TOK1 Is a Volatile Anesthetic Stimulated K+ Channel

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Background: Volatile anesthetic agents can activate the S channel, a baseline potassium (K+) channel, of the marine mollusk *Aplysia.* To investigate whether cloned ion channels with electrophysiologic properties similar to the S channel (potassium selectivity, outward rectification, and activation independent of voltage) also are modulated by volatile anesthetic agents, the authors expressed the cloned yeast ion channel TOK1 (tandem pore domain, outwardly rectifying K+ channel) in *Xenopus* oocytes and studied its sensitivity to volatile agents.

Methods: Standard two-electrode voltage and patch clamp recording methods were used to study TOK1 channels expressed in *Xenopus* oocytes.

Results: Studies with two-electrode voltage clamp at room temperature showed that halothane, isoflurane, and desflurane increased TOK1 outward currents by 48–65% in barium Frog Ringer's perfusate. The concentrations at which 50% potentiation occurred (EC$_{50}$ values) were in the range of 768–814 μM (0.016–0.044 atm) and had a rank order of potency in atm in which halothane > isoflurane > desflurane. The potentiation of TOK1 by volatile anesthetic agents was rapid and reversible (onset and offset, 1–20 s). In contrast, the nonanesthetic 1,2-dichlorohexafluorocyclobutane did not potentiate TOK1 currents in concentrations up to five times the MAC value predicted by the Meyer–Overton hypothesis based on oil/gas partition coefficients. Single TOK1 channel currents were recorded from excised outside-out patches. The single channel open probability increased as much as twofold in the presence of isoflurane and rapidly returned to the baseline values on washout. Volatile anesthetic agents did not alter the TOK1 single channel current-voltage (I-V) relationship, however, suggesting that the site of action does not affect the permeation pathway of the channel.

Conclusion: TOK1 is a potassium channel that is stimulated by volatile anesthetic agents. The concentrations over which potentiation occurred (EC$_{50}$ values) were higher than those commonly used in clinical practice (approximately twice MAC). (Key words: Baseline channels; voltage clamp, *Xenopus* oocyte.)

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VOLATILE anesthetic agents exert actions at several ligand-gated and voltage-gated ion channels (reviewed by Franks and Lieb[1]). An additional class of ion channels, which have not been studied closely for anesthetic sensitivity, are those responsible for the voltage-independent leakage currents first described by Hodgkin and Huxley.[2] Some of these baseline channels are receptor coupled, such as potassium-selective S channels, which are coupled to serotonin receptors in *Aplysia* sensory neurons to regulate neurotransmitter release.[3,4] Winegar *et al.*[5] recently reported that volatile anesthetic agents hyperpolarize *Aplysia* neurons by activation of S channel currents, suggesting that baseline channels may be important sites of volatile anesthetic action.

Because the S channel is highly selective for potassium ions, activation of the channel drives the membrane potential toward the equilibrium potential for potassium. Volatile anesthetic agents reversibly hyperpolarize neurons and block spontaneous activity within abdominal ganglia expressing the S channel.[5] This hyperpolarization of neurons induced by volatile anesthetic agents also has been described in the pond snail,[6] rat hippocampus,[7,8] and human cerebral cortex.[9] Outside the central nervous system, unmyelinated C fiber afferents are hyperpolarized by volatile anesthetic agents through an enhanced potassium conductance.[10]

The S channel is a baseline potassium channel (active at all potentials) that is noninactivating (maintains activity during prolonged membrane depolarization) and exhibits outward rectification (an increase in channel conductance with voltage). Because of its tonic activity near the resting membrane potential, the S channel can modulate the resting potential of *Aplysia* sensory neurons. Baseline potassium channels also contribute to the resting membrane potential of thin myelinated vertebrate axons.[11]

Recently, a potassium channel from yeast has been cloned (TOK1) that also has the properties of baseline activity, noninactivation, and outward rectification.[12] One function of TOK1 in yeast may be to balance charge movement during nutrient transport.[13] The pore regions of potassium channels are characterized by a highly conserved signature sequence that is critical for permeation of potassium ions. TOK1 is the first ion channel cloned that has two pore regions in tandem. This feature may allow channels to form as homodimers.[14,15] Because the *Aplysia* S channel has not yet been cloned, it is not known whether the S channel and TOK1 share common structural features.

Because of the similar electrophysiologic properties that TOK1 shares with the S channel, we tested the hypothesis that TOK1 is also sensitive to volatile anesthetic agents. We found that volatile anesthetic agents potentiate both macroscopic and single-channel currents mediated by these channels. We also found that TOK1 activation potency correlated with volatile agent potency *in vivo*.

