A Study of the Mechanisms Involved in the Pulmonary Arterial Pressor Response to Hypoxia

By Edward H. Bergofsky, M.D., and Seymour Holtzman, M.S.

ABSTRACT
The mechanisms underlying the pulmonary arterial pressor response to hypoxia have been investigated by studying specimens of pulmonary and systemic vascular smooth muscle and the isolated pulmonary and systemic circulations. Hypoxia caused a unique and reversible loss of potassium and gain in sodium by pulmonary arterial smooth muscle that did not occur in pulmonary veins or systemic arteries. These changes in electrolyte concentrations were calculated by the Nernst equation to be associated with depolarization of the smooth muscle cell of the pulmonary artery. Moreover, artificial hyperpolarization and depolarization in the isolated perfused pulmonary circulation were attended by blunting and accentuation, respectively, of the pulmonary arterial pressor response to hypoxia.

Such data suggest that one of the actions of hypoxia on the pulmonary circulation involves a depolarization of the muscle cell so that it is closer to its excitatory threshold and thereby more readily able to diminish the cross-sectional area of the pulmonary vascular bed.

ADDITIONAL KEY WORDS pulmonary vascular resistance vasoconstriction vascular smooth muscle intracellular electrolytes pulmonary veins systemic arteries transmembrane potential cat

One of the important determinants of the resistance to pulmonary blood flow and of the pulmonary arterial pressure is the level of oxygenation in either the blood or air of the lung (1). In most mammals tested, including unanesthetized man (2), hypoxia consistently raises pulmonary arterial pressure; moreover, it has been shown that this pressor response to hypoxia is based on vasoconstriction, inasmuch as left atrial pressure (3), alveolar or pleural pressure (4), and pulmonary blood flow (5) do not change sufficiently to account mechanically for this phenomenon.

Since vasoconstriction is regarded as the most likely explanation of the pulmonary pressor response to hypoxia, considerable attention has been directed toward the mechanisms whereby this vasoconstriction may come about: (a) the autonomic nervous system plays some role but it has not been considered to be the major one, since the pulmonary pressor response to hypoxia occurs after extirpation or blockade of this system (6-8); (b) the mediation of the pulmonary pressor response to hypoxia by various chemical agents, such as catecholamines, histamine, or serotonin does not seem to be the whole explanation of the mechanism in view of the occurrence of this pressor response in the isolated perfused lung as well as in the intact animal following the administration of specific blocking agents (9); and (c) the involvement of local nervous reflexes between various portions of the vascular bed cannot be readily substantiated either anatomically or physiologically (10).

Since a neurohumoral mechanism cannot explain the pulmonary pressor response to

From the Departments of Physiology and Rehabilitation Medicine, School of Medicine, New York University, New York, New York 10016.
This investigation was supported by Grant RT-1 from the Vocational Rehabilitation Administration, Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication March 25, 1967.
Hypoxia under all circumstances, it seems likely that the hypoxic effect can be mediated by a direct effect on the pulmonary vascular smooth muscle. To this end, several investigators have studied the possibility of an intervening chemical reaction; they have considered the effect of increased hydrogen ion concentration which may result if lactic acid should be produced within the smooth muscle cell during hypoxia (11, 12), as well as the action of agents capable of impairing oxygen or electron transport (13). The interpretations of these studies have been hampered by the absence of direct observations on the intracellular environment.

For these reasons, the present study was undertaken to determine whether a direct effect of hypoxia on pulmonary vascular smooth muscle could be detected. We found that hypoxia caused specimens of pulmonary arterial smooth muscle to lose potassium and gain sodium. Since these data suggested that the hypoxic effect on pulmonary arterial smooth muscle might be mediated through changes in cellular membrane potential, which are associated with the losses of cellular potassium, the isolated, perfused pulmonary circulation (and, for comparison, the hind limb systemic circulation) was used to assess the relationship between experimental changes in transmembrane electrolyte concentration, transmembrane electrical potential and the pulmonary pressor response to hypoxia.

Methods

ANALYSIS OF IONIC MOVEMENTS IN PULMONARY VASCULAR SMOOTH MUSCLE DURING HYPOXIA

The general method involved (a) the excision of specimens of smooth muscle from the pulmonary and systemic blood vessels of the cat, (b) the incubation of these specimens in both normally oxygenated and hypoxic Ringer’s solution, and (c) the analysis of electrolyte composition in both the tissue and the Ringer’s solution after a variable period of incubation. Adult cats were lightly anesthetized with pentobarbital (20 mg/kg, intraperitoneally) and then killed by opening the chest; specimens of pectoralis skeletal muscle and jejunal muscularis smooth muscle (about 300 mg each) were excised and treated the same way as blood vessels. Large and small pulmonary arteries (about 60 mg each) and veins were rapidly identified, dissected free, opened lengthwise and divided in half. Specimens of systemic arteries (femoral and carotid) were treated in the same way. All the vessels were stripped of their adventitia and, after opening, their intima was wiped away with gauze so that the specimen consisted chiefly of the muscular media. Each type of blood vessel was divided in half and placed in a series of cuvettes containing 1 ml of Ringer’s solution and continuously aerated with a mixture of 5% CO₂ and 95% O₂. The Ringer’s solution was adapted to duplicate the electrolyte concentrations we found in cat plasma. It contained the following concentrations of electrolytes: Cations: sodium = 135.0 mEq/liter; potassium = 5.5 mEq/liter; calcium = 4.0 mEq/liter; magnesium = 2.0 mEq/liter; anions: chloride = 115.0 mEq/liter; bicarbonate = 28.5 mEq/liter; 100 mg glucose was also added to each 100 ml. When this solution was equilibrated with gas mixtures with CO₂ tensions of approximately 35 mm Hg at 37°C, the pH of the Ringer’s solution was 7.40 ± .05.

