Anesthesiology 2002; 97:1209–17 © 2002 American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins, Inc.

Biphasic Effects of Isoflurane on the Cardiac Action Potential

An Ionic Basis for Anesthetic-induced Changes in Cardiac Electrophysiology

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Background: **The mechanism underlying isoflurane modulation of cardiac electrophysiology is not well understood. In the present study, the authors investigated the effects of isoflurane on the cardiac action potential (AP) characteristics. The results were correlated to modulation of the L-type calcium ($I_{Ca,L}$), the delayed-rectifier potassium (I_{Kdr}), and the inward-rectifier potassium (I_{Kir}) currents.**

Methods: **Single ventricular myocytes were enzymatically isolated from guinea pig hearts. The current clamp and whole cell voltage clamp configurations of the patch clamp technique were used to monitor the cardiac AP and ionic currents, respectively. A dynamic AP voltage protocol that mimicked changes in membrane potential during an AP was used to monitor the $I_{Ca,L}$, I_{Kdr}, and I_{Kir}.**

Results: **Isoflurane produced a concentration-dependent, biphasic effect on the AP duration (APD). At 0.6 mM (1.26 vol%), isoflurane significantly increased APD_{50} and APD_{90} by 50.0 ± 7.6% and 48.9 ± 7.2%, respectively ($P < 0.05$; n = 6). At 1.0 mM (2.09 vol%), isoflurane had no significant effect on APD (n = 6). In contrast, at 1.8 mM (3.77 vol%), isoflurane decreased APD_{50} and APD_{90} by 38.3 ± 5.4% and 32.2 ± 5.5%, respectively ($P < 0.05$; n = 7). The inhibitory effects of isoflurane on I_{Kdr} chord conductance were greater than those on $I_{Ca,L}$ ($P < 0.05$; n = 6/group). Both $I_{Ca,L}$ inactivation and I_{Kdr} activation kinetics were accelerated by isoflurane. Isoflurane had no significant effects on I_{Kir} chord conductance (n = 6).**

Conclusion: **At the lower anesthetic concentration, the prolongation of the APD may be the result of the dominant inhibitory effects of isoflurane on I_{Kdr}. At the higher concentration, the shortening of the APD may be caused by the inhibitory effects on $I_{Ca,L}$ combined with the isoflurane-induced acceleration of $I_{Ca,L}$ inactivation kinetics. Because I_{Kdr} is significantly inhibited by isoflurane, I_{Kir} appears to be the major repolarizing current, which is minimally affected by isoflurane.**

RECENT studies have demonstrated the cardioprotective effects of volatile anesthetic agents. This cardioprotection, termed anesthetic-induced preconditioning, mimics ischemic preconditioning, whereby a short, nonfatal ischemic episode protects the myocardium from a subsequent ischemic injury.[1] In addition to the benefits of reducing contractile dysfunction and infarct size, volatile anesthetic agents also have significant antifibrillatory effects on the heart during and after regional or global ischemia.[2–4] Clinical investigations show that isoflurane can cause fewer incidents of arrhythmia during general anesthesia.[5] Other studies also suggest that isoflurane provides cardiovascular stability and has a beneficial action on predisposed arrhythmia, such as the long QT syndrome (LQTS).[6,7]

The mechanism underlying the effects of isoflurane on cardiac electrophysiology and the contributions of the various ion channels to the observed changes have not been elucidated. Because several ion channel currents modulate the cardiac action potential (AP), the collective effects of isoflurane on these currents will ultimately determine the overall anesthetic action on cardiac rhythm. At the cellular level, studies from our laboratory and others have shown that isoflurane inhibits the cardiac calcium and sodium channel currents.[8–10] Although isoflurane inhibits these cardiac ion channels, the varying degrees of inhibition indicate differential effects of this anesthetic agent.

Voltage clamp studies of volatile anesthetic effects on ion channels are, for the most part, conducted during steady-state conditions. However, during physiologic conditions of a dynamic change in membrane potentials, the kinetics of the ion channels will differ from the steady state. Thus, an alternative approach is to monitor ionic current during voltage changes that mimic the AP profile.[11] This allows for evaluating the changes in the ionic current during a more physiologic voltage setting.

The goal of the present study was to investigate the effects of isoflurane on cardiac electrophysiology by characterizing anesthetic effects on the AP duration (APD). Further, to determine the underlying effects on APD, effects of isoflurane on the L-type Ca channel, the delayed-rectifier K channel, and the inward-rectifier K channel were determined during a dynamic change in membrane potential that mimicked the AP profile.

Materials and Methods

Cell Isolation

The Animal Care and Use Committee at the Medical College of Wisconsin approved all experiments. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. Adult Hartley guinea pigs of either sex weighing 150–300 g (aged 2–4 weeks) were

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Received from the Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin. Submitted for publication February 27, 2002. Accepted for publication July 1, 2002. Supported in part by grants GM-54568 (W.M.K.) and NHLBI-34708 (Z.J.B.) from the National Institutes of Health, Bethesda, Maryland, and by Departments of Anesthesiology, and Critical Care Medicine, Asahikawa Medical College, Asahikawa, Japan (from Prof. Hiroshi Iwasaki). Presented in part at the annual meeting of the American Society of Anesthesiologists, New Orleans, Louisiana, October 13–17, 2001.

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first injected with sodium pentobarbital (250 mg/kg) and 1,000 U heparin intraperitoneally. During deep anesthesia, the thoracic cavity was opened, and the heart was quickly excised. The heart was then mounted on a Langendorff apparatus and perfused *via* the aorta with an oxygenated (95% O_2, 5% CO_2) buffer solution containing Joklik (Gibco-Life Technologies, Grand Island, NY) at 37°C. After the blood was cleared from the heart, it was retrogradely perfused in an enzyme solution containing Joklik, 0.4 mg/ml collagenase (Type II, Gibco-Life Technologies), 0.17 mg/ml protease (Type XIV), and 1 mg/ml bovine serum albumin (BSA; Serologicals Proteins, Kankakee, IL). After approximately 10–14 min of enzyme treatment, the ventricles of the heart were removed and chopped coarsely into small fragments. The ventricular fragments were then shaken in a waterbath for further dispersion for 3–10 min. The cells were then centrifuged and washed three times and stored at room temperature (22–25°C) in a modified Tyrode solution containing the following ingredients: NaCl, 132 mM; KCl, 4.8 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.0 mM; dextrose, 5 mM; HEPES, 10 mM, with pH adjusted to 7.4 with NaOH.

