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Hypoxia Inhibits Contraction but Not Calcium Channel Currents or Changes in Intracellular Calcium in Arteriolar Muscle Cells

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Abstract

Objective—We tested the hypothesis that hypoxia inhibits currents through L-type Ca^{2+} channels and inhibits norepinephrine-induced rises in intracellular Ca^{2+} in cremasteric arteriolar muscle cells, thus accounting for the inhibitory effect of hypoxia on norepinephrine-induced contraction of these cells.

Methods—Single smooth muscle cells were enzymatically isolated from second-order and third-order arterioles from hamster cremaster muscles. The effects of hypoxia (partial pressure of oxygen: 10–15 mm Hg) were examined on Ba^{2+} (10 mM) currents through L-type Ca^{2+} channels by use of the perforated patch clamp technique. Also, the effect of hypoxia on norepinephrine-induced calcium changes was studied using Fura 2 microfluorimetry.

Results—Hypoxia inhibited the norepinephrine-induced (10 μM) contraction of single arteriolar muscle cells by 32.9 ± 5.6% (mean ± SE, n = 4). However, hypoxia had no significant effect on whole-cell currents through L-type Ca^{2+} channels: the peak current densities measured at +20 mV were −3.83 ± 0.40 pA/pF before hypoxia and −3.97 ± 0.36 pA/pF during hypoxia ($n = 15$; $p > 0.05$). In addition, hypoxia did not inhibit Ca^{2+} transients in arteriolar muscle cells elicited by 10 μM norepinephrine. Instead, hypoxia increased basal Ca^{2+} (13.8 ± 3.2%) and augmented peak Ca^{2+} levels (29.4 ± 7.3%) and steady-state Ca^{2+} levels (15.2 ± 5.4%) elicited by 10 μM norepinephrine ($n = 21$; $p < 0.05$).

Conclusions—These data indicate that hypoxia inhibits norepinephrine-induced contraction of single cremasteric arteriolar muscle cells by a mechanism that involves neither L-type Ca^{2+} channels nor norepinephrine-induced Ca^{2+} mobilization. Instead, our findings suggest that hypoxia must inhibit norepinephrine-induced contraction by affecting a component of the signaling pathway that lies downstream from the increases in Ca^{2+} produced by this neurotransmitter.

Keywords

arterioles; calcium ions; contraction; hypoxia; ion channels; microcirculation; oxygen; vascular smooth muscle

INTRODUCTION

Arterioles in the microcirculation are exquisitely sensitive to changes in the partial pressure of oxygen (PO_2) in their environment. Decreases in PO_2 cause arteriolar dilation (7,8,16,20), whereas increases in oxygen tension cause arteriolar constriction (2,3,10,11,14). However, the mechanism by which changes in PO_2 produce changes in arteriolar tone remain unclear.

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Recently, Jackson (12) showed that hypoxia inhibits norepinephrine-induced contraction of single arteriolar muscle cells. This effect of reduced PO_2 was not related to membrane hyperpolarization or activation of outward potassium currents. However, the inhibitory effect of hypoxia could be reversed by agents or conditions that depolarized the arteriolar muscle cells. Therefore, it was hypothesized that reduced PO_2 acted on, or through, a process that was voltage-sensitive.

Hypoxia inhibits currents through L-type calcium channels (Ca_L channels) in some vascular cells (4–6). Norepinephrine-induced contraction of single arteriolar muscle cells depends on Ca^{2+} influx through Ca_L channels (13). Thus, these channels could be the voltage-dependent target of action of hypoxia in arteriolar muscle cells, as previously proposed (12). Therefore, the purpose of the present study was to determine whether hypoxia inhibits norepinephrine-induced contraction of arteriolar muscle cells by inhibiting the function of Ca_L channels.

