

Effects of Inhalational Anesthetics on L-type Ca^{2+} Currents in Human Atrial Cardiomyocytes during β-Adrenergic Stimulation

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Background: **Anesthetics may cause cardiac side effects by their action on L-type Ca^{2+} channels. Direct effects on the channels have not yet been discriminated from an interference with the β-adrenergic channel regulation. The authors therefore studied the effects of halothane, sevoflurane, and xenon on human cardiac Ca^{2+} currents during stimulation with isoproterenol.**

Methods: **Currents through L-type Ca^{2+} channels were measured with the patch clamp technique in atrial cardiomyocytes obtained from patients undergoing cardiac surgery. Cells were superfused with solutions equilibrated with anesthetics at the desired concentrations. Ca^{2+} currents during pulses to 10 mV were evaluated with respect to their peak value (I_{max}) and to the total moved charge (Q).**

Results: **In the absence and in the presence of isoproterenol (1 μM), sevoflurane (0.29 mM, 1 minimum alveolar concentration [MAC]) significantly depressed Q by 37.8 ± 7.2% (mean ± SD) and 40.8 ± 10.3%, respectively. I_{max} was not significantly affected in comparison with control cells never exposed to an anesthetic. Xenon (65%, 1 MAC) did not evoke significant effects. Exposure to halothane (0.39 mM, 1 MAC) during stimulation with isoproterenol significantly reduced Q by 31.3 ± 23.3% (but not I_{max}). After washout of halothane, Q was increased above the level prior to the application of halothane. Moreover, whereas Q promptly declined to baseline levels after washout of isoproterenol in controls, the previous exposure to halothane markedly delayed this decline, leaving Q significantly elevated for several minutes.**

Conclusions: **Halothane exerts a dual effect on Ca^{2+} currents. The long-lasting stimulatory effect may contribute to the proarrhythmic potency of the drug that exceeds that of sevoflurane, which only depressed Ca^{2+} currents.**

CARDIAC side effects of gaseous anesthetics are common and include cardiodepression as well as the induction or facilitation of arrhythmias. An important reason to introduce some recently developed anesthetics into clinical practice is their improved profile of adverse effects on the heart in comparison with older narcotics such as halothane, which is notable as a negative inotropic and proarrhythmic drug.[1–3] Particularly, the noble gas xenon appears to be virtually free of relevant cardiac actions,[4–7] and within the fluorinated compounds, isoflurane, sevoflurane, and desflurane are considered less proarrhythmic than halothane.[8–10]

To elucidate the cellular and molecular basis of the interference of anesthetics with heart function, their influence on cardiac ion channels and currents has frequently been studied in cardiomyocytes.[11–14] Voltage-gated Ca^{2+} currents of the L-type ($I_{Ca,L}$) have attracted much attention because of their fundamental importance not only for the action potential but also for the contractile force. Xenon (1 minimum alveolar concentration [MAC]) did not affect myocardial contractility or the positive inotropic effect of isoproterenol in the guinea pig.[15] Moreover, it did not alter $I_{Ca,L}$ in guinea pig[13] or human cardiomyocytes in contrast to halothane, which induced a marked depression of $I_{Ca,L}$.[14] Sevoflurane also depressed $I_{Ca,L}$ in cardiomyocytes from dogs[11] or guinea pigs.[12] Thus, the effectiveness of the anesthetics on $I_{Ca,L}$ correlates well with the degree by which they cause cardiac complications.

Inhibition of $I_{Ca,L}$ is easily reconciled with the negative inotropy of halothane, although other effects of halothane may also contribute to the phenomenon. The proarrhythmic potency of halothane, however, is harder to explain. Moreover, halothane has not consistently been shown to be cardiodepressive. When cardiac muscle preparations were stimulated with β-adrenergic drugs, halothane decreased the contractile force,[16] whereas it enhanced the positive inotropic effects of catecholamines in others.[17]

Clinically, the cardiac effects of halothane are frequently described as a sensitization of the heart for the sympathetic nervous system.[3,18,19] This prompted us to test the hypothesis that halothane exerts different effects on L-type Ca^{2+} currents in the absence and presence of β-adrenergic stimulation. Accordingly, the present study of human atrial cardiomyocytes addresses the interference of halothane with the isoproterenol-induced stimulation of $I_{Ca,L}$. Halothane was compared with sevoflurane and with xenon and, in the absence of isoproterenol, either inhibited $I_{Ca,L}$ (sevoflurane)[11,12] or left it unaffected (xenon).[13,14] We report dual responses to halothane. Although halothane depressed $I_{Ca,L}$, it considerably prolonged the enhancement of $I_{Ca,L}$ by isoproterenol, even beyond the washout of the β-adrenergic stimulus.

