Oxygen induces electromechanical coupling in arteriolar smooth muscle cells: a role for L-type Ca\(^{2+}\) channels

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Welsh, Donald G., William F. Jackson, and Steven S. Segal. Oxygen induces electromechanical coupling in arteriolar smooth muscle cells: a role for L-type Ca\(^{2+}\) channels. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2018–H2024, 1998.—We tested whether O\(_2\)-induced vasomotor responses of arteriolar vessels correspond to changes in membrane potential (E\(_m\)) of cells in the arteriolar wall. The cheek pouches of anesthetized hamsters were prepared for intravital microscopy and intracellular recording. Microelectrodes containing Lucifer yellow dye were used to label smooth muscle cells (SMC) or endothelial cells (EC) during arteriolar responses to O\(_2\). During low-PO\(_2\) superfusion (~20 Torr; arteriolar diameter 55 ± 2 µm), E\(_m\) of SMC and EC averaged ~37 and ~36 mV, respectively. High-PO\(_2\) superfusion (~150 Torr) depolarized SMC (to ~15 ± 1 mV) with vasodistraction (to 24 ± 2 µm) and diameter cycled with E\(_m\) of SMC during vasodistraction. In contrast, the E\(_m\) of EC did not change with PO\(_2\) nor during vasomotion, yet E\(_m\) depolarized by 21 ± 2 mV when the extracellular K\(^+\) concentration ([K\(^+\)]\(_e\)) was raised to 55 mM. Superfusion with diltiazem (10 µM) or nifedipine (1 µM) abolished vasomotor and electrical responses to PO\(_2\) in SMC but did not eliminate depolarizations to elevated [K\(^+\)]\(_e\). We conclude that, under physiological conditions, electrical and mechanical responses of arteriolar SMC to changes in PO\(_2\) are mediated through L-type Ca\(^{2+}\) channels without corresponding electrical activity in EC.

arteriole; blood flow control; membrane potential; microcirculation; oxygen reactivity; vascular smooth muscle

The goal of the present study was to determine whether O\(_2\)-induced vasomotor responses of arterioles correspond to E\(_m\) changes in cells of the arteriolar wall. In turn, we hypothesized that if Ca\(^{2+}\) influx depends on changes in K\(^+\) conductance, then antagonism of L-type Ca\(^{2+}\) channels should inhibit the vasomotor effects of O\(_2\) with little effect on depolarization. Alternatively, if L-type Ca\(^{2+}\) channel activation underlies both electrical and mechanical responses, then antagonism of these channels should block both responses to O\(_2\).

METHODS

Hamster cheek pouch preparation. All procedures were approved by the Animal Care and Use Committee of The John B. Pierce Laboratory and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC: Natl. Acad. Press, 1996). Male Golden hamsters (90–130 g, n = 18; Charles River Breeding Laboratories) were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized to ensure airway patency. A cannula secured in the left femoral vein enabled continuous replacement of fluids and maintenance of anesthesia throughout experiments (10 mg pentobarbital sodium/ml isotonic saline, infused at 0.41 ml/h). Esophageal temperature was maintained at 37–38°C with conductive heating.

With the use of a stereomicroscope (model DR, Zeiss), the cheek pouch was exteriorized onto a Plexiglas board and superficial connective tissue was removed. The preparation was superfused continuously with a bicarbonate-buffered PSS (37°C; pH 7.4) of the following composition (in mM): 137.0 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 10.0 CaCl\(_2\), and 18.0 NaHCO\(_3\); salts were obtained from Sigma or J. T. Baker. Under control conditions the PSS was equilibrated in a 50-ml reservoir with 5% CO\(_2\)-95% N\(_2\); this condition (referred to as "low" PO\(_2\)) corresponded to a PO\(_2\) of ~20 Torr in the superfusate and ~20–25 Torr in the tissue (5, 15, 16). The preparation was placed on the stage of an intravital microscope (modified model 20T, Zeiss) and transilluminated with a 100-W halogen lamp (condenser NA = 0.32, Zeiss) and observed through a long-working-distance objective (Leitz UM 32; NA = 0.30).

