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Electrophysiologic Mechanism Underlying Action Potential Prolongation by Sevoflurane in Rat Ventricular Myocytes

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Background: **Despite prolongation of the QTc interval in humans during sevoflurane anesthesia, little is known about the mechanisms that underlie these actions. In rat ventricular myocytes, the effect of sevoflurane on action potential duration and underlying electrophysiologic mechanisms were investigated.**

Methods: **The action potential was measured by using a current clamp technique. The transient outward K^+ current was recorded during depolarizing steps from −80 mV, followed by brief depolarization to −40 mV and then depolarization up to +60 mV. The voltage dependence of steady state inactivation was determined by using a standard double-pulse protocol. The sustained outward current was obtained by addition of 5 mM 4-aminopyridine. The inward rectifier K^+ current was recorded from a holding potential of −40 mV before their membrane potential was changed from −130 to 0 mV. Sevoflurane actions on L-type Ca^{2+} current were also obtained.**

Results: **Sevoflurane prolonged action potential duration, whereas the amplitude and resting membrane potential remained unchanged. The peak transient outward K^+ current at +60 mV was reduced by 18 ± 2% ($P < 0.05$) and 24 ± 2% ($P < 0.05$) by 0.35 and 0.7 mM sevoflurane, respectively. Sevoflurane had no effect on the sustained outward current. Whereas 0.7 mM sevoflurane did not shift the steady state inactivation curve, it accelerated the current inactivation ($P < 0.05$). The inward rectifier K^+ current at −130 mV was little altered by 0.7 mM sevoflurane. L-type Ca^{2+} current was reduced by 28 ± 3% ($P < 0.05$) and 33 ± 1% ($P < 0.05$) by 0.35 and 0.7 mM sevoflurane, respectively.**

Conclusions: **Action potential prolongation by clinically relevant concentrations of sevoflurane is due to the suppression of transient outward K^+ current in rat ventricular myocytes.**

SEVOFLURANE has been reported to prolong QTc interval in healthy adults[1,2] and children[3,4] during anesthesia induction. Furthermore, sevoflurane has been reported to markedly prolong the QT intervals in children with congenital long QT syndrome.[5,6] In *in vitro* animal preparations using guinea pig ventricular myocardium[7] and myocytes,[8,9] sevoflurane prolonged the action potential (AP) duration. This seemed to be caused by inhibition of the slowly activating delayed outward K^+ current with minimal effect on the inward rectifier K^+ current (I_{KI}). In contrast to this prolonging effect, shortening of AP duration by sevoflurane has also been observed in guinea pig myocardium,[10] canine,[11] and rat ventricular myocytes.[12]

Cardiac AP duration is determined by a balance between inward and outward membrane currents.[13,14] In most species, the transient outward K^+ current (I_{to}) is responsible for the initial phase of repolarization, and the L-type Ca^{2+} current ($I_{Ca,\ L}$) is the main inward current during the plateau phase. The delayed outward K^+ current (I_K) activation initiates repolarization near the end of the ventricular AP plateau, and the I_{KI} plays an important role in generating the resting membrane potential and in modulating the final repolarization phase of the ventricular AP.[14] The contribution of these currents varies between species and is responsible for the characteristic differences in AP shapes. For example, rat ventricular myocytes possess a prominent I_{to}, the major outward current of the repolarization phase, but little I_K, and have a short AP duration. In contrast, guinea pig ventricular cells lack I_{to}, but have a slowly activating I_k, resulting in a long-lasting AP.[15]

In rat ventricular myocytes, Rithalia *et al.*[12] observed a shortening effect of sevoflurane on AP duration, attributed to the reduced $I_{Ca,\ L}$, with little effect on I_{to}. In another recent study[9] using cloned human cardiac K^+ channels, sevoflurane inhibited Kv4.3 cardiac K^+ channel currents, suggesting inhibition of I_{to}. Based on the similar electrophysiologic characteristics of I_{to} between human and rat ventricular myocytes,[16] we speculated that the I_{to} in rat heart cells may be inhibited by sevoflurane. During preliminary experiments, we observed concentration-dependent prolongation of AP duration in rat ventricular myocytes, which contradicted the results of Rithalia *et al.*[12] Therefore, to elucidate the mechanisms of AP prolongation, we examined the effect of sevoflurane on I_{to} and inward rectifier K^+ current (I_{KI}), as well as $I_{Ca,\ L}$.

