

Temperature-independent Inhibition of L-Type Calcium Currents by Halothane and Sevoflurane in Human Atrial Cardiomyocytes

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Background: **Cardiac L-type calcium currents ($I_{Ca,L}$) are affected by volatile anesthetics, possibly contributing to their side effects. Actions of anesthetics on ion channels are usually studied *in vitro* at room temperature. However, the solubility of anesthetic gases as well as $I_{Ca,L}$ are markedly sensitive to the study temperature. Therefore, temperature-dependent effects of halothane and sevoflurane on cardiac $I_{Ca,L}$ were analyzed.**

Methods: **$I_{Ca,L}$ were studied at 21°C and 36°C with the patch clamp technique in isolated human atrial cardiomyocytes. Concentrations of anesthetics brought into solution by gassing at both temperatures were determined with gas chromatography.**

Results: **The aqueous concentrations of halothane and sevoflurane were linearly related to their concentration in the gas phase (1 to 3 minimum alveolar concentration [MAC]). At 21°C, the slope of this relation was 0.52 and 0.12 mM/vol % for halothane and sevoflurane, respectively, and decreased at 36°C to 0.29 and 0.09 mM/vol %, respectively. $I_{Ca,L}$ displayed significantly higher current amplitudes at 36°C than at 21°C and significantly accelerated time-dependent inactivation. Halothane (1–2 MAC) and sevoflurane (1–3 MAC) evoked stronger inhibitions of $I_{Ca,L}$ at 21°C than at 36°C. In spite of different temperature-dependent current amplitudes, the fractional (percent) inhibition of $I_{Ca,L}$ showed the same linear relationship to the concentrations of halothane and sevoflurane in the bath medium at both temperatures, as revealed from present and previous experiments.**

Conclusions: **Inhibition of $I_{Ca,L}$ by halothane and sevoflurane is determined by the aqueous concentration of the anesthetics, independently of the temperature. Increased solubility may explain the stronger effects of the anesthetics at lower temperatures.**

ANESTHETIC gases may interfere with cardiac ion channels. This interaction may contribute to a dominant part of their cardiac side effects.[1] As one major target of volatile anesthetics in the heart, the L-type Ca^{2+} channel, responsible for the L-type Ca^{2+} current ($I_{Ca,L}$), has been identified.[2–4] A detailed analysis of such an ion channel and its currents requires *in vitro* studies; these are usually carried out at room temperature because physiologic temperatures necessitate considerable more technical efforts and tend to make cells less stable.[5] However, it should be kept in mind that Ca^{2+} currents[6] and the solubility of volatile anesthetics in several biologic media are temperature dependent.[7–9] Specifically, $I_{Ca,L}$, as with most other ion currents in mammals, displays considerably larger amplitudes at higher than at lower temperature in human cardiac cells.[10] This temperature-dependent behavior of calcium currents has also been observed in several other tissues, *e.g.*, in neurons or pancreatic β cells.[11,12] In previous studies on the effects of anesthetic gases on cardiomyocytes from guinea pigs at 37°C,[13–15] $I_{Ca,L}$ was inhibited to a larger degree than was seen in comparable studies at room temperature.[3,16] On the other hand, it has long been known that the solubility of anesthetic gases decreases with rising temperatures.[7] This property of the gases may lead to an attenuated inhibition of currents when the temperature is increased in the presence of a constant concentration of an anesthetic in the gas phase. Finally, a rise in temperature leads to a faster inactivation of $I_{Ca,L}$.[10] Again, this makes it difficult to extrapolate *in vitro* results obtained at room temperature to physiologic temperatures.[17,18]

Studies that directly compared anesthetic-induced changes of cardiac ion currents at different temperatures are rare. Volatile anesthetics suppressed ion currents less at physiologic than at room temperature. For example, in a recent study on voltage-gated slowly activating delayed rectifier K^+ currents (I_{Ks}) in guinea pig cardiomyocytes, the inhibition by isoflurane (0.3 mM) was significantly less pronounced at 36°C than at 22°C.[19] Similar data for cardiac L-type Ca^{2+} currents are not available.

The aim of the present study was to analyze how the modifications of $I_{Ca,L}$ in human atrial cardiomyocytes by halothane and sevoflurane are affected by the temperature, in consideration of the temperature-dependent solubility of the two gases. We prepared solutions gassed with halothane and sevoflurane (1–3 minimum alveolar concentration [MAC]) and controlled the resulting concentrations in the aqueous phase with head space gas chromatography. We report that the inhibition of $I_{Ca,L}$ by halothane and sevoflurane was linearly related to their concentration in solution, independently of the temperature-sensitive current amplitude.