In SMC of coronary (3, 23) and cerebral (8) arteries, a fall in PO\(_2\) has been found to activate ATP-sensitive K\(^+\) channels and Ca\(^{2+}\)-activated K\(^+\) channels (K\(_{Ca}\)), respectively. The resulting hyperpolarization limits Ca\(^{2+}\) influx through voltage-sensitive, L-type Ca\(^{2+}\) channels to produce smooth muscle relaxation. In contrast, O\(_2\) may act directly on L-type Ca\(^{2+}\) channels to regulate Ca\(^{2+}\) entry into SMC independent of changes in K\(^+\) conductance (6, 7). In arterioles, it is unclear whether membrane potential (E\(_m\)) is influenced by the ambient PO\(_2\), particularly under physiological conditions. If so, then changes in PO\(_2\) could regulate arteriolar diameter via electromechanical coupling (22, 25).
yellow dye (lithium salt, 4% solution in deionized H2O; Sigma) and the remainder was filled with 150 mM LiCl2. The microelectrode was secured in a single-axis hydraulic micromanipulator (model MX510, SOMA) that was mounted on a three-way mechanical micromanipulator (model H56, World Precision Instruments).

Membrane potential was measured with an electrometer (Duo 773, World Precision Instruments). To record E_m, a microelectrode was positioned above and parallel to the axis of a second- or third-order arteriole at a penetration angle of ~45°. While observed through the microscope, the tip of the microelectrode was lowered carefully onto the edge of the vessel and a cell was impaled by gradually advancing the hydraulic micromanipulator. The criteria for a successful penetration were 1) sharp, negative deflection of potential on entry, 2) stable recording of E_m for at least 1 min, and 3) sharp, positive deflection on exit; tip potentials on exit averaged <2 mV.

Cell labeling often occurred with diffusion of Lucifer yellow dye from the microelectrode during the recording period (typically 5–20 min); in some experiments, dye was microinjected by passing negative current (5 nA for 1 min) through the recording microelectrode. The cell type recorded from was identified using epifluorescence (75-W Xenon lamp; Zeiss filter set 48 77 05), as viewed through a ×40 immersion objective (NA = 0.75, Zeiss) (24). Internal diameter (ID) of arterioles was measured from the monitor screen with the use of a video caliper (modified model 321; Colorado Video Instruments); spatial resolution was <2 µm.

Simultaneous outputs from the electrometer and video caliper were digitally recorded at 40 Hz (MatLab 8s, AD Instruments). For summary data (Table 1), respective values for E_m and diameter were obtained by averaging data points for a 1,000-ms interval during a stable E_m (~75% of experiments) or for 500-ms intervals coincident with the corresponding peaks of depolarization and vasoconstriction (remaining experiments).

Microiontophoresis. Micropipettes (tip ID 1 µm) were fabricated by using the same capillary tubes and puller as for the recording microelectrode; these were backfilled with phenylephrine hydrochloride (PE, 0.5 M; Sigma). The identity of SMC and EC was tested functionally by microiontophoresis of PE onto the abluminal surface of an arteriole. In the hamster cheek pouch, this selective a1-adrenoceptor agonist depolarizes arteriolar SMC but not EC (24).

Experiment 1: Does O2 influence E_m of cells in the arteriolar wall? Once a stable E_m and vessel diameter were attained (~1 min), a PE micropipette was hydraulically positioned (model MO-102, Narishige) with its tip in close proximity to the recording microelectrode. While E_m and diameter were monitored, a PE stimulus (500-nA, 500-ms pulse) was delivered. After 3–4 min of recovery, the superfuse was equilibrated with gas containing 21% O2 (balance 5% CO2-74% N2, referred to as “high” PO2); this produced a superfuse PO2 of ~150 Torr and a tissue PO2 of ~60–65 Torr (5, 15, 16). In cases where high PO2 recordings were maintained for at least 5 min, the superfuse was then reequilibrated with low PO2. If PE and elevated PO2 elicited vasomotor responses without a corresponding change in E_m (see RESULTS), KCl was added to the 50-mL reservoir to produce an extracellular K⁺ concentration ([K⁺]_o) of ~55 mM in the superfuse; depolarization confirmed successful impalement and intracellular recording (24).

