Reduction of $\text{Ca}^{2+}$ channel activity by hypoxia in human and porcine coronary myocytes

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Abstract

Objective: Oxygen ($O_2$) tension is a major regulator of blood flow in the coronary circulation. Hypoxia can produce vasodilation through activation of ATP regulated K$^+$ (K$_{ATP}$) channels in the myocyte membrane, which leads to hyperpolarization and closure of voltage-gated Ca$^{2+}$ channels. However, there are other O$_2$-sensitive mechanisms intrinsic to the vascular smooth muscle since hypoxia can relax vessels precontracted with high extracellular K$^+$, a condition that prevents hyperpolarization following opening of K$^+$ channels. The objective of the present study was to determine whether inhibition of Ca$^{2+}$ influx through voltage-dependent channels participates in the response of coronary myocytes to hypoxia. Methods: Experiments were performed on porcine anterior descendent coronary arterial rings and on enzymatically dispersed human and porcine myocytes of the same artery. Cytosolic [Ca$^{2+}$] was measured by microfluorimetry and whole-cell currents were recorded with the patch clamp technique. Results: Hypoxia ($O_2$ tension $\leq 20$ mmHg) dilated endothelium-denuded porcine coronary arterial rings precontracted with high K$^+$ in the presence of glibenclamide (5 $\mu$M), a blocker of K$_{ATP}$ channels. In dispersed human and porcine myocytes, low O$_2$ tension decreased basal cytosolic [Ca$^{2+}$] and transmembrane Ca$^{2+}$ influx independently of K$^+$ channel activation. In patch clamped cells, hypoxia reversibly inhibited L-type Ca$^{2+}$ channels. RT±PCR indicated that rHT is the predominant mRNA variant of the $\alpha_1C$ Ca$^{2+}$ channel subunit in human coronary myocytes. Conclusion: Our study demonstrates, for the first time in a human preparation, that voltage-gated Ca$^{2+}$ channels in coronary myocytes are under control of O$_2$ tension. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ca-channel; Calcium (cellular); Coronary circulation; Hypoxia/anoxia; Myocytes

1. Introduction

It is well established that local oxygen tension ($P_{O_2}$) is a major regulator of vessel diameter in most vascular beds (e.g. Refs. [1,2]). Although hypoxic vasoconstriction has been observed in some conditions [3], relaxation is the most common effect of low $P_{O_2}$ on the coronary arteries. This response is of critical physiologic importance because it contributes to adjust the amount of oxygen supplied to the working heart to its metabolic needs. Any imbalance between $O_2$ delivery and demand can lead to angina pectoris and in extreme cases to myocardial infarction. Among the proposed mechanisms to explain coronary hypoxic vasodilation are the release of vasorelaxants, e.g. nitric oxide or prostaglandins, from the endothelium or the production of vasoactive substances (such as adenosine) by the cardiac muscle [4–8]. In addition, $O_2$ can directly influence the excitability and contractility of coronary arterial (CA) myocytes. ATP-regulated K$^+$ ($K_{ATP}$) channels in the vascular smooth muscle (VSM) cell membrane appear to be major contributors to regulation of CA tone. The decrease in coronary resistance by hypoxia in perfused...
hearts is inhibited by the $K_{ATP}$ channel blocker glibenclamide (GLI) and mimicked by the $K_{ATP}$ channel opener cromakalin [9,10]. Moreover, in dispersed CA myocytes, prolonged hypoxic exposures ($>2$ min) induce $K_{ATP}$ channel activity [11]. Therefore, protracted hypoxia or any other circumstance reducing intracellular ATP levels lead to $K_{ATP}$ channel opening, which causes membrane hyperpolarization, closure of voltage-dependent Ca$^{2+}$ channels and relaxation. In other vascular beds, this mechanism appears to be unimportant [12] and in the middle cerebral artery, hypoxia-induced relaxation is mediated by the opening of Ca$^{2+}$-activated maxi-K$^+$ ($K_{Ca}$) channels [13]. Apart from the effects on K$^+$ channels, it is highly likely that Po$_2$ can also directly regulate transmembrane Ca$^{2+}$ influx in VSM cells because systemic arterial relaxation is observed with moderate hypoxia, without compromise of metabolism [14,15] and occurs in vessels precontracted with high extracellular K$^+$ [16,17], a condition that prevents hyperpolarization following opening of K$^+$ channels. It has recently been shown that low Po$_2$ can reduce basal cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) [16,18,19] and decrease the activity of Ca$^{2+}$ channels [20–23] in myocytes from vascular beds that dilate in response to hypoxia. Here, we have tested to see whether a similar mechanism, independent of K$^+$ channel activation, participates in the hypoxic relaxation of human and porcine coronary arteries. This study, to our knowledge the first to describe the responses to hypoxia of isolated human CA myocytes, indicates that in these cells, low Po$_2$ can inhibit the activity of L-type Ca$^{2+}$ channels.