Materials and Methods

Isolation of Single Atrial Myocytes

Right atrial appendages were obtained as surgical specimens from patients (n = 34) undergoing heart surgery.

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Table 1. Summary of the Patient Characteristics at the Time of Surgery

N	Males/Females	Mean Age (Range), yr	ACB	AVR	β-B	ACE-I	NO-don.	Digitalis	DHP
34	25/9	66.8 (44–80)	28	6	33	16	17	1	3

Unless stated otherwise, data are number of patients.

ACB = aortocoronary bypass operation; AVR = aortic valve replacement; ACE-I = angiotensin-converting enzyme inhibitors; β-B = β blockers; DHP = dihydropyridines; NO-don. = nitric oxide donors.

All patients (table 1) were in sinus rhythm and had no evidence of right atrial dysfunction. The cardiovascular medication that most patients received was stopped at least 12 h before surgery. The investigations were performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the local ethical board. All patients gave informed consent prior to surgery.

Cardiomyocytes were prepared according to Hatem *et al.*[20] as modified by Skasa *et al.*[21] In short, the myocardial specimens were cut into chunks (approximately 1 mm^3) and washed in Ca^{2+}-free buffer twice for 5 min. Afterward, the tissue was incubated in 20 ml Ca^{2+}-free buffer that contained protease XXIV (220 U/ml; Sigma, Taufkirchen, Germany). This was followed by incubation in a solution containing fresh collagenase V (100 U/l; Sigma) but no protease XXIV. Incubation was finished as soon as microscopic examination revealed a satisfactory number of intact cardiomyocytes (after approximately 20–45 min). After centrifugation (100*g* for 2 min), cardiomyocytes were resuspended in a buffer containing the following: NaCl, 120 mM; KCl, 5.4 mM; $MgSO_4$, 5 mM; sodium pyruvate, 5 mM; glucose, 20 mM; taurine, 20 mM; HEPES, 10 mM. pH was adjusted to 7.4 with NaOH. Since cell lysis to these cells would occur if a physiologic Ca^{2+} concentration were immediately restituted, gradual recalcification was performed by increasing the $CaCl_2$ concentration every 10 min by 200 μM to a final concentration of 0.8 mM. The cells were stored for 1–5 h before measurements.

Patch Clamp Experiments

The atrial cardiomyocytes were allowed to adhere to a glass coverslip that was transferred into a perfusion chamber. L-type Ca^{2+} currents were recorded with the patch clamp technique in the conventional whole-cell mode.[22] We used an EPC9-amplifier (HEKA, Lambrecht, Germany) and a personal computer equipped with Pulse 8.5 software (HEKA). The patch pipettes, pulled from borosilicate glass (Hilgenberg, Malsfeld, Germany) and fire polished, had a tip resistance of 3–5 MΩ. A holding potential of −60 mV was chosen to minimize Na^+ currents. Ca^{2+} currents were evoked by a series of depolarizing pulses (each 200 ms in duration) to potentials ranging from −50 to +60 mV (in steps of 10 mV). For reasons of time, only single pulses to +10 mV were used to follow $I_{Ca,L}$ over time.

The cardiomyocytes were continuously superfused as described[14] with one of several solutions containing or not containing isoproterenol and/or an anesthetic. In control experiments when no anesthetic was applied, time-matched changes of the superfusate were performed as in the experiments with anesthetics. The experiments were carried out at 21°–23°C.