Materials and Methods

Myocyte Isolation

According to a protocol approved by the Yonsei University College of Medicine Animal Research Committee (Seoul, Korea), the heart was quickly excised from rats (Sprague-Dawley, weighing 250–300 g) after halothane anesthesia. The excised heart was retrogradely perfused by using a Langendorff perfusion system for 5 min at 37°C. The perfusion was at a rate

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of 7 ml/min with modified Tyrode solution containing 143 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM HEPES, and 0.18 mM glucose, pH 7.4. The perfusate was then switched to a nominally Ca^{2+}-free Tyrode solution for 5 min, followed by perfusion with the same solution to which collagenase (0.4 mg/ml, Worthington type II; Worthington Biochemical Corporation, Lakewood, NJ) and hyaluronidase (0.4 mg/ml, Sigma type II; Sigma-Aldrich Co., St. Louis, MO) had been added. After 10–12 min of enzymatic treatment, a final perfusion was performed for 5 min with a Kraftbrühe solution (10 mM taurine, 10 mM oxalic acid, 70 mM glutamic acid, 35 mM KCl, 10 mM H_2PO_4, 11 mM glucose, 0.5 mM EGTA, and 10 mM HEPES, pH 7.4). The ventricles were then cut off, minced with scissors, and agitated in a small beaker of a Kraftbrühe solution. The resulting slurry was filtered through a 200-μm nylon mesh. The isolated ventricular cells were stored in a Kraftbrühe solution for 1 h at room temperature (21°–22°C), then kept at 4°C, and used within a period of 8 h. Only the rod-shaped cells with apparent striations that remained quiescent in the solution containing 2 mM $CaCl_2$ were used for the experiments. All experiments were performed at room temperature.

Electrophysiologic Techniques

Isolated myocytes were allowed to settle to the bottom of a recording chamber, which was mounted onto an inverted microscope where the bathing solutions could be exchanged. The chamber was continuously perfused at a constant rate (2 ml/min). Standard whole cell voltage clamp methods were used.[17] To establish a stable baseline, an interval of 4–6 min was allowed after initiating the whole cell recording configuration. Voltage clamp measurements were performed by using an Axopatch 200B patch clamp amplifier (Axon Instruments Inc., Foster City, CA). Patch electrodes were prepared from a borosilicate glass model KIMAX-51 (American Scientific, Charlotte, NC), which have a typically 2- to 3-MΩ resistance when filled with an internal solution. After fabricating the pipettes with a two-stage micropipette puller, the pipette tips were heat-polished by using a microforge. Data acquisition was performed by using a pCLAMP system version 6.0 (Axon Instruments Inc.) coupled with a Pentium-III personal computer.

Voltage Clamp Protocols

The APS were elicited in current-clamp mode by 5-ms, 800-pA current injections at a frequency of 1 Hz.

To examine I_{to}, from a holding potential of −80 mV, the cells were depolarized to −40 mV for 50 ms to inactivate the Na^+ current and then depolarized to test potentials of up to +60 mV in 10-mV increments for 300 ms. To obtain more information about the possible mechanism of sevoflurane-induced voltage blockade of K^+ currents, the voltage dependence of steady state inactivation was determined by using a standard double-pulse protocol. The membrane potential was initially clamped at −80 mV and then stepped to different potentials ranging from −100 to 0 mV in 10-mV increments for 500 ms followed by a 200-ms test pulse to +80 mV. The voltage clamp protocol was repeated every 2 s. The steady state inactivation data were fitted by a Boltzmann distribution of the following equation: $I/I_{max} = I/\{1 + \exp[(V - V_{1/2})]/\kappa\}$, where I_{max} is the maximal current, $V_{1/2}$ is the membrane potential producing 50% inactivation, and κ is the slope factor.

To test whether the accelerated inactivation of I_{to} in the presence of sevoflurane was associated with time-dependent block of the open channel, the magnitude of current inhibition at various times after the initiation of the depolarizing pulse was determined at the membrane potential of +60 mV. The outward current in the presence of each concentration of sevoflurane, expressed as a proportion of the outward current observed in the control, was plotted as a function of time after the start of depolarization. A hyperbola function $[y = B_{max}(x/(k_d + x))]$ was used to fit the rate of block of I_{to} at each concentration of sevoflurane. B_{max} is the maximum block at drug concentration; K_d is the dissociation constant.

The sustained outward current (I_{sus}) was recorded with the same voltage protocol after addition of 5 mM 4-aminopyridine, which preferentially blocks I_{to}, in modified Tyrode solution. The outward currents activated by depolarizing voltage steps in rat ventricular myocytes consist of a rapidly inactivating component, I_{to}, and a noninactivating, sustained component, I_{sus}.[18,19] The sustained outward currents, comprising I_K and a small time-independent outward current,[18] remain in the presence of 4-aminopyridine. Whereas I_{to} underlies the initial, rapid repolarization phase of the AP, I_{sus} is responsible for the slower phase of AP repolarization back to the resting membrane potential in adult rat ventricular myocytes.[20]

The effect on the I_{KI} was verified by measuring the I_{KI} by step depolarizations from −130 to 0 mV from a holding potential of −40 mV in 10-mV increments, using a 200-ms pulse applied at 5-s intervals.

The voltage-dependent $I_{Ca,\,L}$ was evoked by step depolarizations that lasted 200 ms from a holding potential of −40 mV to +10 mV in one step at a frequency of 0.1 Hz.

