Inhibitors of Oxidative ATP Production
Cause Transient Vasoconstriction and Block
Subsequent Pressor Responses in Rat Lungs

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SUMMARY We wondered if depression of oxidative adenosine triphosphate (ATP) production caused pulmonary vasoconstriction. If so, then several chemically different inhibitors of oxidative ATP production all should cause pulmonary pressor responses. The vascular reactivity of isolated, blood-perfused rat lungs was established by eliciting pressor responses to airway hypoxia and to intraarterial angiotensin II. Then, during normoxia, we added to perfusate one of five chemical inhibitors of oxidative ATP production: 10 mM azide, 1 mM cyanide, 1 mM dinitrophenol, 5 or 10 µM antimycin A, or 0.5 µM rotenone. Each of the five chemical inhibitors, but not their solvents, caused a transient pressor response, followed by loss of vascular reactivity to hypoxia, angiotensin II, and chemical inhibitors. The inhibitor pressor responses were not due to an effect on blood cells, since they also were seen in lungs perfused with plasma. The magnitudes of pressor responses to all metabolic inhibitors except azide correlated with the magnitudes of preceding pressor responses to hypoxia, but not to the preceding angiotensin II responses. When verapamil or calcium chloride was added to perfusate, the hypoxic and inhibitor pressor responses were blunted more than was the angiotensin II response. Thus, five chemically different substances, inhibiting different steps of oxidative ATP production, all caused pressor responses that were blocked readily by verapamil and by increased perfusate calcium chloride. These results support the possibility that depression of oxidative ATP production elicits pulmonary vasoconstriction that is dependent on influx of extracellular calcium. Hypoxia might also be sensed in the pulmonary circulation by decreased oxidative ATP production in some as yet unidentified lung cell.

AIRWAY hypoxia causes pulmonary vasoconstriction. The nature of the oxygen sensor, its intrapulmonary location, and the mechanism by which the sensing of hypoxia is converted to vasoconstriction are unknown (Fishman, 1976). It has been suggested that hypoxia is sensed in the pulmonary circulation through cytochrome P-450 (Sylvester and McGowan, 1978), through direct effects on the vascular smooth muscle cell membrane (Bergofsky and Holtzman, 1967), or through inhibition of oxidative adenosine triphosphate (ATP) production (Fishman, 1976). In support of the latter possibility, two chemical inhibitors of oxidative ATP production have been found to cause pulmonary vasoconstriction. Bergofsky et al. (1963) observed that 2,4-dinitrophenol, an agent that uncouples phosphorylation from electron transport, caused pulmonary vasoconstriction in dogs. Although these investigators believed that the pressor effect of dinitrophenol was due to a fall in pulmonary arterial (mixed venous) Po2, Lloyd (1964, 1965) showed that both dinitrophenol and cyanide, an inhibitor of electron transport, caused pressor responses in isolated dog lungs in the absence of any change in pulmonary arterial Po2. Lloyd, therefore, attributed the pressor effects of dinitrophenol and cyanide to decreased oxidative ATP production in lung tissue. However, these chemicals have effects other than inhibition of ATP production (Hewitt and Nicholas, 1963; Horn and Kumamoto, 1970; Barker and Levitan, 1975), and the question arises as to whether the pulmonary pressor responses to cyanide and dinitrophenol are due to depression of oxidative ATP production or to other actions.

We reasoned that if several chemically different substances, inhibiting different steps of oxidative ATP production, all caused pulmonary pressor responses, then it would be more likely that depression of ATP production, and not other actions of the chemicals, caused pulmonary vasoconstriction. We studied five chemical inhibitors of oxidative ATP production—four inhibitors of electron transport: rotenone, antimycin A, potassium cyanide, and sodium azide—and one uncoupler of electron transport from phosphorylation: dinitrophenol (Fig. 1). We used isolated, blood-perfused rat lungs and tested whether each of the five inhibitors caused pressor responses. To rule out the possibility that...
the metabolic inhibitors were acting on blood cells, rather than on lung tissue, we examined their effects on perfusion pressure in plasma-perfused lungs. We also tested whether airway hypoxia and the chemical inhibitors were eliciting vasoconstriction through similar mechanisms by comparing the susceptibilities of pressor responses to hypoxia, metabolic inhibitors, and angiotensin II to inhibition by verapamil and by increased extracellular calcium chloride, both of which blunt pulmonary vasoconstriction to hypoxia more readily than that to angiotensin II (McMurtry et al., 1976; Voelkel et al., 1978).

