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Am J Physiol Cell Physiol. Author manuscript; available in PMC 2008 February 13.

Published in final edited form as:
Am J Physiol Cell Physiol. 2007 June ; 292(6): C2122–C2128.

Hypoxia reduces K_{Ca} channel activity by inducing Ca^{2+} spark uncoupling in cerebral artery smooth muscle cells

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Abstract

Arterial smooth muscle cell large-conductance Ca^{2+}-activated potassium (K_{Ca}) channels have been implicated in modulating hypoxic dilation of systemic arteries, although this is controversial. K_{Ca} channel activity in arterial smooth muscle cells is controlled by localized intracellular Ca^{2+} transients, termed Ca^{2+} sparks, but hypoxic regulation of Ca^{2+} sparks and K_{Ca} channel activation by Ca^{2+} sparks has not been investigated. We report here that in voltage-clamped (−40 mV) cerebral artery smooth muscle cells, a reduction in dissolved O_2 partial pressure from 150 to 15 mmHg reversibly decreased Ca^{2+} spark-induced transient K_{Ca} current frequency and amplitude to 61% and 76% of control, respectively. In contrast, hypoxia did not alter Ca^{2+} spark frequency, amplitude, global intracellular Ca^{2+} concentration, or sarcoplasmic reticulum Ca^{2+} load. Hypoxia reduced transient K_{Ca} current frequency by decreasing the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current from 89% to 63%. Hypoxia reduced transient K_{Ca} current amplitude by attenuating the amplitude relationship between Ca^{2+} sparks that remained coupled and the evoked transient K_{Ca} currents. Consistent with these data, in inside-out patches at −40 mV hypoxia reduced K_{Ca} channel apparent Ca^{2+} sensitivity and increased the K_d for Ca^{2+} from ~17 to 32 μM, but did not alter single-channel amplitude. In summary, data indicate that hypoxia reduces K_{Ca} channel apparent Ca^{2+} sensitivity via a mechanism that is independent of cytosolic signaling messengers, and this leads to uncoupling of K_{Ca} channels from Ca^{2+} sparks. Transient K_{Ca} current inhibition due to uncoupling would oppose hypoxic cerebrovascular dilation.

Keywords

transient; calcium-activated; potassium; current

CHANGES IN OXYGEN (O_2) partial pressure (P_{O_2}) modulate tissue perfusion and regional blood flow, but reactivity varies depending on the anatomic origin of the vasculature (9,23, 25,30). In general, hypoxia constricts small distal pulmonary arteries and arterioles but dilates small systemic arteries and arterioles, including those in the brain (2,25,31). Hypoxic pulmonary vasoconstriction acts to divert blood supply to O_2-rich regions of the lung. In contrast, in the systemic vasculature hypoxic vasodilation functions to match O_2 supply to metabolic demand. Although O_2 regulation of vascular reactivity serves diverse physiological roles, mechanisms that mediate these responses are poorly understood.

Multiple signaling messengers have been proposed to mediate hypoxia-induced systemic artery dilation, including endothelium-dependent nitric oxide, cyclooxygenase products, adenosine 3′,5′-cyclic monophosphate (cAMP), and guanosine 3′,5′-cyclic monophosphate (cGMP) (12,13,25). Activation of potassium channels, including ATP-sensitive and large-conductance calcium (Ca^{2+})-activated potassium (K_{Ca}) channels, is also proposed to contribute to hypoxic

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vasodilation (3,4,14,16,18,35). However, there are conflicting reports of arterial smooth muscle cell K_{Ca} channel regulation by hypoxia, with studies reporting activation, inhibition, or no modulation (3,16,25,35,41,45). Thus the role of K_{Ca} channels in hypoxic vasodilation is unclear.

In arterial smooth muscle cells, K_{Ca} channels are activated by localized intracellular Ca^{2+} transients, termed Ca^{2+} sparks, which occur because of the opening of ryanodine-sensitive Ca^{2+} release (RyR) channels in the sarcoplasmic reticulum (SR) membrane (21,36). Ca^{2+} sparks generate the micromolar subsarcolemmal intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) elevation necessary for K_{Ca} channel activation and therefore are critical modulators of K_{Ca} channel activity (39,51). Because of their rapid and localized temporal and spatial properties, Ca^{2+} sparks do not contribute directly to $[Ca^{2+}]_i$ (21). Ca^{2+} spark-induced K_{Ca} channel activation causes membrane hyperpolarization, leading to a decrease in voltage-dependent Ca^{2+} influx, a reduction in global $[Ca^{2+}]_i$, and vasodilation. Conversely, Ca^{2+} spark inhibition or a reduction in the coupling of Ca^{2+} sparks to K_{Ca} channels results in vasoconstriction (21, 36).

O_2 regulation of K_{Ca} channels in arterial smooth muscle cells has primarily been studied by measuring single-channel activity or whole cell currents (9,16,20,38). Thus hypoxic regulation of Ca^{2+} sparks and K_{Ca} channels that are under Ca^{2+} spark control is unclear. Since Ca^{2+} sparks are a principal regulator of K_{Ca} channel activity in cerebral artery smooth muscle cells, the present study was undertaken to study hypoxic regulation of Ca^{2+} sparks, K_{Ca} channels, and the coupling relationship between Ca^{2+} sparks and K_{Ca} channels. Our data indicate that in cerebral artery smooth muscle cells hypoxia reduces the apparent micromolar Ca^{2+} sensitivity of K_{Ca} channels, which leads to Ca^{2+} spark to K_{Ca} channel uncoupling and a decrease in the frequency and amplitude of transient K_{Ca} currents. In contrast, in voltage-clamped cells, hypoxia does not alter Ca^{2+} spark frequency or amplitude or global $[Ca^{2+}]_i$. These data suggest that, in response to hypoxia, uncoupling of K_{Ca} channels from Ca^{2+} sparks would oppose the cerebral artery dilation.