Materials and Methods

Isolation of Single Atrial Myocytes

Right atrial appendages were obtained as surgical specimens from patients (n = 21) undergoing heart surgery.

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Table 1. Clinical Characteristics of the Patients in Normal Sinus Rhythm at the Time of Cardiac Surgery

Patient No.	Age (years)	Procedure	Medication	Sex
1	63	CABG + AVR	β-B, N	M
2	74	CABG	β-B, ACE-I, N, Diu	M
3	52	CABG	β-B, ACE-I	M
4	59	CABG	β-B, ACE-I, N	M
5	63	CABG	β-B, ACE-I	M
6	72	CABG	β-B, N	M
7	59	CABG	β-B, N	M
8	62	CABG	β-B, ACE-I, N	M
9	72	CABG	ACE-I, Ca-I	F
10	62	CABG + AVR	β-B	M
11	73	CABG	β-B, ACE-I, N, Diu	F
12	75	CABG	β-B, ACE-I, N, Ca-I	F
13	45	CABG		M
14	51	CABG	β-B, N, Diu	M
15	78	CABG	Dig, Diu, Amio	M
16	79	CABG	β-B	M
17	76	CABG	β-B	F
18	60	CABG	β-B, Diu	M
19	71	CABG + AVR	β-B, ACE-I, N, Diu	M
20	67	CABG	β-B, N, Diu	F
21	69	CABG	β-B, N	F

ACE-I = angiotensin-converting enzyme inhibitor; Amio = amiodarone; AVR = aortic valve replacement; β-B = β-blocker; CABG = coronary artery bypass graft; Ca-I = calcium channel inhibitors; Dig = digoxin; Diu = diuretics; N = NO-donors.

Patient characteristics are summarized in table 1. All patients were in sinus rhythm and had no evidence of right heart failure. The investigations were performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the local institutional review board (Aachen, Germany). All patients gave written informed consent before surgery.

Human atrial cardiomyocytes were prepared according to Hatem *et al.*,[20] as modified and described in detail by Skasa *et al.*[21] Briefly, the myocardial specimens were gently cut into chunks and washed in Ca^{2+}-free buffer twice for approximately 5 min. Afterward, the tissue was incubated in Ca^{2+}-free buffer that contained protease XXIV (Sigma, Germany) and collagenase V (Sigma, Germany). Incubation was finished as soon as microscopic examination revealed intact cardiomyocytes. After centrifugation 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. The pH was adjusted to 7.4 with NaOH. As visible cell damage to these cells would occur if a physiologic Ca^{2+} concentration was immediately restituted, a gradual recalcification was performed. Only well-striated, bleb-free, rod-shaped myocytes were used for the studies, performed within 4 h after the isolation.

Patch Clamp Experiments

Cardiomyocytes were allowed to adhere to a glass coverslip that was transferred into a perfusion chamber. The cells were continuously superfused as described previously.[22]

L-type Ca^{2+} currents ($I_{Ca,L}$) were recorded by means of the whole cell patch clamp technique with use of an amplifier (EPC-9; HEKA, Lambrecht, Germany) and a personal computer equipped with Pulse 8.5 software (HEKA) for data acquisition and analysis. The patch pipettes pulled from borosilicate glass and fire polished had a tip resistance of 3–7 MΩ when they were filled with pipette solution.

A holding potential of −60 mV was chosen to minimize Na^+ currents. L-type Ca^{2+} currents were evoked by a series of depolarization pulses (each 100 or 200 ms in duration) to potentials ranging from −50 to +60 mV. Ca^{2+} currents are given here as peak current amplitude of the respective depolarizing step. For temporal reasons, pulses only to +10 mV were used to evaluate the effects of anesthetics in most experiments.

Temperature Control

Experiments were performed either at room temperature (21–22°C) or 36°C. In the latter case, the temperature of the solution in the double-barreled stainless steel tubes and the bath chamber (volume, 0.4 ml) was kept constant with a feedback-controlled Peltier device (Strothmann, Aachen, Germany). Temperature was continuously monitored with a thermocouple probe mounted in the chamber wall and did not vary by more than 0.3°C during an experiment.

