Oxygen Tension Modulates Inhibition of L-type Calcium Currents by Isoflurane in Human Atrial Cardiomyocytes

Pascal Kowark, M.D.,* Rocco Hünke, M.D.,† Eberhard Jüngling,‡ Rolf Rossaint, M.D.,§ Andreas Luckhoff, M.D.||

Background: Myocardial L-type Ca\(^{2+}\) currents (I\(_{\text{Ca,L}}\)) are inhibited by isoflurane in the presence of a partial pressure of oxygen (Po\(_2\)) of 150 mmHg. In guinea pig cardiomyocytes, I\(_{\text{Ca,L}}\) are inhibited by reduced oxygen tensions. The authors therefore analyzed the effects of Po\(_2\) on I\(_{\text{Ca,L}}\) in human cardiomyocytes and the effects of isoflurane at reduced Po\(_2\).

Methods: Atrial cardiomyocytes were prepared from specimens of patients undergoing open-heart surgery and superfused with either a high or a low Po\(_2\) (150 or 12 ± 1 mmHg) while I\(_{\text{Ca,L}}\) were measured with the whole cell patch clamp technique.

Results: Basal I\(_{\text{Ca,L}}\) were not changed by the Po\(_2\) (range, 9–150 mmHg) at 21° or 36°C. The reducing agent 1,4-dithiothreitol (DTT) left I\(_{\text{Ca,L}}\) unaffected, and the oxidizing agent 5,5’-dithiobis(2-nitrobenzoic acid) (DTNB) irreversibly inhibited I\(_{\text{Ca,L}}\). The Po\(_2\) significantly affected the inhibition of I\(_{\text{Ca,L}}\) by isoflurane (1 minimum alveolar concentration) that decreased I\(_{\text{Ca,L}}\) by 17 ± 2.0% at the high Po\(_2\) but only by 5.8 ± 2.9% (P = 0.037) at the low Po\(_2\). The inhibition of I\(_{\text{Ca,L}}\) by isoflurane was also significantly diminished (P = 0.018) by a low Po\(_2\) when isoflurane effects at both Po\(_2\) conditions were compared in the same cell.

Conclusions: In contrast to the situation in guinea pigs, basal I\(_{\text{Ca,L}}\) in human atrial cardiomyocytes were not sensitive to acute Po\(_2\) changes over a wide range. This might be explained by a lack of oxygen-sensitive splice variants of L-type calcium channel subunits. The Po\(_2\), however, has a decisive role for the effects of isoflurane on I\(_{\text{Ca,L}}\).

MYOCARDIAL contractility is essentially determined by the amount of Ca\(^{2+}\) entering the sarcoplasm during electrical systole and the amount of Ca\(^{2+}\) released from intracellular calcium stores. For both processes, L-type Ca\(^{2+}\) channels are decisive because they provide the main voltage-dependent Ca\(^{2+}\) entry pathway in myocardial cells and because Ca\(^{2+}\) entering through these channels triggers Ca\(^{2+}\)-induced Ca\(^{2+}\) release. Furthermore, the sympathetic regulation of contractile force is, to a major part, mediated by phosphorylation of L-type Ca\(^{2+}\) channels, enabling enhanced Ca\(^{2+}\) influx. Therefore, any interference with these channels is expected to exert immediate effects on myocardial contractility.

ceral inhalational anesthetics such as halothane, isoflurane, and sevoflurane (but not the noble gas xenon) have been demonstrated to reduce L-type Ca\(^{2+}\) currents (I\(_{\text{Ca,L}}\)) in cardiomyocytes. These findings have been used to explain in part the negative inotropic effects of the anesthetics.