Methods

The isolated, blood-perfused rat lung preparation used in these experiments has been described previously (Hauge, 1968; McMurtry et al., 1976). Lungs were isolated from 250- to 500-g, male, Sprague-Dawley rats after intraperitoneal injection of 30 mg of pentobarbital and intracardiac injection of 100 IU of heparin. They were ventilated through a tracheal cannula with a humid mixture of 95% air-5% CO2 (normoxic gas) at a rate of 65 breaths/min using 9 cm H2O-positive inspiratory pressure and 2.5 cm H2O-positive end-expiratory pressure. Thirty to 40 ml of heparinized blood were obtained by cardiac puncture (under ether anesthesia) of three to four adult, male, Sprague-Dawley rats. Lungs to which solvent had been added were cleaned of trachea, heart, and major vessels, gently blotted, and weighed. The lungs then were dried for 3-5 days in an oven at 50°C and their dry weights were determined. These measurements allowed calculation of wet: dry weight ratios as an index of pulmonary edema.

In a separate group of blood-perfused lungs prepared and challenged with hypoxia and angiotensin II as described above, we added one inhibitor twice to at least one lung and measured the effects on perfusion pressure. At least 15 minutes elapsed between the first and second additions of inhibitor. In another experiment, lungs were perfused with hypoxic gas (3% O2-5% CO2-92% N2) and with intraarterial bolus injections of angiotensin II (0.5 µg/0.05 ml of 0.9% NaCl) during normoxia. Effluent blood pH and gas tensions were pH 7.37 ± 0.01, Po2 122 ± 2 mm Hg, and Pco2 34 ± 1 mm Hg during normoxia, and were pH 7.38 ± 0.01, Po2 42 ± 1 mm Hg, and Pco2 36 ± 1 mm Hg during the 4th minute of hypoxia (mean ± SE, n = 25). As has been noted in isolated rat lungs (Hauge, 1968), the pressor responses to hypoxia increased progressively with the first three hypoxic challenges. When the vascular reactivity of the lungs had been established after three hypoxic and three angiotensin II challenges at 90 minutes of perfusion, either sodium azide (n = 4), potassium cyanide (n = 4), 2,4-dinitrophenol (n = 4), antimycin A (n = 5), or rotenone (n = 4) was added to the perfusate reservoir during normoxia. Azide and cyanide were dissolved in 0.9% NaCl, dinitrophenol in a 1:1 mixture of 0.9% NaCl and 7.6% NaHCO3, and antimycin A and rotenone were dissolved in absolute ethyl alcohol, which was then diluted 1:10 into rat plasma. The final perfusate concentrations of inhibitors were 10 mM azide, 1 mM cyanide, 1 mM dinitrophenol, 5-10 µM antimycin A, and 0.5 µM rotenone. These concentrations were based on reports showing inhibition of oxidative ATP production in lung tissue by these chemicals (Bassett and Fisher, 1976; Fisher et al., 1976; Perez-Diaz et al., 1977) and on preliminary experiments to determine what level of chemical inhibitor caused a measurable effect on lung perfusion pressure. An equivalent volume (0.1-0.6 ml) of solvent, 0.9% NaCl (n = 4) or alcohol in plasma (n = 6), was added to the perfusate reservoir of other lungs as a solvent control. The pH of perfusate was 7.33 to 7.44 after the additions. Fifteen minutes after either metabolic inhibitor or solvent had been added to the perfusate, the lungs were challenged again with hypoxia and angiotensin II. In one to four lungs treated with each inhibitor or solvent, blood lactate was measured by the lactate dehydrogenase method (Sigma) on samples of effluent blood obtained after the 30-minute equilibration period and just before and 15 minutes after inhibitor or solvent had been added to the perfusate. After 130 minutes of perfusion, 30 minutes after addition of inhibitor or solvent, five lungs to which solvent had been added and fourteen lungs to which inhibitors had been added were cleaned of trachea, heart, and major vessels, gently blotted, and weighed. The lungs then were dried for 3-5 days in an oven at 50°C and their dry weights were determined. These measurements allowed calculation of wet: dry weight ratios as an index of pulmonary edema.
plasma obtained by centrifugation of 50 ml of heparinized rat blood at 1400 g for 20 minutes. The first 3-5 ml of plasma perfused through the lungs were discarded to minimize contamination with blood cells remaining in the vasculature. After 30 minutes of equilibration, the plasma-perfused lungs were challenged with hypoxia and angiotensin II, as described above. Each metabolic inhibitor was added to two lungs, and the effect on perfusion pressure was determined.