To avoid the evaporation or dilution of the Ringer’s solution during the perfusion, which might alter the concentration of electrolytes, particular care was taken that the perfusing gases were saturated with water vapor and that the temperature throughout the system was constant. Identical weights of the Ringer’s solution before and after the procedure indicated that these conditions had been achieved. After 90 min, a hypoxic gas mixture was introduced into one of each pair of cuvettes by gassing with a mixture of 5% CO₂, 3% O₂ and 92% N₂; the other cuvette was continuously gassed with 5% CO₂ and 95% O₂. Thirty minutes later, all specimens, hypoxic and control, were removed from their Ringer’s solution bath, blotted, weighed and placed in 1 ml of nitric acid for digestion. To determine whether changes in ionic concentrations due to hypoxia were reversible, specimens from two cats were subjected to hypoxia for 30 min and then to 5% CO₂ and 95% O₂ for 30 min before being removed for digestion; they were compared with specimens of the same vessel which had been continuously exposed to 5% CO₂ and 95% O₂ for the same length of time.

The Ringer’s solution in which the equilibration took place and the nitric acid containing the completely digested tissue were each analyzed for their concentrations of sodium, potassium, chloride and calcium. The analyses of sodium utilized a Beckman Model B flame photometer, fueled by hydrogen and oxygen; readings were made at a wave length of 589 mµ using the photomultiplier tube; dilutions of the Ringer’s
and nitric acid solutions were made so that the concentrations in the unknown samples approximated those of the standard calibrating solutions by ± 20 mEq/liter. A similar dilution procedure was followed for potassium analysis, in order that samples approximated calibrating solutions by ± 5 mEq/liter, and these were compared on the flame photometer at a wave length of 768 mµ using a red-sensitive phototube. Known concentrations of sodium and potassium gave the same readings in Ringer's solution and in nitric acid. Measurements of pH were made by a radiometer glass microelectrode and meter. The calcium concentrations were determined by the rapid micromethod of Bett and Frazer (14) and the chloride concentrations by the method of Schales and Schales (15); both these techniques were adapted for use with an Oxford titrator (Oxford Laboratories, San Mateo, California).

The tissue concentrations of the cations were related to cellular concentrations by using an average value of 35% for extracellular space reported by Burnstock (16) for all types of smooth muscle, and were expressed in terms of the wet weight of the tissues before immersion in the Ringer's solution (mEq/kg wet weight). In order to determine whether hypoxia induced changes in the extracellular space of the vascular specimens, a separate series of experiments was used to measure the inulin space in specimens from five cats. Pulmonary arterial and systemic arterial specimens weighing 150 to 400 mg were immersed in 1 ml of Ringer's solution containing inulin levels of 30 mg/100 ml. These solutions were perfused with the same oxygenated and hypoxic gases listed above for the same periods of time. The specimens were then dried with a sponge, teased apart and immersed in inulin-free Ringer's solution for 24 hours, and the inulin concentration of this solution was measured by a resorcinol method (17). The average extracellular space calculated by this method was 38% during oxygenation and 34% during hypoxia for the pulmonary artery. The aortic extracellular spaces were 4% and 5% higher, respectively. These values do not differ significantly from those compiled by Burnstock.

Further evidence that the extracellular space was not materially changed by hypoxia was provided by careful weighing of tissue specimens before and after their immersion in the Ringer's solution. In no specimen, hypoxic or not, did the weight change by more than 1%, an observation which suggests that no large transfer of liquid occurred between the interstitial space and the Ringer's solution.

STUDIES IN THE ISOLATED PERFUSED LUNG OF THE CAT

Adult cats were lightly anesthetized by intraperitoneal injections of pentobarbital (15 mg/kg of body weight) and killed by bleeding from the carotid artery. The blood was heparinized and the plasma was separated by centrifugation. The chest was then opened, all nervous and vascular connections between the lungs and the remaining organs were cut, and the trachea, pulmonary artery and the left atrium were cannulated.

As illustrated by Figure 1, a positive-pressure Harvard apparatus pump was used to ventilate the lungs through the tracheal cannula with gases warmed to 37° and saturated with water vapor. These gases were obtained from tanks; their O₂ and CO₂ concentrations had previously

![FIGURE 1](image)

The isolated, perfused pulmonary circulation of the cat. Since both the mechanical respirator and the constant-flow perfusing pump supply moist gas and perfusate to the lung at 37° C, the isolated lung remains at body temperature. The flaccid membrane constituting the reservoir provided a constant pressure for the venous effluent and therefore a constant left atrial pressure. See text.