A further role of L-type Ca\(^{2+}\) channels has recently become apparent because they exhibited profoundly reduced currents in hypoxic conditions. This was first shown on recombinant L-type channels after expression of the pore-forming \(\alpha_{1C}\) subunits of human cardiac tissue in human embryonic kidney 293 cells. Later, I\(_{\text{Ca,L}}\) in guinea pig cardiomyocytes was demonstrated to be decreased by approximately 22% when the partial pressure of oxygen (Po\(_2\)) was decreased from 150 mmHg to 17 mmHg. In the same line, I\(_{\text{Ca,L}}\) was also inhibited by the thiol-specific reducing agent 1,4-dithiothreitol (DTT), and this effect was antagonized by the thiol-specific oxidizing agent 5,5’-dithio-bis(2-nitrobenzoic acid) (DTNB) and the reducing agent 1,4-dithiothreitol (DTT).

The results on recombinant channels were interpreted as evidence for a structural change of the channels by oxygen tension. Therefore, L-type channels may act as oxygen sensors mediating shortening of action potentials and reduction of contractile force in response to hypoxia.

The anesthetic gas isoflurane has been shown to exert moderate inhibition of I\(_{\text{Ca,L}}\) in voltage clamp experiments on isolated cardiomyocytes, prepared from either animal or human heart tissue. It should be kept in mind that these experiments were all performed at standard experimental conditions that use oxygen tensions in the range of a Po\(_2\) of 150 mmHg. The effects of isoflurane have been attributed to an interaction with channel proteins. In light of the evidence for an interaction of the channels with oxygen, we considered it necessary to reexamine the effects of isoflurane on myocardial I\(_{\text{Ca,L}}\) in the presence of lower oxygen tensions that better represent physiologic and pathophysiologic conditions. The aim of the current study, therefore, was twofold. First, we wished to quantify the modulation of I\(_{\text{Ca,L}}\) by oxygen in human cardiomyocytes. This is be-
cause previous experiments have been performed on cardiomyocytes from guinea pigs, whereas studies on human α1C subunits revealed that only one of three splice variants conferred oxygen sensitivity to recombinant L-type channels.10,19 Although all three variants are reportedly expressed in human hearts,20 their functional contribution is not known. Second, we intended to analyze L-type currents in the presence of isoflurane and reduced oxygen tensions. We report that, surprisingly, no effect of the PO2 on basal I_{Ca,L} can be demonstrated over a range from 9 to 150 mmHg in human atrial cardiomyocytes. However, a lower PO2 dramatically reduced or even abrogated the inhibition of I_{Ca,L} by isoflurane that proved to be quite inert on I_{Ca,L} at a PO2 of approximately 12 mmHg.

Materials and Methods

Isolation of Single Atrial Myocytes

Right atrial appendages were obtained as surgical specimens from patients undergoing heart surgery. The patients (n = 30, 23 men, 7 women) had a mean age of 67 yr (range, 45–85 yr), and all underwent a coronary artery bypass operation (n = 23) or aortic valve replacement (n = 7). All patients were in sinus rhythm and had no evidence of right heart failure. Their obligatory medications included β blockers and nitrates. The examinations were performed in accordance with the principles outlined in the declaration of Helsinki and approved by the local institutional review board (Ethik Kommission, Rheinisch-Westfälische Technische Hochschule Aachen, Germany). All patients gave written informed consent before surgery.

Human atrial cardiomyocytes were prepared according to Hatem et al.,21 as modified and described in detail by Skasa et al.22 Briefly, the myocardial specimens were carefully 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, Deisenhofen, Germany) and collagenase V (Sigma). Incubation was finished as soon as microscopic examination revealed intact single cardiomyocytes. After centrifugation, cardiomyocytes were resuspended in a buffer containing the following: 120 mM NaCl, 5.4 mM KCl, 5 mM MgSO4, 5 mM sodium pyruvate, 20 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH. Because visible cell damage to these cells would occur if a physiologic Ca^{2+} concentration was immediately restored, the CaCl2 concentration was gradually increased to 1.8 mM immediately before measurements of I_{Ca,L}. Only well-striated, bleb-free, rod-shaped myocytes were used for the studies, performed within 4 h after 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.5 I_{Ca,L} were recorded with the whole cell patch clamp technique using 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 3–5 MΩ when filled with pipette solution. A holding potential of −60 mV was chosen to minimize sodium currents. I_{Ca,L} were evoked by a series of depolarization pulses to potentials ranging from −50 to +60 mV. Ca^{2+}-currents are given here as peak current amplitude of the respective depolarizing step. To save time, pulses only to +10 mV were used to evaluate the effects of low PO2 and anesthetics in some experiments.