Electrophysiology

A drop of cells suspended in a modified Tyrode solution was placed in a flow-through chamber mounted on the stage of an inverted microscope (Diaphot 300; Nikon, Tokyo, Japan). Only rod-shaped cells with clear borders and striations were selected for the experiments performed within 12 h after isolation. All experiments, unless otherwise noted, were conducted at room temperature to minimize the rundown and maximize the stability of the various ion channels investigated in this study. Patch pipettes were pulled from borosilicate glass capillaries (Garner, Mornovia, CA) using a multistage puller (Sachs PS-84; Sutter, Novato, CA) and heat polished with a microforge (MF-8 3; Narishige, Tokyo, Japan). The resistance of the recording pipettes when filled with internal solution and immersed in the modified Tyrode solution ranged from 2 to 3 MΩ. Each cell used in the experiment was only exposed to one concentration of anesthetic.

Current Clamp Configuration

The single-electrode current clamp configuration was used to generate cardiac AP from single ventricular myocytes enzymatically isolated from guinea pig hearts. The initial voltage clamp setup was identical to the whole cell configuration of the patch clamp technique. Gigaohm seal and rupture of the membrane by negative pressure was achieved in the modified Tyrode solution. The pipette solution contained the following: K-glutamate, 60 mM, KCl, 50 mM; HEPES, 10 mM; $MgCl_2$, 1 mM; EGTA, 11 mM; $CaCl_2$, 1 mM; K_2-ATP, 5 mM, with pH adjusted to 7.4 with KOH. This ratio of EGTA to $CaCl_2$ results in an estimated free intracellular Ca^{2+} concentration of approximately 10 nM.[12] For all current clamp experiments, the modified Tyrode solution was used for the external buffer. After establishing the whole cell configuration, the voltage clamp was switched to current clamp on the patch clamp amplifier (List EPC-7; Adams and List, Westbury, NY). AP were evoked in response to current pulses of 0.6–1.0 nA (400-μs duration) at a frequency of 0.1 Hz, and the APD were measured at APD_{50} and APD_{90}, defined as the time to 50% and 90% repolarization, respectively, to quantify the effects of isoflurane.

Whole Cell Voltage Clamp Configuration

Standard whole cell configuration of the patch clamp technique was used to measure ion channel currents. After stability of the voltage clamp was established, the external solution was changed to ones that specifically isolated for the Ca^{2+} and K^+ currents, respectively. For the voltage clamp experiments, a digitized AP was used as a command voltage signal ("AP clamp"[11]) created by converting an AP recorded during current clamp into a voltage profile. This voltage protocol mimicked the dynamic changes in membrane potential that occurred during a cardiac AP. For the current clamp and voltage clamp experiments, the pClamp software version 8.0 (Axon Instruments, Foster City, CA) was used for the generation of protocols, data acquisition, and analysis. Additional analyses were performed using ORIGIN (version 6.0; OriginLab, Northampton, MA).

Calcium Current Measurements

For the measurement of the L-type Ca^{2+} current, $I_{Ca,L}$, the standard pipette solution contained the following: CsCl, 110 mM; HEPES, 10 mM; EGTA, 11 mM; K_2-ATP, 5 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1 mM, with pH adjusted to 7.3 with CsOH. The external solution was changed from the modified Tyrode solution to the following solution which isolated for $I_{Ca,L}$, containing NMDG, 132 mM; CsCl, 4.8 mM; HEPES, 10 mM; dextrose, 5 mM; $MgCl_2$, 2 mM; $CaCl_2$, 2 mM, with pH adjusted to 7.4 with HCl. NMDG was a substitute for Na^+ ions and Cs^+ for K^+ ions.

The Ca^{2+} current was monitored during the AP clamp. Because of the single-electrode configuration, the AP generated during a current clamp resulted in an artifact with an overshoot to +50 mV. To prevent the premature inactivation of $I_{Ca,L}$, the overshoot of the digitized AP waveform that was used as a voltage clamp protocol was edited down to +30 mV. This provided a more appropriate measurement of $I_{Ca,L}$. Conductance was calculated as chord conductance (g),[13] determined by $g = I/(V - E_{Ca})$, where I is the peak current amplitude measured during the AP clamp, V is the membrane potential at the peak current, and E_{Ca} is the equilibrium potential for

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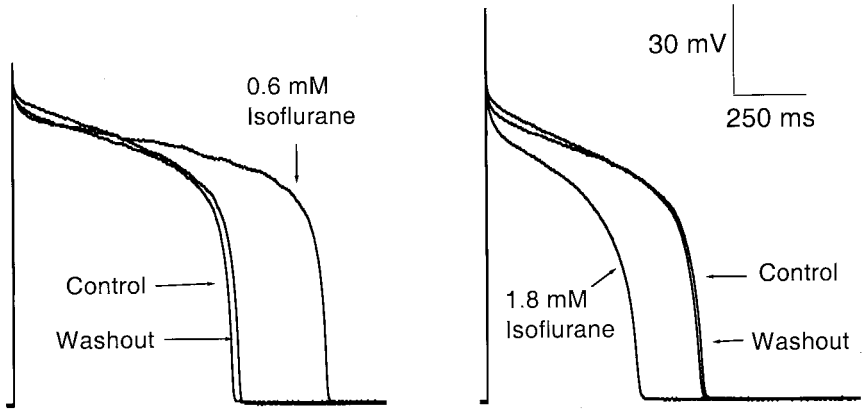

Fig. 1. Effects of isoflurane on the cardiac action potential (AP) recorded from isolated guinea pig myocytes at 22°C. The AP were elicited with a 0.6–1.0 nA current injection (400 μs, 0.1 Hz). Left panel shows sample AP traces recorded in the absence and in the presence of 0.6 mM isoflurane. Right panel shows sample AP traces recorded in the absence and in the presence of 1.8 mM isoflurane. In both cases, the AP traces returned to control levels after washout of isoflurane.

