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Mechanisms Underlying the QT Interval–prolonging Effects of Sevoflurane and Its Interactions with Other QT-prolonging Drugs

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Background: **Sevoflurane prolongs ventricular repolarization in patients, but the mechanisms are not fully characterized. The effects of sevoflurane on many cloned human cardiac ion channels have not been studied, and the interactions between sevoflurane and other drugs that prolong cardiac repolarization have not been detailed.**

Methods: **The effects of sevoflurane on action potentials and L-type Ca^{2+} channels in guinea pig myocytes were examined. Sevoflurane's effects on cloned human cardiac K^+ channels and the cloned human cardiac Na^+ channel were studied. The consequences of combining sevoflurane and the class III antiarrhythmic drugs sotalol or dofetilide on action potential duration were also examined.**

Results: **Sevoflurane produced an increase in action potential duration at concentrations of 0.3–1 mM. Contrary to most drugs that delay ventricular repolarization, sevoflurane was without effect on the human *ether-a-go-go*–related gene cardiac potassium channel but instead produced a reduction in KvLQT1/minK K^+ channel currents and inhibited the Kv4.3 K^+ channel by speeding its apparent rate of inactivation. Sevoflurane had little effect on Na^+ and Ca^{2+} channel currents at concentrations of 1 mM or less. When the authors coadministered sevoflurane with sotalol or dofetilide, synergistic effects on repolarization were observed, resulting in large increases in action potential duration (up to 66%).**

Conclusion: **Prolonged ventricular repolarization observed with administration of sevoflurane results from inhibition of KvLQT1/minK and Kv4.3 cardiac K^+ channels. Combining sevoflurane with class III antiarrhythmic drugs results in supraadditive effects on action potential duration. The results indicate that sevoflurane, when administered with this class of drug, could result in excessive delays in ventricular repolarization. The results suggest the need for further clinical studies.**

DRUG interactions with one or more cardiac ion channels can cause a delay in myocardial repolarization resulting in prolongation of the action potential duration on the cellular level. This in turn prolongs the time interval measured from the start of the Q wave to the end of the T wave (QT interval) recorded on the electrocardiogram. Prolongation of the QT interval may be associated with the generation of the ventricular arrhythmia known as torsades de pointes.[1] It is now well established that virtually all cases of drug-induced QT interval prolongation are a result of blocking one specific cardiac ion channel known as HERG (human *ether-a-go-go*–related gene), the K^+ channel that carries I_{Kr}, the rapid delayed rectifier K^+ current in the human heart.[2–4] Exceptions to this phenomenon are rare but include drugs such as mefloquine, which preferentially blocks KvLQT1/minK, the K^+ channel that carries the slow delayed rectifier current, I_{Ks},[5] and arsenic trioxide and pentamidine, which prolong QT interval *via* a disruption of HERG channel trafficking leading to lower numbers of functional channels on the membrane surface.[6,7]

Sevoflurane is a general anesthetic that produces QT interval prolongation in humans. Sevoflurane has been shown to prolong the QT interval in children,[8,9] women,[10] and the general adult patient population.[11,12] Although sevoflurane has been shown to prolong action potential duration in guinea pig cardiac preparations, the mechanism of this effect is controversial. One publication has indicated that I_{Kr} is the main molecular target for sevoflurane,[13] whereas another has concluded that sevoflurane predominantly blocks I_{Ks}.[14] However, sevoflurane's effects on human cardiac electrical activity are incompletely defined because the drug has never been tested on many cloned human cardiac ion channels, including the human cardiac Na^+ channel (Nav1.5), and the Kv4.3 K^+ channel that underlies the transient outward K^+ current prominent in the human myocardium.[15,16] Furthermore, the consequences of combining sevoflurane with other drugs that prolong ventricular repolarization in humans are not known. The purpose of the current study was to test the effects of sevoflurane on action potential parameters in guinea pig myocytes and then systematically examine the drug's effects on the L-type Ca^{2+} channel as well as the cloned human cardiac Na^+ channel and the cloned human cardiac K^+ channels HERG, KvLQT1/minK and Kv4.3. Because sevoflurane may be administered to patients who are concurrently receiving antiarrhythmic drug therapy, we also tested the effects of the drug on action potential parameters in combination with the prototypical class III antiarrhythmic agents sotalol and dofetilide, two drugs associated with QT interval prolongation in clinical use.[4]

Materials and Methods

Experimental procedures and protocols were approved by the sanofi-aventis Institutional Animal Care and Use Committee (Bridgewater, New Jersey).

