Full Paper

Sevoflurane Inhibition of the Slowly Activating Delayed Rectifier K^+ Current in Guinea Pig Ventricular CellsShigehiro Shibata¹, Kyoichi Ono², and Toshihiko Iijima^{2,*}¹Department of Anesthesiology, Akita City Hospital, Akita 010-0933, Japan²Department of Pharmacology, Akita University School of Medicine, Hondoh 1-1-1, Akita 010-8543, Japan

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Abstract. Single ventricular cells were enzymatically isolated from guinea pig hearts and the effects of sevoflurane on the delayed rectifier K^+ current were investigated by the patch clamp method. The rapidly (I_{Kr}) and slowly activating delayed rectifier K^+ current (I_{Ks}) were isolated using chromanol 293B, a selective blocker for I_{Ks} or E4031 (*N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulfonamide dihydrochloride), a blocker for I_{Kr} . Sevoflurane and halothane decreased I_{Ks} in a concentration-dependent manner with an IC_{50} value of 0.38 mM for sevoflurane and 1.05 mM for halothane. I_{Ks} inhibition was characterized by suppression of maximum conductance with little effect on activation kinetics. Inhibition occurred immediately after anesthetic application and recovered upon wash-out. In contrast to the marked inhibition of I_{Ks} , I_{Kr} was hardly affected by sevoflurane. Under the current clamp, sevoflurane prolonged the action potential duration in a reversible manner and this effect was more marked when I_{Kr} was inhibited by E4031. The results suggest that sevoflurane inhibits I_{Ks} , and not I_{Kr} , in a concentration-dependent manner at clinically relevant concentrations. The resulting prolongation of ventricular repolarization may partly account for the clinical observation of excessive QT prolongation by these anesthetics.

Keywords: QT prolongation, delayed rectifier K^+ current, sevoflurane, chromanol 293B, E4031

Introduction

The delayed rectifier K^+ current (I_K) in cardiac cells consists of fast and slow components, that is, the rapidly activating I_K (I_{Kr}) and the slowly activating I_K (I_{Ks}) (1). Both currents play an important role for repolarization of action potential, and a congenital or acquired prolongation of the QT interval in ECG is often brought about by a loss of function of the above current systems (2). Excessive prolongation of the QT interval may trigger the development of lethal tachyarrhythmia torsade de pointes, resulting in syncope and sudden cardiac death (2). Previous evidence has implicated that clinical use of volatile anesthetics is associated with prolongation of QT interval (3–7). In support of the clinical observations, some volatile anesthetics have been shown to prolong the action potential duration (APD) in cardiac myocytes (8–11). Park et al. showed that in guinea pig

ventricular cells, the prolonged APD is primarily due to the suppression of I_K , although the study did not differentiate between I_{Kr} and I_{Ks} (11). Furthermore, isoflurane and sevoflurane have been shown to inhibit the cloned I_{Ks} coexpressed by KvLQT1 and minK in *Xenopus* oocytes (12). Li and Correa demonstrated that halothane modulated the kinetics of HERG potassium channels, which is partly responsible for cardiac I_{Kr} (13). Thus, it may be possible that inhibition of not only I_{Ks} but also I_{Kr} accounts for QT prolongation.

However, a detailed analysis of the inhibitory action of anesthetics on I_{Ks} and I_{Kr} in native cardiac myocytes has not been carried out. This is particularly important since I_{Ks} and I_{Kr} are known to exhibit pharmacologically distinct properties. I_{Kr} is not only a target of class III antiarrhythmic agents (14) but is also blocked by various, structurally unrelated drugs such as antipsychotics, H_1 -antihistamines, and fluoroquinolones (2). The most common mechanism of QT interval prolongation by pharmaceuticals appears to be inhibition of I_{Kr}

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(2). On the other hand, I_{Ks} is blocked by chromanol derivatives (15, 16) and some general anesthetics including propofol and barbiturates (17). Thus, inhalational anesthetics, when administered in the presence of other agents that block I_{Ks} or I_{Kr} , may trigger excessive QT prolongation, depending on the specificity of the anesthetics on I_{Ks} and I_{Kr} . The present study was therefore designed to determine what sevoflurane targets when causing the delayed repolarization. I_{Ks} and I_{Kr} were isolated electrophysiologically, combined with the use of a selective blocker for each current. Whole-cell patch clamp techniques were used to monitor anesthetic effect on I_{Ks} and I_{Kr} in guinea pig ventricular cells.

