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Hypoxia Does Not Activate ATP-Sensitive K⁺ Channels in Arteriolar Muscle Cells

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Abstract

Objective—To test the hypothesis that hypoxia activates ATP-sensitive K⁺ (K_{ATP}) channels in cremasteric arteriolar muscle cells, resulting in membrane hyperpolarization and inhibition of norepinephrine-induced contraction.

Methods—Arteriolar muscle cells were isolated enzymatically from second- and third-order arterioles that were surgically removed from hamster cremaster muscles. The effects of hypoxia (PO₂ = 12–15 mm Hg) were then examined on norepinephrine-induced contraction, membrane currents, and membrane potential in these cells at room temperature. Whole-cell currents and membrane potential were recorded using the perforated patch technique.

Results—Hypoxia (12–15 mm Hg PO₂) reversibly inhibited norepinephrine-induced contraction to 52 ± 6% of the response in normoxic solutions (156 mm Hg, *n* = 12 digests, *p* < 0.05). These effects of hypoxia could be prevented by superfusion of the cells with either solutions containing the K_{ATP} channel antagonist glibenclamide (1 μM) or solutions containing 35 mM K⁺ to reduce the electrochemical gradient for K⁺ diffusion. Cromakalim, an activator of K_{ATP} channels, also inhibited norepinephrine-induced contraction to a similar extent as hypoxia, and in a glibenclamide and 35 mM K⁺-sensitive manner. These results are consistent with the K_{ATP} channel hypothesis. In contrast, hypoxia had no effect on estimated whole-cell membrane conductance between –40 and –90 mV in voltage-clamp experiments; on holding current measured at –60 mV in cells superfused with 143 mM K⁺ under voltage-clamp conditions; or on membrane potential in current-clamp experiments, despite positive effects of cromakalim in all three protocols. These electrophysiological data lead to rejection of the hypothesis that hypoxia activates K_{ATP} channels.

Conclusions—Hypoxia inhibits norepinephrine-induced contraction of cremasteric arteriolar muscle cells by a mechanism that does not involve K_{ATP} channels. It is speculated that the inhibitory effects of glibenclamide and 35 mM K⁺ on the effects of hypoxia on contraction resulted from depolarization induced by these treatments rather than specific inhibition of K_{ATP} channels.

Keywords

microcirculation; oxygen; norepinephrine; potassium channels; vascular smooth muscle; arterioles; glibenclamide; patch clamp; membrane potential

INTRODUCTION

Oxygen acts as a vasoactive substance in the peripheral microcirculation. Elevation of PO₂ causes vasoconstriction, whereas hypoxia causes vasodilation (6,9–11,16,17,20). Despite the repeated demonstration of this phenomenon, the site and mechanism of action by which

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hyperoxia and hypoxia produce their effects remains unclear. In small arteries or large arterioles from skeletal muscle, several studies have indicated that hypoxia may be sensed by endothelial cells resulting in release of vasodilator prostaglandins that mediate the subsequent vasodilation (6,16,17), consistent with earlier studies by Busse and colleagues (2,3). However, other studies have failed to demonstrate a role for prostaglandins in arteriolar oxygen reactivity *in vivo* (10,20) or *in vitro* (22,23). Most recently, Tateishi and Faber have suggested that hypoxia may be directly sensed by arteriolar muscle cells and act to inhibit α_2 -adrenergic-receptor-mediated tone through a mechanism involving ATP-sensitive K^+ channels (22,23). Therefore, the purpose of the present study was to test the hypothesis that arteriolar muscle cells are intrinsically sensitive to hypoxia and to directly examine the role played by ATP-sensitive K^+ channels in this process.

