Direct Negative Chronotropic Action of Desflurane on Sinoatrial Node Pacemaker Activity in the Guinea Pig Heart

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ABSTRACT

Background: Desflurane inhalation is associated with sympathetic activation and concomitant increase in heart rate in humans and experimental animals. There is, however, little information concerning the direct effects of desflurane on electrical activity of sinoatrial node pacemaker cells that determines the intrinsic heart rate.

Methods: Whole-cell patch-clamp experiments were conducted on guinea pig sinoatrial node pacemaker cells to record spontaneous action potentials and ionic currents contributing to sinoatrial node automaticity, namely, hyperpolarization-activated cation current ($I_{f}$), T-type and L-type Ca$^{2+}$ currents ($I_{Ca,T}$ and $I_{Ca,L}$, respectively), Na$^{+}$/Ca$^{2+}$ exchange current ($I_{NCX}$), and rapidly and slowly activating delayed rectifier K$^{+}$ currents ($I_{Kr}$ and $I_{Ks}$, respectively). Electrocardiograms were recorded from ex vivo Langendorff-perfused hearts and in vivo hearts.

Results: Desflurane at 6 and 12% decreased spontaneous firing rate of sinoatrial node action potentials by 15.9% ($n = 11$) and 27.6% ($n = 10$), respectively, which was associated with 20.4% and 42.5% reductions in diastolic depolarization rate, respectively. Desflurane inhibited $I_{f}$, $I_{Ca,T}$, $I_{Ca,L}$, $I_{NCX}$, and $I_{Kr}$ but had little effect on $I_{Ks}$. The negative chronotropic action of desflurane was reasonably well reproduced in sinoatrial node computer model. Desflurane reduced the heart rate in Langendorff-perfused hearts. High concentration (12%) of desflurane inhalation was associated with transient tachycardia, which was totally abolished by pretreatment with the β-adrenergic blocker propranolol.

Conclusions: Desflurane has a direct negative chronotropic action on sinoatrial node pacemaking activity, which is mediated by its inhibitory action on multiple ionic currents. This direct inhibitory action of desflurane on sinoatrial node automaticity seems to be counteracted by sympathetic activation associated with desflurane inhalation in vivo. (Anesthesiology 2014; 120:1400-13)

T HE heartbeat is normally initiated by an electrical excitation that originates from the sinoatrial node located in the right atrium and then propagates through the conduction system to the ventricles. The primary pacemaker cells in the sinoatrial node thus play an important role in determining the intrinsic rate of heartbeat. The sinoatrial node pacemaker cells exhibit spontaneous electrical activity which depends on a gradual depolarization of membrane potential toward the threshold level for a subsequent action potential, namely, the slow diastolic depolarization (pacemaker potential).1-4 Multiple ionic mechanisms have been implicated in this process: an activation of inward currents such as the hyperpolarization-activated cation current ($I_{f}$),1 T-type and L-type Ca$^{2+}$ currents ($I_{Ca,T}$ and $I_{Ca,L}$, respectively)$^{5-10}$ as well as a time-dependent decay of the delayed rectifier K$^{+}$ current composed of rapid and slow components ($I_{Kr}$ and $I_{Ks}$, respectively).11,12 In addition, recent evidence indicates that local subsarcolemmal Ca$^{2+}$ releases from the sarcoplasmic reticulum also contribute to the rhythmic activity of sinoatrial node. Local subsarcolemmal Ca$^{2+}$ releases are thought to stimulate the forward mode of the electrogenic Na$^{+}$/Ca$^{2+}$ exchange current ($I_{NCX}$) that generates an inward current to depolarize cell membrane toward the action potential threshold.13 At present, the relative contributions of sarcolemmal ion channels and local subsarcolemmal Ca$^{2+}$ releases to sinoatrial node automaticity have yet to be fully elucidated.

What We Already Know about This Topic

- Previous studies have demonstrated desflurane inhalation is associated with sympathetic activation and concomitant increase in heart rate.
- This study determined the direct effects of desflurane on the electrical activity of the sinoatrial node.

What This Article Tells Us That Is New

- Desflurane produces a direct inhibitory action on sinoatrial node pacemaker activity by depressing diastolic depolarization. However, sympathetic activation during desflurane inhalation counteracts the direct inhibitory action of desflurane on the sinoatrial node.
The intrinsic activity of sinoatrial node that controls heart rate is modulated by the autonomic nervous system; sympathetic β-adrenergic stimulation accelerates the electrical activity of sinoatrial node and thereby increases heart rate, whereas parasympathetic muscarinic stimulation decelerates the sinoatrial node activity and heart rate. Some of the ionic currents are targets for regulation by autonomic nervous system. For example, sympathetic activation enhances \( I_f \) and \( I_{Ca,T} \), which seems to be importantly involved in mediating the increases in sinoatrial node automaticity and heart rate.\(^1,4,7\)

Much attention has been given to the effects of volatile anesthetics on heart rate, which could be a major determinant of the myocardial oxygen consumption.\(^14,15\) A number of studies have demonstrated that desflurane administration is accompanied by periods of sympathetic excitation and tachycardia in healthy volunteers,\(^16-19\) patients,\(^20\) and experimental animals\(^21,22\) when the inspired concentration is increased rapidly. Accordingly, it has been demonstrated that the induction of anesthesia with desflurane without opioids is associated with a greater risk of myocardial ischemia in patients undergoing coronary artery bypass surgery compared with the risk in patients given only sufentanil.\(^20\) This action of desflurane is considerably different from that of another halogenated volatile anesthetic, sevoflurane, which exerts a relatively small influence on heart rate.\(^14,15\)

At present, there is little information available regarding the direct effects of desflurane on the intrinsic sinoatrial node automaticity and its underlying ionic mechanisms. We hypothesized that whereas desflurane produces a transient increase in heart rate through sympathetic activation in vivo, desflurane itself has a direct negative chronotropic action on sinoatrial node pacemaking activity by inhibiting multiple ionic currents, such as \( I_f, I_{Ca,T}, I_{Ca,L} \), and \( I_{NCX} \). In this study, the effects of desflurane on cardiac automaticity were systematically investigated in sinoatrial node cells and ex vivo and in vivo hearts.