Ca^{2+} determined by the Nernst equation.[14] The rate of current inactivation during the AP voltage clamp was monitored and fit with a standard double-exponential function to account for the fast and slow components of inactivation.

Potassium Current Measurements

For the measurement of potassium current, the standard pipette solution contained the following: K-glutamate, 60 mM; KCl, 50 mM; HEPES, 10 mM; $MgCl_2$, 1 mM; EGTA, 11 mM; $CaCl_2$, 1 mM; K_2-ATP, 5 mM, with pH adjusted to 7.4 with KOH. The external solution was changed from the modified Tyrode solution to one that isolated for potassium currents and contained the following ingredients: NMDG, 132 mM; $CaCl_2$, 1 mM; $MgCl_2$, 2 mM; HEPES, 10 mM, with pH adjusted to 7.4 with HCl. To eliminate the calcium current, $CdCl_2$ (200 μM) was added to the external solution.

Two types of potassium currents were measured. The cardiac delayed-rectifier potassium current, I_{Kdr} (also known as I_K), was monitored during the AP clamp and measured in 0.1 mM external K concentration to eliminate contribution from the inward-rectifier potassium current, I_{Kir}, in the voltage range of the AP. The rate of I_{Kdr} activation during the AP clamp was best fit with a standard single-exponential function.

The cardiac inward-rectifier potassium current was also monitored. To decrease the contribution by I_{Kdr}, an external K concentration of 4.8 mM was used. This resulted in a significant outward I_{Kir} component at voltages positive and close to the potassium equilibrium potential, where the channel's conductance is high. Simultaneously, the potassium driving force for I_{Kdr} was decreased, resulting in diminished I_{Kdr} amplitude. Current amplitude for I_{Kir} was determined by subtracting the contribution of I_{Kdr} from peak I_{Kir}. The rate of I_{Kir} acti-

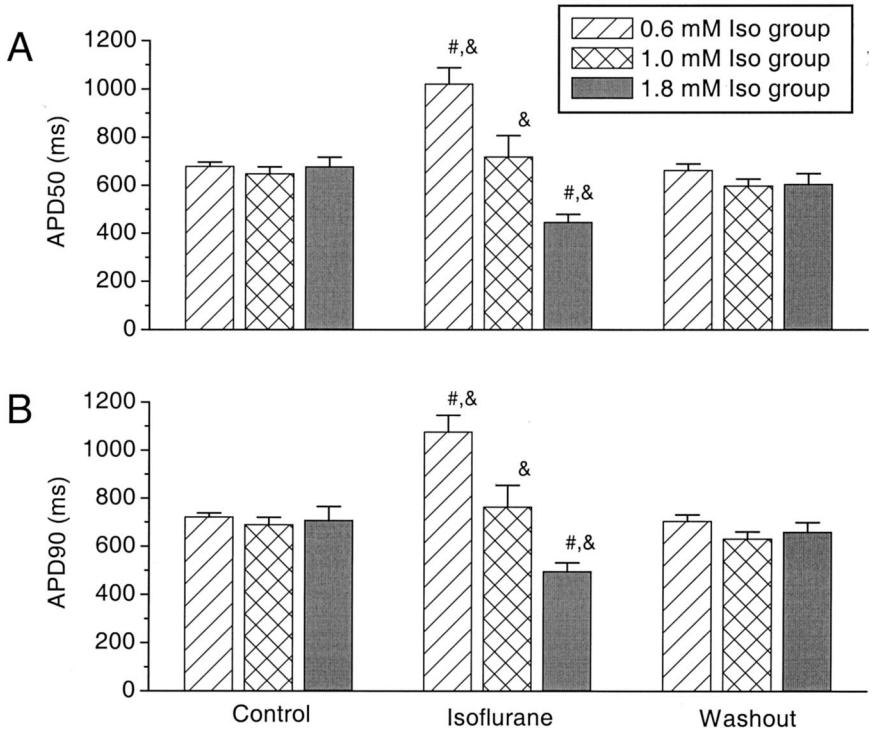

Fig. 2. Summary of the effects of isoflurane on action potential duration (APD). APD was measured at the time to 50% (APD_{50}, *A*), and 90% (APD_{90}, *B*) repolarization. n = 6/experimental group except for 1.8 mM isoflurane group (n = 7). #Significantly different from control, $P < 0.05$. &Significantly different between the anesthetic groups, $P < 0.05$.