Another group of blood-perfused lungs was prepared as described above and challenged twice with hypoxia (6 minutes of 0 or 3% O2) and with angiotensin II (0.25-1.0 μg). The different levels of hypoxia and angiotensin II were used in an attempt to match the magnitudes of these pressor responses to the magnitudes of the inhibitor pressor responses. Ten minutes after the second pressor response to angiotensin II, after 70 minutes of perfusion, either verapamil or calcium chloride in 0.9% NaCl was added to the perfusate reservoir to achieve concentrations of 22 μM verapamil or to increase the blood level of calcium chloride by 10 mM. Ten minutes later, the lungs again were challenged with the same amount of hypoxia and angiotensin II. Metabolic inhibitors then were added to perfusate, as described above, while perfusion pressure was measured. Each of the five inhibitors was added to two lungs for a total of 10 lungs in both the verapamil and calcium chloride groups. A control group of five lungs was treated similarly, except that only 0.9% NaCl was added to perfusate after 70 minutes of perfusion. Each metabolic inhibitor then was added to one lung.

Angiotensin II, sodium azide, rotenone, and antimycin A (type III from Streptomyces Kitazawaeensis mycelia) were obtained from Sigma; 2,4-dinitrophenol from Eastman; potassium cyanide from Allied; and calcium chloride from Baker. Verapamil was supplied by Knoll Pharmaceutical Company.

Results

Table 1 shows pressor responses to hypoxia and to angiotensin II just prior to the addition of either solvent or inhibitor of oxidative ATP production to blood-perfused lungs. In these lungs, each of the five chemical inhibitors, but not the solvents, caused pressor responses following their addition to the perfusate reservoir (Table 1). The small pressor response caused by 10 mM azide was not due to an inappropriate dose, since 1, 25, and 50 mM azide caused pressor responses of 2 mm Hg or less. Typical pressor responses to the five inhibitors are shown in Figure 2. Pulmonary arterial pressure began to rise within 1 minute of adding inhibitor to perfusate, reached a peak, and then gradually re-

### Table 1  Pressor Responses to Hypoxia, Angiotensin II, Inhibitors of Oxidative ATP Production, and Solvents in Lungs Perfused with Blood or Plasma

<table>
<thead>
<tr>
<th>Addition to perfusate</th>
<th>Perfusate</th>
<th>n</th>
<th>Third hypoxic response (3% O2)</th>
<th>Third angiotensin II response (0.5 μg)</th>
<th>Inhibitor or solvent response</th>
<th>Fourth hypoxic response (3% O2)</th>
<th>Fourth angiotensin II response (0.5 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.9%)</td>
<td>B</td>
<td>4</td>
<td>13 ± 3</td>
<td>14 ± 2</td>
<td>0</td>
<td>10 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Alcohol/plasma</td>
<td>B</td>
<td>6</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>0</td>
<td>10 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Azide (10 mM)</td>
<td>B</td>
<td>4</td>
<td>12 ± 2</td>
<td>15 ± 1</td>
<td>4 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cyanide (1 mM)</td>
<td>B</td>
<td>4</td>
<td>13 ± 3</td>
<td>19 ± 3</td>
<td>10 ± 2</td>
<td>0</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Dinitrophenol (1 mM)</td>
<td>B</td>
<td>4</td>
<td>15 ± 3</td>
<td>22 ± 3</td>
<td>15 ± 4</td>
<td>4 ± 4</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Antimycin A (5-10 μM)</td>
<td>B</td>
<td>5</td>
<td>13 ± 2</td>
<td>13 ± 2</td>
<td>8 ± 2</td>
<td>0</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Rotenone (0.5 μM)</td>
<td>B</td>
<td>2</td>
<td>17 ± 3</td>
<td>15 ± 4</td>
<td>12 ± 1</td>
<td>0</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± se; when less than three lungs were studied, the value for each lung is given. Pressor responses are baseline to peak mean pulmonary arterial pressure (∆P, mm Hg). When pressure decreased, a negative number is given. B is blood perfusate; P is plasma perfusate; n is number of lungs.

The third hypoxic and angiotensin II responses were before and the fourth responses were after the addition of inhibitor or solvent to perfusate.
TABLE 2  Pressor Responses to Repeated Additions of Inhibitors of Oxidative ATP Production to Perfusate

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>First inhibitor response</th>
<th>Second inhibitor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azide (2 mM)</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>Cyanide (1 mM)</td>
<td>2</td>
<td>7.6</td>
</tr>
<tr>
<td>Dinitrophenol (1 mM)</td>
<td>2</td>
<td>15.15, 15.19</td>
</tr>
<tr>
<td>Antimycin A (5 µM)</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Rotenone (0.3 µM)</td>
<td>1</td>
<td>18</td>
</tr>
</tbody>
</table>

Pressor responses are baseline to peak mean pulmonary arterial pressure (ΔP, mm Hg). When pressure decreased, a negative number is given. n is number of lungs.