Materials and Methods

Molecular Clones and Complementary Ribonucleic Acid Preparation

The TOK1 clone from *Saccharomyces cerevisiae* (GenBank accession U28005) was a gift from Dr. Steve Goldstein. 5′ Capped complementary ribonucleic acid (cRNA) was synthesized by *in vitro* transcription (T7 mMessage mMachine®; Ambion, Austin, TX) from Not1 linearized and phenol/chloroform purified deoxyribonucleic acid (DNA) templates. cRNA was resuspended in diethylpyrocarbonate-treated oocyte saline (see later), divided in aliquots, and stored at −80°C.

Oocyte Preparation

These studies were approved by the Committee on Animal Research at the University of California, San Francisco. Methods for *Xenopus laevis* oocyte preparation and maintenance were similar to that previously described.[16] Adult frogs (Nasco, Fort Atkinson, WI) were anesthetized with ice-cold 3-aminobenzoic acid ethyl ester (3 g/l). Unfertilized stage V and VI oocytes were surgically removed from the ovaries and slowly agitated for 30–45 min in 2 mg/ml collagenase A or B (Boehringer Mannheim, Indianapolis, IN) in OR-Mg solution (in mM: 82 NaCl, 2 KCl, 20 MgCl$_2$, and 5 HEPES, *p*H 7.4) at room temperature. Oocytes were then washed in enzyme-free OR-Mg and stored at 18°C in ND96 (in mM: 96 NaCl, 2 KCl, 1.8 CaCl$_2$, 1 MgCl$_2$ and 5 HEPES, *p*H 7.5). On the same day they were isolated, oocytes were injected with 5–10 ng of cRNA or oocyte saline (20 mM NaCl, 100 mM KCl in diethylpyrocarbonate-treated water). Oocytes were incubated in ND96 with 50 μg/ml gentamycin, 2.5 mM sodium pyruvate, 5% heat-inactivated horse serum, and 5 mM theophylline at 18°C and gently rotated.

Volatile Agents

Racemic isoflurane (Anaquest, Madison, WI), halothane with 0.01% thymol (weight/weight; Ayerst Laboratories, Inc., Philadelphia, PA, and Halocarbon Laboratories, Augusta, SC), and desflurane (Ohmeda, Liberty Corner, NJ) were used for experiments. The nonanesthetic (see Koblin *et al.*[17]) 1,2-dichlorohexafluorocyclobutane (2N; >97% purity; PCR Inc., Gainesville, FL) was also studied. Ostwald liquid/gas partition coefficients were estimated in triplicate at room temperature for each volatile agent in buffer using standard reextraction methods.[18]

Two-electrode Voltage Clamp Experiments

All electrophysiologic experiments were performed at room temperature (≈22°C) 1–3 days after cRNA or oocyte saline injection. TOK1 currents were measured by two-electrode voltage clamp (Axoclamp 2A; Axon Instruments, Foster City, CA). Electrodes (0.5–1.5 MΩ resistance) were backfilled with 3 M potassium chloride. Signals were filtered with an eight-pole low pass Bessel filter (Frequency Devices, Haverhill, MA) set at a 40- to 100-Hz cutoff before sampling at 100–1,000 Hz. Pulse protocols were performed (except where noted) from a holding potential of −80 mV to target potentials of −140 mV to +40 mV in 20-mV increments. Pulse durations were 1 s, and interpulse intervals were 1.5 s.

Recording Solutions

Two-electrode voltage clamp experiments were performed (except where noted) in barium-containing

Frog Ringer's solution (in mM: 115 NaCl, 2.5 KCl, 1.0 BaCl$_2$, and 10 HEPES) at pH 7.6 at a holding potential of −80 mV. Other recording solutions were low-calcium ND98 (in mM: 98 NaCl, 2.0 KCl, 0.3 CaCl$_2$, and 10 HEPES, pH 7.6) and high-potassium ND98 (in mM: 2 NaCl, 98 KCl, 0.3 CaCl$_2$, and 10 HEPES, pH 7.6).