METHODS

Preparation of Single Arteriolar Muscle Cells

Cremasteric arteriolar muscle cells were isolated enzymatically as described previously with only minor modifications (13). All animal usage was approved by the Institutional Animal Care and Use Committee at Western Michigan University. Briefly, male golden Syrian hamsters (100–160 g) were killed by intra-peritoneal injection of sodium pentobarbital (>150 mg/kg). Cremaster muscles were removed and then rinsed in 4 °C zero Ca^{++} physiological salt solution (PSS, composition in mM: 140 NaCl, 5 KCl, 1 $MgCl_2$, 10 HEPES, 10 glucose; pH = 7.4 with NaOH at room temperature, 295–300 mOsm). The muscles were then placed in a water-jacketed dissection dish maintained at 4 °C and containing 50 ml of zero Ca^{++} PSS to which was added 1 mg/ml bovine serum albumin (BSA), 10 μ M sodium nitroprusside and/or 10 μ M diltiazem. Second- and third-order arterioles were then hand dissected out of the muscles and transferred to 1 ml of dissociation solution (DS, composition in mM: 140 NaCl, 5 KCl, 1 $MgCl_2$, 0.1 mM $CaCl_2$, 10 HEPES, 10 glucose, 1 mg/ml BSA, 10 μ M sodium nitroprusside and/or diltiazem; pH = 7.4 with NaOH at room temperature, 295–300 mOsm) at room temperature. After 10 minutes of incubation, most of this solution was removed and replaced with 1 ml of DS containing 1.5 mg/ml papain and 1 mg/ml dithioerythritol. The arteriolar segments were then incubated in this solution at 37 °C for 35 minutes. At the end of this interval the papain solution was removed and replaced with 1 ml of DS containing 1.5 mg/ml collagenase, 1 mg/ml elastase, and 1 mg/ml soybean trypsin inhibitor, and the segments incubated for an additional 18–22 minutes, at 37 °C. The enzyme-containing solution was then replaced with 4 ml of DS at room temperature and allowed to settle for approximately 10 minutes. This solution was then removed and replaced with 1 ml of fresh DS not containing sodium nitroprusside or diltiazem. Cells were released from the segments by gentle trituration (1–4 strokes) using a 100–1000- μ l Eppendorf-style pipettor and stored in this solution for up to 4 hours at room temperature. In some experiments, cells were stored in Kraft Brü he solution (KB solution, composition in mM: 85 KCl, 30 KH_2PO_4 , 5 $MgSO_4$, 2 Na_2ATP , 0.2 EGTA, 5 sodium pyruvate, 5 succinate, 5 creatine, 20 glucose, 20 taurine; pH = 7.2 and 10 mg/ml BSA) at 4 °C as described previously (12). This latter procedure appeared to decrease the variability of the contractile responses of cells but did not appear to otherwise alter their behavior relative to the goals of the present study.

Single Cell Contraction Studies

Contraction of single cells was assessed as described previously (12,13). Cells were placed in a 1-ml chamber mounted on the stage of an inverted microscope, allowed to settle, and the chamber perfused with bath solution (see below). After a 10 minute equilibration period, the tip of a superfusion pipette (10–50- μ m tip i.d.) filled with a norepinephrine-containing solution (1 μ M) was positioned with a micromanipulator adjacent to a cell and fluid was ejected from the pipette onto the cells by pressurizing the back of the pipette with a water manometer. The

response of the cell was then monitored through the eyepiece of the microscope at 100 \times magnification (10 \times , n.a. 0.2 objective). A positive response was defined as a greater than 30% shortening of a cell within 15 seconds of continuous exposure to norepinephrine as described previously (13). At least 30 cells from several different fields were tested in each aliquot and the final response calculated as the percent of cells responding (13). Three or four such trials were performed for each isolate of cells: two controls in normal bath solution, and one or two trials with inhibitors and/or hypoxic solutions. The order of treatments was randomized and the results from the two control trials were pooled for final display of the data. The bath in these and other experiments was perfused at a rate of 1–3 ml/min by gravity from one of two 50-ml reservoirs connected to the bath by thick-walled, gas-impermeant teflon tubing and plastic stopcock valves. The solutions in these reservoirs were bubbled vigorously with either room air (21% O₂) or N₂ gas (0% O₂). By adjusting the valves, solution from either of these reservoirs could be delivered to the bath chamber. An example of the time course and repeatability of the change in PO₂ when switching from air- to N₂-equilibrated solution is shown in Fig. 1. Bath PO₂ in these experiments was measured with a miniature Clark-type oxygen electrode (Microelectrodes, Inc., Londonderry, NH). The mean PO₂ during the last 3 minutes of hypoxic perfusion was 15 ± 2.1 mm Hg ($n = 6$) in the bath used for contraction studies. This was not significantly different from the PO₂ measured in the patch clamp bath (12 ± 1.0 mm Hg, $n = 5$; $p > 0.05$ compared to contraction bath PO₂). All measurements were performed after cells had been superfused with PSS at the appropriate PO₂ for at least 5 minutes.

Electrophysiology

The perforated-patch technique was used to assess currents (via voltage-clamp) and membrane potential (via current-clamp) in single arteriolar muscle cells to obviate cell dialysis, as described previously (13). An aliquot of cells was placed on the cover-slipped bottom of a 1-ml flow-through chamber, allowed to settle for 5–10 minutes and then superfused with PSS containing 2 mM Ca⁺⁺ and equilibrated with room air (PO₂ = 156 mm Hg at room temperature). Patch-pipettes were constructed from 1-mm i.d. \times 1.5-mm o.d. Corning 7052 glass tubes (Garner Glass Company, Claremont, CA), fire polished, their tips filled with pipette solution (see below) and then back-filled with the same solution containing 240 μ g/ml amphotericin B as described previously (13). Electrical access was gained in 10–15 minutes and was maximal in 15–30 minutes. (Pipette solution contained in mM: 100 K-aspartate, 43 KCl, 1 MgCl₂, 10 HEPES, 0.5 EGTA; pH 7–7.2 adjusted with NaOH, 295–300 mOsm adjusted with sucrose.) Pipettes had tip resistances of 2–5 M Ω when filled with this solution. Seals were made on arteriolar muscle cells by gently touching the fire-polished tip of the pipette to a cell and then applying gentle suction by mouth. Seal resistances were all greater than 10 G Ω for the studies presented and no attempt was made to correct for leakage currents.