**Whole-Cell Patch-Clamp Recordings**

Perforated and conventional (ruptured) whole-cell patch-clamp techniques\(^26,27\) were used to record the spontaneous action potentials and ionic currents, respectively, at \( 36 \pm 1^\circ C.\(^10,11\)

The spontaneous action potential was recorded in normal Tyrode solution containing: 140 mM of NaCl, 5.4 mM of KCl, 1.8 mM of CaCl\(_2\), 0.5 mM of MgCl\(_2\), 0.33 mM of NaH\(_2\)PO\(_4\), 5.5 mM of glucose, and 5 mM of HEPES (pH adjusted to 7.4 with NaOH). The pipette solution contained: 70 mM of potassium aspartate, 50 mM of KCl, 10 mM of KH\(_2\)PO\(_4\), 1 mM of MgSO\(_4\), and 5 mM of HEPES (pH adjusted to 7.2 with KOH), to which amphotericin B (Wako Pure Chemical Industries, Osaka, Japan) was added to obtain a final concentration of 100 μg/ml.\(^10,24\)

\( I_f \) was recorded with a K+-rich pipette solution containing: 70 mM of potassium aspartate, 50 mM of KCl, 10 mM of KH\(_2\)PO\(_4\), 1 mM of MgSO\(_4\), 5 mM of adenosine 5’-triphosphate (disodium salt; Sigma, St Louis, MO), 0.1 mM of guanosine 5’-triphosphate (dilithium salt; Roche Diagnostics GmbH, Mannheim, Germany), 5 mEq of EGTA, 1.2 mM of CaCl\(_2\), and 5 mM of HEPES (pH adjusted to 7.2 with KOH). The bath solution was normal Tyrode solution supplemented with 2 mM NiCl\(_2\) and 0.5 mM BaCl\(_2\), which eliminated the voltage-dependent Ca\(^{2+}\) current and the Ba\(^{2+}\)-sensitive K+ current, respectively. \( I_f \) was elicited by 2,000-ms hyperpolarizing steps applied from a holding potential of \(-40 mV\) to test potentials of \(-50 \) to \(-140 mV\). \( I_f \) was measured as the difference between the instantaneous and steady-state current levels during each voltage step.\(^28\) The \( I_f \) conductance (\( g_f \)) was calculated at each test potential according to the following equation:

\[
g_f = \frac{I_f}{(V_t - V_{rev})},
\]

where \( I_f \) is current density, \( V_t \) is test potential, and \( V_{rev} \) is reversal potential for \( I_f \). The voltage dependence of \( I_f \) activation was assessed by fitting \( g_f \) to a Boltzmann equation:

\[
g_f = g_{max}(1 + exp((-V_t - h)/k)),
\]

where \( g_{max} \) is the fitted maximal conductance of \( I_f \), \( V_t \) is the voltage at half-maximal activation, and \( k \) is the slope factor.

\( I_{Ca,L} \) and \( I_{Ca,T} \) were measured with a Cs+-rich pipette solution containing: 90 mM of cesium aspartate, 30 mM of CsCl, 20 mM of tetraethylammonium chloride, 2 mM of MgCl\(_2\), 5 mM of adenosine 5′-triphosphate (Mg salt; Sigma), 5 mM of phosphocreatine (disodium salt; Sigma), 0.1 mM of guanosine 5′-triphosphate (dilithium salt; Roche), 5 mM of EGTA, and 5 mM of HEPES (pH adjusted to 7.2 with CsOH). The bath solution was a Na+- and K+-free solution containing: 70 mM of potassium aspartate, 50 mM of KCl, 20 mM of tetraethylammonium chloride, 2 mM of MgCl\(_2\), 5 mM of adenosine 5′-triphosphate (Mg salt; Sigma), 5 mM of phosphocreatine (disodium salt; Sigma), 0.1 mM of guanosine 5′-triphosphate (dilithium salt; Roche), 5 mM of EGTA, and 5 mM of HEPES (pH adjusted to 7.2 with CsOH). The bath solution was a Na+- and K+-free solution containing: 140 mM of Tris-hydrochloride, 1.8 mM of CaCl\(_2\), 0.5 mM of MgCl\(_2\), 5.5 mM of glucose, and 5 mM of HEPES (pH adjusted to 7.4 with Tris-base), to which tetrodotoxin (Wako) was added at a concentration of 10 μM to eliminate the possible contamination of the voltage-gated Na+ conductance. Depolarizing voltage steps (200 ms in duration) were initially applied from a holding potential of \(-90 mV\) to test potentials of \(-70 \) to \(+40 mV\) to activate the voltage-dependent Ca\(^{2+}\) current (\( I_{Ca} \), composed of \( I_{Ca,T} \) and \( I_{Ca,L} \)), and then depolarizing voltage steps were applied.

**Materials and Methods**

**Isolation of Guinea Pig Sinoatrial Node Cells**

All experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Shiga University of Medical Science (Otsu, Shiga, Japan; approval number: 2010-12-5), and the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). Single cells were isolated from 5- to 8-week-old female Hartley guinea pigs (250 to 400 g body weight) with the use of an enzymatic dissociation procedure as described previously.\(^23,24\) Cells obtained from the entire sinoatrial node region showed heterogeneity in terms of their morphology, and spindle-shaped cells, which are assumed to be primary pacemaker cells in sinoatrial node,\(^23\) were selected for the present experiments.