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Cell Culture

Chinese hamster ovary cells (American Type Culture Collection, Manassas, VA) were stably transfected with the complementary DNA (cDNA) encoding the human cardiac K^+ channels HERG, Kv4.3, or KvLQT1/minK as previously described.[17–19] Cells were grown in Ham's F-12 media supplemented with 10% fetal bovine serum and 500 μg/ml G418 (Invitrogen, Carlsbad, CA) in an atmosphere of 95% air–5% carbon dioxide. The cDNA encoding SCN5A, the human cardiac Na^+ channel, was stably transfected into human embryonic kidney cells (American Type Culture Collection) as described previously.[19]

Myocyte Isolation

Single ventricular myocytes were isolated from guinea pigs and used to record action potentials and Ca^{2+} channel currents. Single ventricular myocytes were isolated from guinea pigs using a method modified from that described by Salata *et al.*[20] In brief, male Hartley guinea pigs were anesthetized with 5% isoflurane (Baxter Healthcare, Deerfield, IL) in a mixture of nitrous oxide and oxygen (7:3). A thoracotomy was performed, and the heart was removed and immediately transferred to oxygenated cold saline. The heart was perfused retrogradely at 10 ml/min through the aorta with an oxygenated (100% oxygen) Ca^{2+}-free saline at 36°C in three stages: first with standard Ca^{2+}-free saline for 5 min, then with the same solution containing 280 U/ml type II collagenase (Worthington Biochemicals, Freehold, NJ) plus 0.75 U/ml type XIV protease (Sigma Aldrich, St. Louis, MO) for 8 min, and finally with saline containing 0.2 mM $CaCl_2$ for an additional 7 min. The left ventricle was cut into small pieces and was gently shaken at room temperature for approximately 2 min to disperse single myocytes. The isolated myocytes were then maintained at room temperature for electrophysiologic recording within 4–5 h after isolation.

Patch Clamp Recordings

All ionic currents were recorded at 35° ± 1°C using the whole cell configuration of the patch clamp technique.[21] Electrodes (resistance, 1–3 MΩ) were made from TW150F glass capillary tubes (WPI, Sarasota, FL). For all K^+ channel recordings, electrodes were filled with the following solution: 120 mM potassium aspartate, 20 mM KCl, 4 mM disodium adenosine triphosphate, 5 mM HEPES, and 1 mM $MgCl_2$, pH 7.2 with KOH. The external solution contained 130 mM NaCl, 4 mM KCl, 2.8 mM sodium acetate, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, and 1 mM $CaCl_2$, pH 7.4 with NaOH. The internal and external solutions for Na^+ and Ca^{2+} channels recordings have been described previously.[22] All ionic currents were recorded using an Axopatch 200B amplifier (Molecular Devices Corporation, Union City, CA).

Action Potential Recording

Myocytes were placed in a temperature-controlled (35° ± 1°C) chamber and perfused with a modified Tyrode solution containing 132 mM NaCl, 4 mM KCl, 1.2 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.4 with NaOH. Action potentials were recorded using a standard glass microelectrode filled with 3 M KCl (resistance, 20–45 MΩ). Action potentials were amplified using an AxoClamp 2B amplifier (Molecular Devices). Myocytes were paced using an external stimulator and were allowed to equilibrate at a rate of 1 Hz for approximately 20 min before initiation of experiments. All data were collected at a frequency of 1 Hz, and the resting membrane potential, action potential amplitude, and action potential durations at 50% (APD_{50}) and 90% (APD_{90}) repolarization were determined.

Chemicals

Sevoflurane was purchased from Henry Schein, Inc. (Melville, NY) and was formulated as dimethyl sulfoxide stock solutions as previously described.[23] Final dilutions were made in the external buffer solutions just before delivery to the cells. Dofetilide was purchased from Sequoia Research Products (Oxford, United Kingdom). All other reagents were obtained from Sigma Aldrich.

Statistical Analysis

All ionic currents were analyzed using the Clampfit program within the pCLAMP suite of software (Molecular Devices). Statistical differences between treated and untreated groups were analyzed using a repeated-measures analysis of variance followed by a Dunnett test using SigmaStat software, version 2.03 (Systat Software, Inc., Richmond, CA). A P value of less than 0.05 was considered statistically significant. IC_{50} values were obtained by nonlinear least squares fit of the data (GraphPad Software, Inc., San Diego, CA).

Results

Sevoflurane Effects on Action Potential Parameters

Peak plasma levels of sevoflurane achieved during anesthesia have been reported to approximate 0.7 mM.[24] Using concentrations similar to this value, we first examined what effects sevoflurane had on the action potential waveform recorded in single guinea pig myocytes (fig. 1A). Before the addition of drug, the resting membrane potential and action potential amplitude measured −89 ± 0.2 and 129 ± 1 mV, respectively, in these cells (n = 6). Sevoflurane (0.1–1 mM) had no effect on either of these parameters. Action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization averaged 209 ± 9 and 242 ± 11 ms, respectively, before the addition of sevoflurane (n = 6). APD_{50} was significantly ($P < 0.05$) increased by 10 ± 2 and 13 ± 3% after addition of 0.3