Preparation of Solutions

The bath solution contained 136 mM tetraethylammonium chloride, 1.8 mM CaCl2, 1.8 mM MgCl2, 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with tetraethylammonium hydroxide. The pipette solution contained 140 mM CsCl, 2 mM MgCl2, 0.3 mM adenosine 5′-triphosphate, 0.3 mM guanosine 5′-triphosphate, 10 mM EGTA, and 10 mM HEPES, pH-adjusted to 7.2 with CsOH.

Bath solutions were bubbled with either 100% nitrogen or with air in a gastight glass flask equipped with a frit and a membranous septum. Gas could leave the flask through a valve. The PO2 values were measured with a calibrated oxygen-sensitive probe (Unisense, Aarhus, Denmark; tip diameter, 500 μm). Gassing with nitrogen for approximately 30 min resulted in a decrease of the PO2 to approximately 1 mmHg. Bath solutions containing isoflurane were bubbled with an appropriate gas mixture (isoflurane plus air or nitrogen). An anesthetic gas analyzer (Vamos; Dräger, Lübeck, Germany) was used to continuously monitor the concentration of isoflurane in the gas phase. A fraction (10–20 ml) of gassed solutions was taken through the septum of the reservoir into gas-tight syringes (1010 TLL; Hamilton, Bonaduz, Switzerland) and immediately used for experiments. The bath chamber (volume approximately 300 μl) was continuously perfused through high-performance liquid chromatography–grade steel capillaries at a rate of 2–5 ml/min. The surface of the bath was covered with a thin layer of paraffin oil (DAB10; Wasserfuhr, Bonn, Germany) saturated with nitrogen to minimize diffusional exchange of oxygen and isoflurane. The isoflurane concentration in the reservoir as well as in the bath chamber was verified by headspace gas chromatography–mass spectrometry–selected ion monitoring (GC/MS/SIM, SSQ 7000; Finnegan, Bremen, Germany) as described previously.5

To examine the effects of a lowered PO2 on I_{Ca,L}, the myocytes were exposed to a hypoxic solution in the bath chamber by rapid solution exchange, followed by continuous perfusion of the chamber with the same
hypoxic solution. The PO\(_2\) was continuously recorded with an oxygen-sensitive probe (Unisense) placed into the chamber close to the examined cell. A final PO\(_2\) of 12 \(\pm\) 1 mmHg was achieved in the bath chamber. This condition could be held stable throughout the measurements of I\(_{\text{Ca,L}}\) in one myocyte (approximately 5–7 min).

The thiol-specific oxidizing agent DTNB (Sigma) was dissolved in ethanol (100%) to yield a stock solution with 2 mM that was stored at 4°C. Bath solutions contained 200 \(\mu\)M DTNB; in all experiments using DTNB, ethanol was added to control solutions resulting in the same final concentration (1% vol/vol) as the DTNB solutions. The thiol-specific reducing agent DTT (Sigma) was dissolved in water.

**Temperature Control**

Most experiments were carried out at room temperature (21°C). Some experiments were performed at 36°C. In this case, the temperature of the solution in the double-barreled stainless steel tubes and the bath chamber was kept constant with a feedback-controlled Peltier device (Strothmann, Aachen, Germany). The temperature was continuously monitored with a thermocouple probe mounted in the chamber wall.