Preparation of Solutions

For measurements of $I_{Ca,L}$, the bath solution contained tetraethylammonium chloride, 136 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1.8 mM; glucose, 10 mM; and HEPES, 10 mM; this was pH-adjusted to 7.40 with tetraethylammonium hydroxide. The pipette solution contained CsCl, 140 mM;

$MgCl_2$, 2 mM; adenosine 5'-triphosphate, 0.3 mM; guanosine 5'-triphosphate, 0.3 mM; EGTA, 10 mM; HEPES, 10 mM. This was pH-adjusted to 7.20 with CsOH.

Bath solutions containing halothane, sevoflurane, or xenon were prepared by passing an appropriate gas mixture through the solution in a glass flask equipped with a frit and a membranous septum. Gas left the flask through a valve. An anesthetic gas analyzer (Capnomac Ultima; Datex Ohmeda, Duisburg, Germany) was used to continuously monitor the concentrations of halothane and sevoflurane in the gas phase. The glass flask was located in a temperature-controlled water bath (36°C) for experiments at physiologic temperature. After at least 30 min of gassing, a fraction (10 ml) of the solution was taken through the septum into gas-tight glass syringe (1010 TLL; Hamilton, Bonaduz, Switzerland) and immediately used as superfusate of the cells. The preparation of solutions containing volatile anesthetics and xenon and analysis of gas concentrations in solutions by head space gas chromatography were described previously.[22] Standards of anesthetics were prepared by transferring halothane or sevoflurane (in liquid form) into methanol/distilled water at defined concentrations. Samples were taken up into gas-tight flasks. Anesthetic content in the standard solutions were verified immediately afterwards by gas chromatography.[23]

Gassing a solution with halothane, 0.75% (vol/vol in air; corresponding to 1 MAC in humans) at 36°C resulted in a concentration of 0.21 mM halothane. When gassing a solution with sevoflurane, 2.1% (vol/vol in air; 1 MAC in humans), the concentration yielded 0.21 mM at 36°C. Xenon solutions were prepared by gassing with xenon (95%)/O_2 (5%). Some loss of xenon (~30%) occurred during superfusion of the cells; the final concentration of xenon in the immediate neighborhood of the cell was 2.2–2.3 mM at 36°C.

Data Analysis and Statistics

Current amplitude denotes the peak current during one depolarizing pulse. Current densities were calculated by dividing current amplitudes by the whole cell capacitance. Values are expressed as mean ± SD. 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. Details of the analysis have been described previously.[24] In modification of the previous study, the right boundary of the fit was 93 ms after the peak. Fitting was performed with IgorPro 3.15 software (WaveMetrics Inc., Lake Oswego, OR). Statistical comparisons were carried out with Prism 3.02 software (GraphPad Software Inc., San Diego, CA) using the paired Student *t* test. A probability of error of $P < 0.05$ was considered significant.

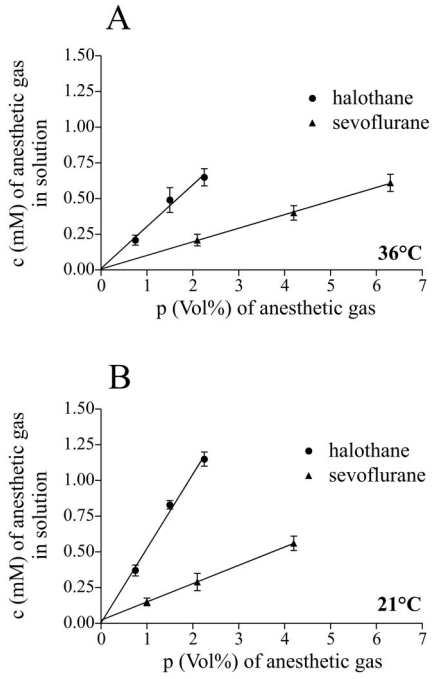

Fig. 1. Concentrations (c in mM) of halothane (●) and sevoflurane (▲) in the aqueous phase (bath solution) after gassing with various concentrations (p in Vol%) of the anesthetics, as measured with head space gas chromatography. The upper panel (*A*) shows the concentrations when the gassing was performed at 36°C; the lower panel (*B*) shows the concentrations when the gassing was performed at 21°C. The lines indicate the fit to a linear relation.