Currents and membrane potential were measured with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) controlled by pClamp software (version 5.7 or 7) running on a generic 233-MHz Pentium computer. Currents were filtered at 1 kHz and sampled at 5 kHz. All currents reported were normalized to cell capacitance to account for any differences in cell size or changes in membrane area. Cell capacitance was estimated by integration of the capacitive transient elicited by stepping from –60 to –70 mV. In 71 cells, cell capacitance averaged 22 ± 0.6 pF under control conditions. Access resistance for these same cells averaged 18 ± 2 M Ω .

Two different voltage-clamp protocols were used to assess the effects of hypoxia on membrane currents. In the first, cells were superfused with PSS containing 2 mM Ca²⁺ (5 mM K⁺) and held at –60 mV. Membrane potential was then stepped for 400 ms, in 10-mV increments, from –90 to 0 mV, and the average current recorded during the last 100 ms of these steps determined. The current–voltage (I–V) relationship for these cells is linear between –90 and –40 mV, with

the slope of the line representing membrane conductance over this voltage range (13). We have previously shown that K_{ATP} channel activity contributes significantly to whole-cell membrane conductance at these potentials. Therefore, the slope conductance between -90 and -40 mV was used to assay for changes in K_{ATP} channel activity produced by hypoxia. Cromakalim, a well-known K_{ATP} channel agonist, was used as a positive control for these experiments (see the Results section).

In the second voltage-clamp assay, cells were superfused with solutions containing 143 mM K^+ (KCl substituted for NaCl in PSS, osmolarity adjusted to 295–300 mOsm with sucrose) and the effects of hypoxia and cromakalim on the holding current recorded at -60 mV were assessed. Under these recording conditions, activation of K_{ATP} channels results in an increase in inward, glibenclamide-sensitive current (see Results). Currents in these experiments were normalized to cell capacitance to compensate for possible differences in cell membrane areas (i.e., cell size).

Materials

Most chemicals and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO). Elastase was purchased from Calbiochem (La Jolla, CA) and cromakalim was a gift from Pharmacia-Upjohn (Portage, MI). Glibenclamide, cromakalim, and amphotericin-B were all dissolved in dimethylsulfoxide (DMSO); other drugs, enzymes, and chemicals were dissolved in 18.2 M Ω reagent-grade water.

Statistics

Data are expressed as mean \pm SE. Mean values were compared by paired and unpaired Student's *t* tests, Wilcoxin's signed-rank test, or by analysis of variance followed by the Student–Newman–Keuls multiple comparison method, as appropriate. Linear regression analysis was used to estimate whole-cell membrane conductance from the current–voltage relationship recorded between -90 and -40 mV membrane potential. Lines were fit to individual I–V data, and the slopes were then averaged for comparison purposes (see Results). All comparisons were performed at the 95% confidence level.

RESULTS

Effects of Hypoxia on Norepinephrine-Induced Contraction

Reduction of bath PO_2 from 156 mm Hg to 12–15 mm Hg inhibited norepinephrine-induced contraction of arteriolar muscle cells (Fig. 2A). If cells were exposed to 156 mm Hg PO_2 solution after exposure to hypoxia, norepinephrine reactivity returned to the control level (control = $59 \pm 6\%$ contraction; reoxygenation = $50 \pm 9.5\%$ contraction; $n = 4$, $p > 0.05$). These data indicate that the effects of hypoxia were reversible.

Effects of 35 mM K^+ and Glibenclamide on Norepinephrine-Induced Contraction

To test the hypothesis that the inhibitory effects of hypoxia described above resulted from activation of K_{ATP} channels, the ability of 35 mM K^+ and of the K_{ATP} channel antagonist glibenclamide (1 μ M) to reverse the effects of hypoxia on norepinephrine reactivity were assessed. Both treatments (Figs. 2B and 2C) prevented hypoxic inhibition of norepinephrine-induced contraction. There was a tendency for both treatments to increase reactivity to norepinephrine (Figs. 2B and 2C); however, this effect did not attain statistical significance ($p > 0.05$).

Effects of Cromakalim on Norepinephrine-Induced Contraction

The K_{ATP} channel agonist, cromakalim, inhibited norepinephrine-induced contraction in a concentration-dependent manner (Fig. 3A). As expected, this effect of cromakalim could be reversed by 35 mM K^+ (Fig. 3B) or glibenclamide (Fig. 3C).

Effects of Hypoxia on Membrane Current and Potential

Hypoxia had no significant effect on the current–voltage relationship elicited by stepping from a holding potential of -60 mV to test potentials from -90 to 0 mV with PSS in the bath (Fig. 4A). If K_{ATP} channels were activated under these conditions, then membrane conductance between -90 and -40 mV should have been increased. This was not the case, as can be seen in Fig. 4C: hypoxia had no significant effect on membrane conductance over this voltage range. Similar results were obtained when the effects of hypoxia were assessed on holding current in cells bathed in 143 mM K^+ , which should amplify any changes in K^+ currents: hypoxia had no effect on holding current under these conditions (see Fig. 6A).