## 2. Methods

### 2.1. Preparation of arterial rings and dispersed myocytes

Experiments were performed on arterial rings and isolated myocytes from epicardial (anterior descendents) human and porcine coronary arteries. Porcine arteries were obtained from anesthetized (sodium pentobarbital, 50 mg/kg, i.v.) animals of weight 25.75±0.3 kg ($n=8$) after exsanguination and cardiectomy. Human arteries were dissected from hearts of patients (52±4 years of age, $n=8$) subjected to orthotopic heart transplantation that had suffered heart failure owing to dilated cardiomyopathy of ischemic origin (stage III–IV of the New York Heart Association). Patients had been chronically treated with anticoagulants. Segments (≈2 cm) of either porcine or human arteries were cleaned of surrounding connective tissue and placed in cold (4°C) oxygenated modified Krebs solution of the following composition (in mM): NaCl 120; CaCl$_2$ 2.5; KCl 3; MgSO$_4$ 1.2; NaHCO$_3$ 28; KH$_2$PO$_4$ 1.2; glucose 5, pH 7.4. Arterial rings (4–5 mm length and 2–4 mm diameter) were mounted on recording chambers and attached to two tungsten wires connected to an isometric force transducer (Cibertec). When desired, the endothelium was denuded by passing a small wire through the lumen. Rings were subjected to a passive tension of 3 g, which yielded maximal contractile response upon exposure to 60 mM KCl, and allowed to stabilize in the standard Krebs solution equilibrated with 95% O$_2$ and 5% CO$_2$ for at least 30 min before the experiments. Exposure to hypoxia was done by bubbling the solution in the chamber with 95% N$_2$ and 5% CO$_2$. A low Po$_2$ value of ≈30 mmHg was obtained in less than 1 min. All the drugs used were dissolved in the Krebs solution. When extracellular K$^+$ was increased, KCl substituted equimolarly NaCl. Experiments were performed at 37°C.

To prepare dispersed CA myocytes the adventitia was carefully removed and the arterial segments cut into 1- to 2-mm pieces and placed in a Petri dish with 5 ml salt solution to which 3–5 mg/ml papain (Sigma), 2–3 mg/ml collagenase (type IA; Sigma) and 1–2 mg/ml bovine serum albumin (fraction V; Sigma) had been added. The salt solution contained (mM): NaCl 125; KCl 5.36; KH$_2$PO$_4$ 0.44; Na$_2$HPO$_4$ 0.34; NaHCO$_3$ 15.5; sucrose 1.45; glucose 10; Heps 10 (pH 7.4). The tissue was stored for ≈14 h at 7°C and afterwards placed for 15 min in a shaker at 37°C. When the first myocytes appeared dispersed in the solution, the tissue was transferred to fresh salt solution at 37°C containing bovine serum albumin (10 mg/50 ml) where the cells were mechanically dispersed using fire polished Pasteur pipettes. After dissociation, the cells were plated on poly-L-lysine-coated glass coverslips and used for the experiments within the next 2–3 h. Myocytes were easily distinguished by their size and typical elongated shape.

### 2.2. Measurement of [Ca$^{2+}]_i$

[Ca$^{2+}]_i$ was measured in dispersed cells either intact or subjected to patch-clamp. In the first case, myocytes were incubated at room temperature (22–25°C) for 15 min with the standard external solution containing 2.5 μM of the membrane permeable form of the Ca$^{2+}$ indicator dye Fura-2 (Fura-2/AM; Molecular Probes). The standard external solution bathing the cells contained (mM): NaCl 140; KCl 2.7; MgCl$_2$ 1; CaCl$_2$ 2.5, and Heps 10 (pH 7.4 and 290 mosm/kg). When KCl was increased it substituted equimolarly NaCl. All drugs were added to this solution. The external solution was continuously applied by superfusion and replaced the recording chamber in less than 30 s. In patch-clamped cells the Fura-2 salt was added to the pipette solution at a concentration of 50 μM. This solution contained, in mM: 110 K aspartate, 20 KCl, 1 MgCl$_2$, 5 Heps, 5 dитриоскватриотетрафосфат, 5 MgATP, pH 7.2. For the experiments, a coverslip with cells attached was placed in a recording chamber (≈0.2 ml) mounted on the stage of an inverted microscope (Axiovert 35, Zeiss) equipped for epifluorescence and photometry. Cytosolic [Ca$^{2+}$] was...
calculated from the ratio \( R \) of fluorescence at 360 and 380 nm according to the formula:
\[
[Ca^{2+}] = \frac{K_{\text{eff}} (R - R_{\min})}{(R_{\max} - R)}
\]