Results

First, we measured the concentrations of halothane and sevoflurane in the standard bath solution after equilibration with various concentrations (corresponding to 1–3 MAC at 37°C) of the anesthetics in the gas phase. The aqueous concentrations differed markedly dependent on whether equilibration was performed at 36°C (fig. 1A) or at room temperature (21°C; fig. 1B). The slope of the relation shown in figure 1 indicates how many mmoles per liter of halothane and sevoflurane are found in the aqueous phase per volume percent of the anesthetics in the gas phase. Respective values are 0.29 and 0.09 mM/vol % for halothane and sevoflurane at 36°C. At room temperature, the corresponding values were 0.52 and 0.12 mM/vol % (n = 3 separate experiments for each concentration).

The L-type Ca^{2+} currents in cardiomyocytes from human right atria exhibited different characteristics at 36°C compared to 21°C (fig. 2A). Most strikingly, the amplitude of the peak current during depolarizing pulses was considerably increased by a rise in the temperature. In atrial cardiomyocytes from four patients measured at

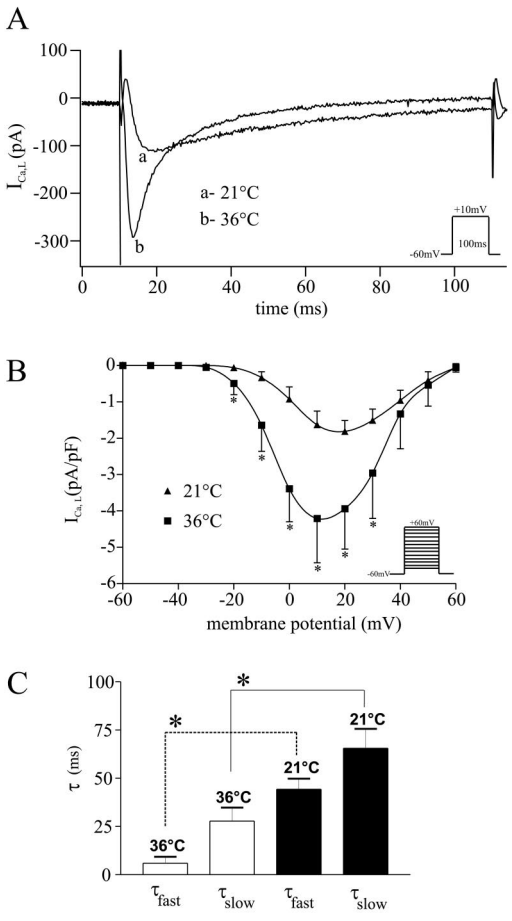

Fig. 2. Amplitudes, current-voltage (I-V) relation and decay kinetics of L-type Ca^{2+} currents ($I_{Ca,L}$) at 21°C and 36°C. (*A*) Current recordings from two cells from the same patient, one studied at 21°C (trace *a*) and the other at 36°C (*b*). Currents were elicited by a depolarizing pulse from −60 to +10 mV (see inset). (*B*) The current-voltage relation (I-V) of the currents at both temperatures. Pulses were applied from a holding membrane potential of −60 mV to various test potentials. Symbols represent mean ± SD of the current density (peak current during one depolarizing pulse, divided by the cell capacitance) of at least five different cells. Currents were significantly larger ($P < 0.05$) at 36°C than at 21°C over a voltage range from −20 to + 30 mV, marked with an asterisk. (*C*) Decay kinetics at both temperatures, as assessed by a fit to a double-exponential function (see Methods). Both, τ_{fast} and τ_{slow}, were significantly lower at 36°C than at 21°C (n = 5; $P < 0.05$).