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from a holding potential of −60 mV to test potentials of −50 to +40 mV to activate $I_{Ca,L}$ in a given cell. $I_{Ca,T}$ was obtained by digitally subtracting $I_{Ca,L}$ from $I_{Ca}$ at each test potential. The voltage dependence of $I_{Ca,T}$ and $I_{Ca,L}$ activation was evaluated by the conductance ($g_{Ca,T}$ or $g_{Ca,L}$)–voltage relationships that were fitted to a Boltzmann equation: $g_{Ca,T} = g_{Ca,L,max}(1 + \exp((V - V_{1/2})/k))$, where $g_{Ca,T,max}$ and $g_{Ca,L,max}$ are the fitted maximal conductances for $I_{Ca,T}$ and $I_{Ca,L}$, respectively.

$I_{NCX}$ was recorded with a modified Cs+-rich pipette solution containing: 90 mM of cesium aspartate, 20 mM of CsCl, 10 mM of NaCl, 20 mM of tetraethylammonium chloride, 2 mM of MgCl$_2$, 5 mM of adenosine 5'-triphosphate (Mg salt; Sigma), 5 mM of 1,2-bis(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid, 1.75 mM of CaCl$_2$, and 10 mM of HEPES (pH adjusted to 7.2 with CsOH; pH 4) and 0.82 ± 0.11 mM (n = 4) for 6 and 12% desflurane, respectively, using gas chromatography.

$E_{Ks}$ was activated during superfusion with normal Tyrode solution supplemented with 1 μM HMR 1556 (Hoechst Marion Roussel, Frankfurt, Germany) and 0.4 μM nisoldipine (Sigma) to minimize $I_{Kr}$ and $I_{Ca,L}$, respectively. HMR 1556 at 1 μM fully inhibits $I_{Kr}$ without producing significant effects on $I_{Kr}$. $I_{Kr}$ was activated by 250-ms depolarizing steps to test potentials of −40 to +40 mV applied from a holding potential of −50 mV. E-4031 was used to confirm that the current recorded under these experimental conditions was due to $I_{Kr}$. $I_{Kr}$ was measured with a K+-rich pipette solution during superfusion with normal Tyrode solution supplemented with 5 μM E-4031 (Wako) and 0.4 μM nisoldipine (Sigma) to eliminate $I_{Ks}$ and $I_{Ca,L}$, respectively. $I_{Kr}$ was activated during 2,000-ms depolarizing steps to test potentials of −40 through +50 mV applied from a holding potential of −50 mV.

The amplitudes of $I_{Kr}$ and $I_{Kr}$ tail currents, elicited on return to the holding potential (−50 mV), reflect the degree of current activation at the preceding depolarizing test potential, and voltage dependence of $I_{Kr}$ and $I_{Kr}$ activation was evaluated by fitting the amplitude of tail current ($I_{tail}$) to a Boltzmann equation: $I_{tail} = I_{tail,max}/(1 + \exp((V - V_{1/2})/k))$, where $I_{tail,max}$ is the fitted maximal tail current density of $I_{Kr}$ or $I_{Kr}$.

The current amplitude was normalized with reference to the cell membrane capacitance and was expressed as current density (in pA/pF). The zero-current level is indicated to the left of the current records by a horizontal line.

Desflurane (Baxter, Deerfield, IL) was equilibrated in bathing solutions in a reservoir by passing air (flow rate, 0.5 l/min) through a calibrated vaporizer for at least 15 min before entering a recording chamber for the patch-clamp experiments. In the present experiments, the effects of desflurane on spontaneous action potentials and ionic currents were examined at 6 and 12%, which have been reported to correspond to approximately 1 and 2 minimum alveolar concentrations, respectively, in guinea pigs. The concentration of desflurane in a recording chamber superfused with normal Tyrode solution at 36 ± 1°C was measured to be 0.35 ± 0.05 mM (n = 4) and 0.82 ± 0.11 mM (n = 4) for 6 and 12% desflurane, respectively, using gas chromatography.

**Measurement of Heart Rate in a Langendorff-perfused Heart Model**

Electrocardiogram was recorded from Langendorff-perfused guinea pig hearts with the use of two silver electrodes attached to the ventricular apex and to the metal aortic cannula, as described previously.

**Measurement of Heart Rate In Vivo**

Guinea pigs were initially anesthetized via an intraperitoneal injection of sodium pentobarbital (80 mg/kg) and were artificially ventilated through a tracheotomy with a respirator (tidal volume, 2.5 ml; rate, 60/min; flow rate, 0.8 l/min of an air–oxygen mixture [60% inspired oxygen]). The surface electrocardiogram was recorded by placing wire electrodes in the subcutaneous spaces in a lead II configuration. A period of approximately 30 min was allowed for the stabilization of surface electrocardiogram recordings, and desflurane was then successively administered in 0.8 l/min of an air–oxygen mixture, at concentrations of 6 and 12% in a randomized order for a period of 10 to 20 min for each concentration. In some experiments, propranolol (Sigma), dissolved in sterile saline, was administered intraperitoneally, at a concentration of 10 mg/kg to induce β-adrenergic blockade before the inhalation of desflurane.