Experiment 2: Are O2-induced responses inhibited by antagonists of L-type Ca²⁺ channels? Superoxide PO2 was increased from low to high to ascertain arteriolar constriction in response to elevated PO2. The cheek pouch was then reequilibrated (~20 min) under low PO2, with diltiazem (10 µM; Sigma) or nifedipine (1 µM; Sigma) in the superfuse. A cell in the arteriolar wall was impaled and a stable recording confirmed (as in experiment 1). Superoxide was raised to high PO2 for 5–10 min and then returned to low PO2 while E_m and vessel diameter were recorded. In light of the effect of L-type Ca²⁺-channel antagonists on the response to high PO2 (see RESULTS), [K⁺]_o was elevated (as in experiment 1) to ascertain the success of recording.

Table 1. Membrane potential and diameter responses to changes in superfusate PO2

<table>
<thead>
<tr>
<th>PO2</th>
<th>E_m, mV</th>
<th>Diameter, µm</th>
<th>E_m, mV</th>
<th>Diameter, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low</td>
<td>-35.7 ± 1.0 (17)</td>
<td>52.3 ± 2.5</td>
<td>-37.1 ± 2.8 (10)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-36.4 ± 0.9</td>
<td>21.9 ± 1.9</td>
<td>-15.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Response</td>
<td>0.7 ± 0.4</td>
<td>30.3 ± 5.2</td>
<td>-21.9 ± 2.6</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Low</td>
<td>-35.8 ± 1.5 (9)</td>
<td>52.3 ± 3.4</td>
<td>-35.3 ± 1.3 (11)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-35.7 ± 1.8</td>
<td>49.6 ± 3.2</td>
<td>-31.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Response</td>
<td>-0.1 ± 0.6</td>
<td>2.6 ± 1.0</td>
<td>-3.9 ± 1.0</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Low</td>
<td>-35.6 ± 2.0 (4)</td>
<td>62.3 ± 1.8</td>
<td>-32.3 ± 0.8 (7)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-35.9 ± 1.9</td>
<td>61.5 ± 1.6</td>
<td>-30.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Response</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.3*</td>
<td>-2.1 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE for number of observations (n) for each experiment, shown in parentheses. Membrane potential (E_m) and arteriolar diameter responses were monitored in separate experiments under control conditions (low PO2) in presence of diltiazem (10 µM) or nifedipine (1 µM) and while superfuse PO2 was increased from ~20 Torr (low) to ~150 Torr (high). "Response" was calculated as difference in values between low and high PO2. See METHODS for details. *Significant difference from control response, P < 0.05.
RESULTS

Cell identification. Cell type was identified anatomically by examining the pattern of dye labeling after intracellular recording (Fig. 1). An SMC appeared as a narrow band wrapped circumferentially around the arteriole, with dye localized to the injected cell. In contrast, EC typically appeared as a narrow band parallel to the vessel axis, with dye spreading from the injected EC into many EC along and around the vessel lumen. This difference in dye-coupling properties between cell types has been reported in vivo (21, 24) and in vitro (17). In nine experiments, cell identity was further confirmed by the nature of intracellular recording during PE microiontophoresis (24). As shown in Figs. 2 and 3, PE consistently depolarized SMC (n = 4) yet had no effect on the E_m of EC (n = 5). Control experiments verified that simply passing current through a microiontophoresis pipette filled with isotonic saline did not produce responses.