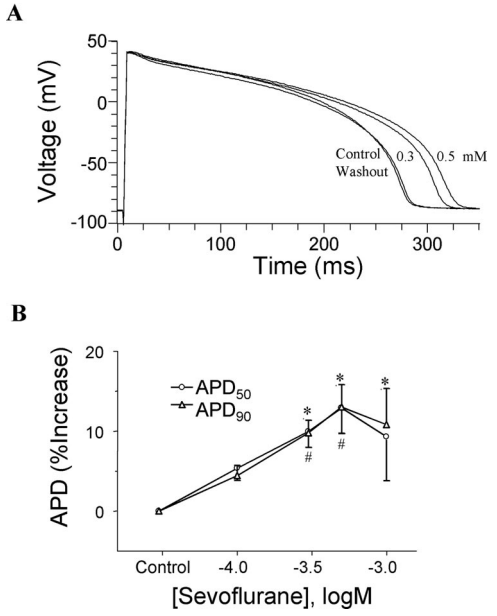

Fig. 1. Effects of sevoflurane on action potentials recorded from single guinea pig myocytes. (*A*) Action potential waveforms in the absence and presence of 0.3 and 0.5 mM sevoflurane and after washout of the drug are shown. (*B*) Effects of sevoflurane on action potential duration (APD). Dose–response relations for the lengthening of the action potential at 50% and 90% repolarization (APD_{50} and APD_{90}, respectively) are illustrated. * (APD_{90}), # (APD_{50}) significantly different from corresponding control values ($P < 0.05$). *Error bars* denote SEM (n = 6).

and 0.5 mM sevoflurane (fig. 1B). APD_{90} was also significantly ($P < 0.05$) prolonged, increasing by 10 ± 2, 13 ± 3, and 11 ± 5% after addition of 0.3, 0.5, and 1.0 mM sevoflurane (fig. 1B). In three of six cells, the action potential prolongation after the addition of 1.0 mM sevoflurane was less compared with that observed after the 0.5-mM treatment.

Effects of Sevoflurane on Ion Channel Currents

To elucidate the mechanisms underlying the observed prolongation in action potential duration, we examined the effects of sevoflurane on the human cardiac K^+ channel HERG and on I_{Kr} recorded from guinea pig myocytes. To record HERG currents, Chinese hamster ovary cells stably expressing HERG were held at −90 mV, depolarized to +10 mV for 300 ms, and then repolarized *via* a −0.5-V/s ramp back to −90 mV (stimulation frequency, 0.2 Hz). Peak outward currents during the repolarizing ramps were recorded in the absence and presence of sevoflurane (fig. 2A). Sevoflurane had little effect on HERG channel currents producing only 17 ± 9% inhibition at a concentration of 3 mM (the solubility limit of the drug under these conditions) and only 2–7% inhibition at concentrations of 0.1–1 mM (fig. 2B). Figure 2C illustrates the effects of sevoflurane on I_{Kr} recorded from guinea pig myocytes. Myocytes were held at −50 mV and depolarized to +10 mV for 300 ms at a rate of 0.2 Hz. Cells were then returned to −50 mV, and dofetilide-sensitive tail currents indicative of I_{Kr} were recorded. Figure 2C shows the effects of 0.5 mM sevoflurane on I_{Kr} as well as the subsequent addition of 1 μM dofetilide. Under these conditions, 0.5 mM sevoflurane had no discernible effect on I_{Kr} (4 ± 2% inhibition).