\( R_{\min} \) and \( R_{\max} \) were calibrated in vitro using the values of the fluorescence ratio in internal solutions containing 2 \( \mu \)M Fura salt and either with 0 Ca\(^{2+}\) (Ca\(^{2+}\)-free plus 5 mM EGTA) or saturated with Ca\(^{2+}\). \( K_{\text{eff}} \) was obtained from a solution with 300 nM Ca\(^{2+}\). Details of the experimental set-up are described in previous reports from our laboratory [19,20].

2.3. Electrophysiological recording

Membrane currents were recorded using the whole-cell configuration of the patch clamp technique as adapted by our laboratory [20,21]. Given the relatively small size of the currents studied we did not systematically use series resistance compensation. The recording solutions used in voltage-clamp experiments contained (in mM): External (140 NaCl, 2.7 KCl, 10 BaC12, 10 Hepes, pH 7.4). Internal (solution in the patch pipette and inside the cell, 100 CsCl, 30 CsF, 1 MgCl2, 10 Hepes, 5 EGTA, 5 BAPTA, 4 MgATP, pH 7.2). Ba\(^{2+}\) was used as charge carrier instead of Ca\(^{2+}\) to favor the flow of current through the Ca\(^{2+}\) channels. ATP was used to prevent the run down of the channels. All the experiments with isolated cells (electrophysiology of microfluorimetry) were conducted at room temperature (22–25°C). The external normoxic solution was equilibrated with air and hypoxia was obtained by bubbling the same solution with \( \text{N}_2 \). \( \text{PO}_2 \) values in the recording chamber \((=150 \text{ and } \approx 20 \text{ mmHg})\) for normoxic and hypoxic solutions, respectively) were estimated by amperometry with a platinum electrode [24].

2.4. RNA purification and RT-PCR

RT-PCR was done using total RNA obtained from human aorta and coronary arteries from three different individuals. RNA was reverse transcribed and amplified products were gel-puriﬁed and cloned into the vector pGEM-Teasy (Promega) for sequencing. The sequences of the oligonucleotides used for RT-PCR (based on numbering of the hHT isoform of the human heart \( \alpha_{1C} \) calcium channel, accession number L04569) are:

Forward (F): 5’-TGGAAGCTCAGCTCAACAG-3’ (6749–6768);
Reverse-1 (R1): 5’-TCCTGGTAGGAGAGCATCTC-3’ (7106–7125);
Reverse-2 (R2): 5’-CAGCCTGCTGGGAGCCAC-TCTC-3’ (6939–6960; specific to hHT).

Unless otherwise noted, all values are given as mean±S.D. and in some cases the number of experiments is given in parentheses. A Student’s \( t \)-test was performed to estimate the significance of the differences between mean values. A value of \( P<0.05 \) was considered significant.

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). Permission for the use of ex vivo human material was granted by the Institutional Review Board.

3. Results

3.1. Reduction of tension in CA rings by hypoxia

Hypoxia-induced relaxation was studied in rings of porcine coronaries precontracted with high external KCl (Fig. 1). The reduction of maximal tension by low \( \text{PO}_2 \) in arteries with endothelium (623 ± 9.2%; \( n = 12 \)) was not significantly different from the values obtained when the endothelium was denuded (56.8 ± 12.12%; \( n = 10 \)) (Fig. 1A). In the presence of 10 \( \mu \)M GLI, a concentration known to block \( K_{\text{ATP}} \) channels [11], hypoxic relaxation of \( K^+ \) evoked contractures in arteries with and without endothelium (58.2 ± 8.3 and 55.7 ± 9.6%, respectively; \( n = 8 \)) was similar and these values were not significantly different with respect to those observed in control conditions (Fig. 1B). These results indicate that precontracted epicardial CAs relax in response to moderate hypoxia by mechanisms that are independent of endothelial factors and that depolarization-induced contraction of CA rings as well as the subsequent relaxation upon exposure to hypoxia are unaffected by blockade of \( K_{\text{ATP}} \) channels with GLI.