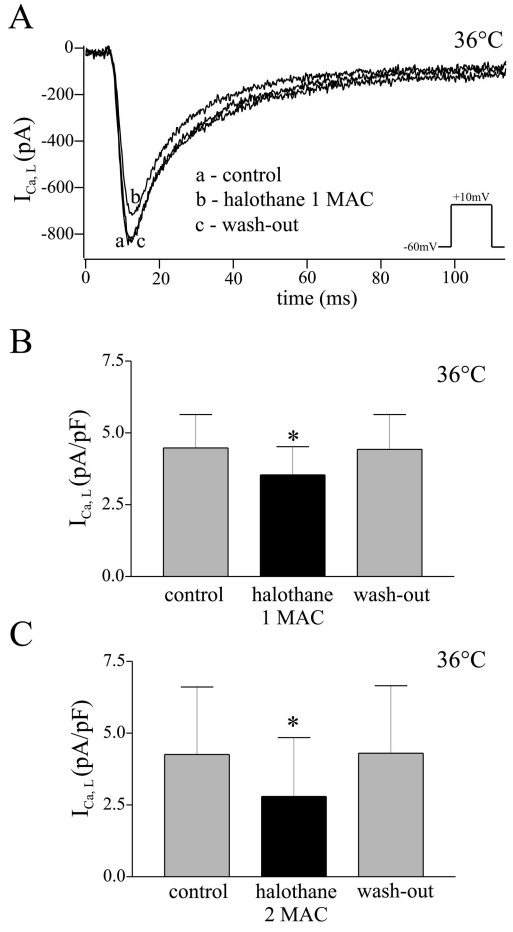

Fig. 3. Effects of halothane on L-type Ca^{2+} currents ($I_{Ca,L}$) at 36°C. (*A*) Original tracings from one cell, before, during, and after application of halothane (1 minimum alveolar concentration or 0.21 mM). (*B*) Summary of the effect of halothane (1 minimum alveolar concentration) on $I_{Ca,L}$ (mean ± SD), as measured in five cells ($P < 0.01$). (*C*) The effect of halothane (2 minimum alveolar concentration or 0.49 mM) on $I_{Ca,L}$ (n = 5, $P < 0.01$).

36°C (n = 5) or at 21°C (n = 5), maximum peak current density was −4.2 ± 1.2 pA/pF at 36°C and −1.8 ± 0.3 pA/pF at 21°C ($P < 0.01$). Moreover, current-voltage relation was slightly shifted to the left at 36°C (fig. 2B) which might, however, indicate a lack of voltage control in the cardiomyocytes. Still, maximal current amplitudes were observed between +10 and +20 mV such that a depolarizing pulse to +10 mV was used as standard stimulus to evoke peak $I_{Ca,L}$.

Finally, the inactivation kinetics of $I_{Ca,L}$ recorded during depolarizing pulses to +10 mV were temperature-sensitive. The decline of $I_{Ca,L}$ after the maximum peak was assessed by a fit of the current amplitudes with a double exponential function (see Methods). The fit yielded two time constants, τ_f and τ_s, that represent the fast and slow inactivating component of $I_{Ca,L}$. The inactivation kinetics were markedly slower at 21°C than at 36°C as both τ_f and τ_s were significantly larger at 21°C. For example, τ_f and τ_s were 5.6 ms and 28.6 ms, respectively, at 36°C for the current tracing labeled with *b* in figure 2A. The corresponding time constants at 21°C were 41.9 ms and 61.6 ms (fig. 2A, tracing *a*). Summarized values (n = 5) for τ_f and τ_s at both temperatures are shown in figure 2C.

In the representative experiment given in figure 3A, halothane (1 MAC) reversibly reduced peak $I_{Ca,L}$ by 18% at 36°C. However, in another cell from the same patient, halothane (1 MAC) evoked a reduction $I_{Ca,L}$ by 30% at 21°C (not shown). In the mean, halothane (1 and 2 MAC) reduced $I_{Ca,L}$ by 21.6 ± 2.9% from −4.5 ± 1.1 pA/pF to −3.5 ± 1.0 pA/pF (n = 5, $P < 0.05$) and by 37.1 ±