**Computer Simulation of Spontaneous Action Potentials in Sinoatrial Node Cell Model**

The Maltsev and Lakatta model was coded using SimBio and was used for a computer simulation study. The spontaneous action potentials of sinoatrial node cells in the presence of desflurane were simulated by decreasing the conductances for $I_{Kr}$, $I_{Ca,T}$, $I_{Ca,L}$, $I_{NCX}$, $I_{Ks}$, and $I_{Kr}$ by the same degree as detected in the present voltage-clamp experiments, without altering any other parameters.
Statistical Analysis

Results are presented as the means ± SD, with the number of animals and experiments indicated by N and n, respectively. Two to three sinoatrial node cells (n) were used for the patch-clamp experiments from one cell isolation (animal, N) in a given protocol. The effects of one or two concentrations of desflurane were measured in each experiment using sinoatrial node cells (patch-clamp), isolated hearts (Langendorff perfusion), or animals (in vivo inhalation), and one measurement was obtained for each concentration of desflurane in a given experiment. The error bars in the figures indicate SD with n given in parentheses. Our previous study showed that the volatile anesthetic sevoflurane (3%) significantly decreases the spontaneous firing rate of guinea pig sinoatrial node cells by 20%.10 A power analysis predicted that a group size of n = 6 was necessary to detect differences of 20% between group means of spontaneous firing rate of sinoatrial node cells and heart rate in ex vivo Langendorff-perfused and in vivo hearts, assuming a statistical power of 0.8 at a significance level (α) of 0.05 (StatMate Version 2.0; GraphPad Software, La Jolla, CA). A group size of n = 4 would allow for the detection of a difference of 30% between group means of ionic currents. Statistical comparisons were performed using a one-way ANOVA, followed by Dunnett test (Prism Version 5.0; GraphPad). We used two-tailed hypothesis testing for all tests. P value less than 0.05 was considered to be statistically significant.

Results

Negative Chronotropic Effects of Desflurane on Spontaneous Activity in Sinoatrial Node Cells

We first examined the effects of desflurane on sinoatrial node automaticity using the whole-cell patch-clamp method. In the experiments shown in figure 1, a spontaneously active sinoatrial node cell was successively exposed to 6 and 12% desflurane for approximately 5 min, with a washout period of approximately 8 min. The firing rate of spontaneous action potentials was reduced by desflurane at both concentrations in a reversible manner (fig. 1, A, lower panel, and B). Under control conditions, the firing rate of spontaneous action potentials averaged 183.2 ± 15.1/min (n = 13, N = 5), which was significantly decreased to 154.1 ± 14.5/min (n = 11, N = 5) and 132.7 ± 20.6/min (n = 10, N = 5) by 6 and 12% desflurane, respectively (fig. 1C).

The sinoatrial node pacemaker cells generate a slow diastolic depolarization (pacemaker potential) that drives the membrane potential toward a threshold level for subsequent action potential (fig. 1B). The slope of the diastolic depolarization, namely the diastolic depolarization rate (DDR), determines the time interval between successive action potentials and acts as the major determinant of the firing rate.41,42 As illustrated in figure 1D, DDR was significantly decreased from the control value of 76.8 ± 16.1 mV/s (n = 13, N = 5) to 61.1 ± 12.3 mV/s (n = 11, N = 5) and 44.2 ± 11.1 mV/s (n = 10, N = 5), during the exposure to 6 and 12% desflurane, respectively. Thus, a decrease in the firing rate was accompanied by a reduction in DDR in spontaneous action potentials of sinoatrial node cells during the administration of desflurane, which suggests that desflurane depresses the DDR and thereby reduces the firing rates of sinoatrial node action potentials.

Effects of Desflurane on I_f in Sinoatrial Node Cells

We then examined the effects of desflurane on the ionic currents that are involved in the electrical activity of sinoatrial node pacemaker cells, namely I_p, I_{CaT}, I_{CaL}, I_{NCX}, I_{Ks}, and
Molecular genetic and pharmacological studies support the view that $I_f$ provides an inward current that determines the DDR, which in turn controls the spontaneous firing rate of sinoatrial node action potentials.\(^{1-4}\)\(^{44}\) Figure 2A illustrates superimposed current traces of $I_f$ recorded during voltage-clamp steps to test potentials of $-50$ through $-140 \text{ mV}$ before and during exposure to 12% desflurane, and after its washout. Desflurane at 12% modestly decreased the amplitude of $I_f$ in a reversible manner. We evaluated the effects of desflurane on $I_f$ by constructing the conductance–voltage relationships (fig. 2B), assuming the reversal potential to be $-24 \text{ mV}$. This analysis confirmed that $I_f$ conductance was modestly but significantly reduced by desflurane at 12% but not at 6% (fig. 2C). Neither the half-activation voltage nor the slope factor was significantly affected by either concentration of desflurane (table 1).

Because $I_f$ is a mixed cationic conductance carried by Na\(^+\) and K\(^+\) under normal physiological conditions, its reversal potential reflects the relative permeability of $I_f$ to Na\(^+\) and K\(^+\).\(^{4,29}\) The reversal potential of $I_f$ was measured by tail currents of fully activated $I_f$ in the absence and presence of desflurane. The membrane was first hyperpolarized to $-130 \text{ mV}$ for 2,000 ms to fully activate $I_f$ and was then clamped back to various test potentials between $+5$ and $-45 \text{ mV}$ (fig. 3). The tail current reversed at approximately $-25 \text{ mV}$ under both conditions (fig. 3A), and this may be more clearly seen in figure 3B, in which amplitude of the tail currents is plotted against the test potentials. There were no appreciable differences in the reversal potentials for $I_f$ in the absence and presence of desflurane ($-24.2 \pm 2.2 \text{ mV}$ vs. $-24.4 \pm 2.1 \text{ mV}$, $n=6$, $N=3$). This observation indicates that desflurane did not appreciably alter the ion selectivity of the channel for Na\(^+\) and K\(^+\).

**Effects of Desflurane on $I_{Ca,T}$ and $I_{Ca,L}$ in Sinoatrial Node Cells**

Experimental evidence has been presented to show that both $I_{Ca,T}$ and $I_{Ca,L}$ contribute to the slow diastolic depolarization and spontaneous activity of sinoatrial node pacemaker cells.\(^{2-4}\)\(^{46}\) $I_{Ca,T}$ and $I_{Ca,L}$ exhibit different voltage dependency for inactivation and activation; $I_{Ca,L}$ is available with a holding potential of $-90 \text{ mV}$ but is fully inactivated at $-60 \text{ mV}$, whereas $I_{Ca,T}$ is available with a holding potential of $-24 \text{ mV}$.