3.2. Changes of [\( Ca^{2+} \)], in individual myocytes exposed to hypoxia

In the normal external solution, most dispersed VSM cells were quiescent and had basal \( [Ca^{2+}] \), of 56 ± 18 nM \( (n = 15 \) porcine myocytes) or 52 ± 13 nM \( (n = 11 \) human myocytes). Exposure to high extracellular \( K^+ \) (60 nM) produced a rise of \( [Ca^{2+}] \), (285 ± 81 nM in eight porcine myocytes, and 163 ± 25 nM in four human myocytes). We also tested \( (n = 3 \) porcine myocytes) that exposure to high extracellular \( K^+ \) (60–100 mM) induced a robust membrane depolarization with a rise of \( [Ca^{2+}] \), presumably due to \( Ca^{2+} \) influx through voltage-dependent channels (Fig. 2A). In one cell tested, the \( K^+ \)-induced rise of \( [Ca^{2+}] \), was blocked by nifedipine (1 \( \mu \)M). Low \( \text{PO}_2 \) did not alter significantly basal \( [Ca^{2+}] \), \( (51 ± 12 \text{ nM in six porcine and 45 ± 14 nM in eight human CA myocytes}) \) but produced a statistically significant reversible reduction of \( [Ca^{2+}] \), in myocytes pre-exposed to 60 mM KCl (Fig. 2B). The reduction of \( [Ca^{2+}] \), by hypoxia was similar in porcine and human CA myocytes with average values of 38.3 ± 21.8% \( (n = 4 \) and 31.8 ± 14% \( (n = 4 \), respectively. These values were also not significantly different from the percentage of
Fig. 1. Reduction of tension by hypoxia in precontracted coronary artery rings. (A) Top. Contraction of an arterial ring by exposure to 30 mM extracellular K⁺ and relaxation by addition of adenosine diphosphate (ADP, 1 mM), an endothelium-dependent inductor of nitric oxide production, sodium nitroprusside (NTP, 100 μM), a nitric oxide donor, and hypoxia. Bottom. Maintenance of the response to NTP and hypoxia in endothelium denuded rings. The lack of endothelial function is evidenced by the disappearance of the response to ADP. (B) The responses of intact (top) and endothelium denuded (bottom) CA rings to hypoxia are maintained in the presence of the K⁺ channel blocker glibenclamide (GLI; 10 μM).

Reduction of [Ca²⁺], seen in porcine myocytes treated with GLI (26.4±9.7, n = 15) (Fig. 2C). Therefore, as it occurs in precontracted arterial rings, depolarization-induced Ca²⁺ influx in individual CA myocytes is inhibited upon exposure to low Po₂.

About 25% of human and porcine myocytes exhibited spontaneous rhythmic global changes of [Ca²⁺], (Ca²⁺ oscillations or spikes) that, as shown in other VSM cells, are primarily due to Ca²⁺ release from the sarcoplasmic reticulum although their amplitude and frequency are regulated by extracellular Ca²⁺ influx [19,25,26]. Typical Ca²⁺ spikes recorded from a human CA myocyte are shown in Fig. 3. Hypoxia induced a reversible decrease in basal [Ca²⁺], (dotted line) paralleled by a reduction of the frequency and increase in the amplitude of the oscillations (Fig. 3A,B). Removal of extracellular Ca²⁺ produced changes similar to those elicited by the decrease in Po₂ and when these two variables occurred together the reduction of cytosolic [Ca²⁺] was sufficient to suppress the oscillations (Fig. 3B). Blockade of L-type Ca²⁺ channels with nifedipine (NIF) also had an effect similar to that of hypoxia (reversible reduction of basal Ca²⁺, decrease in oscillation frequency and increase in the amplitude of individual Ca²⁺ spikes) however in the presence of the Ca²⁺ channel blocker hypoxia had not effect on [Ca²⁺].

These effects of low Po₂ were reproducible and observed in two other human coronary myocytes and four porcine CA myocytes with spontaneous Ca²⁺ oscillations. These responses are also qualitatively similar to those seen in conduit pulmonary VSM cells, which relax in hypoxia [19], thus further suggesting that hypoxia-dependent modifications of [Ca²⁺] in CA myocytes are due to the reduction of transmembrane Ca²⁺ influx. We tested in human CA myocytes that hypoxia does not influence Ca²⁺ release induced by application of caffeine (Fig. 3D). Average values of cytosolic Ca²⁺ transients elicited by 5 mM caffeine were 107±19 nM (n=5, normoxia) and 105±22 nM (n=5, hypoxia).