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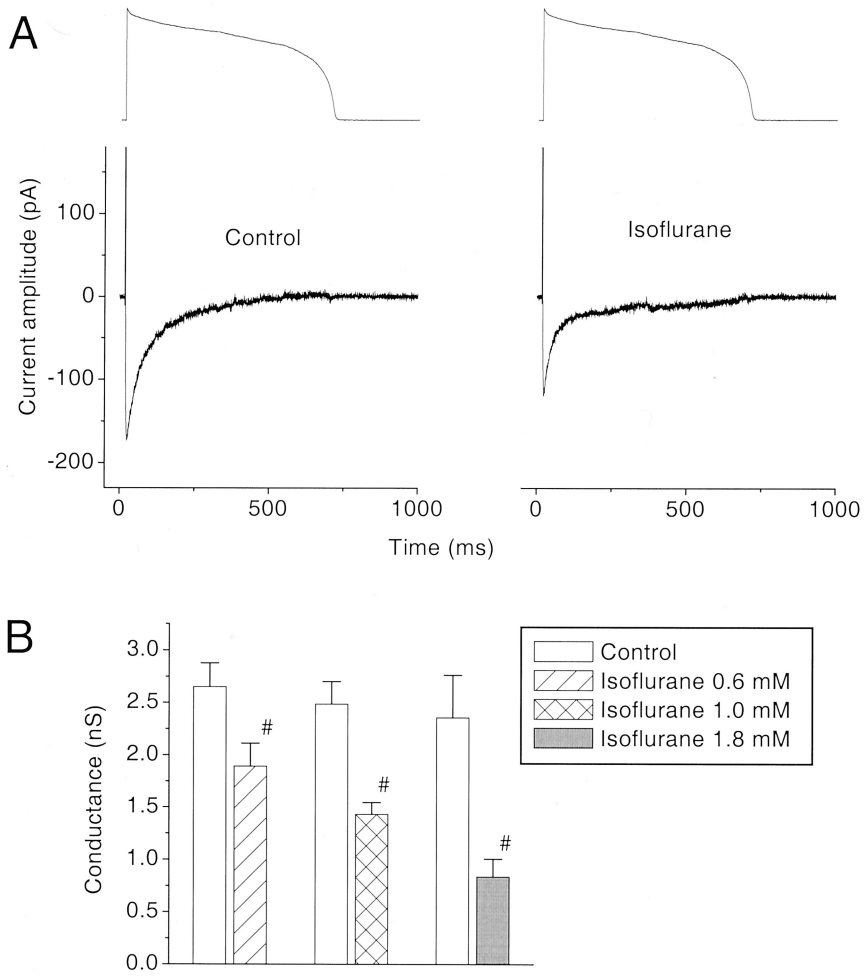

Fig. 3. Effects of isoflurane on the L-type calcium current, $I_{Ca,L}$. (*A*) Sample $I_{Ca,L}$ traces recorded during the action potential (AP) clamp protocol in control and in the presence of 0.6 mM isoflurane. The voltage protocol is depicted in the inset above the current traces. (*B*) Summary of the effects of isoflurane on peak $I_{Ca,L}$ conductance. Isoflurane significantly attenuates $I_{Ca,L}$ conductance in a concentration-dependent manner. n = 6/experimental group. #Significantly different from control and between groups, $P < 0.05$.

vation during the AP clamp was best fit with a standard single-exponential function. From both K^+ current types, chord conductances were calculated.

Volatile Anesthetic

The volatile anesthetic isoflurane was added to the external solution using measured volumes diluted in the appropriate bath solutions contained in 50-ml glass syringes and delivered to the recording chamber at 2 ml/min using a syringe pump. At the end of each experiment, anesthetic samples were obtained from the chamber and analyzed by gas chromatography to verify anesthetic concentrations as previously reported.[15–19]

Statistical Analysis

Data are presented as mean ± SEM. The Student paired and unpaired *t* tests were used to compare means between control and anesthetic treatments and between two groups. For comparisons among three different anesthetic concentration groups, a one-way analysis of variance (ANOVA) with *post hoc* pair-wise correction (Fisher PLSD) was used. Statistical analyses were performed using StatView software version 5.0 (SAS Institute, Cary, NC). A *P* value of less than 0.05 was considered statistically significant.

Results

Concentration-dependent Effects of Isoflurane on the Cardiac Ventricular Action Potential

The effects of isoflurane on the guinea pig ventricular AP were initially monitored. Sample AP measured in control and in the presence of isoflurane are shown in figure 1. The example depicts a biphasic, concentration-dependent effect of isoflurane. At 0.6 mM (1.26 vol%), isoflurane dramatically prolonged the APD. In contrast, at a high concentration of 1.8 mM (3.77 vol%), isoflurane shortened the APD. To quantify the effects of isoflurane, APD_{50} and APD_{90} were measured, and the results are summarized in figure 2. Isoflurane significantly prolonged APD_{50} and APD_{90} at 0.6 mM and significantly shortened both parameters at 1.8 mM. An isoflurane concentration of 1.0 mM (2.09 vol%) did not significantly affect APD_{50} or APD_{90}.

The changes in the APD suggested that either the Ca^{2+} or K^+ current, or both, were modulated by isoflurane.

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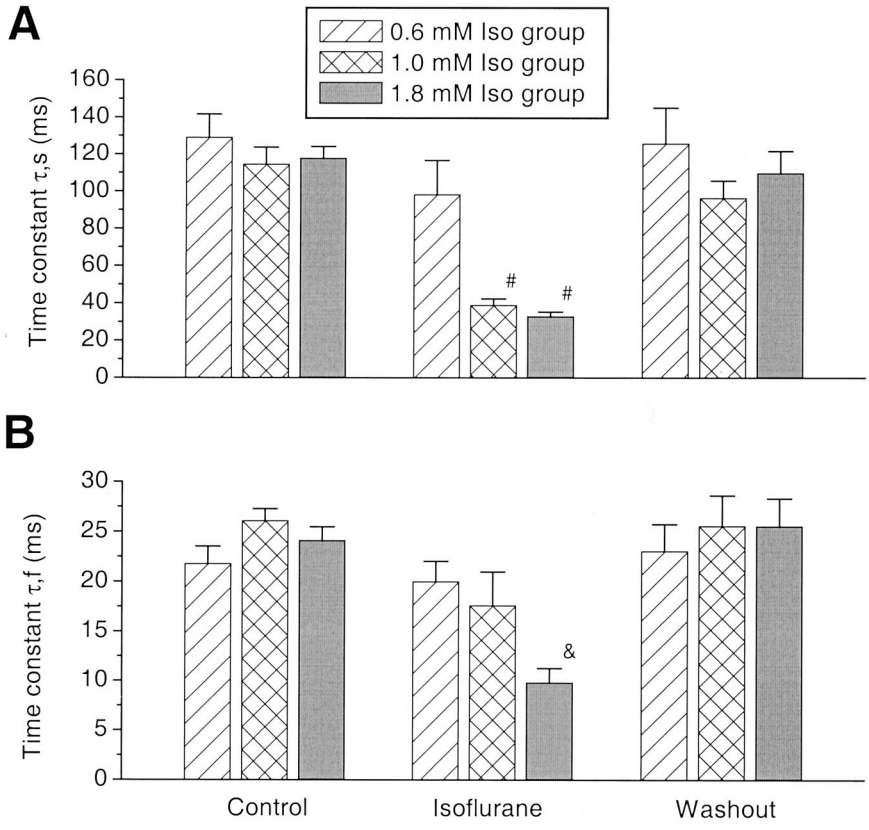