MATERIALS AND METHODS

Arterial smooth muscle cell isolation

Individual smooth muscle cells were enzymatically dissociated as previously described (7, 49,50). Briefly, Sprague-Dawley rats (~200–250 g) of either sex were anesthetized by an intraperitoneally injected overdose of pentobarbital sodium (150 mg/kg body wt). Animal protocols used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee. The brain was then removed and placed into ice-cold (4°C) HEPES-buffered physiological salt solution containing (in mM) 134 NaCl, 6 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.4 with NaOH). Posterior cerebral, middle cerebral, and cerebellar arteries (100–200 μm in diameter) were removed and cleaned of connective tissue. Individual smooth muscle cells were dissociated from arteries with a HEPES-buffered isolation solution containing (in mM) 55 NaCl, 80 sodium glutamate, 5.6 KCl, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.3 with NaOH), which was supplemented with papain (0.7 mg/ml) and collagenase (1.0 mg/ml), as described previously (19). Smooth muscle cells were maintained in ice-cold (4°C) HEPES-buffered isolation solution and used for experiments between 1 and 8 h after isolation.

Patch-clamp electrophysiology

Potassium currents were measured with the perforated-patch or the excised inside-out patch-clamp configuration (Axopatch 200B, Clampex 8.2). For perforated-patch experiments, HEPES-buffered physiological salt solution was used as the bath solution. The pipette solution

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contained (in mM) 110 KAsp, 30 KCl, 10 NaCl, 1 $MgCl_2$, 10 HEPES, and 0.05 EGTA (pH 7.2, KOH). For inside-out patch recordings, the bath solution contained (in mM) 130 KCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, and 1.6 HEDTA (pH 7.2 with KOH), with free Ca^{2+} concentrations of 1, 3, 10, 30, 100, or 300 μM. The pipette solution for inside-out patch experiments contained (in mM) 130 KCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, and 1.6 HEDTA, with 10 μM free Ca^{2+} (pH 7.4 with KOH). Free Ca^{2+} concentrations were measured with a Ca^{2+}-sensitive (Corning no. 476041) and a reference (Corning no. 476370) electrode. Hypoxic solutions were obtained by purging bath solution with 100% N_2 in a gas-impermeant container for at least 1 h before use. Experimental chambers were continuously perfused with normoxic or hypoxic solution at a rate of 5−10 ml/min. Dissolved P_{O_2} was monitored in experimental chambers with an O_2-sensitive electrode (Extech Instruments). Changing the perfusion solution from normoxic to hypoxic reduced the dissolved P_{O_2} in the chamber from ~150 to 15 mmHg. K^+ currents were filtered at 1 kHz and digitized at 4 kHz. K_{Ca} current analysis was performed off-line with custom analysis software or Clampfit 9.2. A transient K_{Ca} current was defined as the simultaneous opening of three K_{Ca} channels, as previously defined (7,27). Single-K_{Ca} channel amplitude was measured in normoxia and hypoxia with histograms.

Confocal Ca^{2+} imaging

Cells were incubated in HEPES-buffered isolation solution and fluo-4 AM (10 μM) for 25 min at room temperature, followed by a 30-min wash. Imaging was performed with HEPES-buffered physiological salt solution in the experimental chamber. Fluo-4 fluorescence was imaged with a Noran Oz laser scanning confocal microscope with a ×60 water-immersion objective (numerical aperture = 1.2) by illuminating with 488-nm light and collecting emitted light >500 nm. Images (256 × 240 pixels, 56.3 × 52.8 μm) were recorded every 8.3 ms (i.e., at 120 images/s). Simultaneous current and fluorescence measurements were synchronized with a light-emitting diode placed above the recording chamber that was triggered during acquisition. Each cell was imaged for at least 10 s under each condition. Ca^{2+} sparks and global Ca^{2+} concentration were analyzed off-line with custom software written with IDL 5.3 that was a kind gift from Dr. M. T. Nelson (University of Vermont, Burlington, VT). Ca^{2+} sparks were detected by dividing off an area 1.54 μm (7 pixels) × 1.54 μm (7 pixels) (i.e., 2.37 μm^2) in each image (F) by a baseline (F_0) that was determined by averaging 10 images without Ca^{2+} spark activity. The entire area of each image was analyzed to detect Ca^{2+} sparks. A Ca^{2+} spark was defined as a local increase in $F/F_0 > 1.2$. Global Ca^{2+} fluorescence was calculated from the same images used for Ca^{2+} spark analysis and was the mean pixel value of 100 different images acquired during a 10-s period (7,8,49,50).

Fura-2 imaging

Isolated smooth muscle cells were incubated in HEPES-buffered isolation solution containing fura-2 AM (5 μM) and 0.05% Pluronic F-127 for 20 min, followed by a 15-min wash. Experiments were performed with HEPES-buffered physiological salt solution in the chamber. Fura-2 was alternately excited at 340 or 380 nm with a PC-driven hyperswitch (Ionoptix, Milton, MA). Background-corrected ratios were collected every 1 s at 510 nm with a Dage MTI integrating CCD camera (Ionoptix). SR Ca^{2+} load ($[Ca^{2+}]_{SR}$) was estimated by measuring the amplitude of caffeine (10 mM)-induced $[Ca^{2+}]_i$ transients (7,8,49,50).