Preparation of Solutions

The desired concentration of the anesthetics was obtained by continuously gassing solutions through a frit in a glass flask for 30 min. Gas concentrations were controlled as described[14] by analysis with head-space gas chromatography.[23] Gassing a solution with halothane, 0.8% (vol/vol) (corresponding to 1 MAC), resulted in a concentration of 0.39 mM halothane. Sevoflurane, 1.1% (1 MAC), yielded 0.29 mM. Xenon solutions were prepared by gassing with xenon (95%)/O_2 (5%). Some loss of xenon occurred during superfusion of the cell; the actual concentration of xenon surrounding the cell was determined as 65%, similarly as in our previous study.[14]

The extracellular solution contained the following: tetraethylammonium chloride, 136 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1.8 mM; glucose, 10 mM; HEPES, 10 mM; EGTA, 0.5 mM. pH was adjusted to 7.4 with tetraethylammonium hydroxide. Isoproterenol (Sigma) was added (prior to gassing) from a stock solution containing isoproterenol (1 mM) and EGTA (1 mM). The intracellular (pipette) solution contained the following: CsCl, 140 mM; $MgCl_2$, 2 mM; adenosine triphosphate, 0.3 mM; EGTA, 10 mM; HEPES, 10 mM. pH was adjusted to 7.2 with CsOH.

Data Analysis and Statistics

For the Ca^{2+} currents during each depolarizing pulse to +10 mV, we calculated (fig. 1) the peak current (I_{max}) and the current time integral, which is equivalent to the moved charge (Q). I_{max} was taken as the maximum value of the current during the pulse interval. I_{max} and Q were calculated using the online analysis provided by the Pulse software.

To analyze the kinetics of the time-dependent inactivation during the pulse, the currents were fitted with the double-exponential decay function:

$$I(t) = I_{fast} \cdot e^{-t/\tau_{fast}} + I_{slow} \cdot e^{-t/\tau_{slow}}$$

where *I* denotes the current; *t*, the time; and τ, a time constant. The subscripts fast and slow refer to the fast and slow components of the total current, respectively. Fitting was performed with IgorPro 3.15 software (WaveMetrics Inc., Lake Oswego, OR). The details of the

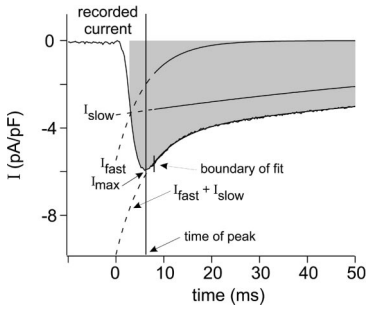

Fig. 1. Evaluation and analysis of Ca^{2+} currents in a human atrial cardiomyocyte. The time course of the recorded current is shown. At time 0, the holding potential was changed from −60 to +10 mV. The time where the current reached its peak (I_{max}) is indicated by a vertical line. The current trace was fitted to a double-exponential function (see Materials and Methods). The boundaries of the fit were 3 ms after the peak and at 200 ms (data not visible). The fit yielded I_{fast} and I_{slow}. I_{fast}, I_{slow}, and the sum of both, $I_{fast} + I_{slow}$, are drawn with *solid lines* within the boundaries of the fit and *dashed lines* outside of the boundaries. The moved charge was calculated as the shaded area with boundaries 4 ms, 0 pA, 200 ms, and current trace. All current values are normalized to the capacitance of the cell (124 pF).

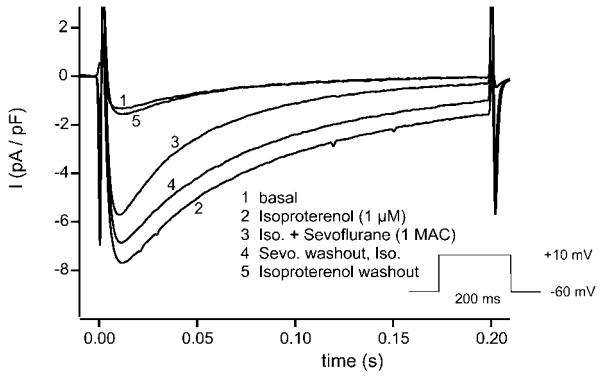

Fig. 2. Effects of sevoflurane on L-type Ca^{2+} currents in human atrial cardiomyocytes prestimulated with isoproterenol. Five traces from one experiment are superposed and recorded under basal conditions, after application of isoproterenol (1 μM), in the presence of sevoflurane (0.29 mM, 1 minimum alveolar concentration [*MAC*]), after washout of sevoflurane but with isoproterenol still present, and also after washout of isoproterenol. The voltage protocol is shown in the inset. The currents are normalized to the capacitance of the cell (78 pF).

analysis and fitting procedure are shown in figure 1. Current amplitudes, I_{max}, and Q were divided by the cell capacitance to normalize them to the size of the cell. Values are expressed as mean ± SD. Statistical comparisons were carried out with Prism 3.02 software (Graphpad Software Inc., San Diego, CA) using the paired *t* test or Dunnett test for multiple comparisons with one control group. A probability of error of $P < 0.05$ was considered significant.

