A Membrane Electrical Mechanism for Hypoxic Vasooconstriction of Small Pulmonary Arteries from Cat*  
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Using specially fabricated muscle myographs, we examined electrical and mechanical responses to reduction of PO2 in small (<300 μm) pulmonary arteries excised from cat lungs. Upon lowering PO2 from 400 to 50 mm Hg, these preparations consistently developed contractile responses concomitant with membrane depolarization and action potential generation. The largest changes in electromechanical responses to reduction of PO2 occurred between 150 and 50 mm Hg. These data strongly suggest that hypoxic activation of small pulmonary arteries is mediated by direct effects of reduced PO2 on muscle cell membrane ionic conductance systems.

The final common pathway for the control of vascular reactivity and, ultimately, blood flow, lies at the level of the vascular muscle cell. Changes in arterial blood gases (PO2 and PCO2) can exert profound effects upon the reactivity and "active state" of arterial muscle. Such responses are heterogeneous between vascular beds, i.e., hypoxia contracts pulmonary arterial muscle and increases pulmonary vascular resistance,1 while dilating cerebral arteries and increasing cerebral blood flow.2 Thus, the effect of PO2 or CO2 on arterial muscle of one vascular bed can not be universally applied to the vasculature as a whole.

It is our purpose to discuss some of the cellular mechanisms by which hypoxia enhances the "active state" of muscle cells within cat pulmonary arteries. Under rigorously controlled conditions, reducing PO2 from 400 to 30 mm Hg consistently results in contraction of small (<300 μm) pulmonary arteries with the largest response occurring between 150 and 30 mm Hg. The membrane electrical response to such changes in PO2 is membrane depolarization and action potential generation. The ionic mechanisms involved in such activation are discussed.

METHODS AND MATERIALS

Mongrel cats were anesthetized with a combination of ketamine and Na-pentobarbital, after which the thoracic cavity was opened and the lungs were removed. Large (>500 μm) and small (<300 μm) pulmonary arteries (measured within excised lungs) were removed following careful dissection of lung parenchymal, bronchial and venous tissue. Small arteries were removed with the aid of a binocular microscope. Arterial segments were mounted in two different ways, one for recording force development and another for recording intracellular electrical activity from arterial muscle cells with glass microelectrodes.

For recording of tension, 3-mm ring segments of pulmonary arteries were threaded by two 22-μ wires which were stretched across two open stainless steel jaws. One jaw was secured to a micrometer and the other connected to a very sensitive load cell (Kulite Semiconductor Inc) as previously described.3 Vessel segments were given a small passive load of 300 mg and allowed to equilibrate for 90 min by being continuously suffused with a physiologic salt solution containing (in mM): Na+, 141; K+, 4.7; Ca2+, 2.5; Mg2+, 1.2; Cl-, 124; H2PO4- 1.7; HCO3-, 25; and glucose, 11. Solutions were aerated with 95 percent O2/5 percent CO2 giving a PO2 of 400 and a PCO2 of 37 to 40 mm Hg as measured within the organ bath. Both gas concentrations and pH were continually monitored by sensing electrodes fixed inside the muscle chamber; PO2 and CO2 by electrodes connected to two radiometer gas analyzers and pH by a microprobe (Biochem International). Gas tensions were maintained within the bathing solutions by filling weather balloons with the desired mixture and pumping it through the aeration system. The entire chamber was covered with a Lucite dome to maintain a constant, uniform gas environment. The entire system was maintained at 37°C.

Electrical measurements were made with glass microelectrodes. For these measurements, 8-mm segments of small pulmonary arteries were threaded onto a glass pipe and tied in place with 22 μ silk suture. The opposite end and side branches were tied off creating a blind sack and transmural pressure adjusted to physiologic levels (5 to 10 mm Hg) as previously described.4 Microelectrode impalements were made from the adventitial surface. Gas tensions were monitored and changed similar to that described above. Similarly, arterial segments were pressurized and suffused with identical solutions as those described above.

RESULTS

Upon continuous reduction of PO2 from control values of 400 to 450 to 30 mm Hg, small pulmonary arteries with a

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Figure 1. Original chart recording depicting O2-dependent contraction and relaxation from a segment of small (250 μm [outer diameter]) pulmonary artery. As can be seen, spontaneous rhythmic activity occurs throughout this particular experiment. Active force development initially occurs at a PO2 of 300 to 250 mm Hg. Note the increase in duration of the rhythmic components as PO2 begins to fall. The steepest portion of the curve falls between 150 and 50 mm Hg. After sustained periods of low PO2 (50 to 30 mm Hg), one observes a small reduction in active force generation. When PO2 is elevated, there is a small "rebond" contraction prior to O2-dependent relaxation.
mean external diameter of around 300 μ exhibit consistent, uniform contractile responses (Fig 1). In 30 such preparations studied thus far, initial active force development begins at around 250 mm Hg. As can be seen in Figure 1, at 150 mm Hg the rate of force development increases sharply. The steepest portion of these responses occurs between the physiologic ranges of PO₂ of 150 to 50 mm Hg. In marked contrast, large pulmonary arteries from the same animals, treated identically as small ones, either showed no consistent response to hypoxia or contracted only slightly at low PO₂ levels (ie, <30 mm Hg).