**Table 1.** Parameters of Voltage-dependent Activation for $I_f$,
$I_{Ca,T}$, $I_{Ca,L}$, $I_{Kr}$, and $I_{Ks}$ in the Absence and Presence of Desflurane

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Data are presented as mean ± SD, and the number of experiments is shown in parenthesis. There are no significant differences in each parameter in the absence and presence of desflurane.

$DE_3 = $ desflurane; $I_{Ca,L} =$ L-type Ca\(^{2+}\) current; $I_{Ca,T} =$ T-type Ca\(^{2+}\) current; $I_f =$ hyperpolarization-activated cation current; $I_{Kr} =$ rapidly activatable delayed rectifier K\(^+\) current; $I_{Ks} =$ slowly activating delayed rectifier K\(^+\) current; $k =$ slope factor; $V_a =$ voltage at half-maximal activation.
whereas \( I_{\text{Ca,L}} \) is activated during depolarizing steps from a holding potential of \(-60\) mV. Therefore, both \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) are measured during depolarizing steps applied from a holding potential of \(-90\) mV, whereas \( I_{\text{Kr}} \) is measured during depolarization from a holding potential of \(-60\) mV.\(^5,6,8–10,29\)

Figure 4A shows the superimposed current traces for \( I_{\text{Ca,L}}, \) \( I_{\text{Ca,T}}, \) and \( I_{\text{Kr}}, \) recorded before and after a 5-min exposure to 12% desflurane at various test potentials. The peak potentials of \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) were \(-30\) mV and \(-10\) mV, respectively, similar to those reported in mouse sinoatrial node cells.\(^6,9,29\) and they were not affected by desflurane (fig. 4, B and C). Desflurane at 6% significantly reduced the maximal conductance for \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) to 81.1 ± 6.1% and 80.2 ± 9.6% (\( n = 4, N = 2 \)) of the control values, respectively. Desflurane at 12% also significantly decreased the maximal conductance for \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) to 70.4 ± 11.1% and 68.6 ± 10.7% (\( n = 4, N = 2 \)) of the control levels, respectively (fig. 4D). However, both the half-activation voltage and the slope factor were not significantly affected by desflurane (table 1).

**Effects of Desflurane on \( I_{\text{NCX}} \) in Sinoatrial Node Cells**

The effects of desflurane on \( I_{\text{NCX}} \), which has also been proposed to contribute to the pacemaker automacity in sinoatrial node cells,\(^9\) were then examined. \( I_{\text{NCX}} \) operates in a bidirectional way under the present ionic conditions, where \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) were present in both the external and pipette solutions. Figure 5 shows results of a representative experiment examining the effect of 12% desflurane on \( I_{\text{NCX}} \). After recording the baseline current, the cell was exposed to 12% desflurane, which was subsequently washed out. The cell was then exposed to 5 mM NiCl\(_2\) to fully block \( I_{\text{NCX}} \) (fig. 5A). Figure 5C illustrates current–voltage relationships for \( I_{\text{NCX}} \) inhibited by 12% desflurane (a–b) and 5 mM NiCl\(_2\) (a–d), obtained by digitally subtracting the two current traces shown in figure 5B. Both current–voltage relationships crossed the voltage axis at approximately \(-30\) mV, which is close to the predicted reversal potential of \( I_{\text{NCX}} \) (\(-33.6\) mV), supporting the view that the measured currents were primarily due to \( I_{\text{NCX}} \).\(^32\) The effects of 6 and 12% desflurane on \( I_{\text{NCX}} \) in the forward and reverse modes were assessed by measuring the fractional block at \( 60\) mV on either side of the reversal potential.\(^33\) As summarized in figure 5D, desflurane at 6 and 12% significantly reduced both the forward and reverse mode \( I_{\text{NCX}} \). It should also be noted that the forward and reverse mode \( I_{\text{NCX}} \) were inhibited by similar degrees by each concentration of desflurane (fig. 5D).

**Effects of Desflurane on \( I_{\text{Kr}} \) in Sinoatrial Node Cells**

We next investigated the effects of desflurane on \( I_{\text{Kr}} \) and \( I_{\text{Kr}} \), which are the major repolarizing outward currents in mammalian sinoatrial node cells.\(^4,10–12\) The effect of desflurane on \( I_{\text{Kr}} \) was examined at test potentials ranging from \(-40\) to \(+40\) mV in the presence of the \( I_{\text{Kr}} \) blocker HMR 1556 (1 \( \mu \)M)\(^35\) and \( I_{\text{Ca,L}} \) blocker nisoldipine (0.4 \( \mu \)M). Figure 6A shows superimposed current traces during 250-ms depolarizing steps to \(-10\) mV applied from a holding potential of \(-50\) mV under control conditions and during administration of 12% desflurane, initially without and then with 5 \( \mu \)M E-4031. \( I_{\text{Kr}} \) was determined as E-4031–sensitive difference current in these experiments. Figure 6B illustrates \( I_{\text{Kr}} \) at a test potential of \(-10\) mV under control conditions and in the presence of desflurane (12%), as obtained by digital subtraction of the two appropriate current traces as indicated. Single exponential fit of the tail current showed that the deactivation kinetics of \( I_{\text{Kr}} \) at \(-50\) mV was not appreciably affected by desflurane (control, \( \tau = 193 ± 23\) ms, \( n = 8, N = 3 \); 12% desflurane, \( \tau = 197 ± 19\) ms, \( n = 6, N = 3 \); fig. 6B, inset). The amplitudes of \( I_{\text{Kr}} \) tail currents at various test potentials in the absence and presence of 6 and 12% desflurane were plotted and fitted with the Boltzmann equation (fig. 6C). As demonstrated in figure 6D, there were no appreciable differences in the peak amplitude of \( I_{\text{Kr}} \) tail currents, as estimated by Boltzmann fit, in the absence (control) and presence of desflurane at 6 and 12%. Furthermore, half-activation voltage and slope factor for \( I_{\text{Kr}} \) were not affected by desflurane (table 1).