Results

L-type Ca^{2+} currents in human atrial cardiomyocytes were evaluated with respect to their peak value I_{max} that was observed 7 to 12 ms after the start of depolarization as well as with respect to their current time integral, which is equivalent to the total moved charge Q and is calculated from the area under the current trace (see Materials and Methods; fig. 1). Q offers the advantage over I_{max} that it is sensitive to changes in the inactivation kinetics of $I_{Ca,L}$ during the depolarizing pulse. The decline of the current during the pulse could well be fitted (fig. 1) to a double-exponential decay function, yielding two time constants τ_{fast} and τ_{slow} or two components (I_{fast} and I_{slow}) of the total current. However, since the calculated values of I_{fast} and I_{slow} depend on the boundaries of the fit (because of the activation kinetics of $I_{Ca,L}$) that must be set arbitrarily, we decided to rely mostly on the value of Q to assess changes of $I_{Ca,L}$ due to altered inactivation kinetics.

Isoproterenol (1 μM) consistently enhanced $I_{Ca,L}$ (figs. 2 and 3). It increased I_{max} by a factor of 3.4 ± 3.5 and Q by a factor of 3.7 ± 2.1 (n = 48; fig. 4). The initial increase observed 10 s after application of isoproterenol was not completely maintained; I_{max} and Q declined over the time (4–6 min) during which isoproterenol was present (data not shown). After washout of isoproterenol, both I_{max} and Q returned to baseline levels (fig. 4, *A*).

Sevoflurane applied at a concentration corresponding to 1 MAC (0.29 mM) led to a depression of $I_{Ca,L}$ already in the absence of isoproterenol (data not shown). Q that was decreased by 37.8 ± 7.2% (n = 4; $P < 0.01$) was more affected than I_{max} that was decreased by 14.7 ± 6.8% (not significant). After prestimulation with isoproterenol, sevoflurane (0.29 mM, 1 MAC) again induced a depression of $I_{Ca,L}$ (fig. 2). Q was reduced by 40.8 ± 10.3% (n = 7; fig. 4, *B*), and I_{max} was reduced by 21.1 ± 9.8% (n = 7; fig. 4, *B*). Control cells were exposed to a protocol that involved exchanges of the superfusates as in the cells treated with sevoflurane, but no sevoflurane was ever present. Since in such isoproterenol-stimulated control cells Q and I_{max} declined over time as in sevoflurane-exposed cells (although to a smaller extent), the

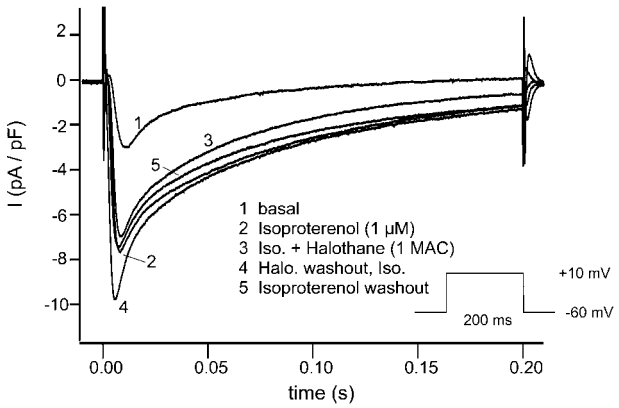

Fig. 3. Effects of halothane on Ca^{2+} currents in cardiomyocytes prestimulated with isoproterenol. Conditions the same as those in Fig. 2, but halothane was used instead of sevoflurane. The cell capacitance was 65 pF.