After the baseline measurements, myocytes were exposed to 1.7% or 3.4% sevoflurane for 2–3 min, and recovery responses were measured after washes for 2–3 min to remove the drugs. A 2-min application of sevoflurane was sufficient to produce a stable and consistent effect in pilot experiments.

Solutions and Chemicals

Before establishing the whole cell recording configuration, modified Tyrode solution, containing 140 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, and 10 mM glucose, adjusted to pH 7.4 with 1N NaOH, was used as an external bathing solution. For K^+ current measurements, a patch pipette solution was used, containing 20 mM KCl, 110 mM K-aspartate, 10 mM EGTA, 10 mM HEPES, 1 mM $MgCl_2$, 5 mM K_2ATP, 1 mM $CaCl_2$, and 10 mM NaCl, adjusted to pH 7.2 with 3N KOH. To measure I_{sus}, 5 mM 4-aminopyridine was added to the modified Tyrode solution. As the pH of the modified Tyrode solution containing 4-aminopyridine revealed 9.04 ± 0.03 (n = 2), the pH was corrected to 7.4 with HCl before the experiment. To eliminate any confounding Ca^{2+} current, 0.2 mM $CdCl_2$ was added to the external solution after establishing the whole cell voltage clamp.

The inward Ca^{2+} current was measured by using a patch pipette solution containing 30 mM CsCl, 100 mM aspartic acid, 100 mM CsOH, 10 mM BAPTA, 10 mM HEPES, 10 mM phosphocreatine, 1 mM Na_2GTP, 5 mM Na_2ATP, 10 mM glucose, and 2 mM $MgCl_2$, adjusted to pH 7.2 with 1 M CsOH. Once whole cell recording was achieved, the bathing solution was exchanged to 140 mM NaCl, 5.4 mM CsCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, adjusted to pH 7.4 with 1 M CsOH. Sevoflurane was purchased from the Abbott Korea Ltd. (Seoul, Korea), and all other chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Before perfusion, sevoflurane was equilibrated in solution in one reservoir by passing 100% O_2 (flow rate: 0.2 l/min) for 15 min through a sevoflurane vaporizer (Sevotec 3; Ohmeda, West Yorkshire, United Kingdom). The end-tidal concentrations of sevoflurane were monitored by using a calibrated gas analyzer (Capnomac; Datex, Helsinki, Finland). As determined by gas chromatographic measurement, sevoflurane concentrations in the room-temperature Tyrode superfusate equilibrated for 15 min were 0.35 mM (n = 4) and 0.7 mM (n = 4) for 1.7% and 3.4% sevoflurane, respectively. With the Tyrode solution/gas partition coefficient of sevoflurane of 0.40 at 22°C,[21] 0.35 and 0.7 mM sevoflurane correspond to gas phase concentrations of 2.12% and 4.24%, respectively.

Statistical Analysis

Repeated measures of analysis of variance followed by the Student-Newman-Keuls test were applied to test for significant differences among control, drug application, and washout. An unpaired *t* test was used to compare the differences in currents of I_{to}, I_{KI}, and $I_{Ca,\ L}$ between 1.7% and 3.4% sevoflurane. All values are expressed as mean ± SEM. A *P* value less than 0.05 was considered significant.

Results

Normal Action Potential

Figure 1 shows concentration-dependent prolongation of AP duration observed in a rat ventricular myocyte. Sevoflurane at 0.35 mM (n = 6) prolonged the APD_{50} and APD_{90} to 129 ± 10% ($P < 0.05$) and 115 ± 2% ($P < 0.05$) of the control, respectively. Sevoflurane at 0.7 mM (n = 7) also prolonged the APD_{50} and APD_{90} to 133 ± 3% ($P <$ 0.05) and 139 ± 6% ($P < 0.05$) of the control, respectively. The AP amplitude and resting membrane potential remained unaltered at either concentration (table 1). The AP duration was completely restored to baseline after washout.

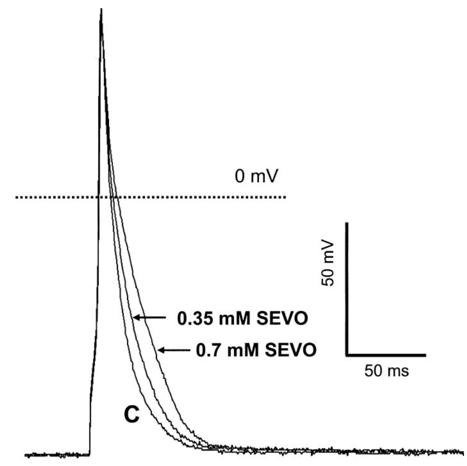

Fig. 1. Effect of sevoflurane (SEVO) on action potential duration in a rat ventricular myocyte. C = control.