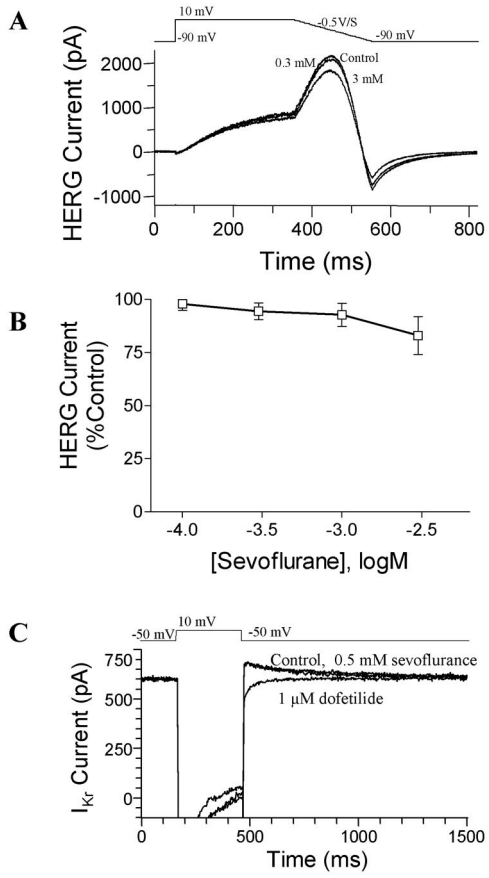

Fig. 2. Effects of sevoflurane on the cloned human *ether-a-go-go*–related gene (HERG) cardiac K^+ channel and the native rapid delayed rectifier potassium current (I_{Kr}). (*A*) Whole cell HERG channel currents were elicited by 300-ms depolarizing pulses to +10 mV from a holding potential of −90 mV every 5 s. Cells were then returned to −90 mV *via* a −0.5-V/s ramp to generate large outward tail currents. Current was measured in picoamperes. The effects of 0.3 and 3 mM sevoflurane are shown. (*B*) Dose–response relation for sevoflurane inhibition of peak HERG tail currents measured during the repolarizing ramp. Sevoflurane had little effect on HERG channel currents producing a maximal inhibition of 17 ± 9% at 3 mM. *Error bars* denote SEM (n = 6). (*C*) Lack of effect of sevoflurane (0.5 mM) on the rapid delayed rectifier K^+ current, I_{Kr}, in guinea pig myocytes. I_{Kr} was elicited by 300-ms depolarizing pulses to +10 mV from a holding potential of −50 mV every 5 s. Cells were then returned to −50 mV, and dofetilide-sensitive tail currents indicative of I_{Kr} were recorded. Tail currents in the presence and absence of 0.5 mM sevoflurane and the subsequent addition of 1 μM dofetilide are shown.

Figure 3 illustrates the effects of sevoflurane on the KvLQT1/minK K^+ channel expressed in Chinese hamster ovary cells and on I_{Ks} recorded from guinea pig myocytes. For KvLQT1/minK recordings, cells were held at −90 mV, and currents were elicited by step depolarizations to +10 mV for 300 ms at a frequency of 0.2 Hz. Sevoflurane produced a concentration-dependent inhibition of KvLQT1/minK currents that

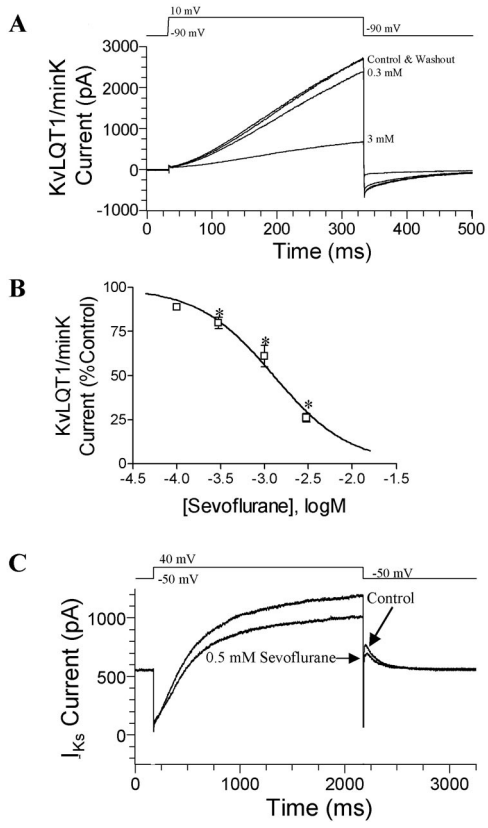

Fig. 3. Sevoflurane inhibition of cloned KvLQT1/mink potassium channels and the native slow delayed rectifier potassium current (I_{Ks}). (*A*) Whole cell KvLQT1/minK currents were generated by 300-ms depolarizing pulses to +10 mV from a holding potential of −90 mV every 5 s. Current was measured in picoamperes. The effects of 0.3 and 3 mM sevoflurane and its subsequent washout are illustrated. (*B*) Dose–response relation for sevoflurane inhibition of peak KvLQT1/minK channel currents. The IC_{50} value for sevoflurane measured 1.3 mM. * Statistically significant from control value ($P < 0.05$). *Error bars* indicate SEM (n = 6). (*C*) Effects of sevoflurane on the slow delayed rectifier K^+ current, I_{Ks}, in guinea pig myocytes. I_{Ks} was elicited by 2-s step depolarizations to +40 mV from a holding potential of −50 mV every 5 s. Cells were then returned to −50 mV, and peak tail currents were measured. The effects of 0.5 mM sevoflurane are shown. The external solution contained 1 μM verapamil and 1 μM dofetilide to suppress L-type Ca^{2+} channel currents and I_{Kr}, respectively.