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**Fig. 3.** Fully activated current–voltage relationships for hyperpolarization-activated cation current \( I_{\text{NCX}} \). (A) The cell membrane was hyperpolarized from a holding potential of \(-40\) mV to \(-130\) mV for 2,000 ms and was then stepped back to various test potentials of \(+5\) to \(-45\) mV in 10-mV steps. Current records were obtained before and after 5-min exposure to 12% desflurane (DES) from the same cell. Voltage-clamp protocol is indicated above the control traces. (B) Current–voltage relationships for \( I_{\text{Kr}} \) tail currents in the absence (black) and presence (blue) of 12% desflurane, obtained from the data shown in A. Note that the reversal potential for \( I_{\text{Kr}} \) was not appreciably changed by the presence of desflurane.
In a separate set of experiments, we measured $I_{Kr}$ at test potentials of $-40$ through $+40$ mV as an E-4031 (5 μM) sensitive current in the absence of desflurane using the same voltage-clamp protocol as shown in figure 6 (see fig. 1, Supplemental Digital Content 1, http://links.lww.com/ALN/B33). The deactivation time constant of $I_{Kr}$, determined in the absence of desflurane, averaged $216 \pm 21$ ms ($n = 6$, $N = 3$; see fig. 1, A and B, Supplemental Digital...
I different from those of N flurane markedly reduced the amplitudes of M nisoldipine. As demonstrated in figure 7A, 12% des-flurane and 0.4 μM E-4031 and 0.4 μM atrial node cells in the presence of 5 mM NCX. I by desflurane was expressed as the percent of the total NCX. I desflurane (table 1). Thus, the properties and amplitudes of I,Kr in the absence of des-flurane (fig. 1C, Supplemental Digital Content 1, http://links.lww.com/ALN/B33), which is not significantly different from that measured for I,Ks in the presence of desflurane. The amplitudes of I,Ks tail currents at each test potential were similar in the absence and presence of desflurane (see fig. 1C, Supplemental Digital Content 1, http://links.lww.com/ALN/B33). In addition, half-activation voltage and slope factor for I,Ks in the presence of desflurane averaged −26.3 ± 2.3 mV and 6.0 ± 0.6 mV (n = 6, N = 3), respectively, and these values were not significantly different from those of I,Ks determined in the presence of desflurane (table 1). Thus, the properties and amplitudes of I,Ks identified as an E-4031 (5 μM)–sensitive current were similar in the absence and presence of desflurane, thereby supporting the view that desflurane has no appreciable effect on the inhibitory actions of E-4031 on I,Ks.

Effects of Desflurane on I,Ks in Sinoatrial Node Cells
We next investigated the effects of desflurane on I,Ks in sinoatrial node cells in the presence of 5 μM E-4031 and 0.4 μM nisoldipine. As demonstrated in figure 7A, 12% desflurane markedly reduced the amplitudes of I,Ks, activated during 2,000-ms depolarizing steps to −40 to +50 mV, without appreciably affecting the deactivation kinetics of I,Ks measured at −50 mV (control, τ = 143 ± 25 ms, n = 8, N = 3; 12% desflurane, τ = 147 ± 29 ms, n = 6, N = 3; fig. 7A, inset). Figure 7B shows the current–voltage relationships for I,Ks tail currents recorded in the absence and presence of 6 and 12% desflurane. Desflurane at 6 and 12% significantly decreased the maximal amplitude of I,Ks tail current from the control value of 7.69 ± 1.97 pA/pF (n = 8, N = 3) to 4.23 ± 0.72 pA/pF (n = 6, N = 3) and 2.08 ± 0.65 pA/pF (n = 6, N = 3), respectively (fig. 7C), without producing appreciable effects on half-activation voltage and slope factor (table 1).

Effects of Desflurane on the Heart Rate in Ex Vivo Langendorff-perfused Hearts and In Vivo Hearts
We conducted experiments to examine the effects of desflurane on the heart rates ex vivo in Langendorff-perfused hearts and in vivo in desflurane-anesthetized guinea pigs (fig. 8). Desflurane significantly reduced the heart rates in the Langendorff-perfused hearts, in a similar way as in sinoatrial node cells (figs. 8, A and D, and 1C). In contrast, in whole animals, the heart rate was transiently and significantly increased on the inhalation of 12% desflurane in vivo (fig. 8, B and D). As demonstrated in figure 8C, in guinea pigs pretreated with the β-adrenergic blocker propranolol (10 mg/kg, via intraperitoneal injection), the heart rate was monotonically and significantly reduced during inhalation of 12% desflurane, without a transient increase (fig. 8D). It should be noted that the basal heart rate in guinea pigs pretreated with propranolol was significantly lower than that without propranolol pretreatment (196.4 ± 6.9/min, n = 12 vs. 220.8 ± 15.2/min, n = 12).