Transient Outward K⁺ Current

At a membrane potential of +60 mV, 0.35 mM sevoflurane reduced the control peak currents of I_{to} (3.82 ± 0.54 nA) by 18 ± 2% (n = 7; $P < 0.05$), and the plateau currents measured at the end of depolarization (1.51 ± 0.12 nA) by 10 ± 3% (n = 7; $P < 0.05$). Sevoflurane, 0.7 mM, reduced peak currents of I_{to} (3.64 ± 0.30 nA) by 24 ± 2% (n = 11; $P < 0.05$) and plateau currents (1.52 ± 0.19 nA) by 10 ± 2% (n = 11; $P < 0.05$) (figs. 2A–C). Complete recovery of peak and plateau currents was observed after washout of either concentration (figs. 2A and B).

Under control conditions, the voltage required for half-inactivation ($V_{1/2}$) and slope factor (κ) for the I_{to} were −31.53 ± 0.68 mV and −6.23 ± 0.60 mV, respectively (n = 7). Sevoflurane, 0.7 mM, did not shift the steady

Table 1. Effects of Sevoflurane on Action Potential Characteristics in Isolated Rat Ventricular Myocytes

	RMP, mV	AMP, mV	APD_{50}, ms	APD_{90}, ms
0.35 mM SEVO (n = 6)				
Control	−74 ± 2	130 ± 5	5.52 ± 1	19.56 ± 2
SEVO	−74 ± 3	127 ± 6	6.87 ± 1*	22.45 ± 2*
Washout	−74 ± 3	128 ± 6	6.27 ± 1	20.24 ± 2
0.7 mM SEVO (n = 7)				
Control	−74 ± 2	134 ± 3	5.29 ± 0	18.49 ± 0
SEVO	−74 ± 2	128 ± 4	7.01 ± 1*	25.58 ± 1*†
Washout	−75 ± 3	129 ± 5	5.27 ± 0	17.02 ± 1

Values represent mean ± SEM.

* $P < 0.05$, different from control and washout values. † $P < 0.05$, different from 0.35 mM sevoflurane (SEVO).

AMP = action potential amplitude; APD_{50} = action potential duration measured at 50% of repolarization; APD_{90} = action potential duration measured at 90% of repolarization; RMP = resting membrane potential.

state inactivation curve ($V_{1/2}$ = −35.04 ± 0.84 mV, κ = −7.40 ± 0.74, n = 7; not significant) (fig. 3).

Because application of 0.7 mM sevoflurane in figure 2A indicated that sevoflurane accelerated the decay of current during the pulse, we evaluated the effect of sevoflurane on the kinetics of inactivation of I_{to}. Individual current records evoked by a test pulse to +60 mV were fitted with a double exponential function. Figures 4A and B show quality of fit of the double exponential function to the inactivation phase of I_{to} under control conditions and in the presence of 0.7 mM sevoflurane, respectively. The control τ_1 (47 ± 3 ms) was reduced to 27 ± 3 ms by 0.7 mM sevoflurane (n = 7; $P < 0.05$). Inactivation kinetics returned to baseline values after washout (47 ± 4 ms) (fig. 4C).

Figure 5 shows the inhibition of I_{to} by 0.35 and 0.7 mM sevoflurane during depolarization. The inhibition increased in a hyperbolic manner during depolarization. Both the magnitude of the maximum inhibition and the rate of development of the maximum inhibition seemed to be concentration dependent. The B_{max} values of 0.35 and 0.7 mM sevoflurane were 0.6 (n = 7) and 0.82 (n = 8), respectively. The K_d values of 0.35 and 0.7 mM sevoflurane were 19.66 ms (n = 7) and 15.84 ms (n = 8), respectively.

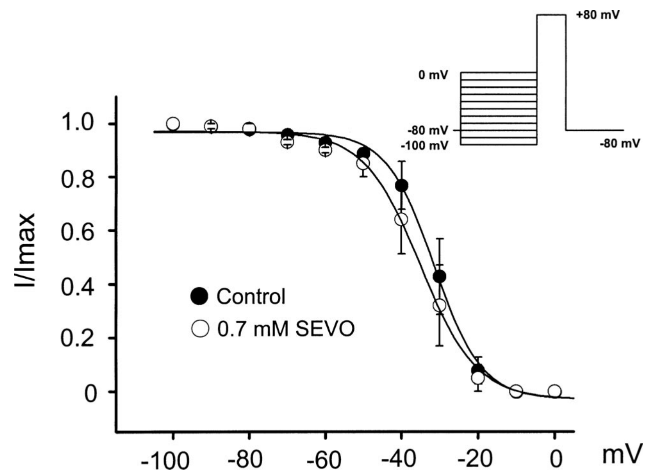

Fig. 3. Steady state inactivation curves of transient outward K^+ currents under control conditions and in the presence of 0.7 mM sevoflurane (SEVO). ***Closed*** **and** ***open circles*** **indicate control and 0.7 mM SEVO, respectively. Data are presented as mean ± SEM for four cells and were fitted with the Boltzmann function. The half-inactivations ($V_{1/2}$) of control and 0.7 mM SEVO were −31.53 ± 0.68 and −35.04 ± 0.84 mV, respectively, which showed no differences.** ***Error bars*** **indicate mean ± SEM.**

