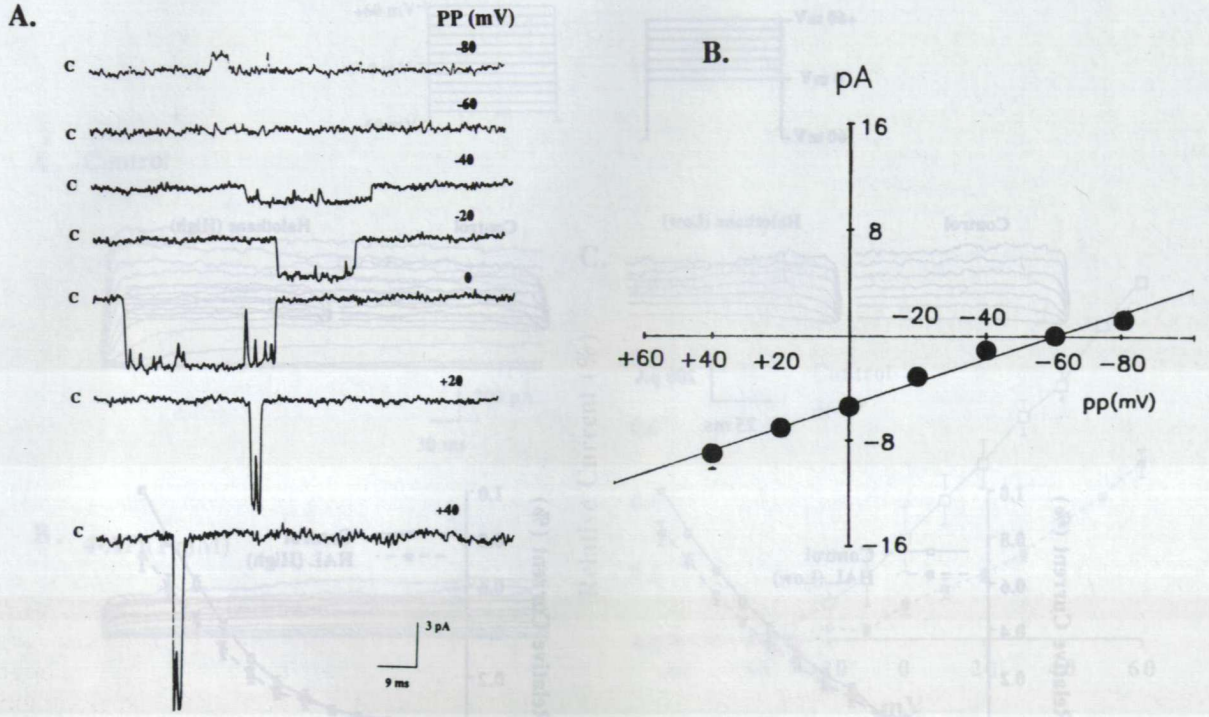

Fig. 5. Effect of changes in pipette potential on single-channel current amplitude in cell-attached patches. (*A*) Representative tracings of single-channel activity taken at various pipette potentials (PP) in patches from two different cerebral arterial muscle cells. c = closed state of the channel. (*B*) The current-voltage (I–V) relationship for this channel obtained from six arterial membrane patches. The slope of the curve determined *via* linear regression analysis was 99 pS. Each point represents the mean ± SEM.

Isoflurane decreased event frequency from 21.0 ± 3.6 to 10.7 ± 3.7 per 2-min recording interval, and the mean open time from 19.1 ± 2.7 ms to 13.0 ± 1.5 ms. The open state probability (NP_o) was decreased from 0.0109 ± 0.0025 to 0.0053 ± 0.0019 in the presence of isoflurane.