Volatile Agent Delivery for Two-electrode Voltage Clamp Experiments

Volatile agents were equilibrated with buffer to form saturated solutions, which were stored in gas-tight glass bottles. New (no previous drug exposure) 100 ml polyvinyl chloride bags (PL146 with di-2-ethylhexyl phthalate plasticizer; Baxter Healthcare Corporation; Deerfield, IL) were used as reservoirs for volatile agent solutions. These bags were filled with buffer, and all air was removed before addition of saturated volatile anesthetic solutions. Teflon® tubing (Nalge Nunc International, Rochester, NY), valves (Automate Scientific Inc., Oakland, CA), and connections (Value Plastics, Inc., Fort Collins, CO) were used to deliver volatile agent solutions to a 25-μl open acrylic recording chamber. Volatile agents were applied at flows of 2–10 ml/min for 2–3 min before voltage pulse protocols. Duplicate samples (two samples in succession) from the recording chamber were drawn into gas-tight glass syringes (B-D Yale; Becton, Dickinson and Co., Franklin Lakes, NJ) and analyzed with gas chromatography (Gow-Mac Instruments Co., Bridgewater, NJ) by standard methods.[18] Reported concentrations are the averages of the duplicate samples.

Kinetics of volatile anesthetic effects also were studied. Onset and offset of volatile anesthetic effects on TOK1 were measured during rapid perfusion (10 ml/min). Pulses of 1 s duration from −80 mV to +40 mV were repeated with interpulse intervals of 1.5 s. Desflurane was applied (or removed) after the first pulse of the set.

Intravenous Anesthetic Agents and Other Lipid Soluble Agents

The following lipid soluble compounds also were studied: propofol parent compound (Zeneca Inc./Stuart Pharmaceuticals, Wilmington, DE), pentobarbital, chlordiazepoxide, and cis-9,10-octadecenoamide (oleic amide; supplied by Dr. Ben Cravatt and Cayman Chemical Co., Ann Arbor, MI).

Single-channel Recording Experiments

Single-channel activity was recorded from outside-out patches using techniques described by Hamill *et al.*[19]

Patch electrodes were pulled from borosilicate capillary tubes (Garner Glass Co., Claremont, CA), the shanks were coated with Sylgard® (Dow Corning, Midland, MI), and the tips were heat polished. Before recording, the oocyte vitelline membrane was removed with a pair of fine forceps after a 10-min incubation in hypertonic saline, containing (in mM) 200 potassium aspartate, 20 KCl, 1 MgCl$_2$, 10 egtazic acid (EGTA), and 10 HEPES, pH 7.4. Currents were measured with a List (Darmstadt, Germany) EPC-7 amplifier and digitally recorded on videotape at a sample rate of 44.1 kHz. The current records were analyzed on an LSI 11/73 computer (Indec Systems, Capitola, CA) after filtering with an eight-pole Bessel filter (−3 dB at 2 kHz) and digitized at 10 kHz.

Single-channel Recording Solutions

The patch electrode filling solution contained (in mM) 108 KCl, 10 HEPES, 10 egtazic acid, and 4 MgCl$_2$, pH 7.4. The bathing solution contained (in mM) 150 potassium aspartate, 10 HEPES, 1 egtazic acid, and 5 MgCl$_2$, pH 7.4. The isotonic potassium bathing solution was used to zero the resting potential of the cell so that the patch potential was the same as the voltage applied to the electrode. Before seal formation, the voltage offset between the patch electrode and the bath solution was adjusted to produce zero current. The recording micropipette resistance ranged from 3–5 MΩ, and seal resistances ranged from 20–40 GΩ.

Delivery of Volatile Anesthetic Agents to Outside-out Patches

Saturated stock solutions of halothane or isoflurane were prepared in gas-tight bottles by dissolving excess volatile anesthetic agent into the bath solution. The stock solutions were diluted to the final experimental concentrations with a calibrated gas-tight syringe (Hamilton Co., Reno, NV) and stored in gas-tight bottles. Experimental solutions were perfused onto the cell or excised patch from a narrow pipette that was placed in the bath. All solutions were delivered at a rate of 25 μl/min from a Rheodyne (Cotati, CA) high-performance liquid chromatograph injector that was connected to an infusion pump. Perfusion with the bathing solution alone had no effect on the mean open probability. The volatile anesthetic concentrations in the stock solutions and the experimental solutions were measured by gas chromatography.

Statistical Analysis

Reported data are obtained from at least three oocytes and from more than one set of injected oocytes. Except

where noted, data are presented as mean $\pm$ SD. A P value <0.05 was assumed to be significant. Concentration–response data were fit to sigmoidal curves with minimum response (b_{min}), maximum response (b_{max}), cooperativity coefficients (n_{Hill}), and the concentrations at which 50% potentiation occurred (EC_{50} values) by standard methods.[20] Normalized response is defined as the average current during voltage pulse from -80 to $+40$ mV relative to control values in the absence of drug.