**Data Analysis And Statistics**

Current amplitudes mark the peak current during one depolarizing pulse. Current densities were calculated by dividing current amplitudes by the whole cell capacitance amounting to 85 \(\pm\) 7 pF in 59 cells. Values are expressed as mean \(\pm\) SEM. Statistical comparisons were performed with SPSS version 12.0 software (SPSS Inc., Chicago, IL). Differences in I\(_{\text{Ca,L}}\) were tested for statistical significance using the Wilcoxon nonparametric test for paired data. Unpaired data were tested using the nonparametric Mann–Whitney U test. A probability of error of \(P < 0.05\) was considered significant.

**Results**

To study effects of the oxygen tension on I\(_{\text{Ca,L}}\) in cardiomyocytes, we continuously measured the PO\(_2\) in the immediate neighborhood of a patch clamped cell. When the superfusate was changed from a solution with a PO\(_2\) of 150 mmHg to a solution gassed with nitrogen, a decrease in PO\(_2\) resulted that fully developed over approximately 1 min. Then a stable plateau of 12 \(\pm\) 1 mmHg (range, 9–15 mmHg) was reached. Lower PO\(_2\) values could not be obtained. When the superfusate was switched back to the original solution with the high PO\(_2\), PO\(_2\) values were restored within 20 s. I\(_{\text{Ca,L}}\) was measured in intervals of approximately 20 s as peak current values after depolarizing steps from a holding potential of \(-60\) mV to \(+10\) mV. Unexpectedly, I\(_{\text{Ca,L}}\) did not exhibit any noticeable changes when the PO\(_2\) was changed from the control conditions (150 mmHg) to 12 mmHg (fig. 1A) and back to 150 mmHg (not shown). In the mean, I\(_{\text{Ca,L}}\) was 2.00 \(\pm\) 0.30 pA/pF (\(n = 11\)) in control conditions, 2.03 \(\pm\) 0.31 during low PO\(_2\), and 1.97 \(\pm\) 0.31 after restoration of the control PO\(_2\) (fig. 1B). I\(_{\text{Ca,L}}\) at other

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membrane potentials ranging from \(-30\) to \(+30\) mV were not significantly altered by changes in the PO2, either (fig. 1C). Moreover, no modifications of \(I_{\text{Ca,L}}\) were observed during the transition phases of the PO2. Likewise, the kinetics of the currents during each depolarizing step were virtually identical in each PO2 condition (fig. 1A).

Although the range over which the PO2 was varied in our experiments was well within the range for which significant changes of \(I_{\text{Ca,L}}\) have previously been reported,\(^9,10\) we reasoned that the PO2 might not be sufficiently low to affect \(I_{\text{Ca,L}}\) in human cardiomyocytes. Because oxygen contamination, probably due to diffusion, led to a failure of our attempts to reduce the PO2 further by oxygen-free superfusion of the cells, we tried to remove oxygen enzymatically\(^23,24\) by adding 100–300 mU/ml Oxyrase (Oxyrase Inc., Mansfield, OH) to the superfusate. Indeed, very low PO2 values could be obtained with Oxyrase (even below 1 mmHg). \(I_{\text{Ca,L}}\) was completely suppressed by these solutions. However, this suppression was irreversible. Moreover, the effects of Oxyrase could not be attributed to PO2 reductions because exposure to Oxyrase evoked the same irreversible inhibition of \(I_{\text{Ca,L}}\) in the presence of a PO2 of 150 mmHg (data not shown).

The experiments reported so far were performed at room temperature. We also tested whether a potential sensitivity of \(I_{\text{Ca,L}}\) to decreased oxygen tensions might reveal in physiologic temperatures (36°C). The problem with experiments at this temperature is that a significant run-down of \(I_{\text{Ca,L}}\) occurs in the majority of experiments, in contrast to those at room temperature when \(I_{\text{Ca,L}}\) was usually stable over 10–15 min. Typically, run-down at 36°C reduces \(I_{\text{Ca,L}}\) by as much as 50% over 8 min, making the unequivocal detection of small inhibitions on top of the run-down impossible. However, in none of the experiments in which a low PO2 was applied to cells at 36°C (\(n = 7\)) was any decrease of \(I_{\text{Ca,L}}\) observed that was not explainable by run-down. Moreover, the restoration of a PO2 of 150 mm did not result in a deceleration of time-dependent run-down of \(I_{\text{Ca,L}}\). In two experiments, run-down was slower than usual (fig. 1D); in these cells, \(I_{\text{Ca,L}}\) was identical in a PO2 of 150 and of 14 or 12 mmHg. Therefore, no evidence was obtained that a low PO2 suppressed \(I_{\text{Ca,L}}\) at 36°C.