Materials and Methods

Cell isolation

The protocols for animal experimentation described in this paper were previously approved by the Animal Research Committee at Akita University. Ventricular cells were isolated from hearts of guinea pigs (body weights of 300–400 g) using the enzymatic dissociation technique (16). Guinea pigs were anesthetized with pentobarbital ($50 \text{ mg} \cdot \text{kg}^{-1}$). The chest was opened and the ascending aorta was cannulated in situ to start coronary perfusion with normal Tyrode solution. The heart was then excised and mounted on a Langendorff apparatus. After perfusing with 100 ml Ca^{2+} -free Tyrode solution, the perfusate was switched to Ca^{2+} -free Tyrode solution containing collagenase (38 U/ml ; Wako, Osaka), and the heart was digested for about 30 min. Then the heart was rinsed by perfusing with a high K^+ , low Cl^- storage solution. All perfusates were kept at $36\text{--}37^\circ\text{C}$ during coronary perfusion. The left ventricle was dissected from the digested heart, chopped with scissors, and stored in the storage solution at 4°C for later use.

A small piece of ventricular tissue was dissected and gently agitated in the recoding chamber filled with normal Tyrode solution. After the cells had settled on the floor of the recoding chamber, they were perfused with normal Tyrode solution at $2\text{--}3 \text{ ml} \cdot \text{min}^{-1}$.

Experiments were carried out at $36\text{--}37^\circ\text{C}$.

Solutions and drugs

Normal Tyrode solution contained 136.9 mM NaCl , 5.4 mM KCl , 1.8 mM CaCl_2 , 0.53 mM MgCl_2 , $0.33 \text{ mM NaH}_2\text{PO}_4$, $5.0 \text{ mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES)}$, and 5.5 mM glucose ($\text{pH} = 7.4$, adjusted with NaOH). The standard external solution was made by adding $0.3 \mu\text{M}$ nisoldipine to the control Tyrode solution in order to block the L-type

Ca^{2+} current (I_{CaL}). The Ca^{2+} -free solution, used for cell isolation, was made by omitting CaCl_2 from Tyrode solution. The high K^+ , low Cl^- storage solution contained 10 mM taurine , $10 \text{ mM oxalic acid}$, $70 \text{ mM L-glutamic acid}$, 25 mM KCl , $10 \text{ mM KH}_2\text{PO}_4$, 0.5 mM EGTA , 11 mM glucose , and 10 mM HEPES ($\text{pH} = 7.4$, adjusted with KOH). The pipette solution used for both the voltage- and current-clamp experiments contained $110 \text{ mM K-aspartate}$, 20 mM KCl , $5 \text{ mM Na}_2\text{ATP}$, 5 mM MgCl_2 , 5 mM HEPES , 5 mM EGTA , $0.1 \text{ mM Na}_2\text{GTP}$ ($\text{pH} = 7.2$, adjusted with KOH).

E4031 (*N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulfonamide dihydrochloride) (Sigma Chemical, St. Louis, MO, USA) was dissolved in distilled water as a 1 mM stock solution. A chromanol derivative, 293B, was kindly supplied by Aventis Co. (Frankfurt, Germany) and dissolved in dimethylsulfoxide as a 10 mM stock solution. Before the experiment, the stock solution was diluted in the external solution to obtain the concentrations described in the text.