MATERIALS AND METHODS

Animal and Tissue Preparation

All animal use was approved by the Institutional Animal Care and Use Committee at Western Michigan University. Hamsters were killed by an overdose of pentobarbital (>150 mg/kg intraperitoneally) or by asphyxiation with CO_2. Single arteriolar muscle cells from the cremaster muscles of male golden Syrian hamsters (weight: 80–160 g; Charles River Laboratories, Wilmington, MA) were isolated enzymatically, as previously described (13). Briefly, the cremaster muscles were removed, rinsed, and placed in a zero Ca^{2+} physiological salt solution (PSS) at 4 °C with the following composition: NaCl 140 mM; KCl 5 mM; $MgCl_2$ 1 mM; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10 mM; glucose 10 mM; pH 7.4 adjusted with NaOH; 295–300 mOsm. Cremaster muscles were transferred to a cooled (4 °C), water-jacketed dissection chamber (Radnoti Glass, Monrovia, CA) containing dissociation solution (DS; PSS containing 0.1 mM Ca^{2+}, 10 μM diltiazem, 10 μM sodium nitroprusside, and 1% albumin). Cremaster muscles were pinned out flat with insect pins on pads made of Sylgard (Dow Corning, Midland, MI) that were placed in the bottom of the dissection chamber. Second-order and third-order arterioles were hand-dissected out of the muscle, cut into lengths of ~800 μm, and incubated in DS containing 26 U/mL papain and 1 mg/mL dithioerythritol at 37 °C for 35 minutes. The arteriolar segments then were incubated in 1 mL of DS containing 1.95 U/mL collagenase, 1 mg/mL trypsin inhibitor, and 75 U/mL elastase at 37 °C for 17–22 minutes. The vessel segments were washed in DS without enzymes, and single arteriolar muscle cells were dispersed by gentle trituration of the arteriolar segments with an Eppendorf-style pipettor. The cell isolate was placed in a 1.5-mL siliconized-polypropylene microcentrifuge tube and stored at room temperature for up to 4 hours. Aliquots of cells from the isolate then were placed in a chamber mounted on the stage of an Eclipse TE300 inverted microscope (Nikon, Tokyo, Japan), with the additional details for each specific set of experiments listed below. During all experiments, flow was maintained through the chamber by gravity from 60 mL reservoirs at a rate of 1–3 mL/minute with bath solutions specific for each protocol (see below). All experiments were performed at room temperature (20–25 °C).

Contraction Assays

The contractility of single arteriolar muscle cells in response to norepinephrine was assessed as previously described (13) with minor modifications. Small coverslips (1 cm^2) were placed in the bottom of the chamber to allow the transfer of sets of cells into and out of the chamber. The coverslips were coated with a cell adhesive (Cell-Tak; Becton Dickinson, Franklin Lakes, NJ) to facilitate the adherence of cells to the coverslips. An aliquot (100 μl) of the cell isolate was placed on the coverslip, and cells were allowed to adhere for 10 minutes. After the cells

adhered, the chamber was perfused with PSS containing 2 mM Ca^{2+} for 10 minutes. The contraction of single smooth muscle cells in response to micropipette-applied norepinephrine (10 μM) then was determined either during control conditions or during hypoxia (see below), as described previously (13). Briefly, a micropipette filled with norepinephrine was positioned adjacent to a cell with a micro-manipulator. Norepinephrine containing PSS was ejected from the pipette by pressurization with a water manometer. The micropipette was moved within the field of view by use of a micromanipulator. The micropipette and the field of view were both moved against the direction of chamber flow. This prevented cells that were not directly exposed to norepinephrine from contracting. At least 30 cells were tested from each aliquot, and cells were counted as contracting if they shortened >30% within 15 seconds after norepinephrine exposure. After determining the number of cells contracting during either the control condition or hypoxia, a second aliquot of cells was placed on a second Cell-Tak-coated cover-slip in the chamber, and the norepinephrine-dependent contractility then was determined for the remaining oxygen tension (only one condition was tested in each aliquot). Thus, if contractility under control conditions was studied in the first aliquot of cells, the contractility under hypoxia was determined in the second aliquot of cells. The order of the treatments was randomized to obviate time-dependent differences in reactivity, and only one oxygen tension was tested for each aliquot. Data were normalized to the fraction of cells that contracted in response to norepinephrine (i.e., the number that contracted divided by the total number of cells tested) under control conditions and were expressed as the percentage of the control, as described previously (13). Bath solutions were bubbled vigorously with room air (control PO_2- 159 mm Hg) or with 100% N_2 (hypoxia PO_2: 10–15 mm Hg measured in the chamber), as previously described (12). Oxygen tensions were measured with a miniature Clark-type oxygen electrode (model # MI-730; Microelectrodes, Inc., Bedford, NH) at the beginning and end of our experiments. Oxygen tensions did not differ between these two time points, nor did they differ from values measured in previous experiments (12).