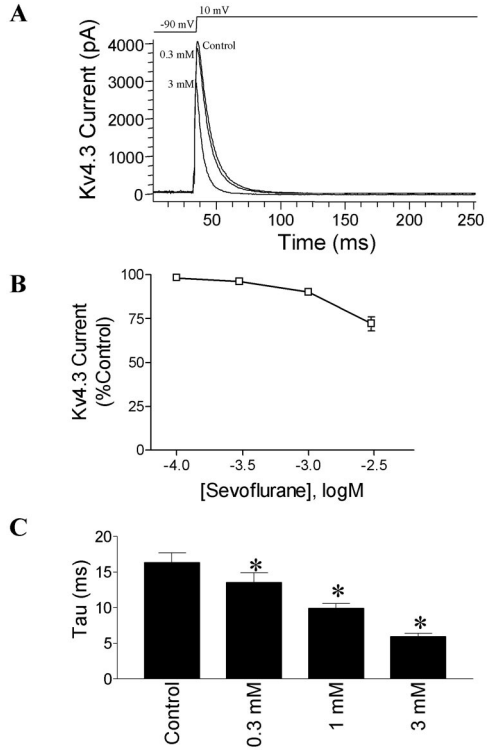

Fig. 4. Effects of sevoflurane on the cloned human cardiac transient outward potassium channel, Kv4.3. (*A*) Whole cell Kv4.3 channel currents were elicited by 300-ms depolarizing pulses to +10 mV from a holding potential of −90 mV every 5 s. Current was measured in picoamperes. The effects of 0.3 and 3 mM sevoflurane on Kv4.3 are shown. (*B*) Dose–response relation for sevoflurane inhibition of peak Kv4.3 channel currents. Maximal inhibition of Kv4.3 by sevoflurane measured 28% at a concentration of 3 mM. *Error bars* denote SEM (n = 8). (*C*) Sevoflurane speeds Kv4.3 current decay. The Kv4.3 current decay time constant (Tau) was fit to a single exponential function and measured 16.3 ms before addition of drug. Sevoflurane accelerated the rate of Kv4.3 current decay at concentrations of 0.3 mM and higher. * Significantly different compared with control values ($P < 0.05$). *Error bars* denote SEM.

was completely reversible upon washing the cell with drug-free solution (fig. 3A). Statistically significant ($P < 0.05$) inhibition was noted at concentrations of 0.3 mM and higher. Figure 3B shows the dose–response relation for sevoflurane's inhibition of KvLQT1/minK. The IC_{50} values for sevoflurane inhibition of KvLQT1/minK measured 1.3 mM (95% confidence limits, 0.7–2.4 mM). Figure 3C illustrates the effects of sevoflurane on I_{Ks} recorded from guinea pig myocytes. Cells were held at −50 mV and depolarized for 2 s to +40 mV at a rate of 0.2 Hz. Cells were returned to −50 mV, and peak I_{Ks} tail currents were recorded. The external solution contained 1 μM dofetilide and 1 μM verapamil to suppress I_{Kr} and the L-type Ca^{2+} current, respectively. Under these conditions, sevoflurane (0.5 mM) reduced I_{Ks} by 44 ± 2% (n = 6).

The effects of sevoflurane on the human cardiac Kv4.3 K^+ channel are shown in figure 4. Sevoflurane had a modest effect on Kv4.3 current amplitude inhibiting peak Kv4.3 currents by 28 ± 4% at 3 mM (figs. 4A and B). However, we noted that sevoflurane accelerated the rate of current decay at much lower concentrations. When fit to a single exponential function, Kv4.3 current decayed with a time constant of 16.3 ± 1.4 ms ($r^2 = 0.98$, n = 9). This value was significantly ($P < 0.05$) reduced in the presence of sevoflurane (0.3 mM and above) and measured 13.5 ± 1.4, 9.9 ± 0.7, and 5.9 ± 0.5 after addition of 0.3, 1, and 3 mM sevoflurane, respectively (fig. 4C).

The effects of sevoflurane on cardiac Ca^{2+} and Na^+ channels are illustrated in figure 5. L-type Ca^{2+} channel currents were recorded from single guinea pig myocytes because we did not have access to the cloned human channel. Cells were held at −40 mV,