3.3. Inhibition of Ca²⁺ channel activity by hypoxia

Inhibition of Ca²⁺ channel activity by low Po₂ was demonstrated in dispersed human coronary VSM cells subjected to patch-clamp under conditions that block outward K⁺ currents. As described before [27], human epicardial coronary myocytes generated voltage-dependent calcium currents (Fig. 4A) with a current–voltage relation that peaked at +10 mV (Fig. 4B). Maximal values of
current, leaving a small fast inactivating current possibly due to T-type Ca channels [20,27], which appeared to be unaffected by low $P_O_2$ (Fig. 4C).

3.4. Molecular characterization of Ca$^{2+}$ channel $\alpha$-subunits in human epicardial CA myocytes

Because it has recently been reported that the recombinant splice variant hHT of the human Ca$^{2+}$ channel $\alpha$-subunit responds selectively to low $P_O_2$ [28], we studied whether this class of channel is expressed in human coronary myocytes. PCR reactions using coronary mRNA and oligos F and R1, which amplify Ca$^{2+}$ channel $\alpha$-subunits hHT and rHT [29], resulted in a single band of 164 bp, corresponding to the rHT isoform (Fig. 5). Identification of this band as the Ca$^{2+}$ channel variant was confirmed by sequencing. In our experimental conditions, the 377-bp fragment, expected if the hHT isoform was present, was not detected (Fig. 5, third lane from the left). This observation was repeated in several experiments using mRNA from three different subjects and in one case we confirmed that, as previously described [29], two bands of the appropriate size corresponding to the rHT (164 bp) and hHT (377 bp) isoforms were observed after amplification of mRNA from human aorta (not shown). To further investigate if the hHT isoform is expressed in epicardial human coronary arteries, we designed a new reverse oligo (R2) to be used with oligo F to specifically amplify the hHT isoform. These PCR reactions detected a product of the appropriate size (212 bp) but with low level and difficult to see in the agarose gel. Reamplification of this product in a second round of PCR yielded a clear band of the expected size, which was shown to belong to the hHT sequence (Fig. 5). These data suggest that at the level of mRNA, rHT is the predominant Ca$^{2+}$ channel variant present in coronary myocytes. The hHT isoform is also expressed but apparently in lower quantity.

4. Discussion

The major findings in this study are: (i) epicardial CAs rings precontracted with high K$^+$ can relax in response to hypoxia by mechanisms not involving the endothelium or $K_ATP$ channel activation; (ii) in single human and pig coronary myocytes, hypoxia directly reduces transmembrane Ca$^{2+}$ influx independently of $K^+$ channel opening, and (iii) in patch-clamped dispersed human CA myocytes, exposure to acute hypoxia leads to reversible inhibition of L-type Ca$^{2+}$ channels. Therefore, as described before in systemic rabbit [20,21] and rat [22,23] arterial VSM cells, as well as in preliminary observations on porcine CA myocytes [21], at moderately depolarized membrane potentials $P_O_2$ regulates the activity of Ca$^{2+}$ channels in the human coronary circulation.

CAs have variable responses to hypoxia which depend
on animal species, vessel size and whether or not the endothelium is present (e.g. Refs. [3,5,6]). Although there is general accord in that hypoxia acts directly on K\textsubscript{ATP} channels to regulate coronary arterial resistance [9–11], there is also evidence suggesting that other mechanisms contribute to this response. For example, it is known that a hypoxia-induced decrease in resting tension of CA rings is unaffected by blockade of K\textsubscript{ATP} channels with GLI [30]. We show here that hypoxia relaxes CA rings precontracted with high external K\textsuperscript{+} and that this is unaffected by GLI. Although hypoxia could activate other K\textsuperscript{+} channels, as it occurs in cerebral VSM cells [13], this cannot explain our results with arterial rings or isolated myocytes depolarized with high external K\textsuperscript{+} because in this condition opening of K\textsuperscript{+} channels facilitates the maintenance of membrane depolarization, Ca\textsuperscript{2+} influx and contraction. We have shown in isolated human myocytes with spontaneous Ca\textsuperscript{2+} oscillations that nifedipine, a blocker of Ca\textsuperscript{2+} channels in human coronary myocytes (Fig. 4C and Ref. [27]), abolishes the effect of hypoxia. Moreover, low P\textsubscript{O\textsubscript{2}} reversibly inhibits the macroscopic Ba\textsuperscript{2+} currents of isolated human CA myocytes. Altogether, these data strongly indicate that in some CA myocytes, L-type Ca\textsuperscript{2+} channels in the plasmalemma are under control of P\textsubscript{O\textsubscript{2}}. In the pulmonary arterial circulation, hypoxia inhibits L-type Ca\textsuperscript{2+} channels in myocytes dispersed from conduit vessels whereas the opposite, potentiation of Ca\textsuperscript{2+} channel activity, is frequently observed in myocytes from small resistance arteries [19,31]. Given the specialized roles of epicardial and transmural coronary arteries, future experimental work should study whether longitudinal differences in Ca\textsuperscript{2+} channel modulation are also manifested in the coronary circulation.