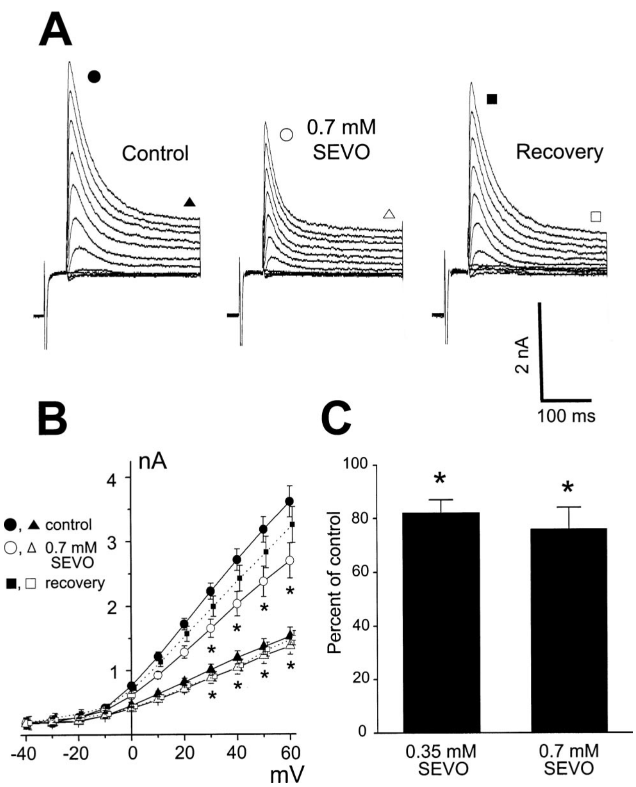

Fig. 2. Effects of sevoflurane (SEVO) on transient outward K^+ currents (I_{to}) in rat ventricular myocytes. (*A*) Recordings of control, 0.7 mM SEVO, and recovery in a rat ventricular myocyte. (*B*) Current–voltage relations of I_{to}. ***Closed*** **and** ***open circles*** **indicate the peak current of I_{to} at every potential in the control and in the presence of 0.7 mM SEVO.** ***Squares*** **are the peak (*closed*) and plateau (*open*) currents after washout.** ***Dotted lines*** **also indicate recovery.** ***Triangles*** **are the plateau current levels at the end of the test pulses before (*closed*) and after (*open*) application of 0.7 mM SEVO. (*C*) Effect of 0.35 and 0.7 mM SEVO on the amplitude of peak I_{to} at +60 mV. * $P < 0.05$ *versus* control.** ***Error bars*** **indicate mean ± SEM.**

Sustained Outward Current

Representative tracings of isolated I_{sus} by application of 5 mM 4-aminopyridine are illustrated in figures 6A and B. Sevoflurane, 0.7 mM, had no effect on the I_{sus} (n = 6) (fig. 6C). Before sevoflurane exposure, the baseline value of I_{sus} at the end of the plateau during test potential of +60 mV was 1.31 ± 0.12 nA (n = 6).

Inward Rectifier K^+ Current

At membrane potential ranges from −130 to 0 mV, 0.7 mM sevoflurane did not alter the I_{KI} (n = 7; not significant) (fig. 7B). The I_{KI} was measured at the end of the pulse duration. Before sevoflurane exposure, the baseline value during test potential of −130 mV was −2.92 ± 0.25 nA.

L-type Ca^{2+} Current

At a membrane potential of +10 mV, 0.35 and 0.7 mM sevoflurane reduced the $I_{Ca,\ L}$ by 28 ± 3% (n = 8; $P <$ 0.05) and 33 ± 1% (n = 7; $P < 0.05$), respectively (fig. 8B). The effect of sevoflurane on $I_{Ca,\ L}$ was completely reversible after washout. The baseline values before exposure to 0.35 and 0.7 mM sevoflurane were 0.75 ± 0.09 and 0.81 ± 0.2 nA, respectively.

Discussion

This study shows that clinically relevant concentrations of sevoflurane prolong the AP duration and significantly inhibit the I_{to} and $I_{Ca, L}$ in isolated rat ventricular myocytes. Although sevoflurane did not shift the steady state inactivation curve, it significantly accelerated inactivation of I_{to}.

Prolongation of the ventricular AP duration by sevoflurane has been observed in guinea pigs,[7–9] whereas other studies using canine ventricular cells[11] or guinea pig papillary muscles[10] have reported shortening of the AP duration. In a study using rat ventricular cells, sevoflurane caused modest but significant shortening of AP duration,[12] a curious difference from our study despite use of the same animal species.