Table 2

Inhibitor was added to the perfusate reservoir at time 0 min.

Representative pressor responses to each chemical inhibitor of oxidative ATP production are shown as mean pulmonary arterial pressure against time. Inhibitor was added to the perfusate reservoir at time 0 min.

Between 30 and 90 minutes of perfusion, before the additions to perfusate, the rate of lactate accumulation in the blood was not different between four lungs to be treated with the solvents and 11 lungs to be treated with the chemical inhibitors (Table 3). However, between 90 and 105 minutes of perfusion, after the additions to perfusate, the rate of lactate accumulation was greater in lungs treated with the inhibitors than in lungs treated with the solvents (Table 3).

After 130 minutes of perfusion, the blood-perfused lungs were not visibly edematous, and wet: dry weight ratios were similar in five lungs to which solvent had been added and 14 lungs to which the chemical inhibitors had been added (Table 3).

The plasma-perfused lungs showed pressor responses to hypoxia, angiotensin II, and each of the five metabolic inhibitors (Table 1). Pressor responses tended to be smaller in plasma-perfused than in blood-perfused lungs. As was the case in blood-perfused lungs, plasma-perfused lungs showed transient pressor responses to the chemical inhibitors and subsequently did not respond to hypoxia or to angiotensin II.

In the blood- and plasma-perfused lungs, the pressor responses to all the inhibitors of oxidative ATP production except azide were correlated with the preceding pressor responses to hypoxia, but not with the preceding responses to angiotensin II (Fig. 3).

Table 4 shows that pressor responses to the chemical inhibitors of oxidative ATP production, as well as those to hypoxia, were markedly decreased after the addition of verapamil or calcium chloride to perfusate, but not after the addition of 0.9% NaCl. The pressor responses to angiotensin II were

Table 3

<table>
<thead>
<tr>
<th>Addition to perfusate</th>
<th>Lactate accumulation (mmol/liter per hr)</th>
<th>n</th>
<th>Wet:dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td></td>
<td>4</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Azide (10 mM)</td>
<td></td>
<td>2</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Cyanide (1 mM)</td>
<td></td>
<td>2</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Dinitrophenol (1 mM)</td>
<td></td>
<td>2</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Antimycin A (5 µM)</td>
<td></td>
<td>2</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Rotenone (0.5 µM)</td>
<td></td>
<td>2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>All inhibitors</td>
<td></td>
<td>12</td>
<td>10.8 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; when less than three lungs were studied, the value for each lung is given. n = number of lungs. Solvents are 0.9% NaCl (n = 2) or absolute ethyl alcohol (n = 2) diluted 1:10 in plasma.

* Different from lungs treated with solvents at P < 0.05.
ATP INHIBITORS CAUSE PULMONARY VASOCONSTRICTION

Discussion

Each of five chemically different inhibitors of oxidative ATP production—azide, cyanide, dinitrophenol, antimycin A, and rotenone—elicited a transient pressor response in isolated, blood-perfused rat lungs. In each case, the pressor response was followed by loss or severe blunting of vascular reactivity to hypoxia, angiotensin II, and a second addition of the inhibitors. Since similar effects were observed in plasma-perfused lungs, the pressor responses apparently were due to actions on lung or vascular tissue, rather than on blood cells. It is likely that the pressor responses were caused by vasoconstriction, since blood flow was constant and pulmonary edema did not occur.

Our results with cyanide and dinitrophenol in isolated rat lungs are similar to those of Lloyd (1964, 1965) who found pressor responses to these agents in isolated dog lungs. Although Lloyd (1964) found that bolus injections of 0.01 mmol of potassium cyanide into the pulmonary artery of the isolated lung caused sustained pressor responses and enhanced subsequent reactivity to hypoxia, he also reported that 1 mmol of cyanide elicited a large, transient pressor response which was followed by complete loss of vascular reactivity. In contrast to our results, Duke and Killick (1952) reported that in isolated cat lungs both sodium cyanide (0.2 mmol) and sodium azide (0.08 mmol) caused vasodilation when injected directly into the pulmonary artery. Duke and Killick (1952) and Said et al. (1976) found that, after the injection of azide, cat lungs were nonresponsive to hypoxia but still showed pressor responses to other vasoconstrictor agents. We do not know what accounts for the discrepancies between our results and those of Duke and Killick and Said et al., but species, dose of metabolic inhibitor, and site of administration of inhibitor are three obvious differences among the studies.