Electrophysiological experiments

The current and voltage signals were recorded using a patch clamp amplifier (EPC-7; List, Darmstadt, Germany) (18). Patch pipettes were pulled with a micropipette puller (Model P-97; Sutter Instrument Co., Novato, CA, USA) from glass capillaries (Corning #7052). The pipettes had a resistance of $3\text{--}5 \text{ M}\Omega$ when filled with the internal solution (see above). After seal formation ($1\text{--}8 \text{ G}\Omega$), the patch membrane was ruptured by applying suction and the series resistance was compensated (approximately 40%) with the patch clamp amplifier. The data were digitized at $2\text{--}10 \text{ kHz}$ into an IBM-compatible computer (Proside, Tokyo) using the pClamp software (Axon Instruments, Foster City, CA, USA).

Sevoflurane (Abbott Laboratories, Tokyo) or halothane (Takeda Chemical Industries, Osaka) was added to the perfusate by passing the air through a vaporizer (ACOMA, Tokyo) prior to bubbling the external solution. The volume of the perfusion chamber was approximately 0.5 ml , and complete exchange of the solution was achieved within 20 s. Anesthetic aqueous concentrations were determined by collecting samples of perfusate from the recording chamber and analyzing by gas chromatography (19). The perfusate contained 0.35 ± 0.02 ($n = 4$), 0.65 ± 0.02 ($n = 4$), and $1.67 \pm 0.05 \text{ mM}$ ($n = 4$) sevoflurane when equilibrated with 1%, 2%, and 5% sevoflurane, respectively. For halothane, the perfusate contained 0.50 ± 0.01 ($n = 4$), 1.13 ± 0.05 ($n = 4$), and $2.87 \pm 0.11 \text{ mM}$ at vaporizer settings of 1%, 2%, and 4%, respectively.

The stability of the recording was important in order to quantitatively measure the inhibitory action of anesthetics. In the present study, response recovery was checked in all experiments, and data showing >90% recovery (for example, see Fig. 3) were used to calculate concentration-dependency of anesthetics.

Statistical analyses

Statistical values are shown as the mean $\pm$ S.E.M. Comparisons between two groups were performed using a paired Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

Effects of sevoflurane on time-dependent outward current

An initial series of experiments was carried out to examine the effects of inhalational anesthetics on total I_K. After setting up the conventional whole-cell clamp in normal Tyrode solution, the external solution was changed to the standard external solution to block I_{CaL}. Under these conditions, square pulses of 3-s duration were applied from a holding potential of -40 mV to various potentials (Fig. 1). The I_K was recognized as a time-dependent outward current upon depolarization and the tail current on repolarization (Fig. 1: Aa and Ba).

During the course of the experiments, total I_K was monitored by applying a 3-s pulse between -40 and $+60$ mV (not illustrated) and either 0.65 mM sevoflurane (Fig. 1A) or 1.13 mM halothane (Fig. 1B) were applied. Usually a steady-state effect was achieved within a few min of anesthetic application (see Fig. 3). After confirming that the anesthetic action had stabilized, square pulses of 3 s were applied from -40 mV to various potentials (Fig. 1: A and B). The results showed that sevoflurane (Fig. 1A) and halothane (Fig. 1B) suppressed total I_K. These effects were reversible upon wash-out (for example, see Fig. 3).

The current-voltage relationship and steady-state activation of I_K

The current-voltage (I-V) relationship was evaluated in four cells using sevoflurane (Fig. 1Ab) and in four cells using halothane (Fig. 1Bb). The current amplitudes measured at the end of the 3-s pulses were plotted against the membrane potential (*b*). Sevoflurane (solid circles in Fig. 1Ab) or halothane (solid circles in Fig. 1Bb) decreased time-dependent outward current at potentials more positive than 0 mV. The current amplitude at $+70$ mV was decreased from 1.70 ± 0.37 to 0.87 ± 0.18 pA/pF by 2% sevoflurane ($P < 0.05$) and from 2.59 ± 0.56 to 0.89 ± 0.17 pA/pF by 2% halothane