Reagents

Unless otherwise specified, all reagents were purchased from Sigma-Aldrich (St. Louis, MO). Fluo-4 AM, fura-2 AM, and Pluronic F-127 were purchased from Molecular Probes (Eugene, OR) and papain from Worthington Biochemical (Lakewood, NJ).

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Statistical analysis

Values are expressed as means ± SE. Student's *t*-test and Student-Newman-Keuls test were used for comparing paired or unpaired data and multiple data sets, respectively. Simultaneous Ca^{2+} spark and transient K_{Ca} current amplitude data were fit with a linear regression function and the slope ± SE of each fit was compared with a Student's *t*-test. The relationship between K_{Ca} channel open probability (P_o) and free Ca^{2+} concentration was fit with a Hill equation, $y = V_{max} * x_n / (x_n + k_n)$, where V_{max} is the maximal P_o of K_{Ca}; n is the Hill coefficient (n_H), and k is the dissociation constant (K_d). V_{max}, n_H, and K_d were compared between normoxia and hypoxia with a Student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

Hypoxia inhibits transient K_{Ca} currents in cerebral artery smooth muscle cells

Transient K_{Ca} currents were measured in isolated smooth muscle cells using the perforated-patch clamp configuration. At −40 mV, a reduction in dissolved P_{O_2} from ~150 (normoxia) to 15 (hypoxia) mmHg reversibly reduced the frequency and amplitude of transient K_{Ca} currents (Fig. 1). Specifically, hypoxia decreased mean transient K_{Ca} current frequency from 0.79 ± 0.16 to 0.48 ± 0.11 Hz, or to ~61% of normoxia ($n = 10$ cells, $P < 0.05$). Hypoxia reduced mean transient K_{Ca} current amplitude from 33.0 ± 6.3 to 25.1 ± 5.7 pA, or to ~76% of normoxia ($n = 10$ cells, $P < 0.05$).

Hypoxia attenuates the coupling ratio and amplitude relationship between Ca^{2+} sparks and transient K_{Ca} currents

To investigate mechanisms mediating transient K_{Ca} current inhibition by hypoxia, simultaneous measurements of Ca^{2+} sparks and transient K_{Ca} currents were obtained by performing confocal Ca^{2+} imaging in combination with patch-clamp electrophysiology. At −40 mV, in normoxia, 89.1 ± 3.5% of Ca^{2+} sparks activated a transient K_{Ca} current ($n = 155$ sparks, 16 cells). In the same cells, hypoxia reduced Ca^{2+} spark coupling to 62.6 ± 9.1% ($n = 123$ sparks). Hypoxia also reduced the slope of the amplitude relationship between coupled Ca^{2+} sparks and evoked transient K_{Ca} currents from 66 ± 4 to 45 ± 5 pA/F/F_0 ($P < 0.05$, Fig. 2*B*). In contrast, hypoxia did not alter mean Ca^{2+} spark frequency (normoxia 0.74 ± 0.13 Hz, hypoxia 0.76 ± 0.17 Hz; $n = 16$ cells, $P > 0.05$) or amplitude (F/F_0, 1.70 ± 0.05 vs. 1.72 ± 0.05; $n = 155$ and 123 sparks, respectively, $P > 0.05$) (Fig. 2, *C* and *D*). In hypoxia, global fluo-4 fluorescence was 1.03 ± 0.04 that in normoxia, indicating that global Ca^{2+} did not change ($n = 16$ cells, $P > 0.05$).

Consistent with the observation that hypoxia did not alter Ca^{2+} spark frequency, hypoxia also did not change $[Ca^{2+}]_{SR}$, as determined by measuring caffeine (10 mM)-induced $[Ca^{2+}]_i$ transients in isolated smooth muscle cells (Fig. 3). Collectively, these data suggest that in cerebral artery smooth muscle cells hypoxia reduces the effective coupling of Ca^{2+} sparks to K_{Ca} channels but does not alter Ca^{2+} spark properties or global $[Ca^{2+}]_i$.

Hypoxia decreases the apparent Ca^{2+} sensitivity of cerebral artery smooth muscle cell K_{Ca} channels

To determine mechanisms mediating hypoxia-induced Ca^{2+} spark uncoupling and attenuation of the amplitude relationship between Ca^{2+} sparks and K_{Ca} channels, K_{Ca} channel activity was measured in cerebral artery smooth muscle cells. To block transient K_{Ca} currents, Ca^{2+} sparks were inhibited with thapsigargin (100 nM), which depletes $[Ca^{2+}]_{SR}$.K_{Ca} channel activity was measured with the perforated-patch clamp configuration with myocytes voltage-clamped at 0 mV to improve the signal-to-noise ratio. In the same smooth muscle cells, hypoxia reduced mean K_{Ca} channel activity (NP_o) from 0.18 ± 0.03 to 0.08 ± 0.03, or to ~44% of that in normoxia

($n = 7$ cells, $P < 0.05$; Fig. 4). In contrast, hypoxia did not alter K_{Ca} channel amplitude (normoxia 5.2 ± 0.3 pA, hypoxia 5.2 ± 0.4 pA; $n = 7$ cells, $P > 0.05$).

We then tested the hypothesis that hypoxia inhibits transient K_{Ca} currents by decreasing K_{Ca} channel apparent Ca^{2+} sensitivity. We also sought to determine whether K_{Ca} channel inhibition by hypoxia requires cytosolic signaling pathways. To test these hypotheses, cerebral artery smooth muscle cell K_{Ca} channel open probability (P_o) was measured over a range of intracellular free Ca^{2+} concentrations from 1 to 300 μM in excised inside-out membrane patches voltage-clamped at −40 mV. Hypoxia increased the mean K_d for Ca^{2+} of K_{Ca} channels from 17.3 ± 1.3 to 31.9 ± 0.81 μM without altering n_H or the maximum P_o (Fig. 5A and B). Relative K_{Ca} channel inhibition by hypoxia was also Ca^{2+} dependent. With 3, 10, and 30 μM free Ca^{2+}, which were the Ca^{2+} concentrations where inhibition was greatest, hypoxia decreased K_{Ca} channel P_o to 29%, 38%, and 65% of normoxia, respectively (Fig. 5*A*).