Computer Simulation of the Effects of Desflurane on the Spontaneous Action Potentials in Sinoatrial Node Cells
Our final investigations explored the implications of desflurane-induced changes in ionic conductances for its negative chronotropic action, using the sinoatrial node cell model of Maltsev and Lakatta (fig. 9). The firing rate of spontaneous action potentials was decreased (fig. 9A) by simulating the inhibitory effects of 6% desflurane on I,Ks, I,Cl,T, I,Cl,L, I,NCX (forward mode), I,Ka, and I,Ks in the sinoatrial node cell model (fig. 9, C–H). It is interesting to note that net inward current during diastolic depolarization phase, which is responsible for DDR, is modestly but appreciably decreased in the computer simulation of desflurane effect on sinoatrial node action potentials (fig. 9B). Figure 9I compares the percent decreases in the firing rate and DDR induced by 6% desflurane between patch-clamp experiments and computer simulations. The sinoatrial node cell model was able to reasonably well simulate the experimental data concerning the inhibitory effects of desflurane on the spontaneous action potentials of sinoatrial node cells (fig. 1).
Discussion

Direct Negative Chronotropic Action of Desflurane Mediated through the Inhibition of Multiple Ionic Currents

The present patch-clamp experiments revealed that desflurane produces a direct inhibitory action on sinoatrial node pacemaker activity by depressing the diastolic depolarization (fig. 1). We previously demonstrated that sevoflurane, another halogenated volatile anesthetic, also suppresses DDR and thereby decelerates the spontaneous activity of sinoatrial node pacemaker cells.10 It is generally accepted that the slow diastolic depolarization is driven by a net inward current produced by a complex but coordinated interaction of multiple inward and outward ionic currents, such as $I_f$, $I_{Ca,T}$, $I_{Ca,L}$, $I_{NCX}$, $I_{Kr}$, and $I_{Ks}$, although the relative contribution of each ionic current remains to be fully elucidated.4 However, clinical evidence has shown that loss-of-function mutations and/or pharmacological blockade of $I_f$,44 $I_{Ca,T}$,46 or $I_{Ca,L}$7,47 result in a sinus dysfunction and/or bradycardia. Experiments using molecular genetic and/or pharmacological approaches have supported the functional significance of $I_f$, $I_{Ca,T}$, $I_{Ca,L}$, and $I_{NCX}$ in the regulation of DDR and spontaneous activity of sinoatrial node cells.1–10,43 It is therefore reasonable to assume that the inhibitory action of desflurane on multiple ionic currents, including $I_f$, $I_{Ca,T}$, $I_{Ca,L}$, and $I_{NCX}$ (figs. 2–7), is responsible for the reduction of DDR and deceleration of spontaneous activity in sinoatrial node cells. The computer simulation study also supports the implication of the desflurane-induced changes in ionic currents for its negative chronotropic action on sinoatrial node cells (fig. 9).
It is generally believed that the inhibitory action of volatile anesthetics on ion channel/transporter arises from direct binding to channel/transporter proteins or is mediated by altering the behavior and dynamics of plasma membrane lipids. In addition, there is good evidence that volatile anesthetics indirectly affect the function of ion channels by modifying regulatory signaling molecules/pathways.48 The present experiments found that the degree of inhibition by desflurane varied among the ionic currents in sinoatrial node cells; desflurane potently inhibited \( I_{\text{Ks}} \) (fig. 7), moderately inhibited \( I_{\text{Ca,L}} \) and \( I_{\text{NCX}} \) (fig. 5), and \( I_{\text{f}} \) (figs. 2 and 3), but had little effect on \( I_{\text{Kr}} \) (fig. 6). Our previous study shows that sevoflurane also inhibits \( I_{\text{f}} \), \( I_{\text{Ca,T}} \), \( I_{\text{Ca,L}} \), and \( I_{\text{Ks}} \) to different degrees in sinoatrial node cells;\(^{10} \) sevoflurane modestly inhibits \( I_{\text{f}} \) whereas potently suppressing \( I_{\text{Ca,T}} \), \( I_{\text{Ca,L}} \), and \( I_{\text{Ks}} \) (see table 1, Supplemental Digital Content 1, http://links.lww.com/ALN/B33). Among these ionic currents, \( I_{\text{f}} \) seems to be less sensitive to inhibition by desflurane and sevoflurane. In contrast, \( I_{\text{Ks}} \) seems to be more sensitive to inhibition by these two volatile anesthetics. These differences in the sensitivity of ionic currents to inhibition by desflurane and sevoflurane suggest that the inhibitory mechanisms of these volatile anesthetics differ among the individual ionic currents. Future studies are needed to elucidate the precise mechanisms underlying the actions of volatile anesthetics, which may include direct...
interaction with ion channels and/or the indirect modification of regulatory signaling molecules/pathways.

Interestingly, $I_{Kr}$ in guinea pig sinoatrial node cells is insensitive to inhibition by desflurane (fig. 6). Previous investigations have also shown that both isoflurane and sevoflurane have a minimal effect on $I_{Kr}$ in guinea pig ventricular myocytes and its molecular correlate human ether-a-go-go-related gene channels exogenously expressed in Chinese hamster ovary cells. Although $I_{Kr}$ and human ether-a-go-go-related gene channels are susceptible to blockade by a wide variety of compounds, including clinical drugs, associated with drug-induced long QT syndrome, it seems likely that $I_{Kr}$ and human ether-a-go-go-related gene channels are less sensitive to inhibition by the halogenated volatile anesthetics.

The negative chronotropic effect of desflurane was also observed in the isolated Langendorff perfusion model (fig. 8), similar to the previous observations. Experimental results obtained from sinoatrial node cells and isolated Langendorff-perfused hearts, where the neural and humoral influences are mostly abolished, strongly supports the view that desflurane has a direct inhibitory effect on the intrinsic cardiac automaticity produced by sinoatrial node pacemaker cells.