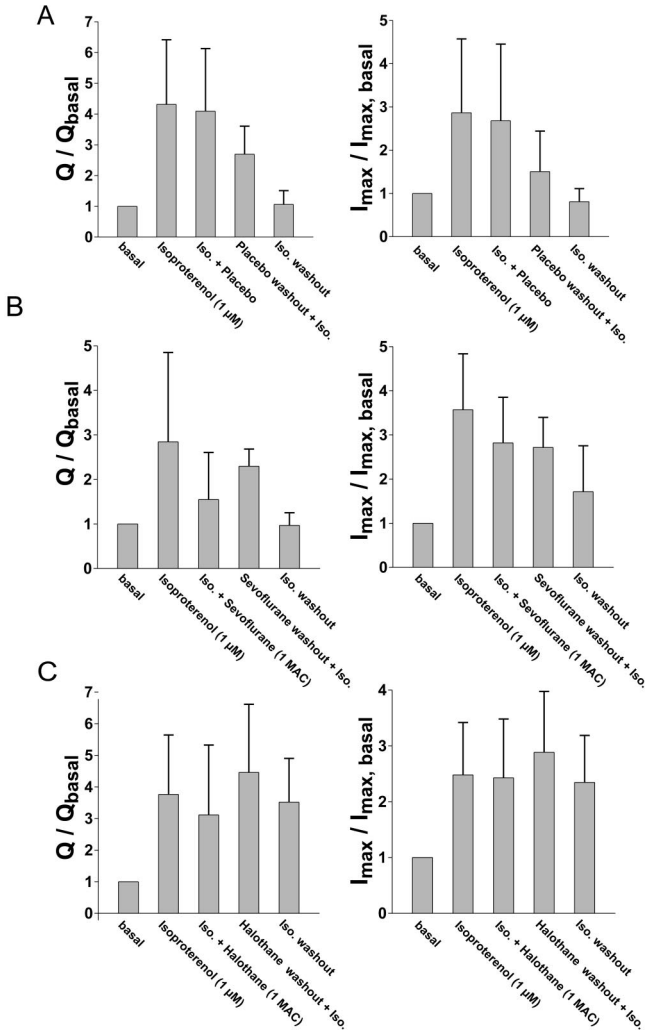

Fig. 4. Effects of sevoflurane and halothane on L-type Ca^{2+} currents in human atrial cardiomyocytes after prestimulation with isoproterenol in comparison with controls (not exposed to an anesthetic). Each ordinate shows either the moved charge (*Q*) or the peak current (I_{max}) in relation to the respective value obtained under basal conditions in the beginning of each experiment. Each cell was first stimulated with isoproterenol (1 µM; *first column in each panel*) and then (*second column*) additionally exposed to placebo (*A*), sevoflurane (0.29 mM, 1 minimum alveolar concentration [MAC]) (*B*), or halothane (0.39 mM, 1 MAC) (*C*). Subsequently, first the anesthetic and then also isoproterenol were washed out (*fourth* and *fifth columns*). Note that statistical evaluation of the data is given in figure 5.

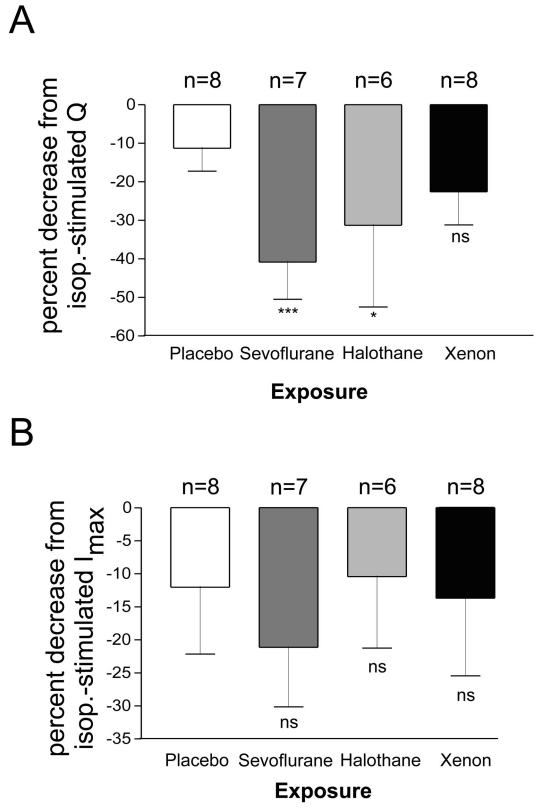

Fig. 5. Effects of sevoflurane, halothane, and xenon (each at 1 minimum alveolar concentration) on isoproterenol (*isop.*)–stimulated L-type Ca^{2+} currents in comparison with controls. (*A*) Effects on the moved charge (*Q*). (*B*) Effects on the peak current (I_{max}). The significance of differences from controls (placebo) is indicated (* $P < 0.05$; *** $P < 0.001$; *ns*, $P > 0.05$; Dunnett test for multiple comparisons with one control group). In all cells including controls (never exposed to an anesthetic), Q and I_{max} derive from measurements after an identically long exposure to isoproterenol (1 µM). The *numbers* denote the number of experiments in each group.