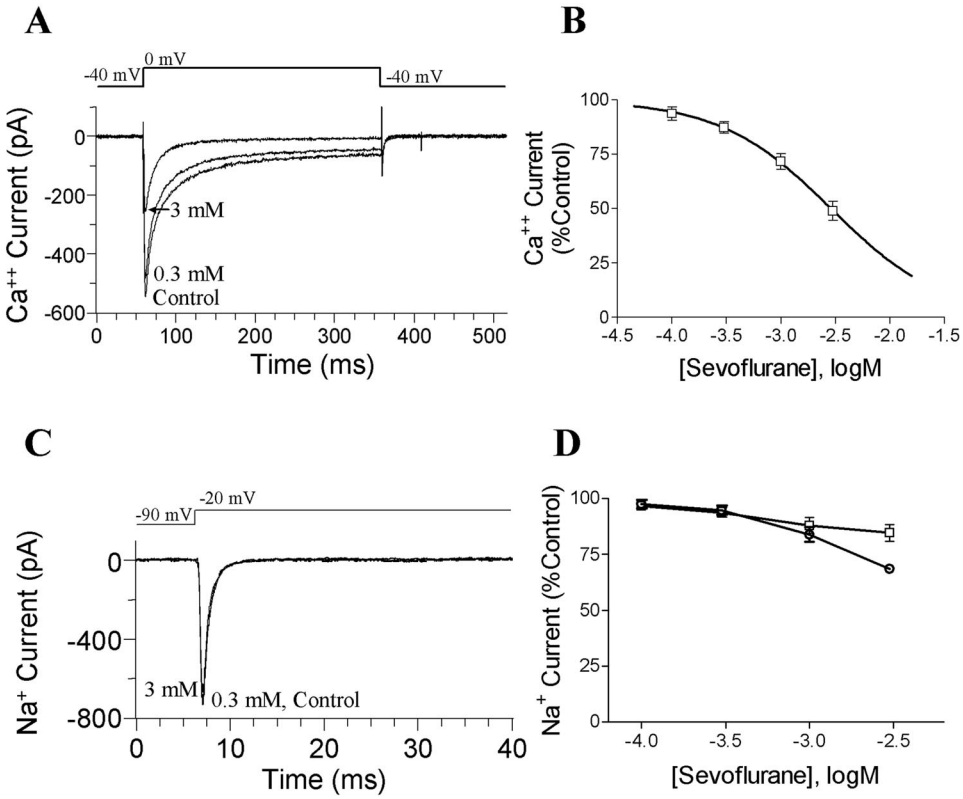

Fig. 5. Effects of sevoflurane on L-type Ca^{2+} channel currents and cloned human cardiac Na^+ channel currents. (*A*) L-type Ca^{2+} channel currents were recorded from guinea pig myocytes. Currents were generated by 300-ms depolarizing pulses to 0 mV from a holding potential of −40 mV every 5 s. The effects of 0.3 and 3 mM sevoflurane are shown. (*B*) Dose–response relation for sevoflurane block of Ca^{2+} channel current. The IC_{50} approximates 3 mM, the solubility limit for sevoflurane under these conditions. *Error bars* indicate SEM (n = 6). (*C*) Human cardiac Na^+ channel (Nav1.5) currents were elicited by 50-ms step depolarizations to −20 mV from a holding potential of −90 mV every 5 s. The effects of 0.3 and 3 mM sevoflurane are shown. (*D*) Dose–response relation for sevoflurane inhibition of peak Na^+ channel currents. Data were obtained from a holding potential of −90 mV (*open squares*) or −70 mV (*open circles*). *Error bars* denote SEM (n = 6).

and currents were elicited by 300-ms step pulses to 0 mV delivered at a rate of 0.2 Hz. Sevoflurane produced a dose-dependent reduction in Ca^{2+} channel current that reached a maximum of 51 ± 4% at a concentration of 3 mM (figs. 5A and B). The effects of sevoflurane on the human cardiac Na^+ channel (Nav1.5) stably transfected into human embryonic kidney cells are shown in figures 5C and D. Sevoflurane was weakly active on sodium channel currents producing only 15 ± 4% inhibition at a concentration of 3 mM and a holding potential of −90 mV. When the holding potential was set at −70 mV, maximal inhibition of Na^+ channel currents reached 31 ± 2% (fig. 5D).

Interactions between Sevoflurane and Class III Antiarrhythmic Drugs

We next examined the effect sevoflurane would have on the action potential in combination with sotalol, a drug known to prolong cardiac repolarization. In this experiment, we exposed single guinea pig myocytes to 0.5 mM sevoflurane. This treatment resulted in a 10% increase in both APD_{50} and APD_{90} (n = 6; figs. 6A–C). After washing out the sevoflurane and returning the action potential duration to within approximately 1% of its original value, we challenged the cells with 100 μM of the class III antiarrhythmic drug sotalol. This produced a 15 ± 2% increase in APD_{50} and a 16 ± 2% increase in APD_{90}. After this, cells were rechallenged with 0.5 mM sevoflurane in the presence of 100 μM sotalol. Rather than producing simple additive effects, the combination of the two drugs resulted in a synergism that prolonged APD_{50} and APD_{90} by 34 ± 3 and 39 ± 2%, respectively (figs. 6B and C).

We extended these findings by next testing the interaction between sevoflurane and another class III antiarrhythmic drug, dofetilide. Again, guinea pig heart cells were challenged with 0.5 mM sevoflurane, and this treatment resulted in a reversible 15% prolongation in both the APD_{50} and APD_{90} (n = 6; figs. 7A–C). After washout of sevoflurane, the cells were exposed to 10 nM dofetilide. This treatment resulted in a 19 ± 4% increase in APD_{50} and a 20 ± 5% increase in APD_{90}. When sevoflurane was then added back to the cells in the presence of 10 nM dofetilide, large increases in both APD_{50} (63 ± 10%) and APD_{90} (66 ± 11%) were observed (figs. 7B and C).