In seven of 30 small pulmonary arteries studied, rhythmic contractions could be seen similar to those depicted in Figure 1. In these preparations, the first notable effect of reducing PO₂ is an increase in frequency of rhythmic contractions. These experiments were carried out by switching aeration gases from control (95 percent O₂/5 percent CO₂) to 95 percent N₂/5 percent CO₂ and measuring the change in bath PO₂. The change in PO₂ was exponential with a T½ of 1.25 minutes.

In a separate set of experiments we monitored the mechanical response of arterial segments to step changes in PO₂ (250, 150, 100, and 50 mm Hg). At each step change in PO₂, the arterial preparations contracted in an O₂ tension-dependent manner. The maximum contractile response occurred at 50 mm Hg, and at a level not significantly different from that observed in Figure 1 for the same PO₂. The force development to a given PO₂ expressed as percent maximum (50 mm Hg) was 4 ± 1 (SE) (250 mm Hg), 22 ± 4 (150 mm Hg) and 59 ± 5 (100 mm Hg). Preparations were re-equilibrated with control (95 percent O₂/5 percent CO₂) gases between stepwise changes.

In an effort to understand some of the cellular mechanisms of hypoxic vasoconstriction in cat pulmonary arteries, intracellular electrical activity was measured under control and hypoxic (50 mm Hg) conditions. To simulate physiologic parameters, these arteries were pressurized at a transmural pressure of 10 mm Hg to prevent variability in any myogenic membrane responses often seen in arterial muscle. At a PO₂ of 300 mm Hg the membrane potential (E_m) was −51 ± 1.4 mV. On reduction of PO₂ to 50 mm Hg the muscle cells within these pulmonary arteries depolarized to −37 ± 2.0 mV, measured in 38 cells from 11 different preparations.

This depolarization occurred within five minutes and did not appear to be due to inhibition of Na-K-ATPase, since it also occurred in the presence of ouabain (10⁻⁴ M). In all of the preparations studied in this manner, membrane depolarization was accompanied by action potential generation (Fig 2). These action potentials possess some of the characteristic properties of the Ca²⁺ dependent action potentials in most other arterial muscles studied (ie, low rate of rise, under-shooting, prominent pre and after potentials).

**Discussion**

These data demonstrate that small isolated pulmonary arteries from cat consistently exhibit contractile responses to hypoxia within physiologic ranges of PO₂. Such contractile behavior is accompanied by muscle membrane depolarization and action potential generation. It is interesting that this type of excitatory behavior was never observed in larger pulmonary arteries greater than 500 μ outer diameter. This difference between large and small pulmonary arteries may be due to differences in membrane properties. Differences in membrane properties between large and small arteries within the same vascular bed have been noted in lung as well as other vascular beds.

Action potential generation in arterial muscle is predominately Ca²⁺ dependent. Our findings that hypoxia induces action potentials suggests that reduction of PO₂ increases Ca²⁺ permeability in cat pulmonary arteries. We have demonstrated that the observed depolarization and subsequent development of spontaneous electrical activity is not due to reduction of outward K⁺ current but rather due to an increased conductance of Ca²⁺ and possibly Na⁺ (unpublished observations). However, we do not yet know whether such activity is due to a direct action on the pulmonary arterial muscle cell membrane, or due to release of substances or transmitters from endothelium or adventitial nerve endings.

Whatever the initial activation (ie, direct or chemically mediated) to hypoxia, if reducing PO₂ does, indeed, increase Ca²⁺ conductance by voltage-dependent mechanisms, the findings are consistent with the concept that Ca²⁺ channel blockers may be useful in treating disease states involving increased pulmonary vascular resistance to hypoxia.

**References**

The Site and Mechanism of Oxygen Sensing for the Pulmonary Vessels*

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Lung vessels are unique in the body in that they react to hypoxia with constriction rather than dilatation. Whether this characteristic is inherent in the lung vessel or is due to an influence from a sensor in the surrounding lung parenchyma is not resolved. Recent data, however, showing that vascular hypoxia as well as airway hypoxia can produce pulmonary vasoconstriction and that the sensor for alveolar hypoxia is upstream in the precapillary vessels, allows but does not prove the precapillary pulmonary artery itself to be the $O_2$ sensor. In addition, with the elimination of the mast cell as a necessary extravascular sensor for hypoxia at least in the mouse, there is no good candidate for an extravascular sensor for hypoxic pulmonary vasoconstriction.

Lung vessels react to hypoxia, in particular to alveolar hypoxia, with constriction which is limited to the hypoxic lung zone whether it be the entire lung or only a lobe. This is in striking contrast to systemic vessels, which dilate with hypoxia to carry more blood to the hypoxic organ. The unique vasoconstrictor response of lung vessels to hypoxic pulmonary vasoconstriction has been attributed to the presence of sensors in the pulmonary vasculature that are different from those present in the systemic circulation. These sensors are believed to be located in the pulmonary arteries and veins and to be involved in the control of pulmonary blood flow.

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