($P < 0.05$). The effects of sevoflurane and halothane on the quasi-steady state activation of total I_K were examined by measuring the tail current upon repolarization (Fig. 1: Ac and Bc). The tail current amplitude was normalized in reference to that of the maximal amplitude in the absence of anesthetic. The relation between the test potential (*V_m*) and the current amplitude was fitted by the Boltzmann equation:

$$I = I_{\max} / (1 + \exp((V_m - V_{1/2}) / S)),$$

where *V_{1/2}* is the half-activated potential and *S* indicates the slope factor. The values of *V_{1/2}* and *S*, determined by a least-squares fit, were 8.0 ± 4.5 and 15.1 ± 1.0 mV in the control and -1.5 ± 2.0 and 12.6 ± 0.9 mV in the presence of 0.65 mM sevoflurane (*n* = 4). *I_{max}* was reduced to $47.0 \pm 10.3\%$ of the control value by sevoflurane. *V_{1/2}* and *S* were 15.4 ± 2.8 and 14.7 ± 1.5 mV in the control and 6.7 ± 2.4 and 17.4 ± 1.6 mV in the presence of 1.13 mM halothane (*n* = 4). *I_{max}* was reduced to $27.3 \pm 8.1\%$ of the control by halothane.

Sevoflurane and halothane block I_{Ks} rather than I_{Kr}

Both I_{Ks} and I_{Kr} exist in guinea pig ventricular cells (1). To examine the relative selectivity of inhalational anesthetics to I_{Ks} and I_{Kr}, double pulses consisting of 300-ms and 3-s square pulses to $+60$ mV were applied from -40 mV every 20 s. Sevoflurane at 0.35 mM decreased both the outward and tail currents irrespective of the pulse duration (Fig. 2A). However, the suppression was significantly less for the tail current at a 300-ms pulse duration, as compared to the outward current or the tail current at a 3-s pulse duration. At a 300-ms duration, sevoflurane decreased the outward current to 0.41 ± 0.03 and the tail current to 0.55 ± 0.02 (*n* = 7) (Fig. 2B). At a 3-s duration, 0.65 mM sevoflurane decreased the outward current to 0.44 ± 0.03 and the tail current to 0.47 ± 0.02 (Fig. 2B). These findings may indicate that I_{Kr} is relatively insensitive to sevoflurane. However, no significant difference was detected when 1.1 mM halothane was applied. At a 300-ms duration, the relative amplitude of the outward and tail current was 0.35 ± 0.02 and 0.38 ± 0.03 (*n* = 4), respectively, in the presence of 1.1 mM halothane. At a 3-s duration, the relative amplitude of the outward and tail current was 0.39 ± 0.04 and 0.38 ± 0.04 , respectively (Fig. 2C).

Concentration-dependent inhibition of I_{Ks} by sevoflurane

The effects of sevoflurane and halothane on I_{Ks} were evaluated in the presence of 5 μ M E4031, which inhibits I_{Kr} (1, 20). The time courses of I_{Ks} inhibition by sevoflurane are shown in Fig. 3. It is evident that sevoflurane inhibited I_{Ks} reversibly in a concentration-dependent manner.

The concentration-response relationship was con-

Fig. 1. Effects of sevoflurane and halothane on total I_K in guinea pig ventricular cells. Aa: Original current recordings in the absence (left) and the presence (right) of 0.65 mM sevoflurane. Traces shown were obtained by applying 3-s depolarizing pulses from a holding potential of -40 mV to various potentials in 10-mV increments. Dashed lines indicate the zero current level. Ba: Current recordings in the absence (left) and the presence (right) of 1.1 mM halothane. I-V relations were measured just before the end of the depolarizing pulses (Ab and Bb) and for the tail current upon repolarization (Ac and Bc) in the absence (open circles) and presence (solid circles) of anesthetics. Data are the average of four cells and vertical bars indicate S.E.M.; no bars are shown when the S.E.M. is smaller than the symbol size. In Ac and Bc, the tail current was normalized in reference to that of the maximal amplitude in the absence of the drug and plotted against the membrane potential. The smooth curves were drawn by the least squares fit with the Boltzmann equation: $I = I_{max} / (1 + \exp((V_m - V_{1/2}) / S))$. See text for details.

structed by measuring the tail current amplitude. The current amplitudes were normalized by referring to those obtained in the control. The results are summarized in Fig. 4. The smooth curves were drawn by the least squares fit using the following equation:

$$I = 1 / (1 + ([X]/IC_{50})^n),$$

where $[X]$, IC_{50} , and n indicate anesthetic concentration, half-inhibitory concentration, and Hill's coefficient, respectively. The IC_{50} values sevoflurane and halothane were 0.38 and 1.05 mM, and the n values were 2.3 and

1.5, respectively.