Patch Clamp Methods

An aliquot (approximately 100 μl) of cell-containing solution was placed in a 1-mL chamber mounted on the stage of an Eclipse TE300 (Nikon) inverted microscope. Cells were allowed to adhere to the bottom of the chamber for approximately 10 minutes. After a 10-minute wash period with 2 mM Ca^{2+}-containing PSS, heat-polished borosilicate patch clamp pipettes (tip resistances: 2–5 MΩ when filled with pipette solution; see below) were placed on the membranes of single arteriolar muscle cells with a hydraulic micromanipulator. Micropipette tips were filled with the following solution: CsCl 143 mM; $MgCl_2$ 1 mM; ethylene glycolbis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.5 mM; HEPES 10 mM; pH 7.2 with CsOH. The micropipettes were back-filled with the same solution also containing 50–240 μg/mL amphotericin B. High-resistance seals of >10 GΩ were obtained, and the perforated patch configuration was achieved using amphotericin B (to obviate cell dialysis) while observing increases in capacitative current. After obtaining stable seals, the chamber was perfused with a bath solution containing: tetraethylammonium chloride (TEACl) 10 mM; 4-aminopyridine 10 mM; NaCl 125 mM; $BaCl_2$ 10 mM; $MgCl_2$ 1 mM; HEPES 10 mM; and glucose 10 mM; pH 7.4 with CsOH. Ba^{2+} was used as the charge carrier to amplify currents through Ca_L channels and to obviate Ca^{2+}-dependent run-down of the currents (18).

The pipette voltage was controlled and the current was measured with an Axopatch 200A amplifier controlled by a computer running pClamp 7 or pClamp 8 software (Axon Instruments, Forest City, CA). Signals were passed through a four-pole Bessel filter with a cutoff frequency of 1 KHz, digitized at 5 KHz, and stored on a computer hard drive for subsequent analysis. Whole-cell Ba^{2+} currents were normalized to cell capacitance to account for differences in cell size or changes in membrane area. Cell capacitance was estimated by integration of the capacitive transient generated by 10 mV hyperpolarizing pulses from the holding potential

after electronic cancellation of pipette-patch capacitance. The mean cell capacitance was 18.0 ± 0.7 pF, and the mean access resistance was 10.0 ± 0.4 MΩ (n = 31).

Two different voltage-clamp protocols were used to assess the effects of hypoxia on Ca_L channels. In the first protocol, to determine the effect of hypoxia on the activation of Ca_L channels, cells were held at −70 mV. The membrane potential was stepped for 200 milliseconds from −90 to +60 mV (in increments of 10 mV), and the peak Ca_L channel currents were measured. In a second voltage-clamp protocol that was designed to study the steady-state inactivation of Ca_L channels, cells were held at −80 mV and were subjected to a conditioning pulse of 1000 milliseconds in duration (starting at −90 mV and increasing in increments of 10 mV up to +60 mV) to inactivate an increasing portion of the Ca_L channels. After the conditioning pulse, the membrane potential was stepped back to −80 mV for 20 milliseconds to deactivate any noninactivated channels before applying the test potential of +20 mV for 200 milliseconds. Peak Ca_L channel currents then were measured at the test potential of +20 mV (this test potential yielded maximal currents in the activation protocol; see RESULTS). In each cell, one of the voltage-clamp protocols was performed under control conditions (room air) and was repeated after 10 minutes of hypoxia. Ba^{2+} currents also were measured in these cells after recovery from hypoxia and did not differ from currents obtained during control conditions (data not shown).

Inactivation data were displayed as I/I_{max}, where I_{max} is the average maximum current amplitude elicited during the test pulse to +20 mV after conditioning potentials that caused no inactivation (i.e., potentials more negative than −40 mV). A Boltzmann distribution curve was fit to the data using the following equation: $I/I_{max} = (1/[1 + \exp(V_{0.5} - V)/k]) + C$, where $V_{0.5}$ is the conditioning potential resulting in 50% inactivation, V is the conditioning potential, k is the slope factor, and C is the noninactivating component.

Measurement of Intracellular Calcium

Aliquots of cells (100 μl) were placed onto Cell-Tak-treated coverslips that were placed in the bottom of the chamber. After allowing the cells to settle and adhere to the coverslips, the cells were loaded with 1 μM Fura 2-(acetyloxy) methyl ester (AM) (in 2 mM $CaCl_2$ PSS with 0.05% dimethyl sulfoxide and 1% bovine serum albumin) for 30 minutes and then were washed for 30 minutes with 2 mM Ca^{2+}-containing PSS.