Results

Effect of Volatile Anesthetic Agents

Xenopus laevis oocytes injected with TOK1 transcript were studied. As controls, uninjected oocytes and oocytes injected with oocyte saline were studied during the same conditions. Volatile anesthetic agents reduced the baseline currents of control oocytes at membrane potentials more positive than the potassium reversal potential. The reduced baseline currents were slightly larger in oocyte saline-injected oocytes than in uninjected oocytes. The potassium channel blockers barium, tetraethylammonium, and mast cell degranulating peptide prevented this shift in baseline current. When the potassium reversal potential was shifted to -15 mV with high extracellular potassium perfusate, the baseline currents were not affected by volatile anesthetic agents at this holding potential. These observations suggest that volatile anesthetic agents inhibit an endogenous baseline potassium channel in oocytes. Therefore, experiments with TOK1-injected oocytes were performed during conditions that minimize these endogenous currents to avoid masking the effect of volatile anesthetic agents on TOK1.

TOK1 expression was manifest as large, outward currents after voltage pulses from a holding potential of -80 mV to positive holding potentials (fig. 1). Isoflurane potentiated these outward TOK1 currents in a concentration-dependent manner. During voltage pulses of longer duration (2–5 s), potentiation of TOK1 currents in barium-containing Frog Ringer's solution was still maintained (data not shown). The onset of volatile anesthetic potentiation of TOK1 was rapid, occurring within seconds (data not shown); washout occurred more slowly, although the kinetics observed may have been limited in part by fluid exchange within the recording chamber. Potentiation of TOK1 by isoflurane was also
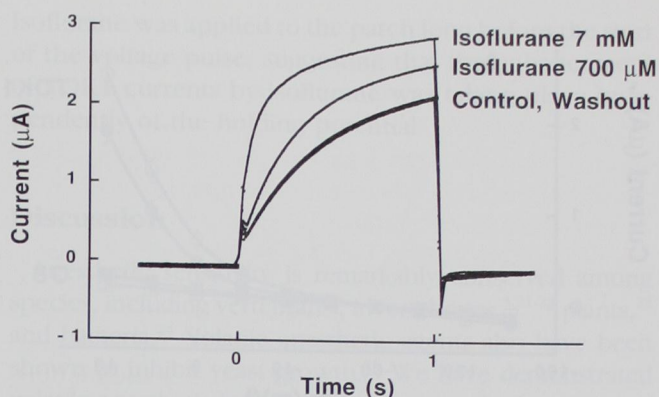

Fig. 1. Currents measured during a voltage pulse (-80 to $+40$ mV) of a TOK1 cRNA-injected oocyte before (control) and after (washout) application of isoflurane in barium Frog Ringer's solution. Tracings are shown without leakage subtraction. Time is relative to the onset of the voltage pulse. The control and washout tracings appear as superimposed curves in the figure.

observed in low-calcium and high-potassium recording solutions (data not shown).

TOK1-injected oocytes showed marked outward rectification in their I–V curves, which was significantly potentiated by isoflurane (700 μM) at the most positive pulse potentials (fig. 2A). At extreme concentrations of isoflurane, all outward currents of TOK1-injected oocytes were significantly increased (fig. 2B). Oocytes not expressing TOK1 (oocyte saline-injected) displayed near linear I–V relations; the application of isoflurane produced a small reduction in their baseline currents. Serial measurements of concentration of the volatile agent showed minimal decreases in concentration with time during experiments ($<5\%$).