DISCUSSION

The major novel findings of our study investigating O_2 regulation of cerebral artery smooth muscle cell K_{Ca} channels that are under Ca^{2+} spark control indicate that hypoxia: *1*) reversibly reduces transient K_{Ca} current frequency and amplitude, *2*) does not alter Ca^{2+} spark frequency or amplitude or change global $[Ca^{2+}]_i$ in voltage-clamped cells, *3*) attenuates both the coupling ratio and effective coupling of Ca^{2+} sparks to K_{Ca} channels, and *4*) reduces the apparent Ca^{2+} sensitivity of K_{Ca} channels through a mechanism that is independent of cytosolic signaling pathways. These data indicate that a reduction in P_{O_2} causes a decrease in K_{Ca} channel apparent Ca^{2+} sensitivity, leading to K_{Ca} channel uncoupling from Ca^{2+} sparks and a decrease in transient K_{Ca} current frequency and amplitude. These data suggest that hypoxia-induced K_{Ca} channel uncoupling would oppose the resulting vasodilation.

Previous studies have demonstrated that hypoxia inhibits K_{Ca} channels in rabbit and lamb pulmonary artery smooth muscle cells, rat cerebral artery smooth muscle cells, and rat carotid body chemoreceptor cells (1,9,20,30,31,38,42,47). Similarly, recombinant human and rat K_{Ca} channels expressed in immortalized cell lines were inhibited by hypoxia (24,26,32,48). In contrast, hypoxia activated cat cerebral artery smooth muscle cell and piglet pial artery K_{Ca} channels, leading to vasodilation (3,4,16). Our data indicate that in rat cerebral artery smooth muscle cells, hypoxia inhibits transient K_{Ca} currents in intact cells and K_{Ca} channels in both intact cells and excised membrane patches. Conceivably, K_{Ca} channel responses to O_2 may be species specific, but further studies will be required to investigate this hypothesis.

Hypoxia may have blocked transient K_{Ca} currents by inhibiting Ca^{2+} sparks. Data obtained with confocal imaging indicated that hypoxia did not alter Ca^{2+} spark frequency or amplitude. An elevation in global $[Ca^{2+}]_i$ activates Ca^{2+} sparks, whereas a reduction in global $[Ca^{2+}]_i$ inhibits Ca^{2+} sparks (21). Hypoxic regulation of global $[Ca^{2+}]_i$ was measured to determine whether mechanisms that regulate Ca^{2+} sparks were altered by a reduction in P_{O_2}. However, hypoxia did not change global $[Ca^{2+}]_i$. Since a $[Ca^{2+}]_{SR}$ reduction inhibits Ca^{2+} sparks, and blocking SR Ca^{2+} release elevates $[Ca^{2+}]_{SR}$, we also measured hypoxic regulation of $[Ca^{2+}]_{SR}$ (6,28). Hypoxia did not alter $[Ca^{2+}]_{SR}$, providing further support for our finding that hypoxia did not regulate Ca^{2+} sparks. Rather, hypoxia decreased transient K_{Ca} current frequency by reducing the percentage of Ca^{2+} sparks that activated a transient K_{Ca} current. Hypoxia also reduced the amplitude relationship between Ca^{2+} sparks and K_{Ca} channels that remained coupled, resulting in a decrease in transient K_{Ca} current amplitude. Therefore, hypoxia reduced K_{Ca} channel activity in cerebral artery smooth muscle cells by reducing both the coupling percentage and the effective coupling of Ca^{2+} sparks to K_{Ca} channels. In smooth muscle cells, Ca^{2+} sparks activate transient K_{Ca} currents by elevating subsarcolemmal $[Ca^{2+}]_i$ to within the micromolar concentration range (39,51). Here, hypoxia reduced K_{Ca}

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channel coupling in intact smooth muscle cells where K_{Ca} channels were exposed to subsarcolemmal micromolar Ca^{2+} concentrations generated by Ca^{2+} sparks. Hypoxia also reduced K_{Ca} channel apparent Ca^{2+} sensitivity in excised membrane patches that were exposed to micromolar Ca^{2+} concentrations. These data demonstrate that hypoxia reduces K_{Ca} channel apparent Ca^{2+} sensitivity via a mechanism that is independent of cytosolic signaling pathways, and this a primary mechanism leading to Ca^{2+} spark uncoupling.