Control E_m and diameter. A summary of E_m and diameter values recorded throughout these experiments is given in Table 1. During low-PO_2 superfusion, resting E_m averaged approximately -36 mV and was not different between EC and SMC.

Experiment 1: Influence of O_2 on cells in arteriolar wall. The elevation of superfusate PO_2 elicited SMC depolarization and arteriolar constriction (Table 1). The mechanical responses to elevated PO_2 were characterized as being essentially sustained (with slight oscillations, 20 of 27 arterioles; Fig. 2) or rhythmically cycling "vasomotion" (2–7 cycles/min, 7 arterioles; Fig. 3). Diameter changes in response to high PO_2 were typically preceded (~2 s) by corresponding changes in the E_m of SMC, with depolarization leading constriction and repolarization leading dilation. In marked contrast, high PO_2 had no effect on the E_m of EC. Nevertheless, EC promptly depolarized (with arteriolar constriction) by 21 ± 2 mV (n = 18) on elevation of [K+]_o. Mechanical and electrical responses to high PO_2 were consistently reversed on restoration of low PO_2 (data not shown).

Experiment 2: Effects of L-type Ca^{2+}-channel antagonists on responses to O_2. During low-PO_2 superfusion, the addition of diltiazem or nifedipine to the superfusate produced a transient (2–3 min) dilation of arterioles, which then recovered. Thus antagonism of L-type Ca^{2+} channels had no lasting effect on resting tone or E_m (Table 1). This finding is consistent with the maintenance of tone in arterioles of the rat cremaster muscle during similar exposure to nifedipine (11). Taken together, these observations indicate pathways for Ca^{2+} entry into arteriolar SMC other than through L-type Ca^{2+} channels. Nevertheless, both diltiazem and nifedipine effectively prevented the depolarization and constriction of SMC in response to high PO_2 (Fig. 4).

In the presence of either diltiazem or nifedipine, raising [K+]_o continued to produce sustained depolarization in SMC and EC. However, in contrast to the accompanying vasoconstriction seen under control conditions (Figs. 2 and 3), these depolarizations were associated with a slow and progressive vasodilation (Fig. 4) from a resting diameter of 57 ± 2 to a peak of 79 ± 2 µm (n = 22; P < 0.05, paired t-test). In approximately one-half of the SMC recordings, elevated [K+]_o initiated a slight, transient hyperpolarization before depolarization (Fig. 4) that occurred whether or not (data not shown) antagonists were present. Control experiments verified that arterioles regained sensitivity to O_2 following the washout of diltiazem or nifedipine (data not shown).

DISCUSSION

We present the first intracellular recordings from the wall of arterioles undergoing vasomotor responses to changes in ambient PO_2. Our findings in the hamster cheek pouch demonstrate that SMC depolarized and arterioles constricted when superfusate PO_2 was increased from ~20 Torr to ~150 Torr and tissue PO_2 was increased by ~40 Torr (5, 16). This correspondence between depolarization and vasoconstriction with elevation of PO_2 indicates that electromechanical coupling is the basis of the vasomotor response to O_2 under physi-
ological conditions. Moreover, both depolarization and contraction of SMC were prevented by antagonism of L-type Ca$^{2+}$ channels, indicating that the O$_2$-induced influx of Ca$^{2+}$ is central to both electrical and mechanical events. Furthermore, EC remained electrically quiescent during changes in PO$_2$, providing additional evidence (24) against electrical coupling between EC and SMC layers during blood flow control.

Specificity of cellular responses. Raising superfusate PO$_2$ constricted arterioles and depolarized SMC, with recordings confirmed anatomically by dye labeling (Fig. 1) and functionally by responses to PE (Figs. 2 and 3). The elevation of superfusate PO$_2$ elicited sustained (with oscillation) vasoconstriction and depolarization in SMC; in contrast, EC had no electrical response to PE or to high PO$_2$, yet prompt depolarization occurred on exposure to 55 mM [K$^+$].