The most sensitive assay for activation of membrane K^+ channels is membrane potential due to the high input impedance of these cells—on the order of 10 G Ω (13). Therefore, the effects of hypoxia on membrane potential also were assessed. Similar to the results obtained in the voltage-clamp experiments presented above, hypoxia had no effect on membrane potential recorded in PSS using current clamp (Fig. 7A).

The effects of hypoxia on membrane current also were examined in cells exposed to 1 μ M norepinephrine to test for possible interactions between hypoxia and adrenergic receptor activation. Norepinephrine significantly reduced holding current (control = -0.99 ± 0.27 pA/pF; 1 μ M norepinephrine = -0.47 ± 0.11 pA/pF; $n = 9$, $p < 0.05$). This effect of norepinephrine on holding current was mostly prevented by pretreatment with 1 μ M glibenclamide (1 μ M glibenclamide = -0.78 ± 0.1 pA/pF; glibenclamide + 1 μ M norepinephrine = -0.58 ± 0.1 pA/pF, $n = 5$; $p > 0.05$). Consistent with the findings presented above, hypoxia was also without effect in the presence of norepinephrine (1 μ M norepinephrine = -0.40 ± 0.08 pA/pF; norepinephrine + hypoxia = -0.39 ± 0.05 pA/pF; $n = 5$, $p > 0.05$).

Effects of Cromakalim on Membrane Current and Potential

In contrast to the lack of effect of hypoxia, cromakalim elicited large increases in slope conductance in cells bathed in PSS (Figs. 4B and 4D) and in holding currents in cells superfused with 143 mM K^+ (Figs. 5A and 6B). Although there was considerable heterogeneity in the magnitude of the cromakalim effect among cells, all cells tested responded to this K_{ATP} channel agonist. The reversal potential of the current activated by cromakalim was -76 ± 3.5 mV, which was not significantly different from the estimated equilibrium potential for K^+ (E_{K^+}) of -84 mV ($p > 0.05$). Supporting results were obtained in current-clamp experiments. Cromakalim caused concentration-dependent hyperpolarization of cremasteric arteriolar muscle cells (Figs. 7B).

Effects of Glibenclamide and 35 mM K^+ on Membrane Current and Potential

We have previously shown that glibenclamide inhibits currents between -60 and -30 mV and decreases membrane conductance over this same potential range (13). Consistent with these findings, glibenclamide significantly decreased holding currents in the present study (Figs. 5B and 6C). This sulfonylurea compound also abolished the effects of cromakalim on holding currents in these cells (Figs. 5B and 6C). Glibenclamide was also able to reverse the effects of cromakalim on membrane potential (Fig. 8A). Similarly, 35 mM K^+ significantly inhibited cromakalim-induced hyperpolarization of cremasteric arteriolar muscle cells (Fig. 8B).

DISCUSSION

This study demonstrates that hypoxia ($PO_2 = 12\text{--}15$ mm Hg) reversibly inhibits norepinephrine-induced contraction of cremasteric arteriolar muscle cells. These data support the findings of Tateishi and Faber (22,23) and indicate that cremasteric arteriolar muscle cells are intrinsically sensitive to oxygen. In addition, we found that the effects of hypoxia could be reversed by a selective inhibitor of K_{ATP} channels, glibenclamide, or by exposure of cells to solutions containing 35 mM K^+ to reduce the electrochemical gradient on K^+ . These functional studies also support the findings of Tateishi and Faber (22,23) and implicate K_{ATP} channels in the mechanism of action of hypoxia on cremasteric arteriolar muscle cells. However, direct electrophysiological recording of membrane currents or potential failed to detect an effect of hypoxia, in the presence or absence of norepinephrine, despite positive evidence for K_{ATP} channel activity in these cells (see below). Therefore, it must be concluded that hypoxia does not activate K_{ATP} channels in cremasteric arteriolar muscle cells and that the inhibitory effects of glibenclamide and 35 mM K^+ observed on cell contraction (present study) or cannulated arteriole studies (22,23) must represent some nonspecific effect of these agents (see below for further discussion of this point).

It is unlikely that the lack of effect of hypoxia on K^+ current and membrane potential resulted from an inability to measure K_{ATP} channel activity. Concentrations of cromakalim that inhibit contraction of cremasteric arteriolar muscle cells to an extent similar to that produced by hypoxia, activated glibenclamide-sensitive currents and hyperpolarized these cells (see Results). These data, along with the observation of glibenclamide-sensitive resting currents in these cells (present study and Ref. 13) confirm the ability to detect K_{ATP} channel activity by the methods used.

Results from the present study do not agree with the findings of Dart and Standen (4), who observed that hypoxia activated K_{ATP} currents in smooth muscle cells isolated from porcine coronary arteries. Most likely, these differences result from species and regional differences in ion channel expression and hypoxia-sensitive mechanisms. It is also possible that subtle methodological differences in the isolation of the cells, in the solutions used during isolation and study of cells, or in pipette solutions, might also be involved. The present study also differs from the recent findings of Gauthier-Rein et al. (8), who found that isolated, cannulated bovine small coronary arteries displayed glibenclamide-sensitive, hypoxia-induced hyperpolarization in the absence of effects of this hyperpolarization on myogenic tone, despite vasodilation and hyperpolarization induced by cromakalim. These data illustrate the complexity of this problem and support the notion that there may be regional and species differences in responses.