The mechanism by which P\textsubscript{O\textsubscript{2}} regulates arterial L-type Ca\textsuperscript{2+} channels is unknown. It has been demonstrated that hypoxia inhibits the recombinant human L-type cardiovascular Ca\textsuperscript{2+} channel \(\alpha\textsubscript{IC}\) subunit expressed in human embryonic kidney (HEK) cells in a manner indistinguishable from that observed in native smooth muscle L-type Ca\textsuperscript{2+} channels [20,21,32]. It has recently been shown that when expressed in heterologous systems, hypoxia inhibits only one of the three naturally occurring splice variants of these channels (hHT isoform [28]). Our study shows that in epicardial myocytes the rHT mRNA predominates over the hHT isoform. Thus, assuming that mRNA levels determine the relative amount of Ca\textsuperscript{2+} channel \(\alpha\)-subunits incorporated into the membrane, the data suggest that a large fraction of the Ca\textsuperscript{2+} current in human coronary myocytes is mediated by rHT. Therefore, it is possible that besides hHT, the rHT isoform in situ can also be regulated

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**Fig. 3.** Response to hypoxia of an undialyzed human coronary myocyte which generated spontaneously Ca\textsuperscript{2+} oscillations. (A) Reduction of basal cytosolic \([\text{Ca}^{2+}]\) (dotted line) and increase in the amplitude of the oscillations by hypoxia and removal of extracellular Ca\textsuperscript{2+} (0 Ca and 1 mM EGTA added). Low P\textsubscript{O\textsubscript{2}} and 0 Ca also decreased the frequency of the oscillations and superposition of these two variables produced complete suppression of the oscillations (B). (C) Blockade of L-type Ca\textsuperscript{2+} channels with nifedipine (NIF, 1 \textmu M) produced the same effect of hypoxia or the removal of external Ca\textsuperscript{2+} (decrease in basal Ca\textsuperscript{2+} and increase in spike amplitude). In these conditions, exposure to hypoxia did not modify the Ca\textsuperscript{2+} oscillations. (D) Ca\textsuperscript{2+} release induced by caffeine (5 mM) in a quiescent human myocyte in normoxic and hypoxic solutions.
Fig. 4. Reversible inhibition by hypoxia of L-type Ca$^{2+}$ channels in human coronary myocytes. (A) Macroscopic Ba$^{2+}$ currents at three different membrane potentials illustrating the voltage-dependent inhibition of current amplitude by hypoxia. (B) Peak Ba$^{2+}$ current−voltage relationship in normoxia (control and recovery) and hypoxia. (C) Blockade of the calcium channel current by nifedipine (0.5 μM). Pulse to +20 mV. In all cases the holding potential was −80 mV and pulse duration 15 ms.

by low P$_O_2$. Both native and recombinant cardiac L-type Ca$^{2+}$ channels are subjected to redox modulation [33–35] and hypoxic regulation of the recombinant channels is lost after treatment of the cells with some sulfhydryl reagents [36]. Therefore, it is likely that some critical cysteine residues in the α$_{1C}$ or in auxiliary subunits are involved in the modulation of the Ca$^{2+}$ channels by hypoxia. Interestingly, a thiol-based redox/O$_2$ sensor has recently been proposed to regulate the function of skeletal muscle Ca$^{2+}$ release channels [37]. Clarification of the mechanisms underlying Ca$^{2+}$-channel modulation by O$_2$ tension in human CAs may be of help in the understanding of coronary pathophysiology as well as in the development of new coronary vasodilators.

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