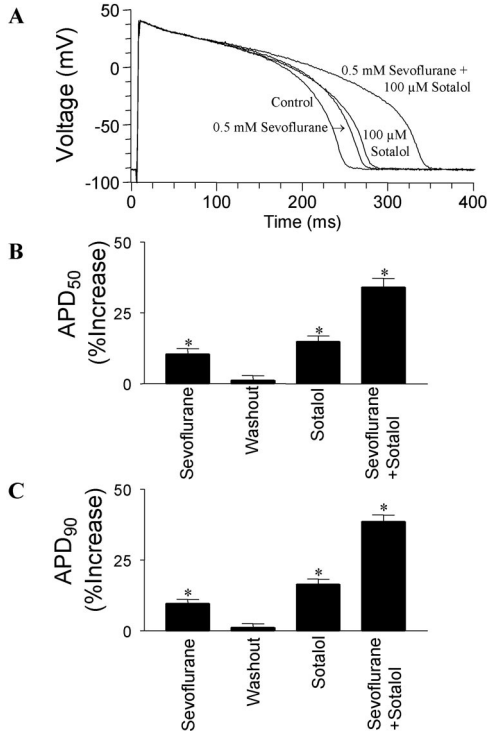

Fig. 6. Effects of sevoflurane and sotalol on action potentials recorded from guinea pig myocytes. (*A*) Action potential waveforms showing the effects of sevoflurane (0.5 mM) alone, sotalol (100 μM) alone, and the combination of the two drugs. (*B*) Summary of the action potential duration at 50% repolarization (APD_{50}) after various treatment conditions. * Significantly different from control ($P < 0.05$). *Error bars* denote SEM (n = 6). (*C*) Summary of the action potential duration at 90% repolarization (APD_{90}) after various treatment conditions. * Significantly different from control value ($P < 0.05$). *Error bars* denote SEM. For *B* and *C*, the data are expressed as the percent increase over the control (pretreatment) value.

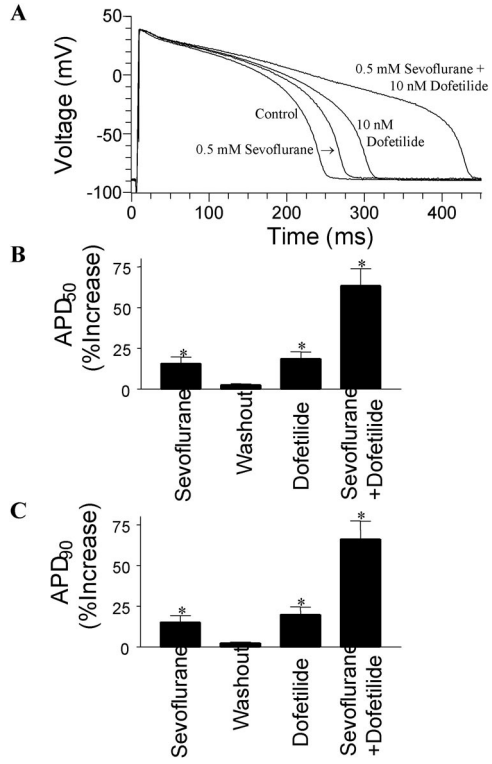

Fig. 7. Effects of sevoflurane and dofetilide on action potentials recorded from guinea pig myocytes. (*A*) Action potential waveforms illustrating the effects of sevoflurane (0.5 mM) alone, dofetilide (10 nM) alone, and the combination of the two drugs. (*B*) Summary of the action potential duration at 50% repolarization (APD_{50}) after various treatment conditions. *Error bars* indicate SEM (n = 6). (*C*) Summary of the action potential duration at 90% repolarization (APD_{90}) after various treatment conditions. * Significantly different from control ($P < 0.05$). *Error bars* indicate SEM. For *B* and *C*, the data are expressed as the percent increase over the control (pretreatment) value.

Discussion

Sevoflurane has previously been shown to prolong the action potential of guinea pig cardiac preparations at concentrations of 0.6–0.7 mM, levels that approximate the plasma concentrations observed clinically.[14,24,25] We confirm these findings and extend them to show that action potential prolongation can be observed at sevoflurane concentrations ranging from 0.3 to 1 mM. Sevoflurane had no effect on the action potential amplitude. This is consistent with our data on the cloned human cardiac Na^+ channel showing that sevoflurane has little effect on Na^+ currents, at near normal membrane potential, even at concentrations as high as 3 mM. Sodium channel blockade was enhanced, albeit slightly, at a more depolarized holding potential (−70 mV), a finding similar to effects seen in guinea pig myocytes.[26] L-type Ca^{2+} channel currents in rat and guinea pig myocytes are inhibited by approximately 15–25% by high micromolar concentrations of sevoflurane.[23,25] Likewise, we find that L-type Ca^{2+} channel currents are inhibited by sevoflurane with an IC_{50} of approximately 3 mM. At a concentration of 1 mM, Ca^{2+} channel currents are inhibited by 27%. Block of Ca^{2+} channels is known to attenuate the action potential prolonging effects of many drugs.[27] We believe that the Ca^{2+} channel block that is apparent after exposure to 1 mM sevoflurane is primarily responsible for limiting further increases in action potential duration beyond the maximum that we observed at 0.5 mM, although we cannot rule out interactions with other ion channels or transporters. A similar biphasic effect of sevoflurane on action potential duration has been described previously.[13]