Fura 2 fluorescence from single cells was measured using a Ratiomaster microscope-based photometry system equipped with a microscope photometer and a DeltaRam high speed multi-illuminator and shutter system (Photon Technologies, Inc., Lawrenceville, NJ). For fluorescence measurements, emission at 510 nm was sampled at 20 Hz for the excitation wavelengths of 340 and 380 nm.

After the subtraction of background fluorescence, the ratio of fluorescence emission for 340/380 nm illumination ($[F_{340} - Background_{340}]/[F_{380} - Background_{380}] = R$) was calculated and used as an index of $[Ca^{2+}]_i$. This was done rather than a calculation of actual $[Ca^{2+}]_i$ because of inherent uncertainties in the conversion of the ratios to Ca^{2+} concentration using an *in vitro* calibration. $[Ca^{2+}]_i$ was estimated for display purposes using the equation $[Ca^{2+}]_i = K_d*\beta;*(R - R_{min})/(R_{max} - R)$, where R_{min} and R_{max} are the minimum and maximum R values, and μ is the ratio of emission intensities at 380 nm in zero Ca^{2+}/saturating Ca^{2+} as determined by *in situ* calibration. A K_d value of 235 nM, as determined by *in vitro* calibration of our system, was used in these calculations.

To test the hypothesis that hypoxia prevents the norepinephrine-induced contraction of single arteriolar muscle cells by inhibiting Ca^{2+} increases, cells were stimulated with micropipette-applied norepinephrine (10 μM) under control conditions and after 10 minutes of hypoxia, and

then were stimulated a third time after cells were re-exposed to room air (recovery). Thus, the effects of all three conditions (i.e., control, hypoxia, and recovery from hypoxia) on norepinephrine-induced contractility were tested in each cell. Preliminary data indicated that the repeated application of norepinephrine under control conditions yielded similar Ca^{2+} transients. On some experimental days, up to four cells were examined for each isolate.

Materials

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) with the following exceptions: elastase was purchased from Calbiochem (La Jolla, CA); bovine serum albumin was purchased from USB (Cleveland, OH); and Fura 2-AM was purchased from Molecular Probes (Eugene, OR). Amphotericin B was dissolved in dimethylsulfoxide, and nifedipine was dissolved in 100% ethanol. All solutions were prepared using 18 MΩ reagent-grade water.

Statistical Analyses

The data are presented as the mean ± SE. Data were analyzed by paired Student's *t* tests or analysis of variance where appropriate. If a treatment effect was found by analysis of variance, the means were compared by Student-Newman-Keuls *post hoc* analysis. Statistical comparisons were performed at the 95% confidence level. For the inactivation data, a Boltzmann distribution curve was fit using KaleidaGraph software (version 3.5; Synergy Software, Reading, PA).

RESULTS

Hypoxia Inhibits Norepinephrine-Induced Contraction

Hypoxia significantly inhibited the contraction of arteriolar muscle cells in response to 10 μM norepinephrine. In contraction assays (four isolates; a total of 412 cells were observed with 205 cells under control conditions and 207 during hypoxia), 83.3 ± 2.7% of the cells contracted in response to norepinephrine under control conditions, and this value was reduced to 56.1 ± 6.2% of cells during hypoxia ($p < 0.05$). Thus, hypoxia reduced the contractility of single arteriolar muscle cells to 67.0 ± 5.6% of the control ($p < 0.05$) (Fig. 1).

Hypoxia Does Not Affect Ca_L Currents in Arteriolar Muscle Cells

Barium currents elicited by stepping from a holding potential of −70 mV to test potentials from −90 mV to +60 mV are shown in Fig. 2. The currents activated at −40 mV and were maximal at +20 mV. Superfusion of the cells with dihyropyridine, nifedipine (1 μM) substantially (>90% inhibition) and reversibly inhibited the currents (Fig. 2A). Figure 2B shows a barium current trace before and during nifedipine application on depolarization of a cell to +20 mV. The voltage-dependent characteristics of these currents, along with the block by nifedipine, confirm that currents induced by step depolarizations were carried through Ca_L channels. No currents were seen during more negative test potentials (i.e., those expected to elicit T-type currents), either in the absence or presence of nifedipine, indicating that T-type calcium channels were absent in these cells (15).