Mechanisms by which acute changes in P_{O_2} regulate K_{Ca} channel activity are unclear. Heme oxygenase-2 (HO-2) is physically coupled to K_{Ca} channels and is proposed to act as an oxygen sensor in carotid body glomus cells (23,48). However, genetic ablation of HO-2 did not change O_2 sensitivity of carotid body glomus cells or chromaffin cells (37). Oxygen sensitivity of K_{Ca} channels may also depend on the presence of a cysteine-rich, stress-regulated exon (STREX) present within the channel COOH terminus (32). However, K_{Ca} channels expressed in rat cerebral artery smooth muscle cells were recently cloned by our group and do not contain a STREX motif (20). Thus O_2 sensing in cerebral artery smooth muscle cell K_{Ca} channels occurs through a STREX-independent mechanism. NADPH oxidase and AMP-activated protein kinase (AMPK) may also act as O_2 sensors in pulmonary artery smooth muscle and carotid body type I cells (10,22,23). In the present study, the membrane-delimited effect of hypoxia on K_{Ca} channels that occurs in excised patches is unlikely to involve soluble signaling messengers or enzymes that require cofactors other than the ions present in the bath solution, arguing against a role for NADPH. In addition, phosphorylation is unlikely to be necessary since ATP was not present, suggesting that AMPK is not involved. Chronic hypoxia also reduces cerebral artery K_{Ca} channel β_1-subunit expression (35), an effect that would reduce K_{Ca} channel apparent Ca^{2+} sensitivity (5). However, in our experiments, hypoxic inhibition of transient K_{Ca} currents and K_{Ca} channels was immediate and unlikely to occur through a reduction in protein expression. Mitochondria are another potential O_2 sensor in vascular smooth muscle cells (33,46). Hypoxia depolarizes mitochondria in renal artery smooth muscle cells but hyperpolarizes mitochondria in pulmonary artery smooth muscle cells (33). A small mitochondrial depolarization, such as that induced by diazoxide, an ATP-sensitive potassium (K_{ATP}) channel opener, or a nanomolar concentration of CCCP, a protonophore, activates Ca^{2+} sparks and transient K_{Ca} currents in cerebral artery smooth muscle cells (49). In contrast, a large mitochondrial depolarization induced by micromolar CCCP or rotenone, an electron transport chain complex I blocker, inhibits Ca^{2+} sparks and transient K_{Ca} currents (8,49). Here, the primary effect of hypoxia was mediated by an effect on K_{Ca} channels, since hypoxia did not change Ca^{2+} spark frequency or amplitude. These data suggest that if hypoxia alters mitochondrial potential in cerebral artery smooth muscle cells, the net effects on Ca^{2+} sparks and K_{Ca} channels are very small and secondary to a direct membrane-delimited effect of the P_{O_2} reduction on K_{Ca} channels. While the K_{Ca} channel O_2 sensor is unclear, data indicate that the interaction between O_2 and K_{Ca} channels can occur in the absence of cytosolic signaling pathways and suggest that the K_{Ca} channel itself or a closely associated regulatory molecule is the O_2 sensor.

In human and porcine coronary and rabbit cerebral artery smooth muscle cells, hypoxia inhibited L-type Ca^{2+} channels and reduced $[Ca^{2+}]_i$ (43). In contrast, in pulmonary artery smooth muscle cells hypoxia stimulated SR Ca^{2+} release and increased cytosolic $[Ca^{2+}]_i$ (11, 17,31,34,44). In fetal sheep pulmonary artery smooth muscle cells, a P_{O_2} elevation stimulated transient K_{Ca} currents (40), whereas in rabbit pulmonary artery smooth muscle cells hypoxia irreversibly blocked transient outward currents (45). While opposing responses to O_2 have been reported in pulmonary artery smooth muscle cells, in the present study hypoxia did not alter either cytosolic $[Ca^{2+}]_i$ in voltage-clamped cerebral artery smooth muscle cells or $[Ca^{2+}]_{SR}$ in isolated myocytes.

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Hypoxia leads to cerebral artery dilation, a response that functions to match blood flow to metabolic requirements (15,25). Although the signaling mechanisms mediating hypoxic vasodilation may depend on the precise reduction in P_{O_2}, hypoxia induces systemic artery hyperpolarization, which would reduce smooth muscle cell voltage-dependent Ca^{2+} channel activity, leading to a reduction in global $[Ca^{2+}]_i$ and vasodilation (13,29,30,43). Data here indicate that a hypoxic reduction in K_{Ca} channel activity due to Ca^{2+} spark uncoupling would attenuate, rather than contribute to, the hypoxic hyperpolarization and vasodilation. In contrast to effects in systemic arteries, hypoxia depolarizes and constricts pulmonary arteries (31). In pulmonary artery smooth muscle cells, hypoxia-induced K_{Ca} channel inhibition would contribute to the membrane depolarization, which would activate voltage-dependent Ca^{2+} channels, leading to vasoconstriction (31,34). It remains to be determined whether hypoxia also modulates Ca^{2+} sparks and the coupling relationship between Ca^{2+} sparks and K_{Ca} channels in pulmonary artery smooth muscle cells, and whether such changes are mediated by the mechanism we describe here.

In summary, data indicate that hypoxia reduces K_{Ca} channel apparent Ca^{2+} sensitivity through a membrane-delimited mechanism, leading to a decrease in the effective coupling of Ca^{2+} sparks to K_{Ca} channels and a reduction in transient K_{Ca} current frequency and amplitude. These data indicate that K_{Ca} channel inhibition caused by uncoupling from Ca^{2+} sparks would oppose the hypoxic vasodilation.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-67061 and HL-77678 to J. H. Jaggar. A. Adebiyi is a recipient of a postdoctoral fellowship from the Southeast Affiliate of the American Heart Association.