Kinetics of I_{Ks}

The steady-state activation was obtained by measuring and normalizing the tail current amplitude in the absence of sevoflurane (Fig. 5). The least squares fit using the Boltzmann equation gave S and $V_{1/2}$ values of 11.3 ± 0.73 and 21.2 ± 1.3 mV in the control and 9.9 ± 0.9 and 19.7 ± 2.7 mV, respectively, in the presence of 0.35 mM sevoflurane ($n = 6$).

Fig. 2. Double pulse experiment. A: Double pulses consisting of 300 ms and 3 s square pulses to +60 mV were applied from -40 mV every 20 s. Current recordings in the absence and presence of 0.65 mM sevoflurane were superimposed. B: Current amplitudes in the presence of 0.65 mM sevoflurane at various pulse durations. The values were normalized in reference to those in the absence of sevoflurane. Open bars, the amplitudes of the time-dependent outward current during the test pulse; hatched bars, the tail current amplitudes. The values are the average of five cells and vertical lines indicate S.E.M. * $P < 0.05$. C: Current amplitudes in the presence of 1.1 mM halothane at various pulse durations.

Fig. 3. Inhibition by sevoflurane of I_{Ks} . Time course of changes in the tail current amplitude during sevoflurane application. Bar above the graph indicates application of 0.17, 0.35, and 0.65 mM sevoflurane (sev). The upper panels show superimposed original current traces obtained at times indicated by the corresponding letters in the bottom graphs. The dotted line indicates the zero current level.

Fig. 4. Concentration-dependent inhibition of I_{Ks} by sevoflurane and halothane. Relationship between the concentration and the relative amplitude of the tail current (open circles: sevoflurane, solid circles: halothane). The smooth curves were drawn by the least squares fit using the equation $I = 1 / (1 + ([\text{sevoflurane}] / IC_{50})^n)$, where IC_{50} and n indicate the half-inhibitory concentration and Hill's coefficient, respectively. The IC_{50} values of sevoflurane and halothane were 0.38 and 1.07 mM and the n values were 2.3 and 1.5, respectively.

A possible change in the gating kinetics was examined by comparing the time course of I_{Ks} activation and deactivation. The current traces in the absence and presence of sevoflurane were peak-adjusted and superimposed (Fig. 6: A and B). It is evident that the activation time course was hardly affected by sevoflurane. However, the deactivation time course appeared to be slowed by sevoflurane. Therefore, we conducted further investigation into the effects of sevoflurane on I_{Ks} deactivation. The time course of deactivation fitted well with the sum of two exponentials. Both the time constants for the fast (Fig. 6C) and slow component (Fig. 6D) were significantly increased by sevoflurane.

Effects of sevoflurane on I_{Kr}

The effects of sevoflurane on I_{Kr} were examined under I_{Ks} blockade. The external solution contained 0.3 μM nisoldipine and 50 μM chromanol 293B, a selective blocker for I_{Ks} (15, 16). The holding potential was set to -50 mV, and a 300-ms depolarizing pulse to 0 mV was applied to evoke I_{Kr} . In the control (Fig. 7A), the current shifted initially to a downward direction upon depolarization in response to the test pulse, due to the lack of the inward rectifier K^+ current

Fig. 5. Effects of sevoflurane in I_{Ks} . A: Whole-cell current of I_{Ks} , measured in the presence of 5 μM E4031. Traces shown were obtained by applying 3-s depolarizing pulses from a holding potential of -40 mV in 10 mV increments in the absence (upper traces) and the presence (lower traces) of 0.65 mM sevoflurane. Dashed lines indicate the zero current level. B: Voltage-dependent activation of I_{Ks} in the absence (open circles) and the presence (solid circles) of 0.65 mM sevoflurane. Data are the average of six cells, and bars indicate S.E.M. Smooth curves were drawn by the least squares fit using the Boltzmann equation.