In rat ventricular myocytes, cardiac voltage-activated K^+ current consists of I_{to} and I_K, sensitive to 4-aminopyridine and tetraethylammonium, respectively.[20] In frog and guinea pig ventricular myocytes, the dominant voltage-activated outward K^+ current is I_K.[22,23] However, in rat, dog, rabbit, and human myocytes, I_{to} is prominent and plays a significant role in the early repolarization phase of the AP.[24–27] In several cardiac tissues, two types of I_{to} have been identified; one is voltage- and Ca^{2+}-independent, and the other is Ca^{2+}-dependent.[28,29] In rat ventricular myocytes, only a Ca^{2+}-independent I_{to} has been identified.[30] The Ca^{2+}-independent I_{to}, carried predominantly by K^+ ions, has been suggested to be a major determinant of the cardiac AP duration because of its large size and pronounced frequency dependence.[29]

Rithalia *et al.*,[12] in their study using rat ventricular subendocardial and subepicardial myocytes, observed no changes of I_{to} by application of 0.6 mM sevoflurane

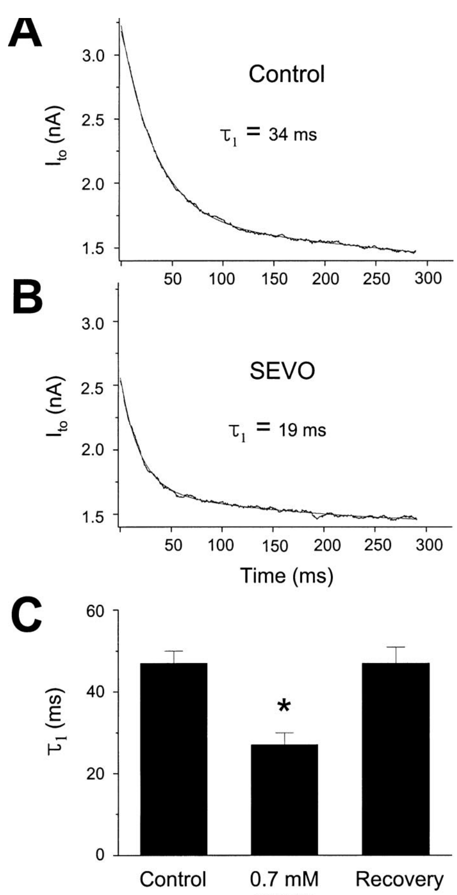

Fig. 4. Effect of sevoflurane (SEVO) on current inactivation. The inactivation phase of transient outward K^+ currents (I_{to}) were best fitted by a double exponential function under control conditions (*A*) and in the presence of 0.7 mM SEVO (*B*) in a rat ventricular myocyte. Mean values (± SEM) of τ_1 (n = 7) under control conditions, in the presence of 0.7 mM SEVO, and after washout (*C*). * $P < 0.05$ *versus* control and recovery. *Error bars* indicate mean ± SEM.

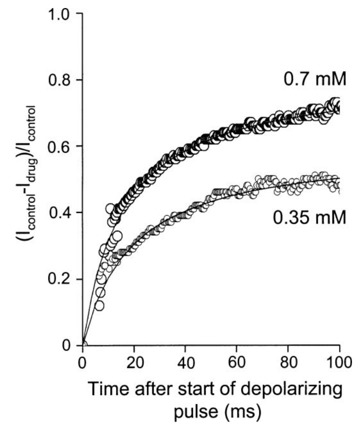

Fig. 5. Time-dependent inhibition of transient outward K^+ currents (I_{to}) by sevoflurane. Time course of the development of the inhibition by 0.35 mM (n = 7) and 0.7 mM (n = 8) sevoflurane after a depolarizing pulse to +60 mV from a holding potential of −40 mV. The reduction of I_{to} in the presence of sevoflurane is expressed as a proportion of the control current at any given time after the start of the depolarizing pulse.

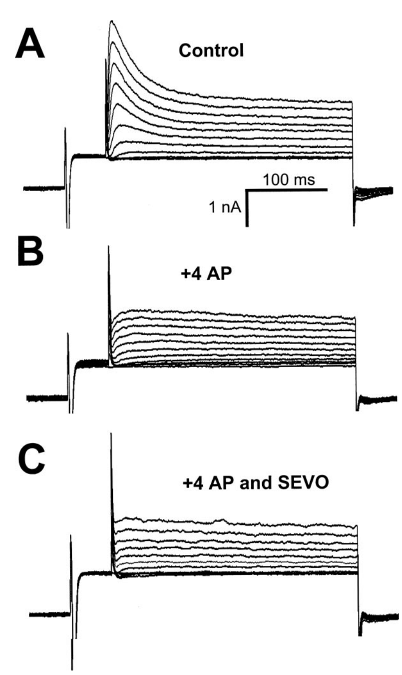

Fig. 6. Effect of sevoflurane (SEVO) on sustained outward currents (I_{sus}) in a rat ventricular myocyte. (*A*) A control recording of transient outward K^+ currents (I_{to}). (*B*) I_{sus} obtained after application of 5 mM 4-aminopyridine (4-AP), which preferentially blocks I_{to}. (*C*) 0.7 mM SEVO exposure after application of 5 mM 4-aminopyridine.