In the absence of electrophysiological evidence for K_{ATP} channel activation, how can the effects of glibenclamide and 35 mM K^+ on the cells' responses to hypoxia be explained? Results from this and a previous study (13) have shown that glibenclamide inhibits K^+ currents around the resting membrane potential (-60 to -30 mV) and thus depolarizes cremasteric arteriolar muscle cells in the presence of physiological K^+ gradients (13). A similar degree of depolarization is observed when these cells are incubated in solutions containing 35 mM K^+ . Therefore, it is hypothesized that this depolarization is responsible for the reversal of the effects of hypoxia on norepinephrine-induced contraction in the present study, and possibly for the effects of glibenclamide on α_2 -adrenergic reactivity in the study by Tateishi and Faber (22, 23), rather than an effect specific for K_{ATP} channels. Membrane potential regulates the open-state probability of voltage-gated Ca^{2+} channels (18), which provide an important source of activator Ca^{2+} in cremasteric arteriolar muscle cells (13), and may also influence the release of Ca^{++} from intracellular stores (7,14,24), as well as the Ca^{++} sensitivity of the contractile apparatus (19). Hypoxia has been demonstrated to inhibit currents through voltage-gated Ca^{2+} channels (5) and may influence other membrane-potential-sensitive processes (21). Thus,

it is possible that depolarization, per se, may antagonize the effects of hypoxia by shifting the voltage-dependent activation of one or more of these processes. Tateishi and Faber (22,23) argued against an effect of hypoxia on L-type Ca^{++} channels in cremaster arterioles, but did not critically test this hypothesis. Further research will be required to resolve this issue and determine the mechanism by which hypoxia inhibits norepinephrine-induced contraction of cremasteric arteriolar muscle cells.

In the present study, norepinephrine was found to decrease holding current at -60 mV, an effect that could be attenuated by pre-exposure of the cells to glibenclamide. These data support the findings of Bonev and Nelson (1) that vasoconstrictors that activate protein kinase C (PKC), such as norepinephrine acting at α -adrenergic receptors, close K_{ATP} channels. These data may also provide an explanation for the decrease in oxygen reactivity that was observed in rat gracilis arteries exposed to norepinephrine or serotonin (6). In those studies, hypoxic dilation most likely resulted from hypoxia-induced release of prostacyclin from the endothelium, which subsequently opens K_{ATP} channels in vascular muscle cells leading to hyperpolarization and vasodilation as has been reported in rat cerebral arteries (15). However, in the presence of norepinephrine or serotonin, both of which act, in part, by activating protein kinase C, the K_{ATP} channels in the vascular muscle would be closed, preventing the effects of prostacyclin on these channels.

Tateishi and Faber (22,23) have argued that activation of $\alpha_{2\text{D}}$ -adrenergic receptors, but not activation of $\alpha_{1\text{D}}$ -adrenergic receptors, closes K_{ATP} channels through an undefined mechanism. Data from the present study neither support nor refute these findings as the specific α -adrenergic receptor subtype involved in the effects of norepinephrine on K_{ATP} currents was not determined. However, studies in other vessels (1) have shown that phenylephrine ($1 \mu\text{M}$), an $\alpha_{1\text{D}}$ -adrenergic agonist, inhibits current through K_{ATP} channels in a PKC-dependent fashion. Thus, it is not clear why both $\alpha_{1\text{D}}$ -adrenergic and $\alpha_{2\text{D}}$ -adrenergic agonists did not display glibenclamide-sensitive responses in the studies by Tateishi and Faber (22,23). The interaction of K_{ATP} channels with specific α -adrenergic receptors and the signal transduction pathways involved in these interactions should be the topic of future investigations.

In conclusion, results from the present study indicate that cremasteric arteriolar muscle cells are intrinsically sensitive to oxygen in that hypoxia can inhibit norepinephrine-induced contraction of these cells. However, this oxygen sensitivity does not involve the activation of arteriolar muscle cell K_{ATP} channels. It is hypothesized that hypoxia acts to modulate some membrane-potential-sensitive process, such that the effects of hypoxia can be limited by prior membrane depolarization. Future studies will be required to determine the precise mechanism by which hypoxia modulates norepinephrine reactivity of these cells.