Effect of Tetraethylammonium on Volatile Anesthetic-induced Vasodilation

In an attempt to examine whether blockade of the Ca^{2+}-activated K^+ channel interferes with anesthetic-induced vasodilation of middle cerebral arteries, the effects of halothane and isoflurane was determined before and after treatment of the vessels with TEA. The vasodilator action of halothane and isoflurane on middle cerebral arterial rings (number of vessels = 28, number of animals = 4) preconstricted with $PGF_{2\alpha}$ (3 μM) in the absence and presence of TEA are summarized in table 1. Treatment of the vessels with 1 or 3 mM TEA, a blocker of the Ca^{2+}-activated K^+ channel,[11,13] produced no significant effect on halothane- or isoflurane-induced vasodilation in canine cerebral arterial vessels. These data suggest that the anesthetic-induced vasodilation in cerebral arterial smooth muscle does not involve Ca^{2+}-dependent K^+ channels.

Discussion

In the current study, using 2.5 mM EGTA and 1.8 mM $CaCl_2$ (intracellular $[Ca^{2+}]$ of 1 μM) in the pipette solution, we have recorded a macroscopic outward K^+ current that was suppressed by 3 mM TEA in dog cerebral arterial muscle cells. The current did not inactivate significantly over a period of 200 ms. The rate of activation of this current became faster at more positive potentials demonstrating voltage-dependence of activation. A similar TEA-sensitive Ca^{2+}-activated macroscopic K^+ current has been described in number of vascular smooth muscle cells including rabbit portal veins[12,18] and rat mesenteric arteries.[11] In dog cerebral arterial muscle cells dialyzed with pipette solution containing zero Ca^{2+} and 10 mM EGTA to buffer the

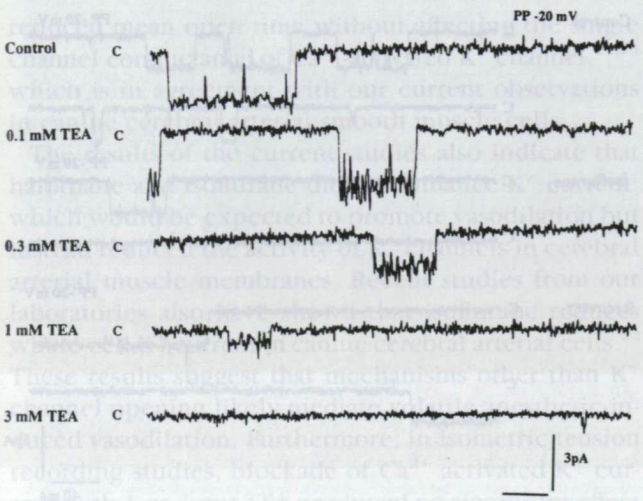

Fig. 6. Representative tracings showing concentration-dependent reduction by tetraethylammonium (TEA) of single-channel amplitude at pipette potential (PP) of −20 mV. c = closed state of the channel.

intracellular Ca^{2+} ($[Ca^{2+}]_i$ of ≤1 nM), we also recorded a macroscopic outward K^+ current that was TEA (3 mM)-insensitive but 4-aminopyridine sensitive, thus displaying properties of a delayed rectifier K^+ current. A similar outward, delayed rectifier K^+ current has been described in cat cerebral[16] and rabbit pulmonary arterial muscle cells.[17]

The inhalational anesthetic halothane similarly depressed these two K^+ current types in canine cerebral arterial cells. Unlike these results, recordings in bovine adrenal chromaffin cells have shown that inhalational

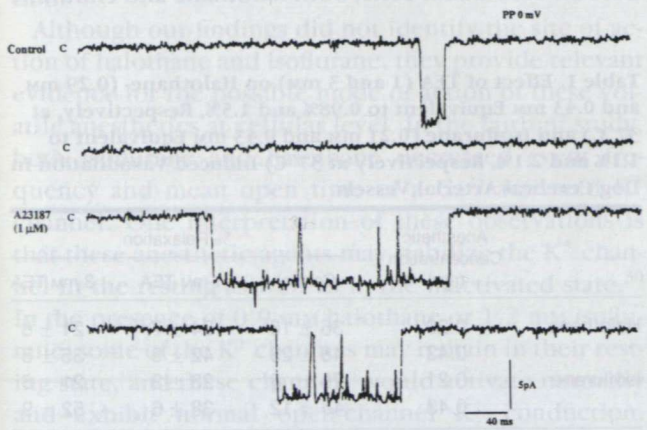

Fig. 7. Representative traces showing the effect of Ca^{2+} ionophore (A23187, 1 μM) on single-channel activity at pipette potential (PP) of 0 mV. c = closed state of the channel.