![FIGURE 2](image)

The isolated, perfused hind limb of the cat. See text.
### TABLE 1

**Effect of Hypoxia on Tissue Sodium and Potassium in Pulmonary and Systemic Vascular Smooth Muscle of the Cat**

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Tissue K⁺</th>
<th>Tissue Na⁺</th>
<th>External K⁺</th>
<th>External Na⁺</th>
<th>External Cl⁻</th>
<th>External Ca⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypoxia</td>
<td>Control</td>
<td>Hypoxia</td>
<td>Control</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Extrapulmonary artery</td>
<td>Avg</td>
<td>47.5</td>
<td>34.1</td>
<td>86.0</td>
<td>105.0</td>
<td>117.8</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.3</td>
<td>3.1</td>
<td>6.4</td>
<td>9.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.10</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intrapulmonary artery</td>
<td>Avg</td>
<td>43.7</td>
<td>37.3</td>
<td>98.5</td>
<td>119.0</td>
<td>133.4</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.8</td>
<td>0.8</td>
<td>6.6</td>
<td>8.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Pulmonary vein</td>
<td>Avg</td>
<td>36.0</td>
<td>36.6</td>
<td>90.0</td>
<td>100.2</td>
<td>131.0</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.1</td>
<td>1.8</td>
<td>7.2</td>
<td>12.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>Avg</td>
<td>39.5</td>
<td>36.5</td>
<td>100.4</td>
<td>110.0</td>
<td>134.0</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.7</td>
<td>3.0</td>
<td>8.0</td>
<td>7.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>Avg</td>
<td>43.0</td>
<td>42.5</td>
<td>95.8</td>
<td>96.8</td>
<td>131.1</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.9</td>
<td>2.4</td>
<td>11.2</td>
<td>12.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Intestine</td>
<td>Avg</td>
<td>66.0</td>
<td>61.0</td>
<td>64.8</td>
<td>55.2</td>
<td>138.0</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5.3</td>
<td>5.5</td>
<td>5.1</td>
<td>4.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Avg</td>
<td>95.0</td>
<td>96.5</td>
<td>49.0</td>
<td>51.0</td>
<td>130.0</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>6.0</td>
<td>7.2</td>
<td>5.2</td>
<td>4.7</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*Tissue" concentrations expressed as mEq/kg; "external" concentrations, i.e., in the Ringer's solution, expressed as mEq/liter. When a significant or possibly significant difference exists between electrolyte concentrations of control and hypoxic muscle, the P values have been listed in the appropriate columns. The differences between all other pairs of control and hypoxic data were not significant.*
been analyzed by the Scholander (18) microgas analyzer. Blood plasma was supplied to the pulmonary artery by a Sigmamotor pump so that the flow rate was constant throughout an entire experiment. These flow rates ranged from 100 to 600 ml/min, depending on the size of the animal, and produced average pulmonary arterial pressures ranging from 10 to 14 mm Hg. Left atrial pressure, or the outflow pressure of the lung, was held constant by means of a reservoir with a flaccid membrane, which is shown in the figure. Gas pressure in the trachea and blood pressure in the pulmonary artery were monitored continuously by strain gauges and a Grass Instruments oscillographic recorder. Only 20 min elapsed from the time of death to the time when artificial perfusion of the lung was established.

In order to maintain a relatively constant pH, all gases supplied to the lung contained 5% CO₂. Periods of normal oxygenation were provided by supplying gases containing 20% O₂, whereas hypoxic periods were obtained by supplying gases containing 3% O₂. This latter O₂ concentration was chosen because it simulated the O₂ tension of mixed venous blood in man when he is breathing gas containing 12% O₂. The concentration of electrolytes in the perfusate was altered by adding small quantities of plasma containing high concentrations of either sodium, potassium, or chloride to the reservoir so that homogeneous mixing could occur before the altered plasma was circulated to the lung. Additions were made and samples removed by means of the reservoir tap shown in the figure. By warming the inspired air and the plasma reservoir to 37°, the temperature of the lung (measured at the venous outflow) was maintained at normal body levels.

**STUDIES IN THE ISOLATED PERFUSED HIND LIMB OF THE CAT**

Cats were killed by the method described above and a hind limb disarticulated at the hip. It was extremely difficult to set up a closed circulation, because of the multiplicity of venous channels, many of which were too tiny to be closed. Accordingly, after the femoral artery was catheterized with polyethylene tubing, the venous effluent was allowed to drain freely into a funnel from which it could be discarded after sampling. Ringer's solution of the same electrolyte composition described above was pumped at a constant flow rate by means of a Sigmamotor pump into the femoral artery from storage bottles wherein the Ringer's solution had been adjusted to 37° and equilibrated with gases of known O₂ and CO₂ tensions. As Figure 2 illustrates, the apparatus was designed with reservoirs of Ringer's solution at different O₂ tensions, so that rapid changes in the gas composition of the perfusate could be obtained. Pressures in the femoral artery were measured by a Statham strain gauge, and the weight of the limb was continuously monitored by a force-displacement transducer. This transducer was calibrated by means of known weights placed at varying distances from the attachment to the limb; the output of both transducers was recorded by a direct-writing Grass oscillograph. The reservoirs contained in the constant temperature water bath provided means of rapidly changing the gaseous and electrolytic composition of the perfusate.