Fig. 4. Summary of the effects of isoflurane on $I_{Ca,L}$ inactivation kinetics. Current inactivation kinetics were fitted with a standard double-exponential function to yield two time constants, τ_f (fast component) and τ_s (slow component). (*A* and *B*) Effects of isoflurane on τ_f and τ_s, respectively, are shown. n = 6/experimental group. #Significantly different from control and from the 0.6 mM isoflurane group, $P < 0.05$. &Significantly different from control and from the 0.6 and 1.0 mM isoflurane groups, $P < 0.05$.

Previous studies have shown that volatile anesthetic agents depress Ca^{2+} and K^+ channel currents to differing degrees.[10,17] Consequently, the net effects of isoflurane on the Ca^{2+} and K^+ currents were determined to investigate the ionic mechanism underlying this biphasic effect.

Effects of Isoflurane on $I_{Ca,L}$

The effects of isoflurane on $I_{Ca,L}$ were determined, and a sample of the L-type Ca^{2+} current traces monitored during the AP clamp in control and in the presence of isoflurane is depicted in figure 3A. Isoflurane, 0.6 mM, significantly attenuated the peak $I_{Ca,L}$, resulting in a decrease in chord conductance. The effects of isoflurane on $I_{Ca,L}$ conductance are summarized in figure 3B. The inhibition of $I_{Ca,L}$ conductance by isoflurane was concentration dependent, and in all cases, the inhibitory effects were reversible.

The rate of inactivation of $I_{Ca,L}$ was also accelerated by isoflurane in a concentration-dependent manner. The results are summarized in figure 4. At 0.6 mM, isoflurane had no significant effect on $I_{Ca,L}$ inactivation kinetics. At a higher concentration of 1.0 mM, isoflurane significantly decreased only the slow time constant (τ_s). Finally, at 1.8 mM, the slow and fast (τ_f) time constants were significantly decreased by isoflurane, indicating an acceleration of current inactivation kinetics. The changes in the time constants returned to control levels on washout of isoflurane.

Effects of Isoflurane on I_{Kdr}

The inhibitory effects of isoflurane on $I_{Ca,L}$ by themselves will result in a shortening of the AP. Consequently, the prolongation of the AP observed at the lower concentration of isoflurane is likely the result, in part, of the anesthetic effects on potassium channel currents. Thus, the effect of isoflurane on I_{Kdr}, a major repolarizing current in the cardiac AP, was investigated. Sample I_{Kdr} current traces monitored during the AP clamp are depicted in figure 5A. In the presence of 0.6 mM isoflurane, I_{Kdr} was markedly inhibited. A summary of the inhibitory effects on I_{Kdr} conductance is shown in figure 5B. Similar to the effects on $I_{Ca,L}$, the inhibitory effects of isoflurane on I_{Kdr} conductance were concentration dependent. However, the degree of inhibition of I_{Kdr} conductance was significantly greater than that of $I_{Ca,L}$. At 0.6 mM, isoflurane inhibited I_{Kdr} conductance by 71.4 ± 3.5%, whereas $I_{Ca,L}$ conductance was depressed by 31.5 ± 3.8%. At 1.0 mM and at 1.8 mM, I_{Kdr} conductance was blocked by 86.5 ± 4.3% and 99.5 ± 0.5%, respectively, whereas $I_{Ca,L}$ conductance was depressed by 45.7 ± 3.7% and 65.0 ± 1.7%, respectively.

The activation kinetics of I_{Kdr} during the AP clamp was also significantly affected by isoflurane, as summarized in figure 5C. In the presence of isoflurane, the time constant of activation decreased significantly in a concentration-dependent manner, indicating an increased rate of current activation. The effects of isoflurane on the acti-

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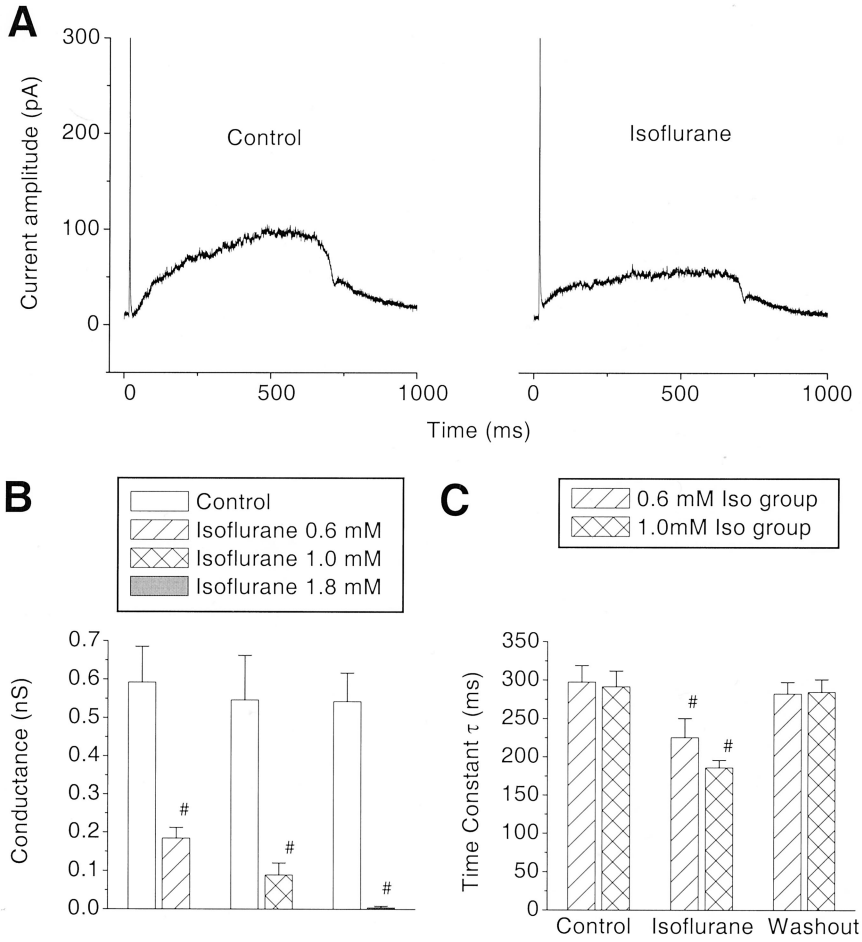