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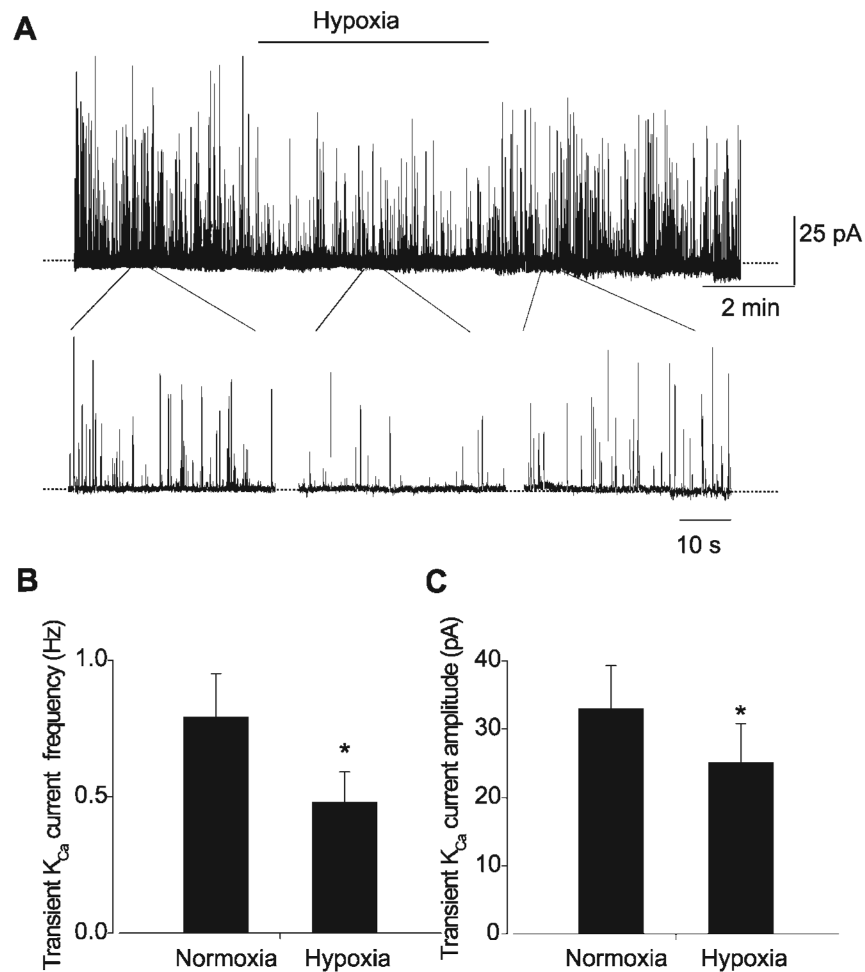

Fig. 1.

Hypoxia inhibits transient Ca^{2+}-activated potassium (K_{Ca}) currents in voltage-clamped cerebral artery smooth muscle cells. *A*: original trace illustrating reversible transient K_{Ca} current inhibition by a reduction in dissolved O_2 from ~150 (normoxia) to ~15 (hypoxia) mmHg. Expanded segments are shown below the full trace to illustrate individual transient K_{Ca} currents in normoxia and hypoxia. *B* and *C*: hypoxia reduces mean transient K_{Ca} current frequency (*B*) and amplitude (*C*) (n = 10 for each). *$P < 0.05$ compared with normoxia.

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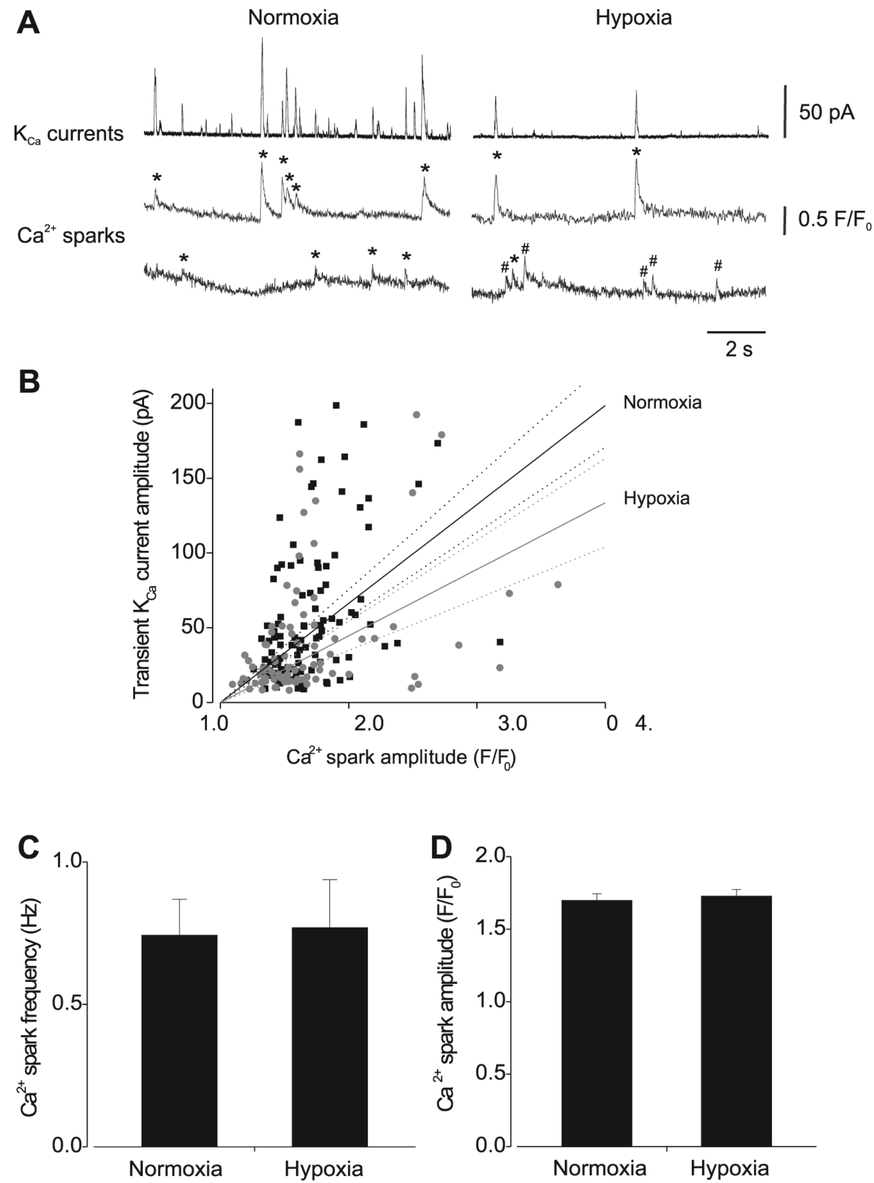