In order to prevent any tissue edema, which itself might modify vascular resistance, the extremity was perfused at a rate to achieve somewhat lower than normal pressures (40 to 50 mm Hg). When perfused with Ringer's solution at an O₂ tension of 160 mm Hg, the venous effluent had an average O₂ tension of 70 mm Hg; during hypoxia, the Ringer's solution entering the limb had an O₂ tension of 60 mm Hg and that leaving the limb had a PO₂ of only 1 mm Hg. It is possible that only the vascular bed, and not the rest of the hind limb, was viable under these conditions.

**Results**

**IONIC MOVEMENTS IN VASCULAR SMOOTH MUSCLE DURING HYPOXIA**

Average data from 7 cats are listed in Table 1. The concentrations of sodium and potassium in the media of the blood vessels differed considerably from either the small intestinal muscularis or the pectoralis skeletal muscle, although all specimens were treated identically. The tissue potassium concentration of blood vessels ranged from 36 to 47.5 mEq/kg; these values were considerably lower than those of the intestine and less than one-half the potassium concentrations of the skeletal muscle. In a similar manner, the sodium concentrations of the vascular smooth muscle ranged from 86 to 100 mEq/kg and were thus twice that of the intestinal smooth muscle or skeletal muscle from the same animal. The relatively low potassium and high sodium concentrations in systemic vascular smooth muscle, compared with concentrations in skeletal muscle, have been noted previously (19).

Between the control and the hypoxic periods, only two statistically significant differences ($P = <0.01$) in ionic concentration occurred: (a) The potassium concentration of both large (extra-) and small (intra-) pul-
Hypoxia and Pulmonary Artery Pressure

Table 2

Reversibility of the Hypoxic Effect on Tissue Potassium and Sodium in Pulmonary Vascular Smooth Muscle

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Tissue K⁺</th>
<th></th>
<th>Tissue Na⁺</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Control 2</td>
<td>Control 1</td>
<td>Control 2</td>
</tr>
<tr>
<td>Large pulm. art.</td>
<td>38.8 35.7</td>
<td>38.0 37.2</td>
<td>110.0 105.5</td>
<td>112.0 106.5</td>
</tr>
<tr>
<td>Small pulm. art.</td>
<td>40.5 38.5</td>
<td>42.5 38.5</td>
<td>95.0 97.5</td>
<td>97.0 98.5</td>
</tr>
<tr>
<td>Pulm. vein</td>
<td>44.0 39.5</td>
<td>42.0 39.5</td>
<td>98.5 98.2</td>
<td>101.5 106.5</td>
</tr>
<tr>
<td>Femoral art.</td>
<td>44.0 40.5</td>
<td>46.0 40.5</td>
<td>93.2 94.0</td>
<td>95.1 92.5</td>
</tr>
</tbody>
</table>

All values in mEq/kg of tissue; 1 and 2 designate specimens from each of two cats. "Control" specimens were kept in oxygenated Ringer’s solution for 120 min; “Control after hypoxia” specimens were immersed in oxygenated Ringer’s solution for 60 min, followed by 30 min of hypoxia and then by 30 min of oxygenation.

Pulmonary arterial smooth muscle specimens fell during hypoxia. Conversely, in the Ringer’s solution, bathing the large pulmonary arteries, a reciprocal rise in potassium concentration occurred during hypoxia. (b) The tissue sodium levels rose for both large and small pulmonary arteries. By way of contrast, in the pulmonary veins, the systemic arteries, the intestine, and the skeletal muscle, the potassium and sodium concentrations remained remarkably constant despite the imposition of hypoxia. The average pH during the oxygenated period was 7.41 and during the hypoxic period was 7.40.

Table 2 contains data bearing on the reversibility of the changes in ionic concentrations of the tissue during hypoxia. The control values for tissue potassium in these vascular specimens ranged from 35.7 to 44.0 mEq/kg and thus are similar to the specimens listed in Table 1. After a portion of each of these tissues was subjected to hypoxia and then returned to a control state of oxygenation for 30 min, analyses revealed essentially the same values for tissue potassium as in the control period. The observations for tissue sodium were similar; the variations between the control specimens and the oxygenated specimens after a period of hypoxia were small, amounting to no more than 3 mEq/kg in all but one specimen.