Fig. 5. Effects of isoflurane on the delayed-rectifier K^+ current, I_{Kdr}. (*A*) Sample I_{Kdr} traces in control and in the presence of 0.6 mM isoflurane monitored during the action potential (AP) clamp protocol similar to the one depicted in figure 3A. (*B*) Summary of the effects of isoflurane on I_{Kdr} conductance. n = 6/experimental group. (*C*) Summary of the effects of isoflurane on I_{Kdr} activation kinetics. The current activation kinetics were fitted with a standard single-exponential function. Isoflurane effects on the time constant of activation are shown. n = 6/experimental group. #Significantly different from control and between groups, $P < 0.05$. Because isoflurane blocked I_{Kdr} by approximately 100%, 1.8 mM group is not shown.

vation kinetics were reversible on washout of the anesthetic agent.

Effects of Isoflurane on I_{Kir}

The cardiac I_{Kir} plays two prominent roles in cell excitability. It sets the resting membrane potential and contributes to the late repolarization of the AP. The latter is because of the characteristic outward current in a limited voltage range positive to the potassium equilibrium potential. Sample I_{Kir} current traces monitored during the AP clamp in control and in the presence of isoflurane are depicted in figure 6A. The contribution of I_{Kir} to the cardiac AP is evident by the outward current activated at membrane potentials corresponding to the latter stages of repolarization. In contrast to the inhibitory effects on $I_{Ca,L}$ and I_{Kdr}, isoflurane had no significant effect on I_{Kir}. The effects of isoflurane on I_{Kir} conductance are summarized in figure 6B. Further, isoflurane had no significant effects on I_{Kir} activation kinetics at each of the concentrations tested (fig. 6B).

Effects of Isoflurane on the Cardiac Ventricular Action Potential at Physiologic Temperature

Although our results show that isoflurane had a concentration-dependent, biphasic effect on APD, these observations were made at hypothermic, room temperature (22°C) conditions. To test whether this observed biphasic effect of isoflurane was also evident at physiologic temperature, additional AP measurements were conducted at 37°C. Sample AP measured at 37°C are shown in figure 7. At 0.6 mM, isoflurane significantly prolonged the APD_{90} from 203 ± 10 ms in control to 239 ± 14 ms ($P < 0.05$; n = 5), and 1.8 mM isoflurane significantly shortened the APD_{90} from 202 ± 10 ms in control to 169 ± 14 ms ($P < 0.05$; n = 5). These results show that similar biphasic effects of isoflurane on APD were observed at room (22°C) and physiologic (37°C) temperatures.

Discussion

The results from the present study indicate that isoflurane has a concentration-dependent, biphasic effect on the cardiac APD recorded from isolated guinea pig ventricular myocytes at 22°C. At a clinically relevant concentration of 0.6 mM, isoflurane increased APD_{50} and APD_{90}. At a higher concentration of 1.0 mM, isoflurane had no significant effect on APD. As the isoflurane concentration was increased to 1.8 mM, APD shortened,

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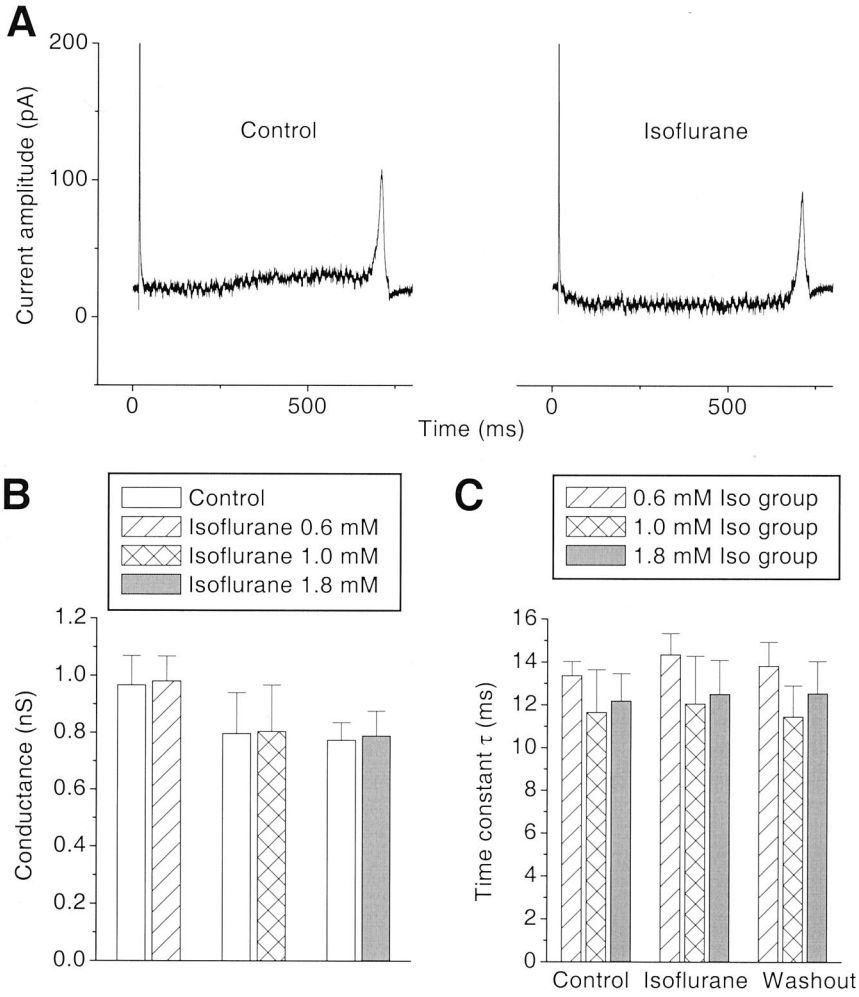