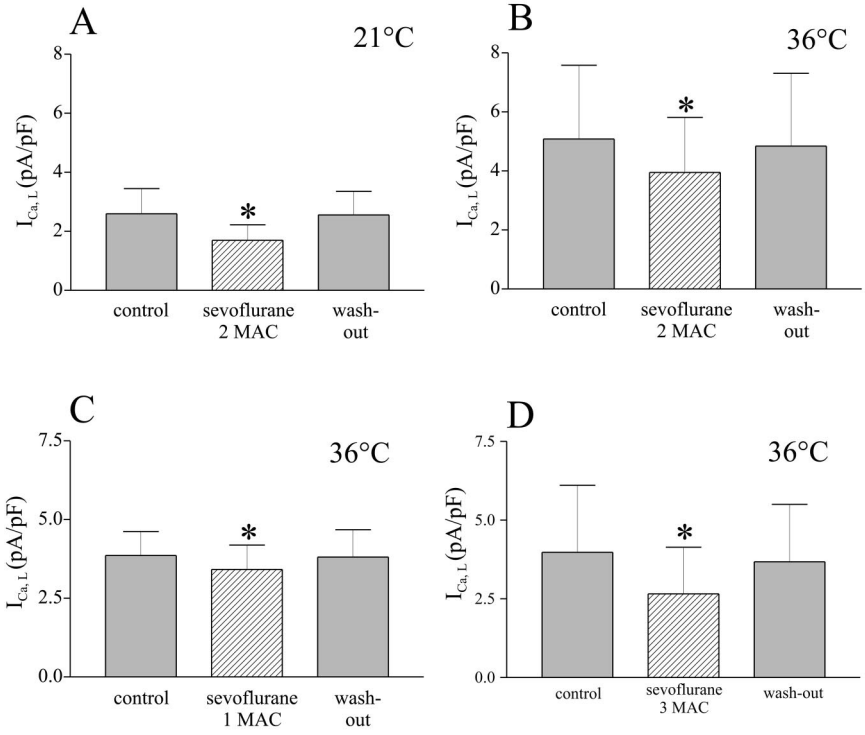

Fig. 4. L-type Ca^{2+} currents ($I_{Ca,L}$) before, during and after application of sevoflurane at 21°C and 36°C. The sevoflurane concentrations were 2 minimum alveolar concentration (0.56 mM) at 21°C in panel *A*, 2 minimum alveolar concentration (0.4 mM) at 36°C in panel *B*, 1 minimum alveolar concentration (0.21 mM) at 36°C in panel *C*, and 3 minimum alveolar concentration (0.61 mM) at 36°C in panel *D*. Significant reductions of the peak current amplitudes are marked with an asterisk.

12.0% from −4.2 ± 2.3 pA/pF to −2.8 ± 2.0 pA/pF (n = 5, $P < 0.05$), respectively, at 36°C (fig. 3B and C).

For comparison, halothane (1 MAC) inhibited peak $I_{Ca,L}$ by 31.3 ± 4.1% from -1.9 ± 0.5 pA/pF to −1.4 ± 0.4 pA/pF (n = 5, $P < 0.05$) at 21°C. These results are similar to those of our previous study performed at room temperature.[22]

Similarly as halothane, sevoflurane reduced the peak amplitude of $I_{Ca,L}$. When we compared the effects of sevoflurane at a concentration of 2 MAC at 21°C *versus* 36°C, the inhibition of $I_{Ca,L}$ was markedly less extensive at the higher temperature (fig. 4A and B). Sevoflurane (2 MAC) inhibited peak $I_{Ca,L}$ by 33.4 ± 11.4% from −2.6 ± 0.8 pA/pF to −1.7 ± 0.5 pA/pF (n = 5, $P < 0.05$) at 21°C and by 21.1 ± 7.8% from −5.1 ± 2.5 pA/pF to −3.9 ± 1.8 pA/pF (n = 6, $P < 0.05$) at 36°C (fig. 4A and B).

We studied two other concentrations of sevoflurane (1 MAC and 3 MAC, fig. 4C and D) at 36°C that reduced peak $I_{Ca,L}$ in a concentration-dependent manner. Sevoflurane inhibited peak $I_{Ca,L}$ by 11.7 ± 6.1% at 1 MAC (n = 5; $P < 0.05$) and by 33.4 ± 9.9% at 3 MAC (n = 5; $P < 0.05$). Again, the wash-out of sevoflurane restored $I_{Ca,L}$ to the amplitudes observed before the application of the anesthetic gas (fig. 3 and 4).

The reduction of $I_{Ca,L}$ (*i.e.*, of the peak amplitude) by halothane and sevoflurane were not accompanied by major effects on the inactivation kinetics. In particular, the major component of the inactivation, τ_{fast}, was not significantly altered. Significant changes were found for the higher concentrations of either anesthetic on τ_{slow} (table 2) but these changes were in opposite directions.

In contrast to halothane and sevoflurane, the noble gas xenon (65%) had not affected $I_{Ca,L}$ in our previous experiments at 21°C.[22] As there was a (insignificant) tendency of xenon to accelerate the inactivation kinetics of $I_{Ca,L}$ after its peak, we reasoned that an effect could be validated at 36°C. Equilibration at 36°C of solutions with gases containing xenon at a concentration of 95% and delivery to the bath chamber (some loss during transport) resulted in a final concentration of 2.2 mM xenon in the aqueous phase. Peak $I_{Ca,L}$ was not affected by xenon at 36°C (not shown). Moreover, xenon did not affect the inactivation kinetics and both time constants remained unchanged (table 2).