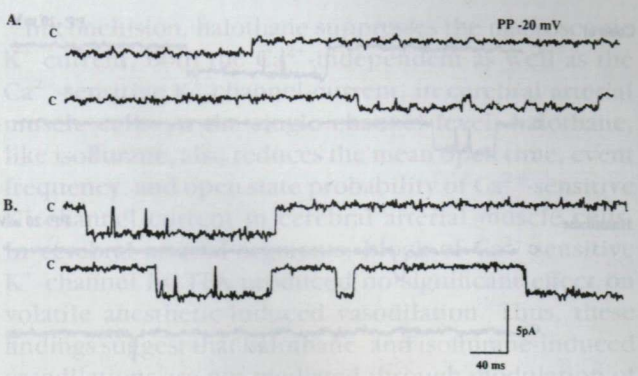

Fig. 8. Two single-channel current amplitudes recorded at pipette potential (PP) of −20 mV in two cell-attached patches from two different canine cerebral arterial muscle cells. (*A*) Channel activity showing small amplitude channel. (*B*) Channel activity showing large amplitude channel. c = closed state of the channel.

anesthetics have a pronounced effect on Ca^{2+}-dependent macroscopic K^+ current as compared to Ca^{2+}-independent K^+ current.[23] These discrepancies might be due to tissue differences as well as methodologic differences, because $[Ca^{2+}]_i$ was fixed in the current study but not in the bovine adrenal chromaffin cell study. In the latter study, blockade of Ca^{2+} influx by anesthetics may have contributed to the more pronounced depression of Ca^{2+}-sensitive K^+ channel current.[23]

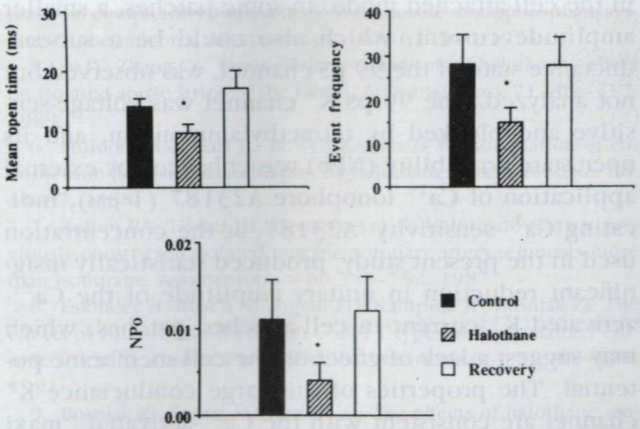

Fig. 9. Summary of statistical analysis of mean open time, open state probability (NPo), and event frequency (pipette potential = −20 mV) single-channel K^+ currents recorded from isolated canine middle cerebral arterial muscle cells before, during, and after halothane (0.9 mM, n = 9). All parameters were decreased significantly in the presence of halothane. This effect of halothane was reversed when the inflow perfusate was changed to the control solution. *Significant difference from control values at $P < 0.05$.