There were no other significant differences between control and hypoxia in the potassium, sodium, chloride, and calcium concentrations of the extracellular phase (Ringer’s solution). However, compared with fresh Ringer’s solution, in which the potassium was 5.5 mEq/liter, the Ringer’s solution after equilibration with the vascular specimens had somewhat higher values, ranging from 5.87 to 6.0 mEq/liter. In a similar manner, whereas fresh Ringer’s solution contained a sodium concentration of 135 mEq/liter, this solution after equilibration with specimens frequently showed decreases of 1 to 4 mEq/liter.

Since these values suggested spontaneous exchanges of electrolyte between the tissues and the bathing Ringer’s solution, a series of analyses was performed to compare stripped specimens of fresh tissues taken directly from the animal without equilibration with identical
specimens of tissue bathed in oxygenated Ringer’s solution for varying periods of time. This experiment required that 5 segments of tissue from the same large pulmonary artery be treated in the following way: specimen 1 was analyzed immediately without immersion, and specimens 2-5 were immersed in oxygenated Ringer’s solution and analyzed in 10, 20, 30 and 60 min, respectively. Figure 3 shows the averages of data from two animals. Before immersion in Ringer’s solution, the tissue potassium concentrations were 40 and 46 mEq/kg, respectively; after 10 min in Ringer’s solution, the average potassium values had dropped to 20 and 26 mEq/kg; in the next hour, there was a gradual recovery, until the potassium level at 60 min of immersion had again approached average control value. The average sodium concentration was 76 mEq/kg before immersion, rose to 110 mEq/kg and then fell back toward normal. However, the sodium concentration did not return as closely to the control values as did the potassium concentrations, and sodium analyses of these tissues taken 90 min after immersion, as shown in Table 1, still ranged between 80 and 85 mEq/kg. When specimens from these two animals were analyzed at 120 min in the course of hypoxia experiments, oxygenated specimens still had values averaging 42.5 mEq/kg and 84 mEq/kg for K⁺ and Na⁺, respectively. These data are similar to those of Hagemeijer (20) and form the basis for beginning the experiments with hypoxia only after 90 min of equilibration in oxygenated Ringer’s solution.

ISOLATED PERFUSED LUNG

Since it was found in this preparation that the only perfusate which would prevent pulmonary edema was the cat’s own plasma, and since this was in short supply, all changes in the concentration of electrolyte in the perfusate were made by adding salts. A series of preliminary experiments were undertaken to assess the effect of increasing the osmolarity in the perfusate. One experiment is illustrated in Figure 4. Identical quantities (0.001 moles) of two different salts were added to the perfusate (average volume = 100 ± 10 ml). The potassium chloride produced a slight rise in pressure, whereas the sodium chloride produced no change or a slight fall.

\[ P_{PA} \] (mmHg)

\[ 0.001 \text{M KCl} \]

\[ 0.001 \text{M NaCl} \]

\[ 0.001 \text{M KCl} \]

\[ 1 \text{ min} \]

**FIGURE 4**

Effect of osmotic changes in perfusate on pulmonary arterial pressure. At each arrow, 0.001 moles of salt (abbreviated as M) is added to the perfusate reservoir in an isolated perfused lung preparation. Each time the potassium chloride is added, the pressure rises, the response becoming more prominent as the potassium concentration of perfusate rises. However, 0.001 moles of sodium chloride produces a fall in pressure. See text.

**FIGURE 5**

Effect of initial transmembrane potential of smooth muscle on pulmonary pressor response to hypoxia. All tracings of pulmonary arterial pressure are from the same isolated cat lung. At the arrows, hypoxia (3% O₂, 5% CO₂ in inspired gas) is imposed. The middle and lower tracings show the effect of previous hyperpolarization and partial depolarization in blunting and accentuating, respectively, the pressor response to hypoxia.
### TABLE 3

**Average Effect of Changes in Electrolyte Composition on the Pressor Response to Hypoxia in the Isolated Perfused Cat Lung**