Because the resulting concentrations of the anesthetics halothane and sevoflurane in the aqueous phase after gassing with a given partial concentration in the gas phase and the inhibition of $I_{Ca,L}$ by either anesthetic

Table 2. Effects of Volatile Anesthetics and Xenon (± SD) on the Inactivation Kinetics of $I_{Ca,L}$ at 36°C

	τ_{fast}	τ_{slow}
Control	6.8 ± 2.4 ms	27.10 ± 8.3 ms
Halothane 1 MAC (n = 5)	5.9 ± 4.6 ms	25.40 ± 7.2 ms
Control	9.2 ± 4.1 ms	29.20 ± 4.5 ms
Halothane 2 MAC (n = 5)	8.2 ± 3.7 ms	17.08 ± 6.2 ms*
Control	6.0 ± 2.4 ms	30.50 ± 8.0 ms
Sevoflurane 1 MAC (n = 5)	6.7 ± 4.4 ms	31.50 ± 9.8 ms
Control	7.1 ± 4.5 ms	28.10 ± 9.5 ms
Sevoflurane 2 MAC (n = 6)	10.7 ± 5.7 ms	65.10 ± 10.6 ms*
Control	8.6 ± 3.9 ms	34.3 ± 9.60 ms
Xenon 65 Vol%; 1 MAC (n = 5)	9.4 ± 3.7 ms	38.8 ± 10.5 ms

* Significant changes of the time constant τ ($P < 0.05$).

MAC = minimum alveolar concentration.

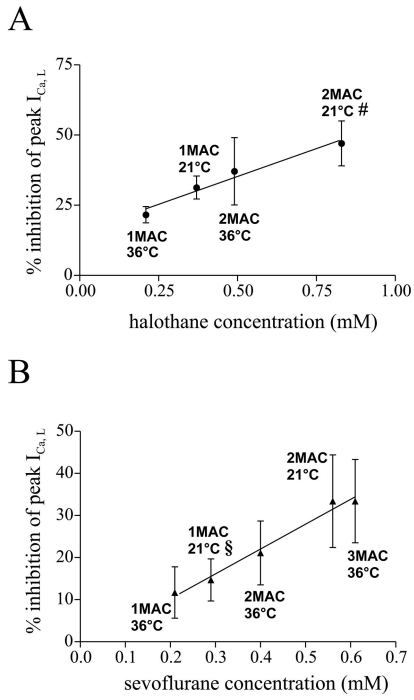

Fig. 5. Concentration-dependence of the inhibition of L-type Ca^{2+} currents ($I_{Ca,L}$) by halothane (*A*) and sevoflurane (*B*). For each point, the concentration of the anesthetic used for gassing of the solutions as well as the temperature is indicated. Data marked with # are from our previous study.[22] Data marked with § are from our previous study.[24] The lines indicate the fit to a linear relation.

decreased considerably when the temperature was raised from 21°C to 36°C, we plotted the fractional (percent) inhibition of $I_{Ca,L}$ against the concentrations of the anesthetics in the aqueous phase (fig. 5 A and B). In this figure, some data are included that derive from our previous studies[22,24] in which $I_{Ca,L}$ in human atrial cardiomyocytes were measured at room temperature (21°C) under identical conditions and with the same set-up as in the present study. Despite the fact that the current amplitudes differed markedly, dependent on the temperature, the plot revealed an almost linear dependence of the percent inhibition of $I_{Ca,L}$ on the concentration of halothane or sevoflurane in the bath solution.

Discussion

This study addresses the question of how temperature affects L-type Ca^{2+} currents and their modification by anesthetic gases in human atrial cardiomyocytes. The main findings are that the amplitudes, inactivation kinetics, and inhibition of $I_{Ca,L}$ by halothane and sevoflurane differed strikingly between 36°C and 21°C. However, the percent inhibition of peak $I_{Ca,L}$ showed an almost linear relationship to the concentrations of the volatile anesthetics in the bathing medium. This relationship was the same at either temperature. These results indicate that the concentration of an anesthetic in the aqueous phase, but not in the gas phase, determines its percent inhibition of $I_{Ca,L}$, independently of the temperature.