Fig. 2.
Hypoxic regulation of Ca^{2+} sparks and the effective coupling of Ca^{2+} sparks to K_{Ca} channels. *A*: original simultaneous recordings of transient K_{Ca} currents (*top*) and Ca^{2+} sparks that occurred at 2 locations (*bottom*) in the same cell voltage-clamped at −40 mV in normoxia (*left*) and hypoxia (*right*). * and #, coupled and uncoupled Ca^{2+} sparks, respectively. *B*: amplitude correlation of coupled Ca^{2+} sparks and evoked transient K_{Ca} currents in normoxia (black squares; n = 134) and hypoxia (gray circles; n = 86) with linear regression and 95% confidence bands fit to each data set (slope ± SE: normoxia 66 ± 4, hypoxia 45 ± 5). Hypoxia decreased the effective coupling of Ca^{2+} sparks to K_{Ca} channels (P = 0.0037). *C* and *D*: hypoxia did not change mean Ca^{2+} spark frequency (*C*) or amplitude (*D*) (n = 155 and 123 sparks in normoxia and hypoxia, respectively; n = 16 cells).

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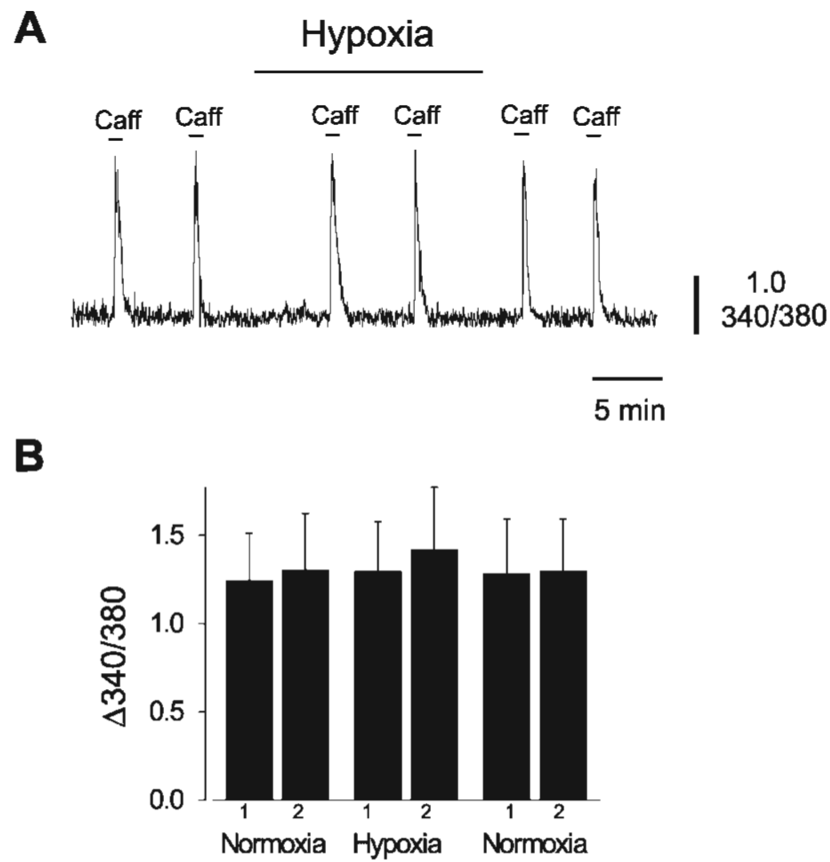

Fig. 3.

Hypoxia does not alter cerebral artery smooth muscle cell sarcoplasmic reticulum (SR) Ca^{2+} load. *A*: caffeine (10 mM)-induced intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) transients were similar in normoxia and hypoxia in an isolated cerebral artery smooth muscle cell. 340/380, 340- to 380-nm ratio. *B*: mean change in fura-2 ratio in normoxia and hypoxia (n = 6 cells).

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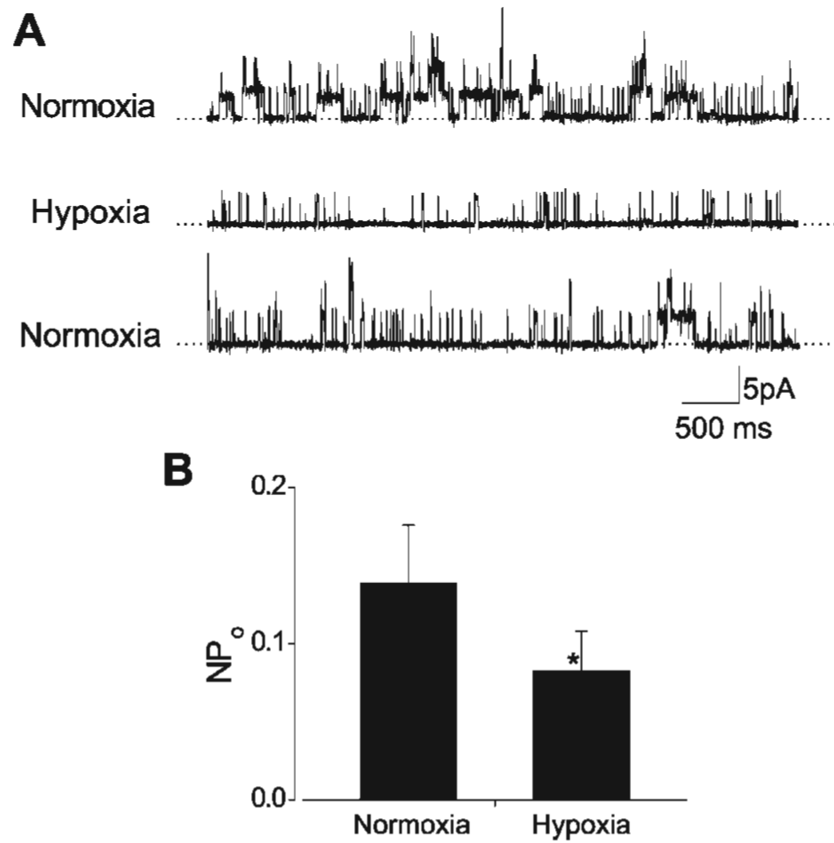