<table>
<thead>
<tr>
<th>Oxygenation</th>
<th>Electrolyte</th>
<th>$P_{PA}$ (mm Hg)</th>
<th>$[K^+]_e$ (mEq/liter)</th>
<th>$[Cl^-]_e$ (mEq/liter)</th>
<th>pH</th>
<th>PVR (mm Hg/ml/sec)</th>
<th>$P$</th>
<th>$\Delta PVR$ (mm Hg/ml/sec)</th>
<th>$\Delta PVR$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>8.17</td>
<td>5.1 ± 0.26</td>
<td>133 ± 3.88</td>
<td>7.38 ± 0.01</td>
<td>8.94 ± 1.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Control</td>
<td>13.18</td>
<td>4.9 ± 0.19</td>
<td>129 ± 4.42</td>
<td>7.39 ± 0.02</td>
<td>12.78 ± 2.18</td>
<td>4.88</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>6.98</td>
<td>4.9 ± 0.21</td>
<td>128 ± 2.68</td>
<td>7.35 ± 0.02</td>
<td>6.87 ± 1.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>$\uparrow [Cl^-]_e$</td>
<td>5.76</td>
<td>4.9 ± 0.22</td>
<td>143 ± 1.88</td>
<td>7.32 ± 0.02</td>
<td>5.57 ± 1.28</td>
<td>2.38</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$\uparrow [Cl^-]_e$</td>
<td>5.81</td>
<td>5.1 ± 0.28</td>
<td>147 ± 3.72</td>
<td>7.31 ± 0.02</td>
<td>5.52 ± 1.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>$\uparrow [K^+]_e$</td>
<td>9.48</td>
<td>18.0 ± 3.81</td>
<td>157 ± 5.04</td>
<td>7.31 ± 0.02</td>
<td>9.91 ± 2.69</td>
<td>4.27</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$\uparrow [K^+]_e$</td>
<td>16.01</td>
<td>15.7 ± 2.92</td>
<td>155 ± 4.45</td>
<td>7.32 ± 0.02</td>
<td>14.09 ± 2.53</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$P_{PA} =$ mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; $[K^+]_e, [Cl^-]_e =$ serum potassium and chloride concentration of perfusate; $P =$ significance determined by Student's t-test, the $P$ value appears in this table between the two average resistances being compared; $\Delta PVR =$ percent change in resistance during hypoxia compared with average of prehypoxic and posthypoxic control periods.
in pressure. Similar studies were undertaken to compare the effects of increases in potassium chloride concentrations with osmotically equal increases in choline chloride or lithium chloride. When the salts were added to fresh preparations without other previously induced alterations in electrolytes, 0.001 moles of potassium chloride induced an average increase in pulmonary arterial pressure in 5 animals of 2.5 mm Hg, whereas 0.001 moles of either sodium, lithium, or choline chloride in 7 animals induced an average decrease in pressure of 1.5 mm Hg. That potassium chloride invariably raised pressure and choline or lithium chloride invariably lowered it suggests that these effects on vascular pressure are related to specific alterations of potassium and chloride concentrations, rather than to nonspecific effects of changes in osmolality. Moreover, Figure 4 indicates that the pressure increments in the pulmonary circulation were approximately proportional to the increases in the external potassium concentration: the upper panel showed a rise in pressure of 1.5 mm Hg in association with an increase in potassium concentration of the perfusate from 5.5 to 9.5 mEq/liter; the lower panel shows a rise in pressure of 6 mm Hg associated with an increase in perfusate potassium from 7.0 to 12.5 mEq/liter.

The effects of hypoxia alone and of hypoxia imposed during an extracellular electrolyte change on the pulmonary arterial pressure are illustrated for 1 animal by Figure 5. Under ordinary conditions, a prompt rise in pulmonary arterial pressure occurs within 2 min after 3% O_2 is substituted for 21% O_2 in the inspired air mixture (upper panel); although not shown, when normal oxygenation was resumed, the pressure returned to control levels at the same rate at which it rose.

In the middle panel, the same isolated perfused lung was first prepared by adding sufficient lithium chloride to increase the chloride concentration of the perfusing plasma by 12 mEq/liter. The administration of 3% O_2 now appears less effective in eliciting a pressor response than previously; in particular, the rate of the rise in pressure was slower though the magnitude of the pressure

<table>
<thead>
<tr>
<th>Oxygenation</th>
<th>Electrolyte</th>
<th>( P_{\text{FA}} ) (mm Hg)</th>
<th>( [K^+]_a ) (mEq/liter)</th>
<th>( P_{\text{PVR}} ) (mm Hg/ml/sec)</th>
<th>( \Delta P_{\text{PVR}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>44</td>
<td>3.3</td>
<td>62 ± 2.1</td>
<td>8</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Control</td>
<td>40</td>
<td>57 ± 2.4</td>
<td>62 ± 2.3</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>( \uparrow ) [K^+]_a</td>
<td>47</td>
<td>5.3</td>
<td>66 ± 3.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>( \uparrow ) [K^+]_a</td>
<td>40</td>
<td>56 ± 3.3</td>
<td>66 ± 3.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>( \uparrow ) [K^+]_a</td>
<td>51</td>
<td>70 ± 5.3</td>
<td>66 ± 5.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>( \uparrow ) [K^+]_a</td>
<td>43</td>
<td>3.9</td>
<td>70 ± 4.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>( \uparrow ) [K^+]_a</td>
<td>39</td>
<td>63 ± 4.3</td>
<td>70 ± 4.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>( \uparrow ) [K^+]_a</td>
<td>43</td>
<td>68 ± 4.8</td>
<td>70 ± 4.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>( \uparrow ) [K^+]_a</td>
<td>49</td>
<td>10.3</td>
<td>79 ± 6.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>( \uparrow ) [K^+]_a</td>
<td>41</td>
<td>66 ± 9.0</td>
<td>79 ± 6.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>( \uparrow ) [K^+]_a</td>
<td>50</td>
<td>80 ± 7.2</td>
<td>79 ± 6.8</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

\( P_{\text{FA}} \) = femoral arterial pressure; \( F_{\text{VR}} \) = femoral regional vascular resistance; \( \Delta F_{\text{VR}} \) = percent change in vascular resistance; other abbreviations as in Table 3.
rise was also slightly less (by 0.5 mm Hg). In the lower panel, the same isolated perfused lung is prepared by adding potassium chloride to the perfusate, so as to raise the potassium level of the perfusate from 5.0 to 15.2 mEq/liter. The pressor response to hypoxia was thereupon restored to normal and even, in this particular case, enhanced.