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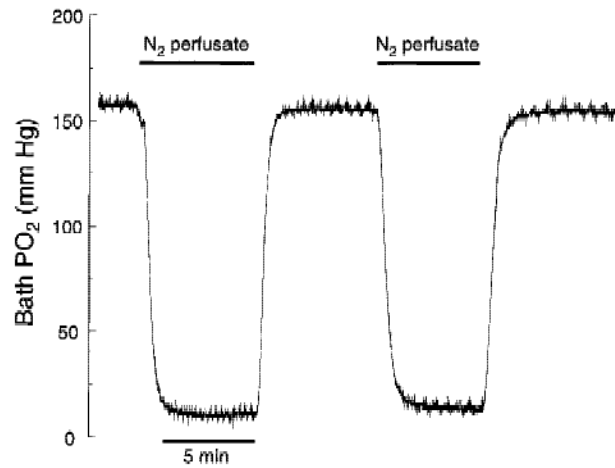


Figure 1.

Time course of PO₂ change in patch clamp bath. Shown in a typical record of bath PO₂ measured with a miniature Clark-type oxygen electrode (Microelectrodes, Inc., Londonderry, NH). As indicated by the solid line, the perfusate through the bath was rapidly switched from one equilibrated with room air (PO₂ = 156 mm Hg) to one equilibrated with N₂ (PO₂ = 12 ± 1 mm Hg, measured in the bath in five experiments similar to that shown in the figure). The flow through the bath in the experiment shown was 2–3 ml/min.

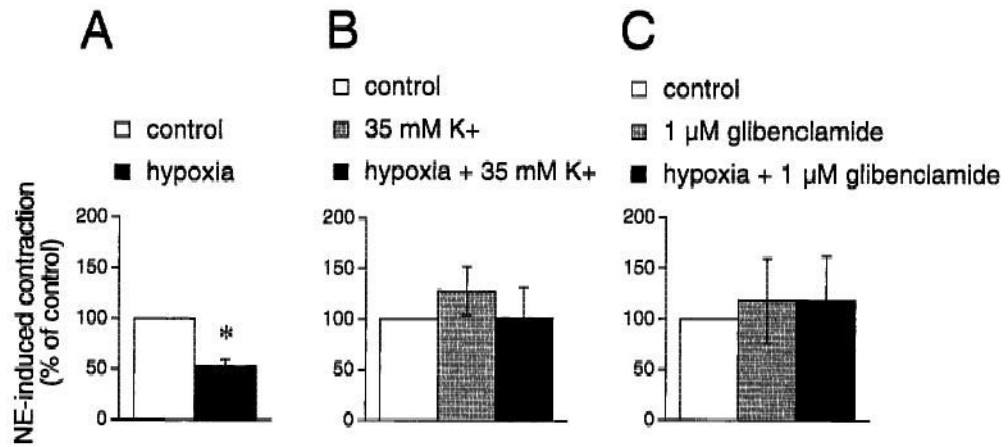


Figure 2.

Hypoxia-induced inhibition of contraction is prevented by 35 mM K^+ or glibenclamide. Data are mean \pm SE percentage of cells contracting to pipette application of 1 μ M norepinephrine (NE), normalized to the response of cells to control PSS. (A) Hypoxia (12–15 mm Hg PO_2) inhibits norepinephrine-induced contraction, $n = 12$; (B) 35 mM K^+ prevents effects of hypoxia, $n = 5$; and (C) glibenclamide prevents effects of hypoxia, $n = 4$. (* = significantly different from control, $p < 0.05$.)

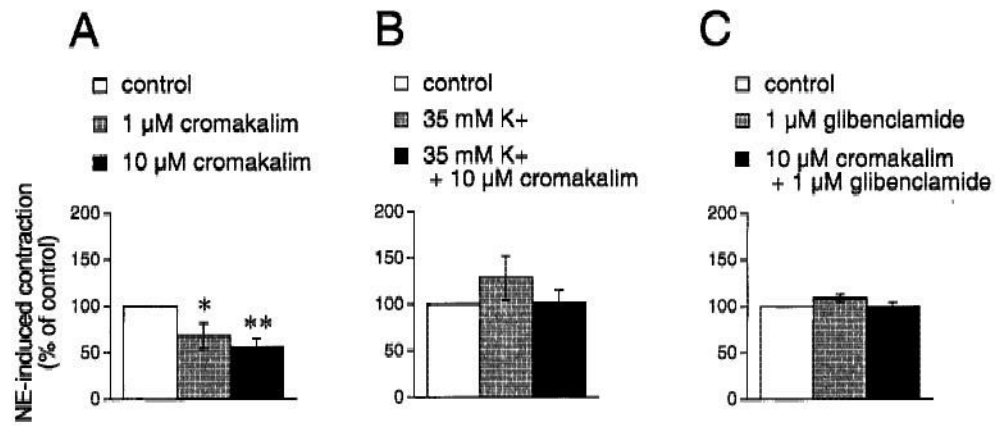


Figure 3. Cromakalim-induced inhibition of contraction is prevented by 35 mM K^+ or glibenclamide. Data are mean \pm SE percentage of cells contracting to pipette application of 1 μM norepinephrine (NE), normalized to the response of cells to control PSS, as in Fig. 1. (A) Cromakalim inhibits norepinephrine-induced contraction in a concentration-dependent fashion, $n = 6-7$; (B) 35 mM K^+ prevents effects of 10 μM cromakalim, $n = 3$; (C) glibenclamide prevents effects of 10 μM cromakalim, $n = 6$. (* = significantly different from control; ** = significantly different from control and 1 μM cromakalim, $p < 0.05$.)