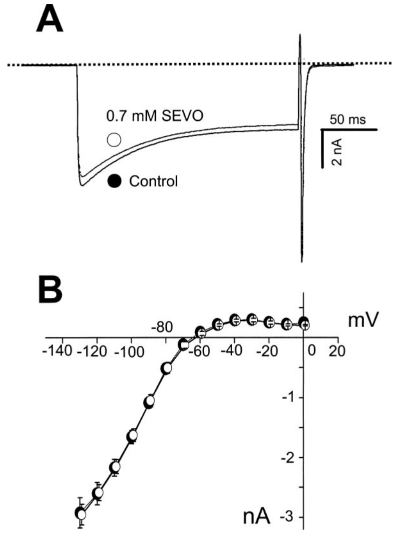

Fig. 7. Effect of sevoflurane (SEVO) on inward rectifier K^+ current (I_{KI}) in rat ventricular myocytes. (*A*) Closed and open circles indicate control and 0.7 mM SEVO in a rat ventricular myocyte, respectively, at a membrane potential of −130 mV. (*B*) Current–voltage relations for I_{KI} before and after addition of 0.7 mM SEVO (n = 7). *Closed* and *open circles* indicate control and 0.7 mM SEVO, respectively. *Error bars* indicate mean ± SEM.

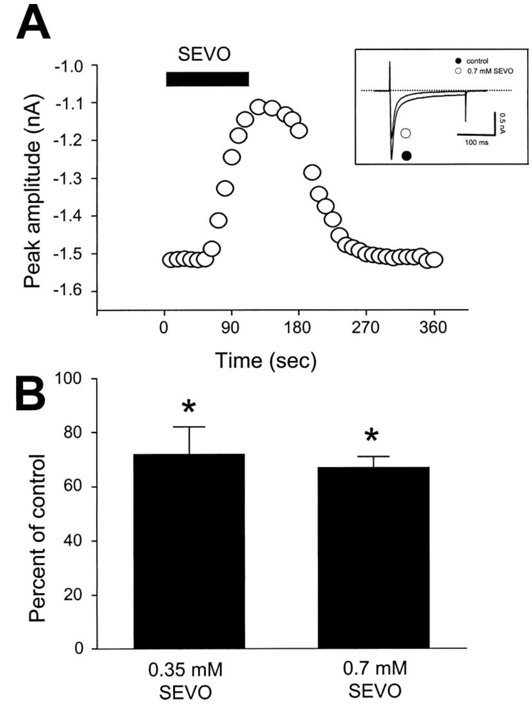

Fig. 8. Effect of sevoflurane (SEVO) on L-type Ca^{2+} current ($I_{Ca, L}$). (*A*) A representative example of the effect of SEVO on $I_{Ca, L}$ in a rat ventricular myocyte. The *open circles* represent the peak of an individual current record. The *horizontal bar* indicates the period when SEVO was applied. (*Inset*) An example of individual currents recorded in the presence of 0.7 mM SEVO. (*B*) Depression of $I_{Ca, L}$ after application of 0.35 and 0.7 mM SEVO, respectively. * $P < 0.05$ *versus* control. *Error bars* indicate mean ± SEM.

(30°C). In contrast, we found that sevoflurane caused a modest but significant depression of approximately 24% by 0.7 mM sevoflurane (22°C). In a recent study using cloned human cardiac K^+ channels, 3 mM sevoflurane inhibited the Kv4.3 cardiac K^+ channel currents by approximately 28% (35°C), suggesting inhibition of I_{to}.[9] Kv4.3 channel has been reported to underlie a significant fraction of I_{to} in the heart of several species, including rat, canine, and human.[31] Considering lower solubility at higher temperatures, the 0.6 mM sevoflurane at 30°C is estimated to be around 0.7 mM at 22°C.[7] Despite application of a similar concentration in the same animal species, it remains unclear why a disparity exists between the result of Rithalia *et al.* and ours. In the above study using cloned human cardiac K^+ channels, 0.43 mM sevoflurane at 37°C will be approximately 0.7 mM at 22°C.[7] Although a 0.43 mM concentration of sevoflurane caused modest depression of the Kv4.3 cardiac K^+ channel currents, significant acceleration of the rate of decay was shown at this concentration,[9] indicating a possible significant open channel inhibition in clinically relevant concentrations of sevoflurane. Considering the similar electrophysiologic characteristics of I_{to} between human and rat ventricular myocytes,[16] reduction of I_{to} in our results seems to correspond to that of Kang *et al.*[9] The prolongation of AP duration as a result of inhibition of I_{to} has been demonstrated with tedisamil, a blocker of I_{to} and I_K in isolated rat ventricular myocytes,[32] and was similar to that reported in isolated rat papillary muscles.[33] Our findings of approximately 24% reduction of I_{to} appear as a small fractional reduction. However, considering the large current density in rat ventricular myocytes, 19.9 ± 2.8 pA/pF at the membrane potential of +60 mV,[16] the effect on the plateau phase will be greater, resulting in AP prolongation.