Specificity of Volatile Agents

The concentration–response curves for several volatile agents in barium-containing Frog Ringer's solution are shown in figure 3 (fig. 3A, in atm; fig. 3B, in μM). Isoflurane, halothane, and desflurane produced concentration-dependent potentiation of outward TOK1 currents. For isoflurane, the concentration–response curve was fit to a sigmoidal curve (estimated parameters: $b_{min} = 0.98$, $b_{max} = 1.55$, n_{Hill} 2.7, and $EC_{50} = 769$ μM $= 0.025$ atm). For halothane, the concentration–response estimated parameters were $b_{min} = 1.00$, $b_{max} = 1.65$, n_{Hill} 3.3, and $EC_{50} = 814$ μM $= 0.016$ atm. For desflurane, the estimated parameters were $b_{min} = 0.95$, $b_{max} = 1.48$, n_{Hill} 2.7, and $EC_{50} = 768$ μM $= 0.044$ atm. In contrast, the nonanesthetic 2N did not potentiate TOK1 currents
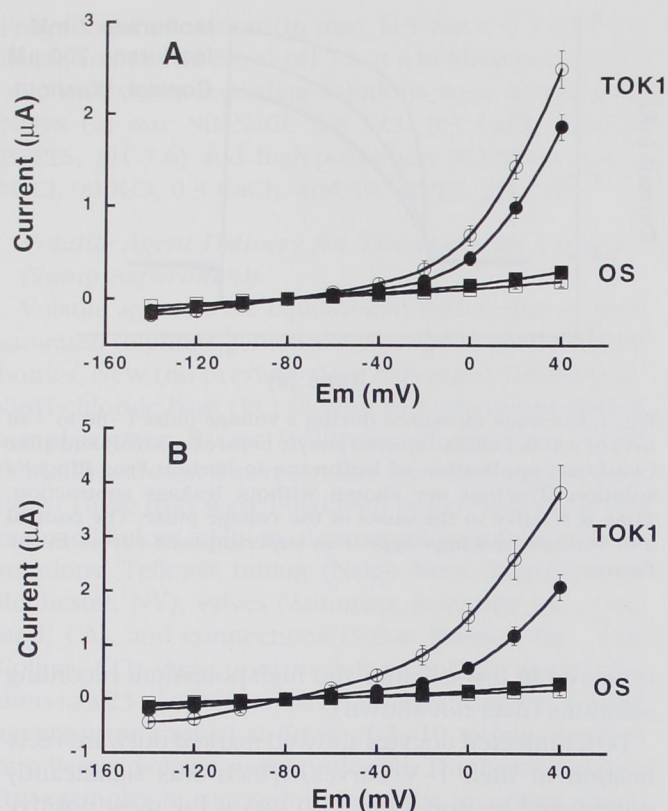
Fig. 2. Response to TOK1 as a function of voltage in the presence (*open circles*) or absence (*filled circles*) of 700 μM (*A*) or 7 mM (*B*) isoflurane. The average current during a voltage pulse is shown. The voltage represented is the target voltage. Also shown is the response of oocyte saline-injected oocytes in the presence (*open squares*) or absence (*filled squares*) of anesthetic agent. Mean values are shown with the standard error of the measured currents.

at concentrations of 0.06–0.19 atm (approximately one to five times the Meyer–Overton predicted minimum alveolar concentration [MAC][17]), although a small degree of inhibition (normalized response 0.95 ± 0.09) was seen at 0.19 atm 2N (fig. 3).

Lipid-soluble Agents

Among the lipid-soluble compounds tested for modulatory activity on TOK1, the intravenous anesthetic agents (propofol: 100 μM with 0.06% ethanol; pentobarbital: 1, 10, and 100 μM; chlordiazepoxide: 10 μM) and an endogenous sleep-inducing compound (oleic amide: 500 nM with 0.06% ethanol) had no effect on TOK1 currents. The halothane preservative thymol (2 μM with 0.002% ethanol) did not alter TOK1 currents in barium-containing Frog Ringer's solution (data not shown).

Single TOK1 Channels Are Activated by Isoflurane

Single TOK1 channel currents were recorded from excised outside-out patches. Channel openings exhibited the same elementary properties previously described for TOK1.[12,13] Channel activity was noninactivating and outwardly rectifying with no measurable inward currents at negative holding potentials. The single-channel conductance during our recording conditions was approximately 19 pS. Single-channel activity in excised patches was increased in the presence of halothane and isoflurane. Figure 4 shows representative currents recorded in the presence of isoflurane. Channel openings became more frequent while the patch was perfused with bath solution containing 2 mM (0.065
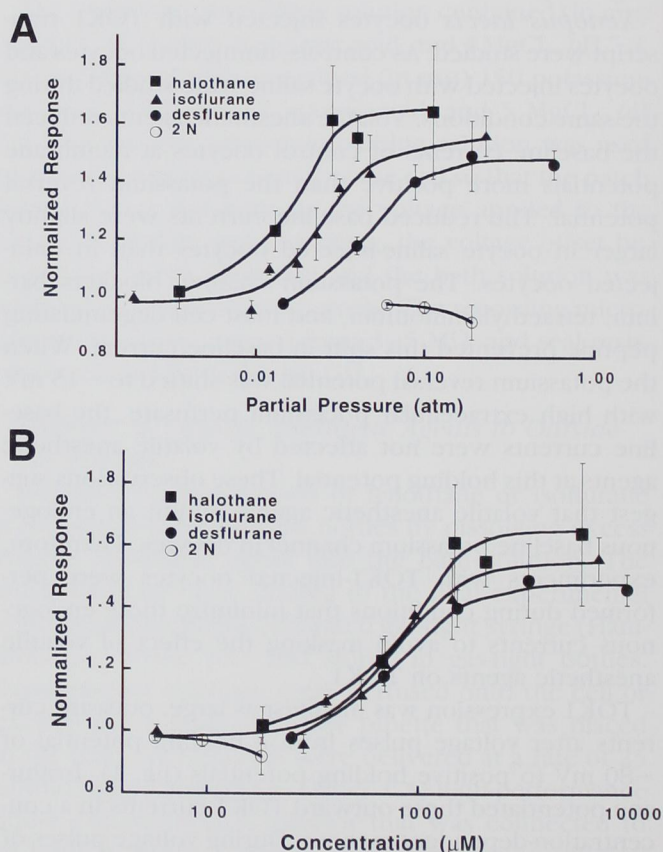