Fig. 2. Membrane potential ($E_m$) and diameter responses in arterioles exposed to high PO$_2$. SMC and EC were impaled during low PO$_2$; responses to phenylephrine (PE), high PO$_2$, or 55 mM extracellular K$^+$ concentration ([K$^+$]$_o$) were then monitored (see METHODS and RESULTS for details). In these recordings, high PO$_2$ elicited sustained (with oscillation) vasoconstriction and depolarization in SMC; in contrast, EC had no electrical response to PE or to high PO$_2$, yet prompt depolarization occurred on exposure to 55 mM [K$^+$].

Fig. 3. Rhythmic cycling of $E_m$ and arteriolar diameter in response to high PO$_2$ (see METHODS and RESULTS for details). In SMC, note correspondence between cycling of $E_m$ and diameter; mechanical responses lag electrical responses by ~2 s. In contrast, $E_m$ of EC was unaffected by high PO$_2$ in presence of vasomotion. EC promptly depolarized on exposure to 55 mM [K$^+$].
analogous vasomotor responses to elevated \( P O_2 \) have been documented (5, 13, 15, 18, 20), this is the first study performed in vivo that demonstrates the correspondence between \( O_2 \)-induced changes in arteriolar diameter and the electrical activity of SMC in the arteriolar wall.

The \( E_m \) of EC [confirmed by dye labeling (Fig. 1) and by lack of response to PE] was unaffected by changes in superfusate \( P O_2 \) (Figs. 2 and 3). This lack of response cannot be explained by the inability of EC to depolarize, because elevating \([K^+]_o\) to 55 mM reduced the \( E_m \) of EC by an average of 21 mV. Our finding that electrical responses to \( O_2 \) were confined to SMC argues further that SMC are not electrically coupled to EC under physiological conditions (24). This conclusion is supported by recent in vitro studies of rat iridial arterioles (12) yet contrasts with the proposal of heterologous coupling between EC and SMC in isolated cheek pouch arterioles (17, 26).

The changes in \( E_m \) with constriction and dilation could reflect a piezoelectric effect associated with bending of the microelectrode tip during intracellular recording. If this were true, however, then \( O_2 \)-induced vasomotion should elicit similar changes in \( E_m \), irrespective of the cell type from which they were recorded. As noted earlier, recordings from EC were consistently stable during pronounced changes in diameter (Figs. 2 and 3). Dissociation of mechanical and electrical events was also observed when \([K^+]_o\) was raised; cells consistently depolarized, yet arterioles constricted under control conditions and dilated in the presence of diltiazem or nifedipine. Furthermore, changes in \( E_m \) consistently preceded those in diameter by \( \sim 2 \) s (Ref. 24 and present data), which is inconsistent with an electrical event arising from mechanical deformation. Lastly, control experiments in which the tip of a microelectrode was positioned in the tissue and moved to approximate displacement during vasomotion had no effect on the potential recorded. We conclude that our electrophysiological measurements are free from mechanical artifact.

**Role for L-type \( Ca^{2+} \) channels in arteriolar smooth muscle responses to \( O_2 \).** In coronary (3, 23) and cerebral (8) arteries, SMC depolarize in response to elevated \( PO_2 \). This depolarization, which activates L-type \( Ca^{2+} \) channels and elicits vasoconstriction, has been attributed to reductions in \( K^+ \)-channel conductance (\( g_K \)) (3, 8, 23). In the present study, if alterations in \( g_K \) were the basis of \( O_2 \)-induced depolarization of arteriolar SMC, then depolarization should still have occurred, even if contraction were prevented by the inhibition of \( Ca^{2+} \) influx. Figure 4 shows that nifedipine abolished both vasomotor and electrical responses to elevated \( O_2 \); similar results were obtained with diltiazem (Table 1). Such consistent findings with both antagonists support the conclusion that electrical and mechanical responses of arteriolar SMC to \( O_2 \) reflect the primary involvement of L-type \( Ca^{2+} \) channels, independent from changes in \( g_K \).