Average data for 9 cats appears in Table 3. As may be seen, hypoxia elicits under ordinary conditions a substantial rise in pulmonary arterial pressure (5 mm Hg) and a 62% increase in pulmonary vascular resistance. When the extracellular chloride level was raised by about 10 mEq/liter by adding either lithium or choline chloride, a moderate, but significant, decrease in pulmonary arterial pressure and pulmonary vascular resistance occurred. Moreover, under these circumstances, hypoxia elicited a smaller pressor response and the pulmonary vascular resistance rose only 42% during the hypoxic period.

Table 3 also lists the effects of raising the extracellular potassium level by adding potassium chloride to the perfusate. Tripling the potassium concentration caused substantial rises in pulmonary arterial pressure and pulmonary vascular resistance which were indefinitely sustained. Under these circumstances, large increments in pulmonary arterial pressure were again elicited by hypoxia; the increments in pulmonary vascular resistance to hypoxia, however, were not as great as during the control periods with normal extracellular electrolyte concentrations. It is apparent that high plasma chloride levels not only lower the pulmonary vascular resistance, but diminish the rise in resistance during hypoxia. On the other hand, high extracellular potassium levels raise the pulmonary vascular resistance substantially and tend to restore the rise in resistance due to hypoxia toward normal.

**Discussion**

The results of the present study support the frequently expressed idea that the pulmonary arterial pressor response to hypoxia is mediated by a direct effect of the hypoxia on the pulmonary arterial smooth muscle. Strips of pulmonary arterial smooth muscle when subjected to hypoxia consistently lost potassium and gained sodium, and these electrolyte changes were reversible when normal oxygenation was restored, despite similar treatment, however, hypoxia appeared to have no such effect on smooth muscle from large pulmonary veins or the systemic arteries.

On the assumption that pulmonary vascular smooth muscle behaves similarly to other tissues in which transmembrane potentials have actually been measured (21, 22), these potassium concentrations in the pulmonary arterial specimens were used as an index of intracellular concentrations, and
those in the Ringer's solution bathing the specimens were used as an index of extracellular concentrations in order to estimate the average resting transmembrane (intracellular) potential for the pulmonary arterial smooth muscle during the control and the hypoxic situations. For this purpose, the Nernst relationship (21) was employed; use of the factors of the universal gas constant, mammalian body temperature, ionic valency, charge per mole and conversion of the natural logarithm to the base of 10 yielded a constant of 60 mv. The results of such calculations are averaged in Figure 6. The mean ratios of intracellular and extracellular potassium concentrations which were used are shown along with the calculated transmembrane potential. As indicated, a fall in the average intracellular potassium concentration during hypoxia from 45.6 to 35.7 mEq/kg yields a corresponding change in the calculated potential from $-57$ to $-51$ mv. Since the plasma membrane of most smooth muscle has been known to function only partially as a potassium electrode (22), a degree of caution is warranted in accepting these present calculated transmembrane potentials as exact values; they do, however, suggest the direction and magnitude of change in the potential. In the light of experience with systemic vascular smooth muscle in the turtle (23), artificial depolarization of this approximate magnitude, i.e., 6 to 10 mv, can be expected to increase the frequency of action potentials and to produce a sustained increase in passive tension. The present data, therefore, suggest that hypoxia exerts its effects on the small pulmonary arteries by producing reversible depolarization of the smooth muscle so that the resting negative intracellular potential moves closer to zero and to its threshold for the production of action potentials and active contraction.

The experience with the isolated perfused lung in the present study tends to support this hypothesis:

First, pulmonary arterial smooth muscle apparently relaxes and contracts in accord with the level of its transmembrane potential. Thus, when the extracellular potassium was raised, a procedure applied in order to depolarize the cell, i.e., to bring the resting negative transmembrane potential toward zero, contraction of pulmonary vascular smooth muscle and increased pulmonary vascular resistance occurred. Conversely, raising the extracellular chloride concentration, a procedure applied to hyperpolarize the cell, i.e., to bring the negative transmembrane potential away from the excitation threshold, was accompanied by relaxation of smooth muscle and a fall in pulmonary resistance. Nor were very large changes in transmembrane potential necessary before well marked changes in vascular resistance occurred. As Figure 7 indicates, a change in potential toward zero of only 10 mv was sufficient to produce a 50% increment in vascular resistance; these data are similar to those of Holman, who studied intestinal smooth muscle (22). Moreover, these shifts in potential, which were produced artificially, were of the same order as those calculated from the analyses of cellular potassium concentrations to occur during hypoxia. Such studies, therefore, suggest (a) that pulmonary vascular smooth