statistical evaluation (fig. 5) of the effects of sevoflurane was performed by comparing Q and I_{max} in the presence of sevoflurane with the corresponding values in control cells (placebo treated in fig. 5). The analysis revealed that Q was significantly reduced ($P < 0.001$), whereas the depression of I_{max} was not significant from the control. After washout of sevoflurane but with isoproterenol still present, Q increased again (figs. 2 and 4, *B*), as is expected after removal of a depressing agent. When isoproterenol also was removed, Q returned to baseline levels (figs. 2 and 4, *B*). Experiments with concentrations of sevoflurane lower and higher than 1 MAC demonstrated a dose-dependent effect of the anesthetic on Q in the presence of isoproterenol. Specifically, sevoflurane decreased Q by 38.1 ± 10.3% (n = 5) at a concentration of 0.5 MAC (0.15 mM) and by 51.5 ± 2.8% (n = 2) at 2 MAC (0.56 mM).

Xenon did not affect $I_{Ca,L}$ in unstimulated atrial cardiomyocytes in our previous study.[14] In the present experiments performed during stimulation with isoproterenol, xenon (65%, 1 MAC) again did not significantly alter I_{max} or Q. It appears from figure 5, *A* that Q decreased more than expected from the time-dependent rundown in control cells; the difference, however, was not significant.

Halothane previously[14] evoked a significant depression of $I_{Ca,L}$ (with respect to the peak current and the time integral of the current) in atrial cardiomyocytes not stimulated with β-adrenergic agonists. In the present study when the experiments were performed in the presence of isoproterenol, I_{max} only slightly declined after application of halothane (0.39 mM, 1 MAC; fig. 3, fig. 4, *C*, and fig. 5), similarly as in control cells. Q was decreased by

31.3 ± 23.3% (n = 6; fig. 4, *C*). In comparison (fig. 5) with cells exposed only to isoproterenol but not to halothane (placebo treated in fig. 5), the difference of Q was significant ($P < 0.05$). After washout of halothane, I_{max} (n = 6; $P < 0.05$) and Q (n = 6; $P < 0.02$) were both significantly raised above the level reached after the initial exposure to isoproterenol (figs. 3 and 4, *C*) in contrast to controls, where the effectiveness of isoproterenol declined over time. The most striking results, however, were obtained when isoproterenol also was removed. In distinct divergence to experiments with cells never exposed to halothane (which displayed a prompt decline of $I_{Ca,L}$ to baseline levels after removal of isoproterenol), a preceding exposure to halothane prevented the decline of $I_{Ca,L}$ after washout of isoproterenol (figs. 3 and 4, *C*). Three hundred sixty seconds after washout of the catecholamine, Q was still increased 3.5-fold ± 1.38-fold (n = 4; $P < 0.02$) over the initial baseline value. The corresponding increase of I_{max} was 2.34 ± 0.84-fold ($P < 0.04$). In cells that could be kept intact in the whole-cell configuration over extended periods, the persisting current enhancement by halothane was observed up to 15 min after isoproterenol removal, until the experiment was finished.

As in the case of sevoflurane, the effect of halothane on Q in the presence of isoproterenol was dependent on the concentration of the anesthetic. Halothane decreased Q by 22.3 ± 9.0% (n = 3) at a concentration of 0.5 MAC and by 42.4 ± 18.7% (n = 8) at 2 MAC (0.85 mM).