The action potential studies clearly show that sevoflurane can prolong cardiac repolarization consistent with the findings that sevoflurane anesthesia produces QT interval prolongation in clinical use.[8,10,12] We therefore examined the effects of sevoflurane on several cloned human cardiac K^+ channels to elucidate the mechanisms underlying these findings. The HERG cardiac K^+ channel is the main molecular target for virtually all drugs that prolong QT interval in humans.[3] Inhibition of HERG is believed to be the primary mechanism that underlies the QT interval prolonging effects of another volatile anesthetic, halothane.[28] Furthermore, block of I_{Kr} in guinea pig myocytes has previously been shown to be the

mechanism underlying the QT interval prolonging effects of sevoflurane.[13] A recent publication has shown HERG, expressed in *Xenopus* oocytes, to be partially inhibited, presumably in the inactivated state, by sevoflurane.[29] We were therefore surprised to find that sevoflurane had no significant effects on HERG channel currents up to concentrations of 1 mM. This is despite the fact that the step/ramp protocol used in this study allows for significant channel inactivation and has also been shown to be the most sensitive protocol for detecting HERG channel blockers.[30] Furthermore, we found no effects of sevoflurane (0.5 mM) on I_{Kr} in guinea pig myocytes using a standard step/step protocol. In contrast, KvLQT1/minK was effectively inhibited by sevoflurane and this inhibition occurred over the exact concentration range where we observed action potential prolongation. I_{Ks} recorded from guinea pig myocytes was also effectively inhibited by 0.5 mM sevoflurane. These findings are similar to reports suggesting I_{Ks} and KvLQT1/minK as the molecular target for sevoflurane-induced QT interval prolongation.[14,31] Previously, sevoflurane has been shown to be without effect on the transient outward K^+ current in rat ventricular myocytes.[23] However, the actions of sevoflurane on the K^+ channel that carries the human transient outward current have not been studied. We find that sevoflurane inhibits human cardiac Kv4.3 channel currents and that this inhibition is mainly the result of speeding the rate of Kv4.3 current decay at concentrations of 0.3 mM and higher. Although the transient outward K^+ current is not present in guinea pig myocytes under normal physiologic conditions,[32] it is an important repolarizing channel in the human heart, where it is a major determinant of the phase 1 notch.[15,16] A reduction in Kv4.3 channel current is believed to underlie the delayed ventricular repolarization that is observed in patients with heart failure.[33] Taking these findings together, we believe that the QT interval prolonging effects of sevoflurane in humans are primarily a result of inhibition of KvLQT1/minK and Kv4.3 K^+ channels but, contrary to previous reports and to most other drugs that delay ventricular repolarization, blockade of the HERG channel is not involved.

Sevoflurane is used to induce and maintain anesthesia in patients undergoing many forms of cardiac surgery.[34–36] Conceivably, these or other patients could be under concurrent therapy with standard class III antiarrhythmic drugs such as sotalol and dofetilide that block HERG/I_{Kr} as their mechanism of action. We were therefore interested in testing the effects of these drugs on action potential duration in combination with sevoflurane. We found that combining sevoflurane with sotalol or dofetilide resulted not in additive effects, but instead in a synergy that led to an unexpectedly large prolongation of the action potential duration. Synergistic effects on action potential duration have been described for the combination of the specific I_{Ks} blocker chromanol 293B and the I_{Kr} blocking drugs dofetilide and E-4031.[37,38] From these studies, it was concluded that the simultaneous inhibition of both I_{Kr} and I_{Ks} leads to a reduction in repolarization reserve resulting in marked prolongation of the action potential duration. We believe a similar mechanism underlies the effects observed in this study, with sevoflurane acting as the I_{Ks} inhibitor in this case. Although there are differences in the ion channel makeup of guinea pig myocytes and human myocytes, our *in vitro* results currently suggest that the use of sevoflurane anesthesia in patients receiving class III antiarrhythmic drug therapy could result in disproportionate QT interval prolongation and, potentially, the risk of ventricular arrhythmia. However, the Ca^{2+} channel block observed with sevoflurane could help limit the occurrence of arrhythmias by reducing the development of early afterdepolarizations, even in a setting of a prolonged QT interval. It should be possible to examine the electrocardiographic effects of sevoflurane anesthesia in patients concurrently receiving I_{Kr} blocking drugs to determine whether the electrophysiologic effects reported here have any demonstrable clinical consequences.

In summary, this report examines the effects of sevoflurane on some of the major ion channels that contribute to electrical activity in the human heart. The ability of sevoflurane to prolong action potential duration when administered alone confirms clinical findings that the drug produces QT interval prolongation. In contrast to virtually all other drugs known to cause acquired long QT syndrome, sevoflurane does not block the HERG cardiac potassium channel, but rather inhibits KvLQT1/minK and Kv4.3, the channels that carry I_{Ks} and the transient outward K^+ current in the human heart, respectively. This study therefore reveals sevoflurane as one of the few clinically used drugs that prolongs QT interval without direct effects on HERG. This unusual mechanism may confer upon sevoflurane the ability to produce excessive delays in ventricular repolarization when it is coadministered in the presence of standard class III antiarrhythmic drugs. By extension, this same response may occur in patients who have congenital long QT syndromes, especially the type II form of the disease in which HERG is mutated leading to a reduction in I_{Kr}.[39] Case reports have already demonstrated marked prolongation of the QT interval or torsades de pointe arrhythmia when sevoflurane is administered to these patients.[40,41] Our results suggest caution when sevoflurane is administered in these cases and may indicate the need for further clinical studies.

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