---

1Exact calculations of membrane potential changes were particularly difficult for a variety of reasons. Uncertainty about permeability of the various ions, as well as the assumptions regarding intracellular and extracellular concentrations from data on tissue and Ringer's solution concentrations. All of the calculations are meant only as approximations and estimates of directional changes, rather than commitments to exact values. For instance, in the case of chloride, estimations were made by the Goldman equation from the magnitude of the change of the ratios of extracellular to intracellular chloride concentrations, assuming an average permeability of all types of smooth muscle for the chloride to be somewhat lower than that of potassium (16). That relaxation of the smooth muscle was actually due to hyperpolarization induced by the increased chloride concentration rather than to any specific effects of the choline or other cations is suggested by the fact that all three chloride compounds—sodium chloride, lithium chloride, and choline chloride—produced similar degrees of relaxation when added to the extracellular fluid in equimolar amounts, and this assumption is supported by the experimental work of Bulbring et al. (24) in taenia coli, which demonstrated that changes in extracellular chloride elicited changes in intracellular potential.
Average effects of hypoxia on the intracellular (tissue specimen) and extracellular (Ringer's solution) potassium concentrations in pulmonary arterial smooth muscle of the cat. Bar graphs at the top of this figure illustrate potassium concentrations; the fractions just below represent the ratios of internal to external potassium concentrations from which the membrane potentials (Vm) were calculated from the Nernst equation, shown at bottom of figure. Base of arrow is the calculated membrane potential during control and arrow head represents value during hypoxia.

muscle readily contracts when partially depolarized by artificial electrolyte changes and (b) that the depolarization caused by hypoxia is of the magnitude required to reproduce this phenomenon.

Second, artificial hyperpolarizations of the pulmonary vascular smooth muscle by excess extracellular chloride levels tended to inhibit the pulmonary pressor response to hypoxia (both in absolute values and in terms of percentile changes in pulmonary vascular resistance). Conversely, partial depolarizations by augmented extracellular potassium levels tended to restore and perhaps accentuate the pressor response to hypoxia. Such a phenomenon is further evidence that hypoxia exerts its effect through bringing the transmembrane potential of the pulmonary arterial smooth muscle cell toward its excitation threshold; the consequence of this relationship would be our experimental observation of an inverse relation between the magnitude of the pulmonary pressor response to hypoxia and the estimated resting transmembrane potentials of the smooth muscle cell (Fig. 7), derived from data of the present study.

These relations, however, do not rule out a role for hypoxia in regulating the contractile mechanism or the excitation-coupling in the muscle cell. Nor do these data bear on whether or not other stimuli, either nervous or mechanical, are needed to facilitate this process. It is possible that a steady inflow to the pulmonary arteries by way of the autonomic nervous system is always present and that this inflow elicits greater contractile responses in the presence of partial depolarization of the smooth muscle. On the other hand, the autonomic nervous system or humoral agents need not be necessary; mechanical stretch (25) may be all that is needed as a stimulus in the presence of partial depolarization by hypoxia; a counterpart for this mechanical stretch exists in the intact pulmonary circulation, where the pulsatile expansion of blood vessels with every heart beat may be resisted more strongly during hypoxia than...
during normal oxygenation and thus facilitate the augmentation of vascular resistance.

The manner in which potassium is lost from the smooth muscle cell under the influence of hypoxia is not certain. However, the simultaneous increase in the intracellular sodium concentration suggests (a) that hypoxia disturbs the function of an active transport system at the cell membrane in which sodium and potassium are linked and (b) that this system in pulmonary arterial smooth muscle is easily disrupted by hypoxia.

The differences in response to hypoxia between pulmonary arterial muscle and that of other blood vessels in this study were striking; the present data, however, do not explain the basis for this difference, other than the conjecture that the energy supply of the cationic active transport system or the permeability characteristics for potassium of the membranes in the two types of blood vessel muscle may differ in their sensitivity to hypoxia. The validity of such a proposition would require that this transport system in pulmonary arterial muscle become partially inhibited during moderate hypoxia while the rest of the cell metabolism, including the contractile mechanism, remain intact; in the systemic vessels, on the other hand, the ionic transport mechanism and the transmembrane resting potential may resist the effects of progressive degrees of hypoxia until long after the rest of the cell is also affected and no longer able to maintain tension.

With respect to systemic blood vessels, this study in the isolated limb has confirmed that which was long known: that hypoxia, in the absence of autonomic nervous influence to the contrary, will elicit systemic vasodilation. Although we found no evidence that hypoxia produced changes in the cellular electrolyte concentrations of this type of smooth muscle, such evidence is still insufficient to indicate whether hypoxia affects the systemic blood vessels directly or indirectly through receptors of unknown type, humoral agents, or residual fibers of the autonomic nervous system. In our studies, the systemic blood vessels reacted to changes in ionic concentra-

**References**

HYPOXIA AND PULMONARY ARTERY PRESSURE


A Study of the Mechanisms Involved in the Pulmonary Arterial Pressor Response to Hypoxia
EDWARD H. BERGOFSKY and SEYMOUR HOLTZMAN

Circ Res. 1967;20:506-519
doi: 10.1161/01.RES.20.5.506
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1967 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/20/5/506

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/