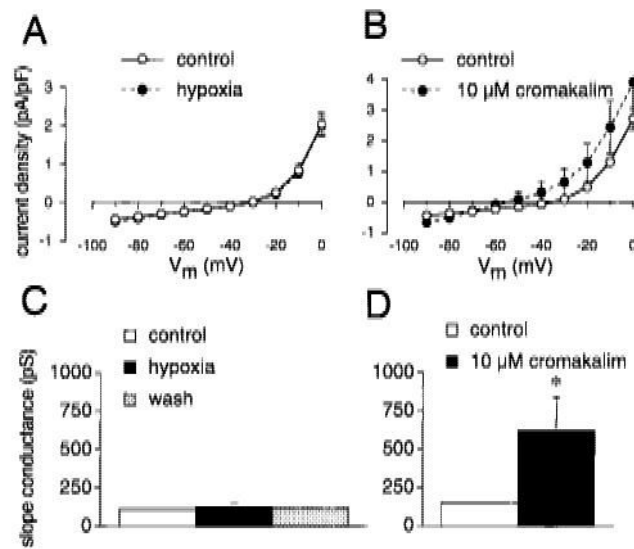


Figure 4.

Hypoxia has no effect on current–voltage relationship or membrane conductance in cremasteric arteriolar muscle cells. (A) Data are mean \pm SE current densities for eight different cells. Bath solution contained, in mM: 140 NaCl, 5 KCl, 1 MgCl₂, 2 mM CaCl₂, 10 HEPES, 10 glucose; pH = 7.4 with NaOH at room temperature, 295–300 mOsm. Pipette solution contained, in mM: 100 K-aspartate, 43 KCl, MgCl₂, 10 HEPES, 0.5 EGTA; pH 7–7.2 adjusted with NaOH, 295–300 mOsm adjusted with sucrose. Two-way analysis of variance indicated a significant effect of voltage on current density ($p < 0.05$), but no significant effect of hypoxia on current density ($p > 0.05$). (B) Mean \pm SE current densities for seven different cells in the absence (control) and presence of 10 μ M cromakalim. Solutions as in (A). Two-way analysis of variance indicated a significant effect of both voltage and cromakalim on current densities ($p < 0.05$). (C) Mean whole-cell membrane conductance \pm SE estimated from the slope of the line fitted to currents measured from –90 to –40 mV in eight cells before (control), during, and after (wash) exposure of the cells to hypoxia. Analysis of variance indicated no significant differences among the means ($p > 0.05$). (D) Mean whole-cell conductance as in (C), before (control) and during exposure of seven cells to 10 μ M cromakalim. In every experiment, cromakalim increased whole-cell conductance. (* = significantly different from control, $p < 0.05$).

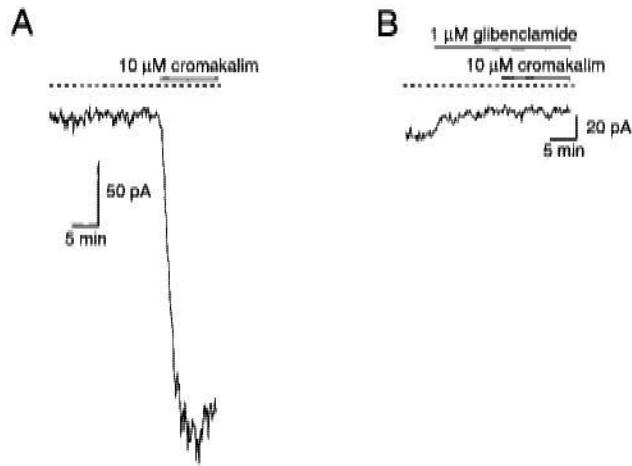


Figure 5.

Effects of cromakalim and glibenclamide on holding current. Shown are digitized records of holding currents in two different cells held at -60 and superfused with solutions containing, in mM: 143 KCl, 1 MgCl₂, 2 mM CaCl₂, 10 HEPES, 10 glucose; pH = 7.4 with NaOH at room temperature, 295–300 mOsm adjusted with sucrose. Pipette solution contained, in mM: 100 K-aspartate, 43 KCl, 1 MgCl₂, 10 HEPES, 0.5 EGTA; pH 7–7.2 adjusted with NaOH, 295–300 mOsm adjusted with sucrose. Currents were digitized at 5 kHz, and then smoothed by taking a box car average every 100 ms. (A) Cromakalim (10 μM) produced a large increase in current; (B) glibenclamide (1 μM) decreased holding current and abolished effects of cromakalim. The dashed line in both panels represents the zero current level. See text and Fig. 6 for more information.