Fig. 6. Effects of isoflurane on the inward-rectifier K^+ current, I_{Kir}. (*A*) Sample I_{Kir} traces in control and in the presence of 0.6 mM isoflurane monitored during a dynamic voltage protocol similar to the one depicted in figure 3A. No significant change in current amplitude was observed in the presence of isoflurane. (*B*) Summary of the effects of isoflurane on I_{Kir} conductance. (*C*) Summary of the effect of isoflurane on I_{Kir} activation kinetics. Current activation was fitted with a standard single-exponential function. Time constants of activation obtained in control and in the presence of isoflurane are shown. n = 6/experimental group.

decreasing APD_{50} and APD_{90}. The ionic mechanism underlying the biphasic effect of isoflurane on the APD is most likely the result of differential effects on the Ca^{2+} and K^+ currents. At a physiologic temperature of 37°C, a similar biphasic effect of isoflurane on APD was observed.

There are few published reports on the effects of volatile anesthetic agents on cardiac APD, and the results are varied. For example, isoflurane prolonged cardiac APD in rats.[20] Halothane also prolonged APD in rats[20] but shortened it in guinea pigs.[21] However, sevoflurane prolonged APD in guinea pigs[22] but shortened APD in dogs.[23] The mechanism underlying these differential anesthetic effects is not known but is likely the result of species differences and the differential expressions of various ion channel proteins. Volatile anesthetic effects on the specific cardiac ion channels have also been reported. In most cases, the anesthetic effects on the various ionic currents are inhibitory. Studies have shown that isoflurane and halothane inhibit $I_{Ca,L}$ in dog Purkinje fibers[24] and guinea pig ventricular myocytes.[9,10] The cardiac sodium channel is also inhibited by volatile anesthetic agents.[15] Our previous studies have reported on the voltage-dependent effect of volatile anesthetic agents on the cardiac inward-rectifier K^+ current.[17] The effect of anesthetic drugs on I_{Kdr} is not well documented, but halothane has been reported to inhibit the current.[25] Yet, information on the combined effects of volatile anesthetic agents on these individual channels in influencing the cardiac AP profile is limited.

In our study using the guinea pig myocytes, the AP clamp, a dynamic voltage protocol mimicking the voltage change in an AP, was used to better characterize the contributions of the Ca^{2+} and K^+ currents in the isoflurane-induced changes in APD. Ionic current measured during the AP clamp differs from that elicited by a conventional steady-state voltage clamp pulse.[26,27] Our results showed that the observed biphasic effect is the result of the differential effects of isoflurane on the L-type Ca^{2+} current and the delayed-rectifier K^+ current.

The prolongation of the cardiac AP in the presence of 0.6 mM isoflurane is likely caused by the inhibition of I_{Kdr}. Even though $I_{Ca,L}$ is also depressed by isoflurane, the degree of inhibition was significantly less than that of I_{Kdr}.

At 1.0 mM isoflurane, no significant changes in APD were observed even though inhibition of I_{Kdr} and $I_{Ca,L}$ occurred. This suggests that at this concentration, the net effects of isoflurane on I_{Kdr} and $I_{Ca,L}$ appeared to have canceled out the individual effects on these two

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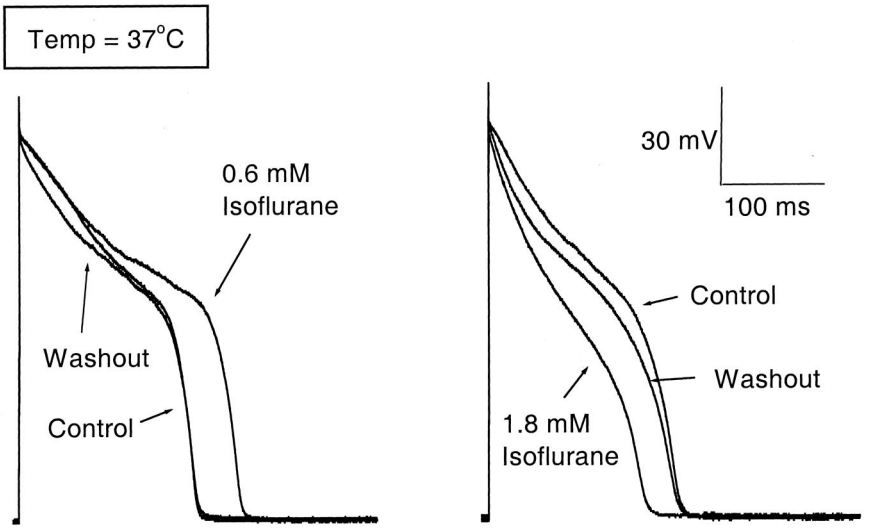

Fig. 7. Effects of isoflurane on the cardiac action potential (AP) recorded at 37°C. AP was elicited as described in figure 1. Left panel shows sample AP traces recorded in the absence and in the presence of 0.6 mM isoflurane. Right panel shows sample AP traces recorded in the absence and in the presence of 1.8 mM isoflurane. In both cases, the AP traces returned to control levels after washout of isoflurane. Note that the control AP durations are shorter at 37°C than at 22°C (fig. 1). Temp = temperature.

ionic currents. However, the changes in the conductances of I_{Kdr} and $I_{Ca,L}$ were not identical at this concentration. The inhibition of I_{Kdr} conductance was greater than that of $I_{Ca,L}$, similar to the results obtained at 0.6 mM. This suggests that not only the channel conductance but also the changes in current kinetics caused by isoflurane had an important role in determining APD and likely contributed to the effect observed at 1.0 mM and also 1.8 mM isoflurane.