Hypoxia did not alter the activation of Ba^{2+} currents through Ca_L channels in any manner. Current amplitudes were similar at all voltages before and during superfusion of the cells with hypoxic solutions (Fig. 3A). Peak current densities at +20 mV were −3.83 ± 0.40 pA/pF before hypoxia and −3.97 ± 0.36 pA/pF during hypoxia ($n = 15$; $p > 0.05$). Neither did hypoxia affect the steady-state inactivation of Ca_L channels in single arteriolar muscle cells (Fig. 3B). Hypoxia had no effect on the currents measured, or on the current–voltage relationship in any of the cells tested. Under control conditions, the test potential resulting in 50% inactivation ($V_{0.5}$)

was −4.6 ± 1.4 mV, a value that was not significantly different from the value of −2.9 ± 1.9 mV that was observed in the same cells during hypoxia ($n = 8$; $p > 0.05$).

Hypoxia Does Not Inhibit Norepinephrine-Induced Calcium Changes in Arteriolar Muscle Cells

A representative trace of norepinephrine-induced changes in Fura 2 fluorescence during control conditions is shown in Fig. 4. Similar to the responses of other smooth muscles (25,26), the norepinephrine-induced calcium transients in arteriolar smooth muscle cells could be described by the following three components: a baseline, followed by a peak on application of norepinephrine, and a plateau that was maintained until norepinephrine removal. The Fura 2 ratio returned to baseline values on norepinephrine removal.

Hypoxia did not inhibit norepinephrine-induced changes in the Fura 2 ratio in arteriolar muscle cells (Fig. 5). Instead, exposure to hypoxia slightly augmented the norepinephrine-induced changes in the ratio. The mean control Fura 2 ratio values for the baseline, peak, and plateau during norepinephrine exposure were 0.65 ± 0.03, 2.22 ± 0.13, 1.21 ± 0.06, respectively (21 cells), whereas during hypoxia the values for the baseline, peak, and plateau were 0.73 ± 0.02, 2.83 ± 0.21, and 1.38 ± 0.07, respectively ($p < 0.05$). On returning to control conditions (recovery), the mean baseline ratio (0.68 ± 0.02) and the mean norepinephrine-induced plateau (1.24 ± 0.07) returned to values that were not significantly different from the control value. However, the norepinephrine-induced peak remained elevated (2.73 ± 0.22; $p < 0.05$ versus control). It should be noted that in all of the cells tested, hypoxia either augmented (16 cells) or did not change (5 cells) the norepinephrine-induced Fura 2 ratios. In none of the cells tested did hypoxia inhibit the norepinephrine-induced changes in the Fura 2 ratio.

It has been reported that hypoxia may lead to an increase in the Fura 2 ratio by eliminating O_2-induced quenching of Fura 2 fluorescence (23). To test whether changes in PO_2 may alter the Fura 2 ratio independent from changes in intracellular Ca^{2+}, single arteriolar muscle cells were loaded with Fura 2-AM (see METHODS) and permeabilized with 10 μM ionomycin, and the extracellular Ca^{2+} concentration was set at 500 nM (to fix $[Ca^{2+}]_i$ at the same level). Fura 2 fluorescence remained unaltered while changing from room air to hypoxia and then back to room air (data not shown). These data suggest that changes in oxygen tension did not directly affect Fura 2 fluorescence. Thus, norepinephrine-induced changes in the Fura 2 ratio were due to changes in $[Ca^{2+}]_i$ and likely were not due to a direct effect of the changes in oxygen tension.

DISCUSSION

This study demonstrates that hypoxia, although inhibiting the contractility of arteriolar muscle cells, did not inhibit currents through Ca_L channels, nor their voltage-dependent activation or inactivation. Hypoxia also did not inhibit $[Ca^{2+}]_i$ changes elicited by norepinephrine. Thus, the inhibitory effects of hypoxia on norepinephrine-induced contraction must occur at steps downstream from norepinephrine-induced Ca^{2+} mobilization.