The mechanism by which molecular \( O_2 \) acts on L-type \( Ca^{2+} \) channels to govern the contractile activity of arteriolar SMC remains unclear. It is possible that these channels are involved in the release of paracrine factors from parenchymal cells (5, 15), EC (18, 20), or red blood cells (5a) in response to changing \( P O_2 \), with such factors eliciting electrical responses in SMC. Alterna-
tively, O_2 may directly interact with L-type Ca^{2+} channels in the SMC membrane and influence their gating characteristics, as recently reported for SMC isolated from arteries (6, 7). Nevertheless, the correspondence between vasomotor and electrophysiological events in arteriolar SMC (e.g., Fig. 3) indicates that the actions of P_O2 were ultimately expressed via electromechanical coupling (22, 25). In turn, the phase lag between electrical and mechanical responses reflects the intracellular events that occur between membrane excitation and contraction coupling (22).

The activation of L-type Ca^{2+} channels during high P_O2 could depolarize SMC by different mechanisms. First, the inward Ca^{2+} current could produce depolarization. If current through L-type Ca^{2+} channels was the basis of SMC depolarization, then the Ca^{2+} equilibrium potential must be relatively high under the conditions of our experiments. However, given the small magnitude of this current under physiological conditions [1–2 pA; (10)], depolarization would only occur if the input resistance of SMC was sufficiently high or if global changes in P_O2 activated a sufficient proportion of the available channels. Alternatively, increases in intracellular Ca^{2+} could indirectly depolarize SMC by influencing the conductance of other ion channels. For example, physiological increases in intracellular Ca^{2+} (e.g., from 100 to 500 nM) may limit the activity of voltage-activated K^+ channels (9) as well as augmenting the conductance of Ca^{2+}-activated Cl^- channels (19). Such events would lead to SMC depolarization, particularly if the rise in intracellular Ca^{2+} did not activate K_{Ca}. This latter condition appears likely because the Ca^{2+} threshold for activating K_{Ca} isolated from arteriolar SMC (~3 µM) is above physiological levels (14). It is clear that resolving the specific actions of O_2 on the membrane properties of arteriolar SMC requires additional study.

The elevation of [K^+]_o consistently depolarized SMC and EC. However, in contrast to the vasoconstriction observed under control conditions (Figs. 2 and 3), depolarization in the presence of L-type Ca^{2+}-channel antagonists was accompanied by slow and progressive vasodilation. Although the nature of this response is beyond the focus of this study, we offer the following explanation. The discontinuity between electrical and mechanical responses during high [K^+]_o may have been induced by the release of vasodilator peptides from depolarized sensory nerves (which are insensitive to diltiazem or nifedipine) that course through the cheek pouch (J. L. Morris, D. J. Grasby, and S. S. Segal, unpublished observations). Alternatively, the depolarization of EC by elevated [K^+]_o may have increased intracellular Ca^{2+} (1, 2), thereby stimulating nitric oxide production and SMC relaxation (4).

In summary, we have recorded E_m from defined cells in the wall of arterioles controlling blood flow to the hamster cheek pouch in response to physiological changes in ambient P_O2. Elevating superfusate P_O2 produced SMC depolarization and vasconstriction; when vasomotion was induced, arteriolar diameter cycled with the E_m of SMC. Remarkably, the E_m of EC remained stable during O_2-induced constriction and vasomotion, indicating that respective cell layers are electrically isolated from each other in vivo. Antagonism of L-type Ca^{2+} channels with diltiazem or nifedipine prevented SMC depolarization, constriction, and vasomotion in response to elevated P_O2. Thus O_2 (or a P_O2-linked substance) may regulate the flux of Ca^{2+} into SMC through L-type Ca^{2+} channels. The correspondence between O_2-induced changes in smooth muscle E_m and the diameter of arterioles in vivo indicates that electromechanical coupling is integral to the physiological control of tissue blood flow.

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