The effects of isoflurane-induced acceleration of I_{Kdr} activation kinetics would result in an earlier start in the repolarization process. Acceleration of $I_{Ca,L}$ inactivation kinetics by isoflurane would lead to a shortening of APD. The contribution of the accelerated $I_{Ca,L}$ inactivation kinetics in shortening the plateau phase of the AP has also been previously hypothesized.[10] In addition, the apparent indifference at 1.0 mM isoflurane and the shortening of APD at 1.8 mM reveals the importance of I_{Kir} as a major repolarizing current in the presence of an anesthetic. The relative insensitivity of I_{Kir} to isoflurane appears, in essence, to be crucial to AP repolarization in the presence of an anesthetic. Even at the highest concentration of isoflurane tested, I_{Kir} conductance and activation kinetics were not affected.

The result that I_{Kir} monitored during the AP clamp was insensitive to isoflurane is not in agreement with our previous results obtained during steady-state voltage recording of I_{Kir}.[17] In that study, isoflurane induced a small, but significant, increase in I_{Kir} at membrane potentials positive to the potassium equilibrium potential. A major difference in the voltage protocol used in our present study is that activation of I_{Kir} observed during the AP clamp occurred during a rapid change in membrane potential. This may have decreased the time of interaction between the anesthetic agent and the channel protein, which was not evident during a steady-state voltage pulse of 100 ms. Nevertheless, the increase of I_{Kir} by isoflurane observed during a steady-state voltage protocol would have enhanced the role of I_{Kir} during repolarization.

However, the changes in kinetics and preserved I_{Kir} function at the higher anesthetic concentration may not be sufficient to explain the unexpected APD shortening at 1.8 mM. Other contributing factors are likely involved. The sodium channel (I_{Na}) was not investigated in our model because of the limitation of the AP profile generated from a single-electrode current clamp where the injected current results in an initial artifact of voltage change. The I_{Na} plays an important role in phase 0 of the AP profile and is also modulated by anesthetic agents. A previous study by Hirota *et al.*[28] has correlated their findings of APD shortening and depression of overshoot by halothane with an observed decrease in the Na^+ current. Although isoflurane has a less inhibitory effect on I_{Na} compared with halothane,[15] I_{Na} could be significantly suppressed by isoflurane (at a supraclinical concentration) to contribute to the observed APD shortening.

Several limitations must be considered in interpreting the results of the present study. First, the characteristic of the AP profile is different among species and tissues because of differential expressions of functional channels and modulation of these channels. Thus, the results from the guinea pig heart may not be easily extrapolated to the human heart. Second, the autonomic nervous system regulation and other factors modify the AP profile, conditions that were not included in our *in vitro* study. Third, the $[Ca^{2+}]_i$-activated current system (such as the nonspecific cation channel[29] and sodium–calcium exchanger[30]), which is thought to contribute additional inward current to the AP, was absent in our experimental condition because the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was maintained at the same level (approximately 10 nM) throughout the study. This fixed cytosolic free Ca^{2+} current may also alter Ca^{2+}-dependent ion channel behavior. In general, volatile anesthetic effects on $[Ca^{2+}]_i$-activated current system and other exchang-

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ers are not well understood and remain to be established. Fourth, I_{Kdr} is now recognized to be composed of two different channels, the rapid and slow delayed rectifier channel.[31] Although we did not distinguish between these two currents in our experiment, a detailed study will give additional information about the mechanism underlying observed APD changes.

The implications of the effects of isoflurane on APD with regard to cardiac rhythm are uncertain. Volatile anesthetic agents, such as halothane and isoflurane, have been reported to prolong the QT interval.[32] Our APD prolongation result at a clinically relevant concentration of 0.6 mM may partially support these observations. A prolongation of the APD, thus prolongation of the refractory period, can possibly result in an antiarrhythmic effect similar to class III antiarrhythmic agents. The early afterdepolarization (EAD) is thought to be an important factor in polymorphic ventricular tachyarrhythmia, such as *torsades de pointes* in LQTS, and calcium overload can lead to the development of EAD. The inhibition of $I_{Ca,L}$ we observed itself can suppress EAD formation.[33]

On the other hand, excessive APD prolongation may also allow the reactivation of $I_{Ca,L}$ during the plateau phase, leading to the development of EAD. In addition, the abnormal dispersion of refractoriness is critical for the development of arrhythmia. A recent study showed that volatile anesthetic agents had differential effects on APD across the ventricular wall and caused abnormal transmural gradient in APD.[34] Therefore, more detailed studies and correlated clinical investigations are needed to fully characterize the effects of isoflurane on cardiac rhythm.

In summary, isoflurane had a concentration-dependent biphasic effect on the cardiac AP recorded from guinea pig ventricular myocytes. Isoflurane prolonged and shortened the APD at 0.6 and 1.8 mM, respectively. The differential effects of isoflurane on the current amplitude and kinetics of I_{Kdr} and $I_{Ca,L}$ contributed to the biphasic effect. Further, in the presence of isoflurane, I_{Kir} emerged as a major repolarizing current because of its relative insensitivity to the anesthetic agent.

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