Fig. 3. Anesthetic concentration–response data for halothane (*filled squares*), isoflurane (*filled triangles*), and desflurane (*filled circles*) in barium-containing Frog Ringer's solution. Data for the nonanesthetic agent 2N are shown with open circles. Measured concentrations are plotted in atmospheres (*A*) and micromoles per liter (*B*). Normalized response is the ratio of response of TOK1 cRNA-injected oocytes to voltage pulses (−80 to +40 mV) in the presence or absence of anesthetic agent. Mean values of normalized response are shown with standard errors of the means and best fit curves.
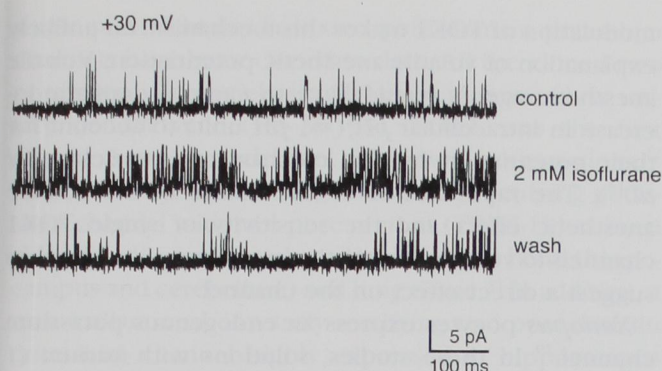
+30 mV

control

2 mM isoflurane

wash

5 pA
100 ms

Fig. 4. TOK1 openings are more frequent in the presence of isoflurane. An experiment in a excised, outside-out patch with 108 mM KCl in the pipette solution and 150 mM K-aspartate in the bath solution. Single-channel activity in the presence of isoflurane increased more than twofold in the presence of isoflurane and returned to the baseline values on washout.

atm) isoflurane. The increased level of activity persisted during the entire 3-min perfusion period, then returned to the baseline level soon after wash with isoflurane-free bath solution. In addition, volatile anesthetic agents did not affect the single channel I-V relationship, suggesting no effect of these agents on ion permeation through the channel.

Effects of Isoflurane on TOK1 Currents in a Multichannel Patch

Because TOK1 channel expression was very high on the second and third day after oocyte injection, most excised patches from these oocytes contained large numbers of active TOK1 channels. Figure 5A shows macroscopic currents from an excised outside-out patch that contained ≈20–30 TOK1 channels. The channel activity had similar properties previously reported from voltage clamp experiments on oocytes injected with TOK1 transcript,[12,13] with outward currents flowing instantaneously on depolarization and exhibiting a delayed increase in amplitude, which has been attributed to a closed state with a slow transition to the open state.[13] The macroscopic TOK1 currents reversibly increased ≈50% in the presence of 4 mM isoflurane, similar to the maximum potentiation seen in whole oocytes.

Figure 5B shows current records that were recorded in the presence of isoflurane with the control sweeps subtracted. Both concentrations of isoflurane increased the instantaneous and slow current components that normally are present in macroscopic TOK1 currents.

Isoflurane was applied to the patch long before the start of the voltage pulse, suggesting that the enhancement of TOK1 currents by isoflurane was taking place independently of the holding potential.