The solutions were prepared by gassing with air enriched with the volatile anesthetics at a gas concentration corresponding to 1–3 MAC in patients at 37°C. When the resulting concentrations in the aqueous phase were measured with head space gas chromatography, a temperature dependence was confirmed that has been reported in previous studies for halothane and sevoflurane in various types of solutions.[9,17,25,26] Similar findings have also been reported for xenon.[27] The solubility of the anesthetics decreased strongly with increasing temperatures. It has been previously suggested that concentrations of anesthetics in solutions used for experimental *in vitro* studies should be given in moles per liter rather than in volume percent in the gas phase.[2,17] The relevance of this suggestion becomes clear in light of the temperature dependency of the solubility and the fact that most *in vitro* studies are performed at room temperature. The present study is, to our knowledge, the first one that explores the effects of temperature on $I_{Ca,L}$ in human atrial cardiomyocytes. The current amplitudes were more than doubled by a rise in the temperature from 21°C to 36°C. On the other hand, the inactivation kinetics of $I_{Ca,L}$ important for the duration of the plateau phase of the action potential were markedly accelerated by an increased temperature. For example (fig. 2A), 25 ms after the peak of $I_{Ca,L}$, current amplitudes at 36°C had receded below a level observed at 21°C at the same time point, despite the fact that the maximal current (observed 25 ms previously) had shown a much larger amplitude. Therefore, it is obvious that the usual approach to perform experiments at room temperature does not sufficiently take into account the temperature effects on ion currents in the heart. As long as inhibitory effects on current amplitudes are in the experimental focus, studies at room temperature may be sufficiently relevant, in the light of the present results. If, however, data on current kinetics are to be interpreted in a physiologic context, the experiments should be carried out at physiologic temperatures, although this is technically demanding. In our experience, the stability of the cells over time decreases dramatically with higher temperatures. This means that considerably more attempts must be made before sufficient data for a valid analysis are sampled.

Moderate hypothermia is known to exert a positive inotropic effect on the heart that is independent of the negative chronotropic one.[28] The enhanced contractility is explained by an increased Ca^{2+} sensitivity of the myofilaments, whereas the temperature-dependent modifications of $I_{Ca,L}$ do not fit to the positive inotropic effects of hypothermia.

Xenon had no affected $I_{Ca,L}$ in our previous studies when it was tested at room temperature.[22] In the

present study, it once more failed to produce any significant modification of $I_{Ca,L}$ even when studied at 36°C and analyzed in terms of peak amplitude and inactivation kinetics. Hence, xenon seems to represent an anesthetic with the favorable property of being inert to cardiac $I_{Ca,L}$.

An anesthetic-induced change in the fast component of inactivation kinetics was not observed in our study, in agreement with previous results by Pancrazio.[3] However, halothane and sevoflurane had opposite effects on the slow component τ_{slow} of the time-dependent inactivation of $I_{Ca,L}$ at 36°C. Halothane significantly accelerated the slow component of inactivation at 2 MAC, consistent with other work,[3] whereas sevoflurane delayed the slow component of inactivation. This observation is not in line with findings in single bullfrog atrial myocytes by Hirota *et al.*, who demonstrated that sevoflurane (5.0 vol%) reduced the time constant of $I_{Ca,L}$ inactivation by 25% at room temperature.[29] However, this effect was analyzed with a monoexponential fit of the decay of $I_{Ca,L}$. The calculation of τ_{slow} in our study was mainly performed because the current decay of $I_{Ca,L}$ cannot be properly fitted to a single exponential function. The fast component τ_{fast} is far more decisive for the process of inactivation and determines the total amount of transsarcolemmal moved Ca^{2+} (*i.e.*, the current-time integral) over the time of an action potential. We do not think that effects on τ_{slow} can be interpreted in terms of specific interactions of the two anesthetics with the L-type Ca^{2+} channel.

Halothane may act on L-type Ca^{2+} channels by a direct binding of the anesthetic to the protein at its dihydropyridine binding site.[30] In general, binding of anesthetics to ion channels may be the principal mechanisms relevant for their narcotic action.[31–33] In our study, decreased availability of the anesthetic gases in the bath solution with rising temperature would be in accordance to the attenuated inhibition of $I_{Ca,L}$ if the anesthetics acted from the extracellular side. However, it is not known whether temperature influences the fractional amount of anesthetics that are taken up from the bath into the membrane of cardiac cells. Thus, our study design cannot exclude that the gases act on Ca^{2+} channels after accumulation within the cell membrane and that the temperature effects are related to alterations in the rate of membrane accumulation and binding to channel proteins.

From a clinical point of view, it is a common experience that the required concentrations for anesthesia by gases decrease in the presence of reduced body temperatures in animals and humans.[34–38] For example, hypothermia decreases the required concentration of isoflurane in children by 5.1% per each degree Celsius that the body temperature is lowered.[38] The present study suggests as one possible explanation for this phenomenon that smaller concentrations of the anesthetics in the gaseous phase are effective modulators of ion channels when the temperature is lower as a result of increased solubility.

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