Next, we studied the interaction between oxygen and isoflurane with respect to their effects on L-type calcium channels. Isoflurane was added to superfusates at a final concentration of 1.2% (vol/vol), resulting in 0.38 mM and corresponding to a minimum alveolar concentration of 1 at 21°C. Previous experiments at a PO2 of 150 mmHg had shown that isoflurane evokes a rapid, stable, and quickly reversible inhibition of \(I_{\text{Ca,L}}\).\(^5,17,18\) In the current experiments, cells were kept at 150 mmHg PO2 (control conditions) and then exposed either to isoflurane at a low PO2 (9–15 mmHg) or to isoflurane at a control PO2 (150 mmHg). Afterward, the original control conditions were restored. The results (fig. 2) show a weaker inhibition of \(I_{\text{Ca,L}}\) by isoflurane at the lower than the control PO2. Isoflurane reduced \(I_{\text{Ca,L}}\) by 17 ± 2.0% at the control PO2 but only by 5.8 ± 2.9% at the low PO2. When the decreases in \(I_{\text{Ca,L}}\) induced by isoflurane at a PO2 of 150 mmHg (\(n = 8\)) were compared with those at 9–15 mmHg (\(n = 7\)), a slightly significant difference was revealed (\(P = 0.037\), Mann–Whitney U test). To demon-

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**Fig. 2.** Effects of the partial pressure of oxygen (PO2) on the inhibition of L-type Ca\(^{2+}\) currents induced by the anesthetic gas isoflurane. (A) Traces from one experiment in which the cell was kept at a PO2 of 150 mmHg. After obtaining a control trace without the addition of isoflurane, isoflurane (at a minimum alveolar concentration of 1) was applied, followed by restitution of control conditions. (B) Traces from one experiment in which isoflurane was applied during a PO2 of 150 mmHg. The control traces before and after the addition of isoflurane were obtained at a PO2 of 12 mmHg. (C) Summary of the experiments with the protocol of A and B. Note that all controls were performed at a PO2 of 150 mmHg. The inhibition by isoflurane was stronger (\(P = 0.037\)) when the gas was applied at a PO2 of 150 mmHg (\(n = 8\)) than at a PO2 of 12–15 mmHg (\(n = 7\)).
strate more conclusively that isoflurane-mediated inhibitions were dependent on the oxygen tension, we designed a protocol in which the effect of two PO2 values on isoflurane-induced current inhibitions was studied in each cell, allowing the application of a paired test for statistical significance. Isoflurane was applied at a PO2 of 9–15 mmHg to cells kept previously at 150 mmHg PO2. Then, with isoflurane still present in the superfusate, the PO2 was increased to 150 mmHg. Finally, isoflurane was removed while the PO2 was kept at 150 mmHg (fig. 3). In these experiments, there was a significant stronger inhibition of Ica,L by isoflurane at 150 than at 12 mmHg PO2 (P = 0.018, Wilcoxon rank test, n = 7). The data furthermore show that hardly any inhibition of Ica,L was evoked by isoflurane at the low PO2. Washout of isoflurane consistently led to a full recovery of Ica,L.