The current study shows that sevoflurane decreased the peak I_{to} and also accelerated the rate of decay. The acceleration of the rate of decay could be explained by an anesthetic-induced acceleration of the normal conversion of open channels to an inactivated state with sustained depolarization. Sevoflurane could preferentially inhibit the open state of the channel. These findings are similar to that previously reported in rat ventricular I_{to} for quinidine (class Ia antiarrhythmic agent),[34] clofilium,[35] tedisamil (class III antiarrhythmic agent),[32] bupivacaine,[36] and imipramine in rabbit atrial I_{to}.[37]

In the current study, whereas there was no inhibition of I_{to} at the onset of the depolarizing pulses, we observed the inhibition of I_{to} upon continued depolarization. These results may indicate that sevoflurane does not bind to the resting state of the channel in an inhibitory fashion, but rather inhibits the open channel in a hyperbolic manner during continued depolarization. The lack of any effect on the voltage dependence of the steady state inactivation suggests that sevoflurane does not bind to the inactivated state of the channel.

The sustained outward currents, comprising I_K and a small time-independent outward current,[18] remain in the presence of 4-aminopyridine and tetraethylammonium. I_{sus} contributes to the overall repolarization process in rat ventricular myocytes. Whereas 0.7 mM sevoflurane has been reported to significantly depress the I_K in guinea pig ventricular cells, approximately 60%,[7] I_{sus} was not affected by sevoflurane. Although suppression of I_K by sevoflurane in guinea pig ventricular myocytes is mainly responsible for the AP prolongation, I_{sus} does not likely contribute to the prolongation of AP duration by sevoflurane in rat ventricular myocytes. Presumably, little I_{sus} change by sevoflurane may be attributed to marked variations among cells in rat ventricle in the relative amplitudes of I_{to} and I_K (I_{to}/I_K),[21] significant inhibition of I_K by 4-aminopyridine at concentrations above 1 mM,[18] and/or species differences.

The I_{KI} is the primary current responsible for maintaining a stable cardiac resting membrane potential near the K^+ equilibrium potential. Inhibition of I_{KI} can result in diastolic depolarization, which can increase cardiac excitability[38] and lead to dysrhythmias and abnormal automaticity.[39] Both sevoflurane and isoflurane have been reported to cause significant depression of inward component and enhancement of outward component of I_{KI} in guinea pig ventricular myocytes.[40,41] However, the actual differences from baseline values seemed to be modest, suggesting minimal effect on I_{KI} by these anesthetics. Modest effects on inward and outward components of I_{KI} by sevoflurane have been reported in guinea pig ventricular myocytes,[7] and our results in rat ventricular myocytes also showed no depression of the inward component of I_{KI} by sevoflurane. The lack of change in the resting membrane potential after application of 0.7 mM sevoflurane in our results may also reflect the lack of effect on I_{KI}.

In the current study on steady state inactivation of I_{to}, approximately 80% of the channels seemed to be available for activation at −40 mV. This indicates that the outward component of I_{KI} above −40 mV may include I_{to} and thus can influence the outward component of I_{KI} in this preparation.

Our whole cell voltage clamp studies revealed a reduction of peak $I_{Ca,\ L}$ despite the prolongation of AP duration. Suppression of $I_{Ca,\ L}$ by sevoflurane has been reported in various animal preparations.[7,9,11,12] Inhibition of $I_{Ca,\ L}$ can lead to a shortening of AP duration, however, the reduction of I_{to} would seem to have a greater effect, resulting in lengthening of the AP duration.

In human ventricular myocytes, although the current density of I_{to} has been reported to be two or three times smaller than that of the rat myocytes (8.2 ± 0.7 pA/pF at +60 mV), it is, nonetheless, a major outward current in human myocytes.[16] Small changes of I_{to} during the early phase of the AP can profoundly affect the activation of other plateau currents, such as Ca^{2+} and the delayed outward K^+ currents, influencing the AP duration and Ca^{2+} influx.[27]

In conclusion, prolongation of AP duration, induced by clinically relevant concentrations of sevoflurane, appears due to the suppression of I_{to} in rat ventricular myocytes. Considering that the voltage and time dependence of I_{to} in human ventricular myocytes are similar to those found in rat heart cells[16] and prolongation of AP duration in failing human heart seems to be prominently caused by a reduction in the level of Kv4.3 mRNA, resulting in down-regulation of Ca^{2+}-independent I_{to},[42] suppression of I_{to} by sevoflurane may partly account for the clinical observation of QTc prolongation in humans. In addition, considering the presence of I_{ks} in healthy human ventricle,[43,44] inhibition of I_{ks} by sevoflurane[8] may also contribute to QTc prolongation.

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