Fig. 6. Kinetics of I_{Ks} . A: The current traces in the absence and presence of 0.65 mM sevoflurane were peak-adjusted and superimposed. B: Peak-adjusted tail currents in the absence and presence of 0.65 mM sevoflurane. C, D: Time constants of the slow (C) and fast (D) component, where c indicates the control and the numerals indicate the concentration of sevoflurane in mM. The values are the average of four or five cells, and vertical lines indicate S.E.M. * $P < 0.05$.

Fig. 7. Effects of sevoflurane on I_{Kr} . A: Superimposed current traces were obtained by applying a 300-ms depolarizing pulse from -50 to 0 mV in the control, in the presence of either 0.65 mM sevoflurane or $5 \mu\text{M}$ E4031. The dotted line indicates the zero current level. The lower panel shows the tail currents after the subtraction of a time-independent component at the holding potential. B: Tail current amplitudes in the absence (open circles) and presence (solid circles) of 0.65 mM sevoflurane were plotted against the membrane potential. The smooth curves were drawn by the least squares fit with the Boltzmann equation.

Fig. 8. Effects of sevoflurane on action potentials. Time course of changes in APD₅₀ (open circles) and APD₉₀ (solid circles) during sevoflurane application. The action potential was elicited every 5 s. Bars above the graph indicate the time of 0.65 mM sevoflurane (sev) or 5 μ M E4031 application. The right panels show superimposed original action potential traces obtained at times indicated in the left graphs. For each example, four consecutive action potentials were superimposed. The dotted line indicates the 0 mV level. Note that the action potential was markedly prolonged when E4031 was applied with sevoflurane (B).

at 0 mV, and thereafter activation of I_{Kr} was observed. Application of 0.65 mM sevoflurane shifted the holding current to a negative direction, while the time-dependent outward current upon depolarization and the tail current were hardly affected. Subsequent application of E4031, a selective blocker for I_{Kr} , inhibited both the time-dependent outward current upon depolarization and the tail current. The lower panel of Fig. 8A confirms little or no change of the tail current, as the tail current was almost superimposable with the control trace after the subtraction of the time-independent component. A slight negative shift of the holding current was a consistent finding in seven examined cells.

Steady state activation was analyzed by measuring the amplitude of the tail current and normalized to that in response to the test pulse to -40 mV in the absence of sevoflurane (Fig. 7B). In the seven examined cells, the least squares fit with the Boltzmann equation gave I_{max} , $V_{1/2}$, and S values of 1.03 ± 0.04 , -10.3 ± 2.8 mV, and 5.9 ± 0.6 mV, respectively, in the control and 0.91 ± 0.1 , -10.1 ± 2.5 mV, and 5.4 ± 0.5 mV, respectively, in the presence of 0.65 mM sevoflurane. These values were not statistically significant.

Effects of sevoflurane on action potentials

We then investigated the effects of sevoflurane on action potentials in normal Tyrode solution. The action potential was elicited every 5 s under the current clamp mode of the conventional whole-cell clamp condition. Application of 0.65 mM sevoflurane prolonged the APD, while the amplitude of the action potential and the resting potentials remained unchanged. The effect was reversed upon wash-out (Fig. 8A). In ten experiments, APD₅₀ and APD₉₀ values were increased from 298.7 ± 12.0 and 320.2 ± 12.8 ms to 459.7 ± 26.1 and 486.1 ± 27.2 ms, respectively, by 0.65 mM sevoflurane. In another series of experiments, E4031 (5 μ M) was applied after the duration of the action potential was prolonged by 0.65 mM sevoflurane (Fig. 8B). E4031 further prolonged the action potential. In four experiments, APD₅₀ was 288.5 ± 15.2 , 472.6 ± 49.1 , and 598.5 ± 52.5 ms in the control, in the presence of 0.65 mM sevoflurane, and in the presence of both sevoflurane and E4031, respectively. The value of APD₉₀ was 309.7 ± 16.9 , 499.1 ± 51.7 , and 631.2 ± 50.2 ms in the control, in the presence of 0.65 mM sevoflurane, and in the presence of both sevoflurane and E4031, respectively.