The results so far are consistent with the view that not so much isoflurane but rather the high PO2 (because 150 mmHg represents an oxygen tension considerably exceeding the physiologic values) is the decisive factor that accounts for the reduction of Ica,L when isoflurane is applied at a PO2 of 150 mmHg. Because the PO2 might affect membrane proteins such as calcium channels through oxidation and reduction of sulfhydryl groups, we tested the thiol-specific oxidant DTNB in comparison with the thiol-specific reducing agent DTT. The reducing agent DTT (2 mM) did not affect Ica,L at basic conditions (fig. 4A) or in the presence of isoflurane (fig. 4B). Specifically, DTT did not change Ica,L when the agent was added to a standard bath (3.41 ± 1.38 vs. 3.46 ± 1.36 pA/pF, n = 6). Furthermore, when a control value of Ica,L was determined in a standard bath and then the known inhibition by isoflurane was provoked, the further addition of DTT (in the continuous presence of isoflurane) left Ica,L at the same level as in the presence of isoflurane alone (2.80 ± 0.88 vs. 2.86 ± 0.94 pA/pF, n = 5). In contrast to DTT, the oxidizing agent DTNB (200 μM) had profound effects on Ica,L (figs. 4C and D). Addition of the agent to a standard bath evoked an immediate reduction of Ica,L by 11 ± 2% (n = 10). However, this effect was irreversible; after extended washout, Ica,L remained on the diminished level for several minutes, demonstrating that inhibitions of Ica,L by DTNB cannot be attributed to run-down of Ica,L (fig. 4C). Therefore, Ica,L in human cardiomyocytes are more sensitive to oxidation and high oxygen tensions than to reduction and low oxygen tensions.

Discussion

In this study, we have examined how oxygen tension affects Ica,L in human atrial cardiomyocytes at basal conditions as well as in the presence of the anesthetic gas isoflurane, previously shown to inhibit these currents at high PO2 values.5 As main findings, a PO2 between 9 and 150 mmHg did not affect Ica,L in the absence of isoflurane, in discrepancy to previous findings on guinea pig cardiomyocytes. However, the PO2 exerted significant effects on the inhibition of Ica,L by isoflurane. Only at high PO2 levels were inhibitory effects of the anesthetic gas observed. Inhibition of Ica,L was also evoked by the...
oxidizing agent DTNB, whereas the reducing agent DTT did not modify $I_{Ca,L}$.

Our results indicate that $I_{Ca,L}$ in the human heart are inhibited by oxidizing agents and by high $P_O2$ levels (in the presence of isoflurane) rather than by reducing agents and low $P_O2$. What could the reasons be for the apparent discrepancy of the current results, when compared with those obtained in guinea pig cardiomyocytes? First, one could assume that the temperature is relevant. The author of the study in guinea pig cells chose a temperature of 37°C, and such experimental conditions are more difficult to establish than room temperature. From our experience, experiments at physiologic temperatures have a high failure rate due to rapid run-down of $I_{Ca,L}$.

The second reason for the discrepancy could be the existence of species-dependent (atria vs. ventricles) differences in the oxygen sensitivity of cardiomyocytes. In this context, it is noteworthy that no strict correlation between the extent of $P_O2$ reductions and the effects on $I_{Ca,L}$ was reported in guinea pig cardiomyocytes. On the other hand, such a correlation was clearly established in human embryonic kidney 293 cells expressing $\alpha_{1C}$ subunits. Moreover, no saturation of the $P_O2$-induced $I_{Ca,L}$ suppression was evident in the latter cell model, even at the lowest attainable $P_O2$ values. Therefore, the oxygen-sensing capability of a cell may apply to different ranges of $P_O2$ variations. Unfortunately, we could not achieve hypoxic conditions with $P_O2$ values considerably lower than 12 mmHg. Our attempts with Oxyrase did not allow us to attribute the observed marked alterations of $I_{Ca,L}$ to hypoxia but rather to some oxygen-independent effects of the enzyme. However, it should be stressed that our experiments tested a range of $P_O2$ values that produced dramatic changes of $I_{Ca,L}$ in both guinea pig cardiomyocytes and human embryonic kidney 293 cells expressing human $\alpha_{1C}$ subunits, again pointing to distinctly different $P_O2$ sensitivity in human cardiomyocytes than in these other models.