Fig. 4.

Hypoxia inhibits K_{Ca} channels in rat cerebral artery smooth muscle cells. *A*: original traces obtained from the same smooth muscle cell illustrating reversible K_{Ca} channel inhibition by hypoxia at 0 mV. Transient K_{Ca} currents were blocked by pretreatment with thapsigargin (100 nM), to deplete SR Ca^{2+}. *B*: mean data illustrating hypoxic inhibition of K_{Ca} channel activity (NP_o, n = 7 cells). *$P < 0.05$ compared with normoxia.

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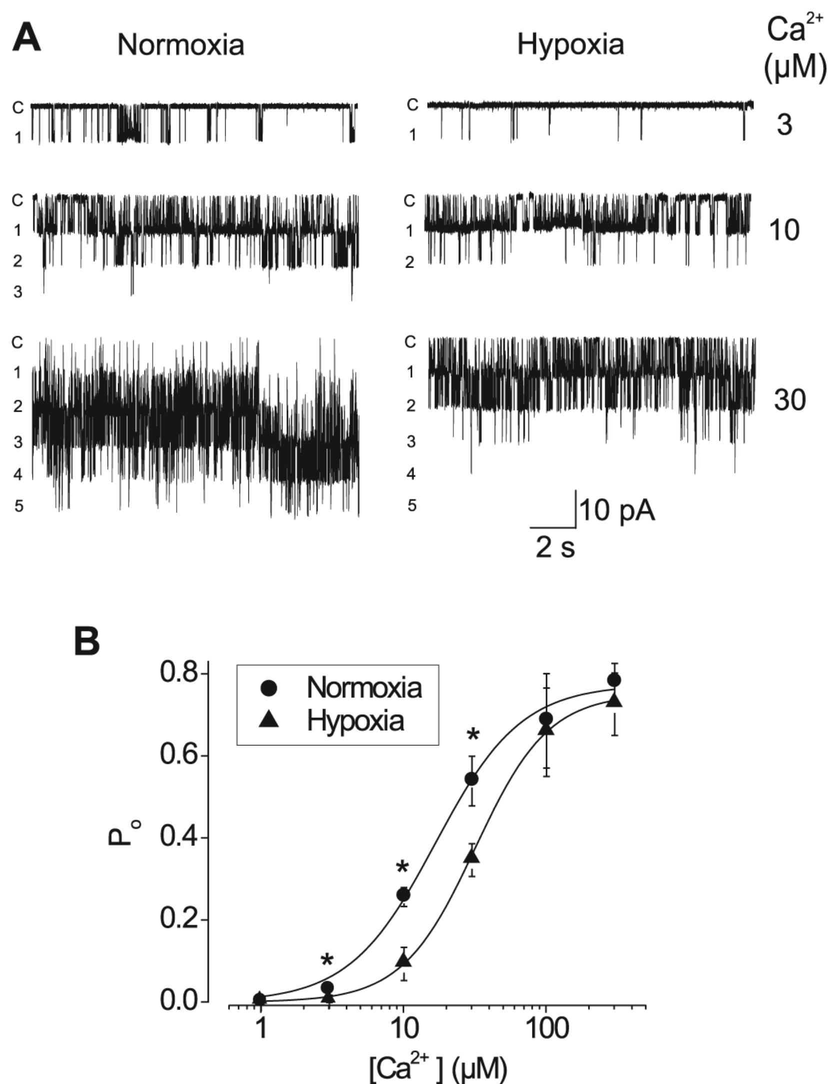

Fig. 5.

Hypoxia decreases the apparent Ca^{2+} sensitivity of K_{Ca} channels in excised membrane patches from rat cerebral artery smooth muscle cells. *A*: original recordings obtained from the same membrane patch voltage-clamped at −40 mV, illustrating K_{Ca} channel inhibition by hypoxia at 3, 10, and 30 μM Ca^{2+}. c, Closed level, with number of open levels also shown. *B*: average single-K_{Ca} channel open probability (P_o) vs. free [Ca^{2+}] in normoxia and hypoxia. Data were fit with a Hill equation. In normoxia, the mean apparent dissociation constant (K_d) for Ca^{2+} at −40 mV was 17.3 ± 1.29 μM, with a Hill coefficient of 1.52 ± 0.14 and a maximum P_o of 0.80 ± 0.02 (n = 3−6 cells). In the same patches, hypoxia increased the mean K_d for Ca^{2+} of K_{Ca} channels to 31.9 ± 0.81 μM but did not alter the Hill coefficient (1.68 ± 0.06) or the maximum P_o (0.75 ± 0.01).