Wu et al. (27) reported that rat cremasteric arteriolar muscle cells display Ba^{2+} currents that seemed to be carried through Ca_L channels. Our findings confirm and extend these observations. We found that Ba^{2+} currents in hamster cremasteric arteriolar muscle cells were inhibited by nifedipine and were voltage-dependent, activating at −40 mV with peak amplitudes at +20 mV. These characteristics are consistent with the expression of high-voltage-activated, dihydropyridine-sensitive Ca_L channels (15). In addition, we report here for the first time the steady-state voltage-dependent inactivation of Ca_L channels in hamster cremasteric arteriolar muscle cells. Inactivation began at −20 mV, with half-maximal inactivation occurring at −4.6 ± 1.4 mV. These data are similar to those seen in other studies of vascular smooth muscle when 10 mM barium was used as the charge carrier (21,22).

Ca_L channel current amplitudes, and voltage-dependent activation and inactivation were unaffected by hypoxia. These data are in direct opposition to those from several studies in which hypoxia was found to reversibly inhibit Ca_L channels in smooth muscle from conduit arteries (5,6) and in cardiac myocytes (4). However, our data are in agreement with the findings of Tateishi and Faber (24), who found that hypoxia did not reverse constrictions induced by the direct activation of Ca_L channels with KCl or the Ca_L channel agonist SDZ-202-791 in rat cremaster arterioles. It may be that the Ca_L channels expressed in arteriolar muscle cells differ somehow from those expressed in larger vessels or elsewhere in the cardiovascular system. Nonetheless, our data suggest that the inhibitory effect of hypoxia on norepinephrine-induced contraction in arteriolar muscle cells does not involve the hypoxia-induced inhibition of currents through Ca_L channels.

The application of norepinephrine to single arteriolar smooth muscle cells induced calcium changes that were similar to those seen in other smooth muscle cells (25,26). Hypoxia did not inhibit these Ca^{2+} transients in any of the cells tested. Instead, we found that hypoxia increased resting Ca^{2+} levels and augmented norepinephrine-induced increases in intracellular Ca^{2+} in most of the cells. These observations suggest that the inhibition of norepinephrine-induced contraction produced by hypoxia must occur downstream from norepinephrine-induced mobilization of Ca^{2+} and suggest that hypoxia decreased the Ca^{2+} sensitivity of the arteriolar muscle cells.

There are a number of studies indicating that hypoxia can desensitize vascular smooth muscle to agonist-induced increases in $[Ca^{2+}]_i$ (i.e., the Ca^{2+} response is not changed, but the agonist-induced contraction is inhibited). In rat cerebral and small mesenteric arteries, hypoxia reduced arginine vasopressin-induced force, but $[Ca^{2+}]_i$ changes were essentially unchanged (1). In porcine coronary arteries, hypoxia inhibited force but not increases in $[Ca^{2+}]_i$ in response to KCl or the thromboxane analog U46619 (19). Hypoxia caused a significant dilation in pressurized cerebral arteries without decreasing $[Ca^{2+}]_i$ (9), suggesting that, in addition to agonist-induced tone, pressure-induced vascular tone may also be inhibited by hypoxia without a reduction in $[Ca^{2+}]_i$. In other nonvascular smooth muscle, hypoxia also inhibits agonist-induced force production without inhibiting $[Ca^{2+}]_i$ changes (17).

Taken together, these data suggest that hypoxia can inhibit force in smooth muscle cells independent of decreases in intracellular calcium. Our finding that norepinephrine-induced $[Ca^{2+}]_i$ changes in arteriolar muscle cells were not reduced despite a reduction in norepinephrine-dependent contractility is consistent with the above findings. The mechanisms responsible for the hypoxia-induced change in Ca^{2+} sensitivity remain to be established.

In conclusion, hypoxia inhibits norepinephrine-induced contraction of smooth muscle cells isolated from skeletal muscle arterioles. However, this effect cannot be attributed to a reduction in Ca^{2+} mobilization because hypoxia did not inhibit Ca_L channel currents, nor did hypoxia inhibit norepinephrine-induced Ca^{2+} transients. These findings suggest that hypoxia may inhibit the contraction of arteriolar muscle cells by exerting effects downstream from the increases in Ca^{2+} such that the cells become desensitized to $[Ca^{2+}]_i$.