Because the half-maximal effects of isoproterenol on cardiac tissue preparations occurred with concentrations in the low nanomolar range,[24] the standard concentration (1 μM) used in this study is expected to be maximal. When a lower concentration (10 nM) of isoproterenol was used (fig. 6, *A*), an exposure to halothane again slowed down the return of $I_{Ca,L}$ to baseline levels after washout of both halothane and isoproterenol (n = 3). In control experiments (fig. 6, *B*) in which no halothane was applied, I_{max} was increased 2.7 ± 0.6-fold (n = 4), and Q was increased 2.9 ± 0.8-fold (n = 4) by isoproterenol (10 nM). After washout of isoproterenol, the initial baseline level of Q was reached 75 s later. Afterward, Q declined even further, probably due to a slight rundown. When cells were exposed to halothane (fig. 6, *A*) after prestimulation with isoproterenol (10 nM) and both agents were subsequently removed, Q remained elevated for an extended period. In the experiment of figure 6, *A*, Q was still larger than at baseline for a period of 260 s after washout of isoproterenol. For a statistical evaluation, we calculated the increase of Q from baseline to the level observed prior to the washout of isoproterenol. Then we calculated the time required for this increase to decline by 90%. This time was 282 ± 164 s in cells previously exposed to halothane (n = 3) and 98 ± 39 s in controls (n = 5; $P < 0.05$).

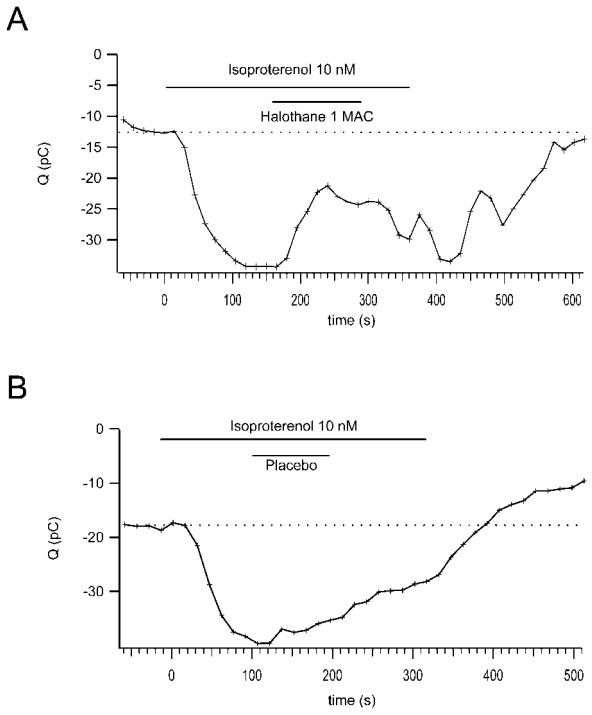

Fig. 6. Time courses of Ca^{2+} currents in two experiments evaluating the effects of halothane and of placebo after prestimulation with a submaximal concentration of isoproterenol. Each point represents one value of the moved charge (*Q*) (not normalized to the cell capacitance). Isoproterenol (10 nM) and halothane (0.39 mM, 1 minimum alveolar concentration [*MAC*]) (*A*) or placebo (*B*) were present in the superfusate during the times indicated by horizontal lines.

We confirmed in further experiments (n = 4) in which no isoproterenol was used our previous finding[14] that halothane depressed basal $I_{Ca,L}$ (I_{max} as well as Q) and that the washout of halothane did not increase $I_{Ca,L}$ above baseline. Therefore, the enhancing action of halothane on $I_{Ca,L}$ was strictly dependent on isoproterenol and was best observed after removal of this stimulus.

Discussion

The present study analyzed the effects of the anesthetic gases xenon, sevoflurane, and halothane on Ca^{2+} currents in human cardiomyocytes during β-adrenergic stimulation with isoproterenol. Xenon did not show significant effects, and sevoflurane evoked similar depressions in the presence of isoproterenol as in the absence. Thus, β-adrenergic stimulation did not relevantly change the action of xenon and sevoflurane. This was different with halothane. Although Ca^{2+} currents were initially reduced, they increased after washout of halothane to levels higher than prior to the application of the anesthetic. Furthermore, the preceding exposure to halothane considerably slowed down the decline of Ca^{2+} currents after washout of isoproterenol.

For the evaluation of the effects of the anesthetics on $I_{Ca,L}$, we considered not only the conventional value

I_{max}, the peak value of $I_{Ca,L}$ after a depolarization, but also Q, the total moved charge during a depolarizing pulse. For the assessment of the effects of isoproterenol, both parameters could be used because both were increased by a similar factor. Sevoflurane and halothane, however, affected Q more strongly than I_{max}. Indeed, significant effects of either anesthetic in the presence of isoproterenol were demonstrated exclusively on Q. This suggests that sevoflurane and halothane preferentially depress the slow rather than the fast inactivating component of $I_{Ca,L}$.