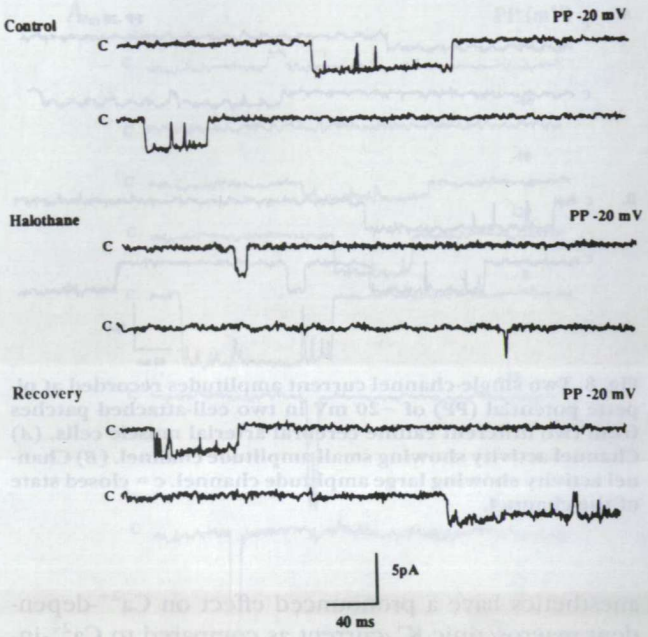

Fig. 10. Representative traces of single-channel activity before, during, and after exposure to halothane (0.9 mM, equivalent to 1.5%) at pipette potential (PP) of −20 mV. c = closed current state.

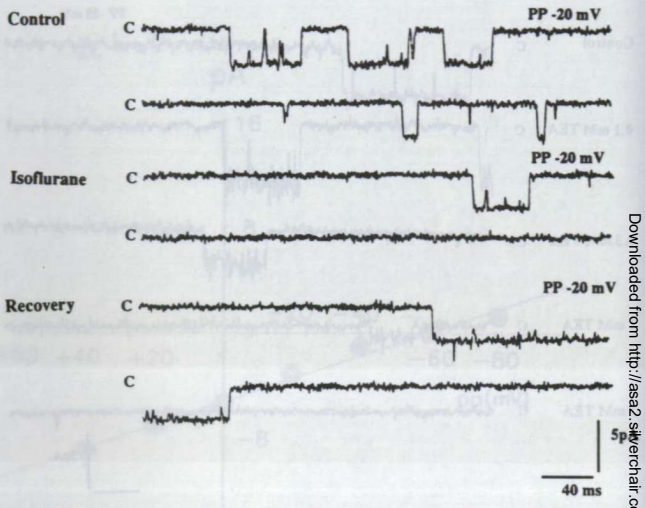

Fig. 11. Representative traces of single-channel activity before, during, and after exposure to isoflurane (1.2 mM, equivalent to 2.7%) at pipette potential (PP) of −20 mV. c = closed channel state.

In the current study, using 145 mM KCl in the patch pipette and 5.2 mM KCl in the bath, we recorded a predominant potassium channel type with a unitary conductance of 99 pS from dog cerebral arterial muscle in the cell-attached mode. In some patches, a smaller amplitude current, which also could be a subconductance state of the 99 pS channel, was observed but not analyzed. The 99 pS K^+ channel was voltage-sensitive and blocked by tetraethylammonium, and its open state probability (NPo) was enhanced by external application of Ca^{2+} ionophore A23187 (1 μM), indicating Ca^{2+}-sensitivity. A23187, at the concentration used in the present study, produced statistically insignificant reduction in unitary amplitude of the Ca^{2+}-activated K^+ current in cell-attached patches, which may suggest a lack of effect on the cell membrane potential. The properties of this large conductance K^+ channel are consistent with the Ca^{2+}-activated "maxi K^+" channel described in a number of smooth muscle cell types.[11,13]

The major findings of the current study is that halothane and isoflurane, at clinically relevant concentrations, reduced the open state probability of the predominant Ca^{2+}-activated K^+ channel without changing its single-channel conductance. This decrease in open state probability appears to have resulted from a decrease in both the frequency of channel opening and the mean open time. Although the present study is the first to show the effect of halothane and isoflurane on the activity of single channel K^+ current in vascular muscle membranes, the effects of anesthetics on Ca^{2+}-activated K^+ channel with similar conductance properties have been reported on rat glioma C6 cells,[24] Chara australis,[25] and bovine adrenal chromaffin cells.[23,26] In rat glioma C6 cells, volatile anesthetics, including halothane and isoflurane, reduced rubidium efflux across Ca^{2+}-activated K^+ channels.[24] In bovine adrenal chromaffin cells, both halothane and enflurane