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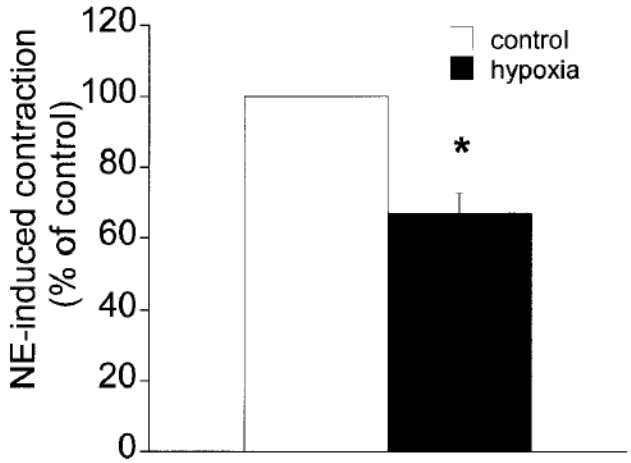

Figure 1.

Norepinephrine-induced contraction of arteriolar muscle cells is inhibited by hypoxia. The data are given as the mean ± SE. The fraction of cells, expressed as a percentage of the control fraction, that contracted in response to micropipette-applied norepinephrine (10 μM; n = 4). * denotes significant difference from the control value ($p < 0.05$).

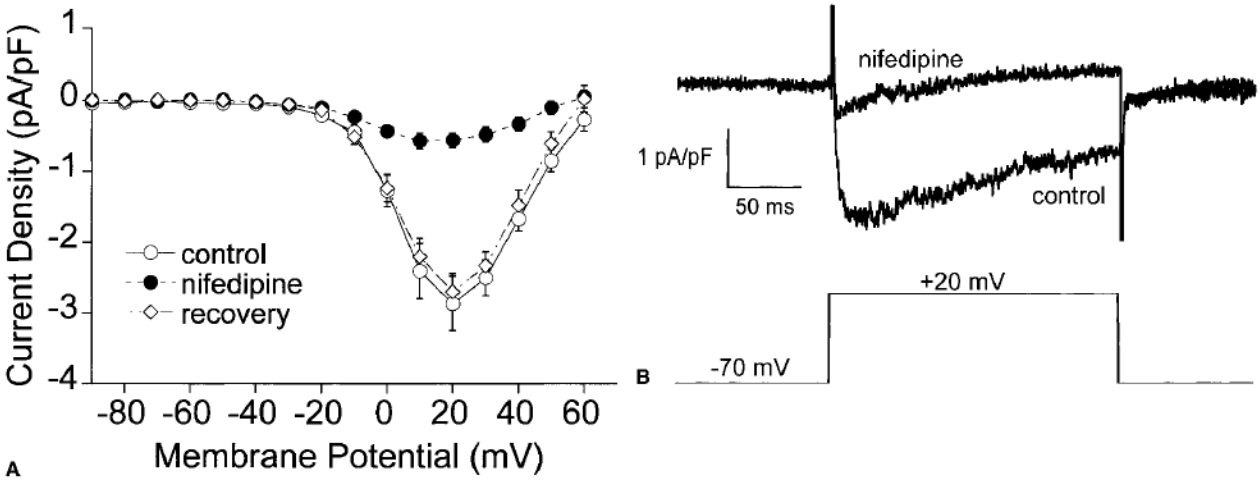

Figure 2.

Nifedipine inhibits Ca_L currents in arteriolar muscle cells. The data are given as the mean ± SE. (A) The mean current-value (I-V) relationship (n = 8) for Ca_L channel currents measured at the peak of test pulses in the absence (○), presence (•), and after washout of 1 μM nifedipine (◊). (B) Raw current traces (top) from a single smooth muscle cell in the presence and absence of nifedipine during a depolarization from −70 mV to +20 mV (bottom).