Discussion

Anesthetic sensitivity is remarkably conserved among species, including vertebrates, invertebrates,[5,21,22] plants,[23] and bacteria.[24] Volatile anesthetic agents also have been shown to inhibit yeast growth.[25] We have demonstrated volatile anesthetic-induced potentiation of a baseline po-

A

4 mM isoflurane
2 mM isoflurane
control & wash

+30 mV

-30 mV

20 pA
100 ms

B

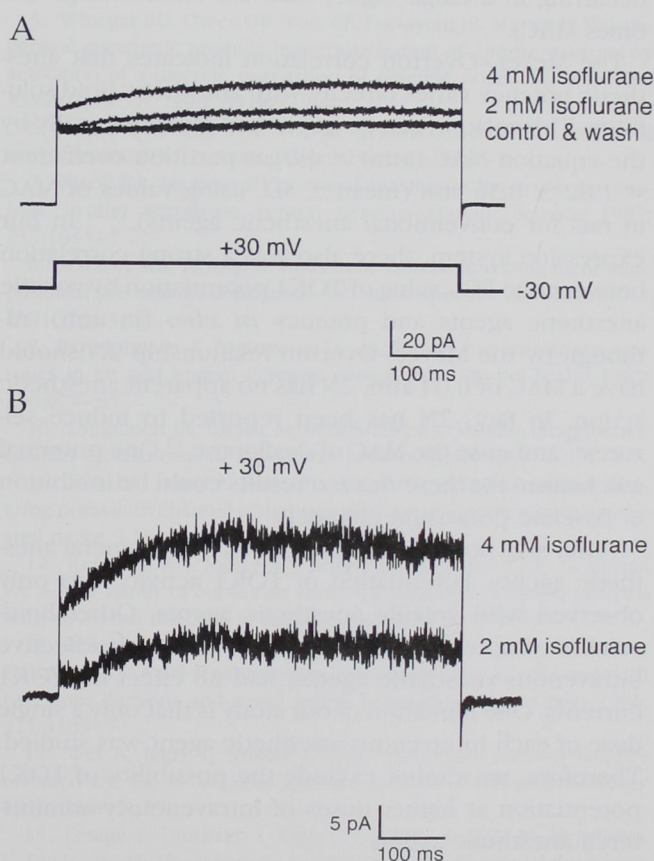+ 30 mV

4 mM isoflurane

2 mM isoflurane

5 pA
100 ms

Fig. 5. Effect of isoflurane on TOK1 channels in a multichannel patch. (A) Reversible activation of TOK1 currents by isoflurane. Each averaged sweep (n = 20) shows outward currents from an excised, outside-out patch that contained a large number of TOK1 channels. Channel activity was increased by isoflurane and returned to the baseline level on washout. (B) TOK1 difference currents. Control currents were subtracted from the currents recorded in the presence of isoflurane. The slope of the difference currents shows that isoflurane has a greater effect on the fast component of TOK1 currents than on the slow component at the beginning of the voltage pulse.

tassium channel, TOK1, cloned from the eukaryotic budding yeast *Saccharomyces cerevisiae*. The maximal increase of TOK1 currents was similar for isoflurane, desflurane, and halothane (b_{max} = 1.55, 1.48, and 1.65, respectively), although there was a trend that less potent volatile anesthetic agents had lower maximal effect. A similar maximal effect also was seen in the macro patch containing 20–30 TOK1 channels. The high Hill coefficients of anesthetic concentration–response (range, 2.7–3.3) may indicate positive cooperativity at anesthetic agent–receptor binding sites. The EC_{50} values for TOK1 potentiation by halothane and isoflurane were twice the concentrations that prevent movement in response to noxious stimulation (MAC), with maximal potentiation occurring in a range higher than the clinical range (20 times MAC).

The Meyer–Overton correlation indicates that anesthetic potency varies directly with anesthetic lipid solubility. This relation can be expressed mathematically by the equation MAC (atm) × oil/gas partition coefficient = 1.82 ± 0.56 atm (mean ± SD, using values of MAC in rats for conventional anesthetic agents).[17,26] In our expression system, there also was a strong correlation between the EC_{50} value of TOK1 potentiation by volatile anesthetic agents and potency *in vivo* (in atm). Although by the Meyer–Overton relationship 2N should have a MAC of 0.04 atm, 2N has no apparent anesthetic action. In fact, 2N has been reported to induce seizures[27] and raise the MAC of desflurane.[17] One potential mechanism for these *in vivo* results could be inhibition of baseline potassium channels.

TOK1 was not strongly modulated by all general anesthetic agents. Potentiation of TOK1 activity was only observed with volatile anesthetic agents. Other lipid-soluble compounds, some of which are highly effective intravenous anesthetic agents, had no effect on TOK1 currents. One limitation of our study is that only a single dose of each intravenous anesthetic agent was studied. Therefore, we cannot exclude the possibility of TOK1 potentiation at higher doses of intravenously administered anesthetic agents.