Discussion

The present study has demonstrated that sevoflurane inhibits I_{Ks} in guinea pig ventricular cells, while I_{Kr} remained almost unaffected. The minimum alveolar concentration (MAC) of sevoflurane has been reported to be approximately 2% (0.79 mM). Given the blood/gas partition coefficient of 0.69 (21), the concentration in the blood equivalent to MAC would be 0.54 mM. In the present study, the IC₅₀ value of sevoflurane to inhibit I_{Ks} was 0.38 mM, indicating that sevoflurane depresses I_{Ks} at clinically relevant concentrations. It should be noted, however, that the amplitude of I_{Ks} was sometimes very large in the present study (more than 1 nA, for example see Fig. 1), and therefore the voltage error might have occurred under the voltage-clamp condition, particularly at positive potentials. Although we always compensated the series resistance after the rupture of patch membrane using the patch amplifier, such voltage error might have caused overestimation or underestimation of IC₅₀ values for I_{Ks} inhibition. Nevertheless, the inhibition of I_{Ks} was a consistent finding and it occurred in both reversible and concentration-dependent manners. Thus, we consider that the inhibitory action of sevoflurane on I_{Ks} has been successfully characterized in the present study.

The following findings support the lack of an obvious effect on I_{Kr}. Firstly, the tail current at -40 mV is relatively insensitive to sevoflurane, compared to the outwardly developing current during depolarization (Fig. 2). This should be true if sevoflurane inhibits I_{Ks} preferentially, since I_{Kr} inactivates quickly at positive potentials. The outwardly developing current is chiefly attributable to I_{Ks} at strong depolarization. However, the tail current includes both I_{Kr} and I_{Ks}, and the contribution of I_{Kr} to the tail current is relatively large with a short pulse duration and decreases with a prolonged pulse duration (1). Secondly, under chromanol 293B blockage of I_{Ks}, the remaining current was hardly affected by sevoflurane, while the subsequent application of E4031 abolished the current (Fig. 7). Finally, the action potential duration, prolonged by sevoflurane, was further prolonged by the additional application of E4031 (Fig. 8).

In contrast to selective inhibition by sevoflurane, halothane appeared to inhibit both I_{Ks} and I_{Kr}, when analyzed by the double-pulse protocols (Fig. 2). The finding is consistent with the recent report that halothane inhibited HERG (human ether-a-go-go-related gene) channels, which are partly responsible for cardiac I_{Kr} (22). Sevoflurane and halothane may act on I_{Kr} channels in a different manner. Also, the finding may be in line with the view that volatile anesthetics mainly alter the

function of ion channels by a direct action on proteins, rather than through indirect effects caused by a disturbance of the lipid environment around ionic channels (23).

Previous studies indicated an inhibitory effect of inhalational anesthetics on I_{Ks}, although these studies did not separate I_{Ks} and I_{Kr}. Hirota et al. reported that 2% halothane reduced I_K to 46% in guinea pig ventricular cells (9). Park et al. demonstrated that 0.35 mM sevoflurane reduced the time-dependent outward current at +90 mV in guinea pig ventricular cells (11). The IC₅₀ values obtained in the present study are consistent with these reports and the recent finding that in *Xenopus* oocytes, 1.5 MAC sevoflurane (approximately 0.8 mM) inhibited K⁺ currents produced by heterologous expression of KvLQT1 and KCNE1 to approximately 50% (12). Converting the IC₅₀ value to MAC gives 0.7 MAC for sevoflurane and 2.5 MAC for halothane, indicating that sevoflurane induced a more marked inhibition of I_{Ks} than halothane at equi-potent concentration. The inhibition of I_{Ks} by sevoflurane is characterized by decreased maximum conductance in the present study. The time course of activation appeared unaffected by sevoflurane. However, the deactivation time course was delayed slightly but significantly by sevoflurane. The finding is in contrast to the recent report by Chen et al., where sevoflurane accelerated the deactivation of KvLQT1 plus minK channels (12). The acceleration of I_{Ks} deactivation has also been reported when halothane was applied to the minK current in *Xenopus* oocytes (24). The discrepancy might be due to different experimental conditions. In the previous studies, the experiments were carried out at room temperature (22–24°C) and the deactivation was measured at -60 mV (12, 24). In the present study, the time course of deactivation was analyzed at -40 mV at 35–37°C. It is well known that I_{Ks} is enhanced by activation of protein kinase A and protein kinase C in a temperature-dependent fashion (25). The action of volatile anesthetics on the I_{Ks} deactivation might differ at different temperatures and/or at various membrane potentials. Another possibility is that volatile anesthetics may affect the voltage-dependent gates of I_{Ks} differently in native cells than in *Xenopus* oocytes. It has been reported that volatile anesthetics act on multiple cellular sites including ionic channels, neurotransmitter receptors, membrane proteins, intracellular second messenger pathways, and lipid metabolism (26–29).