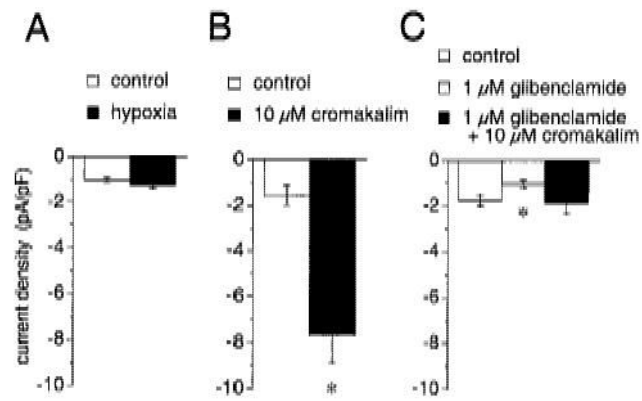


Figure 6.

Effects of hypoxia and cromakalim on membrane holding current. Data are mean \pm SE, holding currents measured at -60 mV in symmetrical 143 mM K^+ . Bath solution contained, in mM: 143 KCl, 1 $MgCl_2$, 2 mM $CaCl_2$, 10 HEPES, 10 glucose; pH = 7.4 with NaOH at room temperature, 295 – 300 mOsm adjusted with sucrose. Pipette solution contained, in mM: 100 K-aspartate, 43 KCl, 1 $MgCl_2$, 10 HEPES, 0.5 EGTA; pH 7 – 7.2 adjusted with NaOH, 295 – 300 mOsm adjusted with sucrose. (A) Lack of effect of hypoxia, $n = 9$; (B) cromakalim increases holding current at -60 mV in a concentration-dependent fashion, $n = 7$; (C) glibenclamide decreases holding current at rest, and prevents effects of cromakalim, $n = 5$. (* = significantly different from control, $p < 0.05$.)

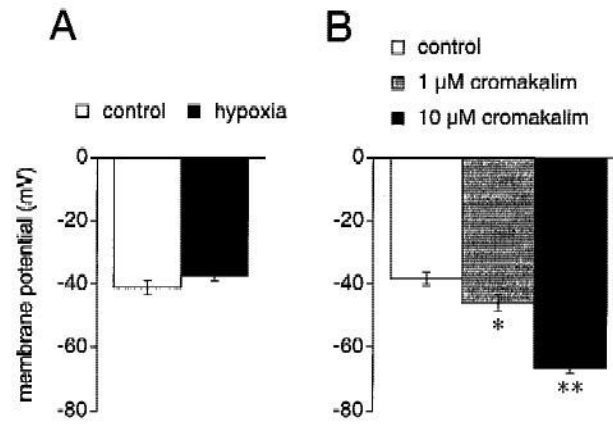


Figure 7.

Effects of hypoxia and cromakalim on membrane potential. Data are mean \pm SE membrane potentials recorded in current clamp. Bath solution contained, in mM: 140 NaCl, 5 KCl, 1 MgCl₂, 2 mM CaCl₂, 10 HEPES, 10 glucose; pH = 7.4 with NaOH at room temperature, 295–300 mOsm. Pipette solution contained, in mM: 100 K-aspartate, 43 KCl, 1 MgCl₂, 10 HEPES, 0.5 EGTA; pH 7–7.2 adjusted with NaOH, 295–300 mOsm adjusted with sucrose. (A) Lack of effect of hypoxia on membrane potential, $n = 9$; (B) cromakalim hyperpolarizes cremasteric arteriolar muscle cells in a concentration-dependent fashion, $n = 15–18$. (* = significantly different from control; ** = significantly different from control and 1 μ M cromakalim, $p < 0.05$.)

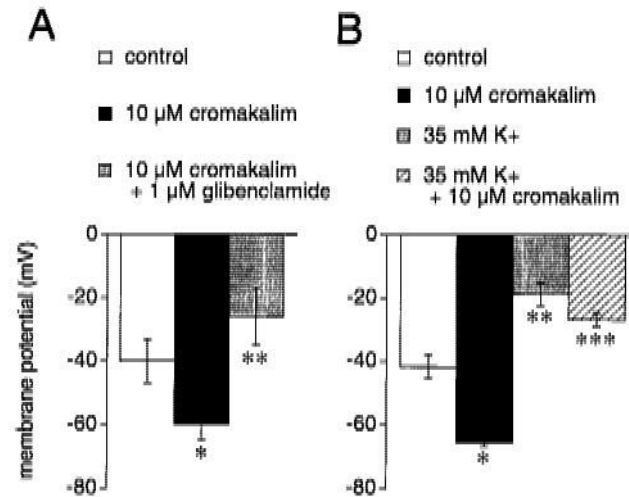


Figure 8.

Glibenclamide and 35 mM K^+ inhibit effects of cromakalim on membrane potential. Data are mean \pm SE membrane potential. Bath and pipette solutions as in Fig. 7. (A) glibenclamide inhibits effects of 10 μM cromakalim, $n = 5$; (B) 35 mM K^+ depolarizes cells and blunts effects of 10 μM cromakalim, $n = 3$. (* = significantly different from control; ** = significantly different from control and 10 μM cromakalim, $p < 0.05$; *** = significantly different from 35 mM K^+ and 10 μM cromakalim, $p < 0.05$.)