Finally, the results that have been reported from studies on different isoforms of $Ca^{2+}$ channel $\alpha_{1C}$ subunits deserve consideration. Only one of three isoforms, or splice variants, of the proteins conferred oxygen sensitivity to $I_{Ca,L}$ in human embryonic kidney 293 cells. Although all three isoforms are likely to exist in the human heart, a quantitative or functional analysis has not yet been performed. Such an analysis should also...
consider regional differences, in particular between atria and ventricles. Currently, a quantitative determination of the expression pattern and of \( I_{\text{Ca,L}} \) could not be performed simultaneously and in identical cells. If there were a preponderance of oxygen-insensitive over oxygen-sensitive \( \text{Ca}^{2+} \) channel proteins in our cells, the experimental results would be explained.

The molecular basis of how the two gases oxygen and isoflurane modulate ion currents has not been fully elucidated. An interaction of isoflurane with channel proteins is likely and may be a predominant factor in many conditions. Similarly, oxygen may interact with L-type \( \text{Ca}^{2+} \) channels directly, as proposed on the basis of the experiment of heterologously expressed channels and of results obtained with thiol-specific reducing and oxidizing agents. It has been concluded that the open probability of L-type \( \text{Ca}^{2+} \) and other channels is critically dependent on the oxidative state of various sulfhydryl groups which are differently amenable to redox-modulating compounds. However, the literature on effects of thiol-reducing and oxidizing agents is full of inconsistencies, as summarized in a recent review. For example, in guinea pigs where \( I_{\text{Ca,L}} \) is sensitive to decreased \( \text{PO}_2 \) values, the reducing agent DTT mimicked the effects of low \( \text{PO}_2 \), whereas the oxidizing agent DTNB partly reversed the inhibition by low oxygen tensions. Mostly in line with these results, DTT attenuated the effects of low \( \text{PO}_2 \) whereas the oxidizing agent p-hydroxy-mercuric-phenylsulfonic acid, reduced rather than increased \( \text{PO}_2 \) values. The current experiments reveal a striking functional difference of \( I_{\text{Ca,L}} \) in human atrial cardiomyocytes, in comparison with cells from guinea pig ventricles, because \( I_{\text{Ca,L}} \) was not modulated by \( \text{PO}_2 \) in human cells in the way demonstrated for guinea pig cells. Our results might be explained by a predominant expression of oxygen-insensitive \( \alpha_{1C} \) subunits. Regional differences of the expression pattern of \( \alpha_{1C} \) subunits may be present in the human heart, but species differences seem to be more relevant. Furthermore, the study demonstrates a clear dependence of the isoflurane-induced inhibition of \( I_{\text{Ca,L}} \) on \( \text{PO}_2 \). An inhibition occurs mostly at standard experimental \( \text{PO}_2 \) conditions, but these are likely to represent hyperoxia not normally present in the microenvironment of living cells.

In conclusion, the current experiments reveal a striking functional difference of \( I_{\text{Ca,L}} \) in human atrial cardiomyocytes, in comparison with cells from guinea pig ventricles, because \( I_{\text{Ca,L}} \) was not modulated by \( \text{PO}_2 \) in human cells in the way demonstrated for guinea pig cells. Our results might be explained by a predominant expression of oxygen-insensitive \( \alpha_{1C} \) subunits. Regional differences of the expression pattern of \( \alpha_{1C} \) subunits may be present in the human heart, but species differences seem to be more relevant. Furthermore, the study demonstrates a clear dependence of the isoflurane-induced inhibition of \( I_{\text{Ca,L}} \) on \( \text{PO}_2 \). An inhibition occurs mostly at standard experimental \( \text{PO}_2 \) conditions, but these are likely to represent hyperoxia not normally present in the microenvironment of living cells.

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