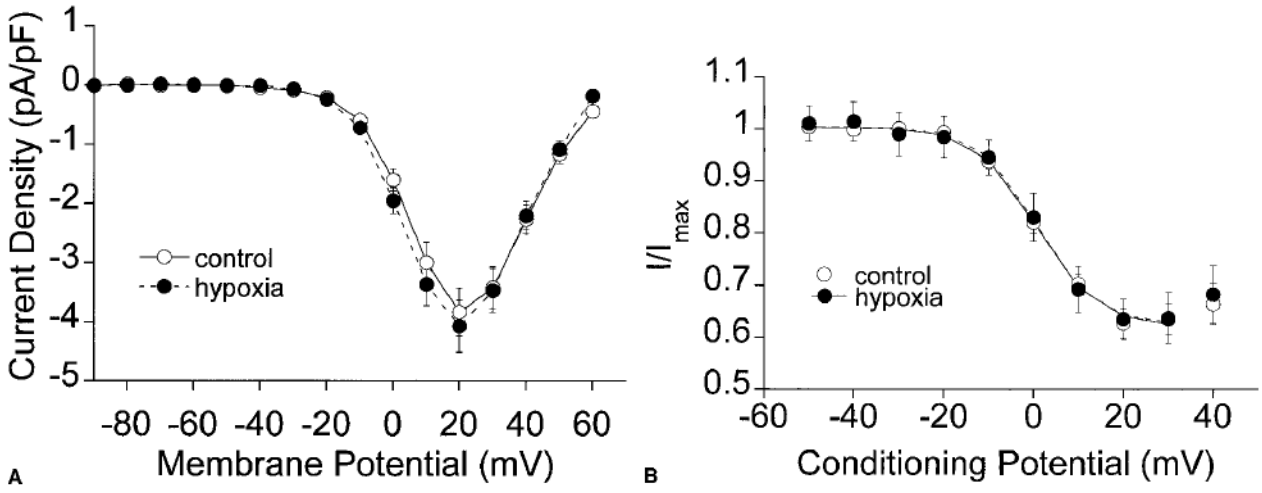

Figure 3.

Hypoxia does not inhibit currents through Ca_L channels in arteriolar muscle cells. All data are given as the mean ± SE. (A) Mean current–voltage relationship (n = 15) of Ca_L channel currents during the activation protocol before (○;) and during hypoxia (•). (B) Normalized inactivation (n = 8) of Ca_L in arteriolar smooth muscle cells before (○) and during hypoxia (•).

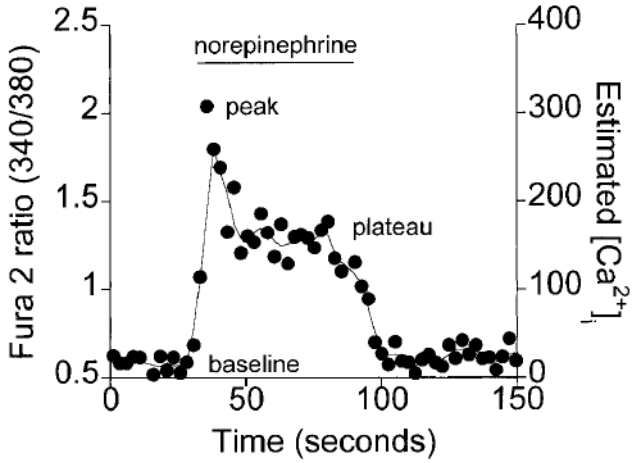

Figure 4.
Norepinephrine-induced Ca^{2+} transient in an arteriolar muscle cell, as indicated by a representative Fura 2 ratio trace. The Fura 2 ratio changes in response to 60 seconds of norepinephrine were characterized by a pre-norepinephrine baseline, a peak on norepinephrine application, followed by a maintained plateau until norepinephrine removal.

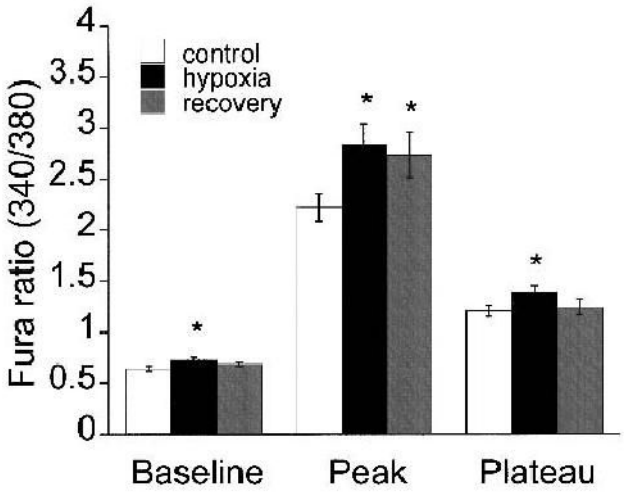

Figure 5.

Hypoxia does not inhibit norepinephrine-induced Ca^{2+} in arteriolar muscle cells. All data are given as the mean ± SE. Summary (21 cells) of the baseline, peak, and plateau Fura 2 ratios from arteriolar muscle cells in response to norepinephrine before (white bars), during (black bars), and after recovery (gray bars) from hypoxia. * denotes a significant difference from control value ($p < 0.05$).