Table 1. Effect of TEA (1 and 3 mM) on Halothane- (0.29 mM and 0.43 mM Equivalent to 0.98% and 1.5%, Respectively, at 37°C) and Isoflurane (0.21 mM and 0.43 mM Equivalent to 1.1% and 2.1%, Respectively at 37°C)-Induced Vasodilation in Dog Cerebral Arterial Vessels

	Anesthetic Concentration (mM)	% Relaxation		
		Control	1 mM TEA	3 mM TEA
Halothane	0.29	36 ± 16	24 ± 7	21 ± 6
	0.43	48 ± 23	42 ± 9	35 ± 8
Isoflurane	0.21	26 ± 6	23 ± 3	21 ± 6
	0.43	45 ± 12	38 ± 6	52 ± 9

Vessel relaxation is expressed as the percentage reduction in tension generated by $PGF_{2\alpha}$ (3 μM). Values are expressed as mean ± SEM (number of vessel segments = 28, number of dogs = 4).

reduced mean open time without affecting the single channel conductance of Ca^{2+}-activated K^+ channel,[23,26] which is in agreement with our current observations in canine cerebral arterial smooth muscle cells.

The results of the current studies also indicate that halothane and isoflurane did not enhance K^+ current, which would be expected to promote vasodilation but instead reduced the activity of K^+ channels in cerebral arterial muscle membranes. Recent studies from our laboratories also have shown that isoflurane reduces whole-cell K^+ current in canine cerebral arterial cells.[22] These results suggest that mechanisms other than K^+ channel opening likely mediate volatile anesthetic-induced vasodilation. Furthermore, in isometric tension recording studies, blockade of Ca^{2+} activated K^+ current with 1 or 3 mM TEA produced no significant effect on halothane- or isoflurane-induced vasodilation. However, recent studies from our laboratories and others have shown that volatile anesthetics reduce whole-cell Ca^{2+} current in cardiac[8,9] as well as in vascular smooth muscle cells.[10] This suppression of Ca^{2+} current may be one of several mechanisms by which halothane and isoflurane relax cerebral arterial muscle. Halothane also has been reported to decrease Ca^{2+} accumulation in the sarcoplasmic reticulum and attenuate the Ca^{2+} activation of contractile proteins in rabbit aortic strips,[5] thus resulting in less Ca^{2+} contractile protein interaction, which is responsible for arterial contraction. Halothane also was reported to increase tissue cGMP levels in mouse heart[27] and canine cerebral arteries,[28] which might be an alternative mechanism for anesthetic-induced vasodilation, as an increase in tissue cGMP level is associated with vascular muscle relaxation.[29]

Although our findings did not identify the site of action of halothane and isoflurane, they provide relevant evidence for the possible mode of action of these volatile anesthetics at cellular level. In the current study, both isoflurane and halothane decreased event frequency and mean open time of a Ca^{2+}-activated K^+ channel. One interpretation of these observations is that these anesthetic agents may stabilize the K^+ channel in the resting as well as in the inactivated state.[30] In the presence of 0.9 mM halothane or 1.2 mM isoflurane, some of the K^+ channels may remain in their resting state, and these channels would activate normally and exhibit normal open-channel K^+ conduction. However, after opening, the channel may go into the inactivated conformation fast or close more rapidly, leading to a net reduction in the open state duration.

In conclusion, halothane suppresses the macroscopic K^+ current, both the Ca^{2+}-independent as well as the Ca^{2+}-sensitive K^+ channel current, in cerebral arterial muscle cells. At the single channel level, halothane, like isoflurane, also reduces the mean open time, event frequency, and open state probability of Ca^{2+}-sensitive K^+ channel current in cerebral arterial muscle cells. In cerebral arterial segments, block of Ca^{2+}-sensitive K^+ channel by TEA produced no significant effect on volatile anesthetic-induced vasodilation. Thus, these findings suggest that halothane- and isoflurane-induced vasodilations are not mediated through modulation of K^+ channels in canine cerebral arterial cells.

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