Various modulators of TOK1 have been proposed, including intracellular divalent cations,[12] protein kinase C,[13] and intracellular pH.[13] Therefore, it is possible that volatile anesthetic agents potentiate TOK1 activity through one or more of these mechanisms. Magnesium and calcium in the bath of excised membrane patches have been found in one study to block TOK1 activity.[12] Other studies have not observed this modulation, however.[13,28] The slow ($\approx$10 min) onset of protein kinase C

modulation of TOK1 makes this mechanism an unlikely explanation of volatile anesthetic potentiation. Volatile anesthetic agents would have to cause a dramatic increase in intracellular pH ($\approx$1 pH unit) to account for their potentiation by this mechanism (see Lesage *et al.*[13]). The rapid and reversible nature of the volatile anesthetic effect and the sensitivity of single TOK1 channels to volatile anesthetic agents in excised patches suggest a direct effect on the channel.

Xenopus oocytes express an endogenous potassium channel.[29] In these studies, solutions with barium (1 mM) were used to quantify the anesthetic concentration–response relation because barium decreased endogenous potassium channel activity of oocytes. In addition, TOK1 currents were more stable with repeat pulse stimulations in barium. Although extracellular barium is a known modulator of TOK1,[12] potentiation of TOK1 by volatile anesthetic agents still occurred in barium-containing solutions. TOK1 potentiation also was observed in barium-free solutions, suggesting that relief of divalent cation blockade is not a mechanism of volatile anesthetic potentiation.

TOK1 channels have been reported to have two kinetically distinct current components: a slow component that is voltage-dependent and a fast component independent of voltage.[13] The current traces in figure 5B clearly show that both current components are present during exposure to isoflurane. Control traces recorded from the same patch in the absence of isoflurane were subtracted from traces recorded during exposure to isoflurane (fig. 5B). These difference currents illustrate that isoflurane increased both the instantaneous currents at the start of the voltage pulse and the slow currents that slowly increased during the sweep. We conclude that the enhancement of TOK1 currents by isoflurane is a generalized effect that is nonselective for the kinetic state of the channel.

Baseline potassium channels have several physiologic roles. Hodgkin and Huxley first postulated the existence of nongated (leakage) channels with high potassium permeability.[2,30] These channels are always open and therefore contribute to the resting membrane potential. Because of their baseline activity, these potassium channels may be the main source of potassium leak currents.[31] In contrast, voltage-gated channels are closed at rest, but when they are opened by a change in voltage they contribute to the action potential profile.

The major families of cloned potassium channels are the inward rectifiers and voltage-gated channels (reviewed by Doupnik *et al.*[32]). These channels have a

single pore domain. Few two pore domain potassium channels have been cloned to date. All the cloned two pore domain channels are flickery and have a low unitary conductance (14–35 pS). TOK1 was the first cloned potassium channel with two pore regions.[12] More recently, a mammalian outwardly rectifying potassium channel with two pore regions (TREK-1) has been cloned and is expressed predominantly in brain (hippocampus and cerebellum) and lung tissue.[33] In addition, a mammalian weak inwardly rectifying two pore domain potassium channel highly expressed in hippocampus and cortex has been cloned (TWIK-1, Lesage *et al.*[34]). Therefore, mammalian two pore domain channels are expressed in brain regions critical to memory and learning. Based on information emerging from the *C. elegans* genome project, there may be many other members of this family. For example, with ≈30% of the *C. elegans* genome sequenced, 23 of the 40 putative potassium channels were found have two pore domains.[14] Therefore, two pore domain channels may constitute the largest family of potassium channels.

In general, the effect of volatile and intravenously administered anesthetic agents on voltage-gated and inward rectifier potassium channels is modest inhibition.[1,35,36] Adenosine triphosphate regulated potassium channels also are stimulated by volatile anesthetic agents.[37] Other ion channels with electrophysiologic properties similar to TOK1 and the *Aplysia* S channel include RCK channels,[38] the M channel,[39] the flicker channel,[11] and outwardly rectifying potassium channels expressed in the retina[40] and pulmonary artery.[41] These channels also may exhibit volatile anesthetic sensitivity.

Summary

The opening frequency of an outwardly rectifying potassium channel of yeast (TOK1), when expressed in *Xenopus* oocytes, was increased by volatile anesthetic agents. Remarkably, TOK1 sensitivity to volatile agents exhibited the same rank order of potency as observed with clinical anesthesia. In addition, TOK1 potentiation was not observed with a nonanesthetic volatile agent. We propose that TOK1, like the *Aplysia* S channel, is a member of a new family of potassium channels stimulated by volatile anesthetic agents. It remains to be determined whether there are other tandem pore domain potassium channels that are stimulated by volatile anesthetic agents.

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