Previous electrophysiologic studies on the effects of anesthetics on cardiomyocytes have mostly been performed in the absence of β-adrenergic stimulation. The use of isoproterenol in the present study revealed actions of halothane that were not evident in unstimulated cells. In contrast to sevoflurane and xenon, halothane interfered with the β-adrenergic regulation of $I_{Ca,L}$. This interference was long lasting and exceeded the time over which the cells were exposed to halothane. Indeed, it became apparent only after washout of the anesthetic because application of halothane, as an immediate and prominent effect, led to a marked depression of $I_{Ca,L}$. This depression vanished as soon as halothane was removed and gave way to an enhancement of $I_{Ca,L}$. Remarkably, the enhancement prevailed even after removal of isoproterenol. It appeared that halothane made the stimulatory action of isoproterenol on $I_{Ca,L}$ permanent over an extended period.

When β-adrenergic agonists are studied in human cardiac tissue, the state of the β receptors needs careful consideration. The use of β blockers may up-regulate their density and enhance the responsiveness to β-adrenergic agonists after washout of the blockers.[25] A drug-free interval of 12 h prior to operation is hardly sufficient to reverse such changes. This may in part explain the considerable scatter of the effectiveness of isoproterenol in our study, although nearly all (33 of 34) patients had received β blockers.

As a potential explanation of the dual effect of halothane in the presence of isoproterenol, an interference with several different proteins should be considered. Halothane has been proposed to bind directly to the L-type Ca^{2+} channel because it shifted the binding curve of the dihydropyridine BayK 8644 to the right.[26] These results suggest that halothane is a ligand of the dihydropyridine binding site of the channel and may thereby attenuate Ca^{2+} influx independently of β-adrenergic stimulation. Furthermore, an inhibition of the inhibitory G protein (G_i) by halothane has been described.[19,27] G_i antagonizes the production of cyclic adenosine monophosphate induced by activated β-adrenergic receptors. Inhibition of G_i, therefore, would be expected to augment and extend the action of isoproterenol. Unfortunately, the time course over which halothane alters G_i has not been determined.

Clinically, the noble gas xenon is thought to be virtually free of cardiac side effects such as cardiodepression or induction of arrhythmias. Experimentally, it did not modify several types of voltage-gated currents in cardiomyocytes.[13,14] The present study confirms that xenon does not significantly affect Ca^{2+} currents, not even during stimulation with isoproterenol. Hence, the *in vitro* data and clinical observations agree.

On the other hand, fluorinated anesthetic compounds such as sevoflurane and halothane are known to be cardiodepressive and proarrhythmogenic. Although the cardiodepression evoked by these drugs does not importantly differ, the risk of arrhythmias is considerably higher during anesthesia with halothane than with sevoflurane.[28,29] As a potential explanation, halothane is often described to sensitize the heart for catecholamines and β-adrenergic drugs.[3,18] No such property has been attributed to sevoflurane or xenon. In the present study, the inhibitions of $I_{Ca,L}$ evoked by sevoflurane and by halothane were quantitatively similar; if any difference existed, sevoflurane was the more effective inhibitor. Therefore, it is tempting to speculate that the demonstrated enhancing effects of halothane on $I_{Ca,L}$ are responsible for a sizable part of its proarrhythmogenic side effects.

It should be noted also for sevoflurane that evidence has been provided that it potentiates the positive inotropic effects of β-adrenergic stimulation on the heart.[30] These experiments have been performed in the rat and are not in line with the clinical impressions in humans. Experiments with cardiac tissues and dobutamine[31] have not confirmed the results. Unfortunately, no data on the inotropy of sevoflurane are available for isolated human myocardium. The present data on human atrial cardiomyocytes suggest that in humans the effects of sevoflurane on cardiac Ca^{2+} currents are confined to attenuation, no matter whether these currents are stimulated by β-adrenergic agonists.

In conclusion, we demonstrated a dual effect of halothane on L-type Ca^{2+} currents in human atrial cardiomyocytes prestimulated with isoproterenol. Halothane depressed $I_{Ca,L}$ but also increased isoproterenol-stimulated currents, as became apparent after washout of the anesthetic. This enhancing effect was not shared by sevoflurane or by xenon and may be an important factor for the induction of arrhythmias by halothane.

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