One might speculate that the inhibition of cardiac I_{Ks}, described in the present study, not only represents a side effect of sevoflurane but also is related to the anesthetic action in the central nervous system. Many studies suggest the involvement of ligand-gated ionic channels

in mediating the action of volatile anesthetics (26, 28). Also a unique structural class of K^+ channels with two pore-forming sequences in tandem (2P K^+ channels), which contributes significantly to background K^+ currents in neuronal tissues, has recently emerged as plausible targets for volatile anesthetics (30, 31). However, little is known about whether delayed rectifier K^+ currents, which are sensitive to volatile anesthetics, is present in the central nervous system. It has been reported that KVLQT1 is strongly expressed in human heart and pancreas (32), and KCNE1 mRNA is abundant in the kidney, submandibular gland, and the uterus (33, 34). However, both are hardly expressed in the brain. Furthermore, mutations of the KVLQT1 or KCNE1 gene, which are involved in long QT syndrome, do not produce any obvious neuronal dysfunction, except in hearing disturbance (35). These findings indicate that the anesthetic action of sevoflurane is probably caused by a mechanism other than I_{Ks} inhibition.

It has been shown that sevoflurane or halothane affects not only I_K but also various ionic currents in cardiac myocytes. Halothane inhibits the fast Na^+ current (36), the I_{CaL} , and T-type Ca^{2+} current (I_{CaT}) (11) in guinea pig ventricles and the transient outward K^+ current in rat ventricles (37). Conversely, only a slight change or no effects were reported on the inward rectifier K^+ current (10, 11, 38). The repolarization of action potential would be determined by a balance of the above inward and outward current systems. In this respect, effects of volatile anesthetics on repolarization vary among different experimental conditions. For example, halothane depressed APD in human atrium (39) and guinea pig ventricle (10), whereas it increased it in frog atrium (40). Sevoflurane has been shown to prolong APD in guinea pig ventricular cells (11), whereas it shortened APD in canine ventricular cells (41) and guinea pig papillary muscle (42). Inhibition of I_{Ks} prolongs APD, whereas I_{CaL} inhibition would shorten it. We thus speculate that the effects of volatile anesthetics on the action potential may vary, depending on the species or the density of ionic channels within a region of the heart. Nonetheless, there is evidence that clinical use of volatile anesthetics is often associated with prolongation of QT interval in the surface electrocardiogram (3–7). All these findings, together with the evidence that I_{Ks} plays a functional role in the human heart (21), indicate that the inhibitory effect of sevoflurane on I_{Ks} may, at least in part, contribute to the clinical observations of the QT prolongation by the anesthetics.

In conclusion, by using native cardiac myocytes and selective inhibitors for I_{Kr} and I_{Ks} , we demonstrated that sevoflurane, at clinically relevant concentrations,

preferentially inhibits I_{Ks} and thereby prolongs APD. The prolongation of APD was enhanced when I_{Kr} was simultaneously blocked. It might be speculated that clinically relevant doses of sevoflurane or even low doses may trigger excessive QT prolongation, particularly when administered with other agents that block I_{Kr} .

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