Implication of Sympathetic Activation in the Desflurane-induced Increase in Heart Rate

Both sympathetic and parasympathetic branches of the autonomic nervous system densely innervate sinoatrial node, and thereby control the electrical activity of the pacemaker cells in the sinoatrial node. The in vivo heart rate is therefore determined by the interaction of the autonomic nervous tone with the intrinsic electrical activity of sinoatrial node

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**Fig. 8.** Effects of desflurane administration on the heart rates ex vivo and in vivo. (A) Time course of changes in heart rates during the administration of 6 and 12% desflurane (DES) in ex vivo Langendorff-perfused hearts, as measured by electrocardiogram recordings. Electrocardiograms recorded at time points indicated by letters (a–e). (B) Time course of changes in heart rates during the inhalation of 12% desflurane (in vivo). Electrocardiograms recorded at time points indicated by letters (a–d). (C) Time course of changes in heart rates during the inhalation of 12% desflurane after intraperitoneal injection of 10 mg/kg propranolol (in vivo with propranolol). (D) Heart rates in ex vivo Langendorff-perfused hearts and in vivo hearts without and with propranolol. Data are expressed as percent deviation (Δ) from their respective control values. *$P < 0.05$ compared with their respective control values (Langendorff perfusion, 202.0 ± 17.0/min, $n = 12$, $N = 12$; in vivo without propranolol, 220.8 ± 15.2/min, $n = 12$, $N = 12$; in vivo with propranolol, 196.4 ± 6.9/min, $n = 12$, $N = 12$), and #$P < 0.05$ compared with values within the same concentration of desflurane. NS = not significant.
Fig. 9. Computer simulations of the negative chronotropic action of desflurane on sinoatrial node automaticity. (A and B) Spontaneous action potentials (A) and total membrane current (I_total; B) in the sinoatrial node cell model of Maltsev and Lakatta under control conditions (black) and in the presence of 6% desflurane (DES; blue), obtained by simulating the decreases in the conductances for hyperpolarization-activated cation current (I_h), T-type Ca^{2+} current (I_{Ca,T}), L-type Ca^{2+} current (I_{Ca,L}), forward mode Na^{+}/Ca^{2+} exchange current (I_{NCX}), rapidly activating delayed rectifier K^{+} current (I_{Kur}), and slowly activating delayed rectifier K^{+} current (I_{Ks}) detected in the presence of 6% desflurane (see below) by the voltage-clamp experiments. The time points of the maximal diastolic potential of first action potentials in control and in the presence of desflurane are superimposed and denoted by vertical dotted lines. V_m represents the membrane potential. Note that total membrane current (B) is shown on an expanded scale to clarify the changes in membrane current during the diastolic depolarization phase (peaks of both inward and outward currents are out of scale), and the red arrow denotes a decrease in inward current in the presence of desflurane. (C–H) Changes in I_h (C), I_{Ca,T} (D), I_{Ca,L} (E), I_{NCX} (F), I_{Kur} (G), and I_{Ks} (H) during spontaneous action potentials in sinoatrial node cell model, obtained by decreasing the conductance for each current by the same degree as in voltage-clamp experiments with 6% desflurane (5.4% reduction in I_h; 19.8% reduction in I_{Ca,T}; 19.8% reduction in I_{Ca,L}; 25.6% reduction in I_{NCX}; 1.3% reduction in I_{Kur}; or 46.8% reduction in I_{Ks}). (I) A comparison of percent decreases in the firing rate and diastolic depolarization rate (DDR) in spontaneous action potentials by 6% desflurane in current-clamp experiments and simulation studies. The experimental data were obtained from the same data shown in figure 1.

Clinical Implications

Several mechanisms have been proposed to explain the desflurane-induced augmentation in sympathetic outflow that occurs upon the rapid increase in the inspired concentrations of desflurane, including (1) a reflex response initiated by irritant receptors in the airway, (2) a baroreflex response to the lower arterial pressure caused by higher concentrations of desflurane, and (3) a direct stimulation of the sympathetic medullary centers. In the clinical settings, the desflurane-induced increases in heart rate and arterial pressure can be effectively attenuated by the administration of the drugs that interfere with sympathetic responses, namely, the β-adrenergic blocker esmolol, α_2-adrenergic agonist clonidine, and μ-opioid fentanyl. β-adrenergic blockers have been shown to have a direct peripheral action, whereas α_2-adrenergic agonists suppress the sympathetic impulses arising from the brain. However, fentanyl has been suggested to attenuate the desflurane-induced increases in heart rate and blood pressure by producing a vagomimetic action (vagal-sympathetic-accentuated antagonism) and/or by reducing the efferent sympathetic activity to the heart. Because clinically used concentrations of desflurane have a

pacemaker cells. A number of investigations have demonstrated that a rapid increase in desflurane concentration elicits a transient increase in heart rate and mean arterial pressure in both humans and experimental animals, which is ascribed to the activation of sympathetic nervous system. When the autonomic nervous system is pharmacologically blocked in dogs, desflurane inhalation even at high concentrations (1.75 minimum alveolar concentration) produces negative chronotropic and inotropic actions. In the present experiments, the heart rate in guinea pig was transiently and significantly increased during inhalation of 12% desflurane, whereas heart rate was monotonically and significantly decreased during desflurane inhalation after β-adrenergic blockade (fig. 8), indicating that β-adrenergic-mediated increase in the heart rate occurs in guinea pig during higher inspired concentration of desflurane. These findings suggest that guinea pig also represents a suitable animal model for investigating the mechanisms for desflurane-induced changes in heart rate, like other experimental animal models such as dog and swine. It therefore seems reasonable to extrapolate the present results to explain the action of desflurane on heart automaticity in the clinical settings.
direct negative chronotropic effect on the heart (figs. 1 and 8), it seems reasonable to blunt the tachycardia induced by higher concentrations of desflurane by reducing the sympathetic influence to the heart. The present study may also draw attention to the possibility that the negative chronotropic action of desflurane could be significant in patients being treated with pharmacological blockers for ion channels involved in sinoatrial node pacemaking or associated with compromised channel functions caused by gene mutations.

In conclusion, the present investigation demonstrates that clinically used concentrations of desflurane have a direct inhibitory effect on sinoatrial node automaticity, which is mediated through the inhibition of ionic currents, and may provide an electrophysiological basis for the effectiveness of drugs that prevent the sympathetic influences to the heart in blunting desflurane-induced tachycardia in clinical practice.

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Competing Interests

The authors declare no competing interests.

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