Our results showed that the five chemicals—azide, cyanide, dinitrophenol, antimycin A, and rotenone—had similar effects on the vascular reactivity of rat lungs. The one action that these substances are known to have in common is inhibition of oxidative ATP production (Lehninger, 1970). We did not make direct measurements of oxidative

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**Table 4** Pressor Responses to Hypoxia, Angiotensin II, and Chemical Inhibitors of Oxidative ATP Production after Addition of NaCl, Verapamil, or CaCl₂ to Perfusate

<table>
<thead>
<tr>
<th>Addition to perfusate</th>
<th>Hypoxia (0 or 3% O₂)</th>
<th>Angiotensin II (0.25–1.0 μg)</th>
<th>Azide (10 μM)</th>
<th>Cyanide (1 μM)</th>
<th>Dinitrophenol (1 μM)</th>
<th>Antimycin A (5 or 10 μM)</th>
<th>Rotenone (0.5 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (0.9%)</td>
<td>18 ± 2 (5)</td>
<td>15 ± 2 (5)</td>
<td>4</td>
<td>6</td>
<td>13</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Verapamil (22 μM)</td>
<td>2 ± 1 (10)</td>
<td>8 ± 1 (10)</td>
<td>−2.1</td>
<td>1.1</td>
<td>1.2</td>
<td>1.2</td>
<td>−1.3</td>
</tr>
<tr>
<td>CaCl₂ (10 mM)</td>
<td>1 ± 1 (10)</td>
<td>8 ± 1 (10)</td>
<td>1.1</td>
<td>0.1</td>
<td>2.2</td>
<td>1.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Values are mean ± SE except where one or two lungs were studied, and these results are presented individually. The number in parentheses is the number of lungs.

Pressor responses are baseline to peak mean pulmonary arterial pressure (ΔP, mm Hg). When pressure decreased, a negative number is given.
metabolism, but the increased rate of lactate accumulation in the perfusate of lungs treated with the metabolic inhibitors suggested that oxidative ATP production had been depressed. In perfused rat lungs or in isolated rat lung cells, cyanide, dinitrophenol, antimycin A, and rotenone inhibited oxidative metabolism when added in concentrations similar to those used in the present study (Bassett and Fisher, 1976; Fisher et al., 1976; Perez-Diaz et al., 1977). We are not aware of studies of the effect of azide on oxidative metabolism in lung tissue.

We do not know how a depression of ATP production might have caused the vasoconstriction. It is likely that calcium influx into cells was involved, since the inhibitor pressor responses were blunted by verapamil and by an increased level of extracellular calcium chloride, both of which are thought to reduce transmembrane calcium influx (Hurwitz, 1964; Rosenberger and Triggle, 1978). It may be that decreased ATP production altered membrane permeability and allowed calcium influx into vascular smooth muscle to initiate a contraction or into a secretory cell to release a vasoconstrictor. The transience of the inhibitor pressor responses and the subsequent loss of vascular reactivity to the same and other stimuli might have been due to such severe depletion of high energy stores in the vascular smooth muscle that contraction was no longer possible, or to the release of a vasodilator from the lung tissue. Both vasoconstriction and subsequent blunting of vascular reactivity could have been caused by inhibition of ATP production in vascular smooth muscle if the inhibition required a few minutes to become maximal and if early, partial inhibition caused contraction, while later, severe inhibition caused relaxation. Alternatively, the biphasic response also could be accounted for if inhibition of ATP production and release of a vasoconstrictor from some secretory cell occurred more rapidly than did depletion of ATP and loss of contractile activity in the vascular smooth muscle. If relaxation and blunting of vascular reactivity were caused by release of a vasodilator, then it would have to be that such a process occurred more slowly in response to inhibition of ATP production than did the series of events leading to the initial vasoconstriction. Of course, it is also possible that one or both of the vascular effects of the inhibitors were due to some action other than inhibition of oxidative ATP production. We are not aware of actions of antimycin A or rotenone on tissues other than inhibition of ATP production, but cyanide and azide inhibit numerous enzymes (Hewitt and Nicholas, 1963) and dinitrophenol may interfere directly with ion transport across cell membranes (Horn and Kumamoto, 1970; Barker and Levitan, 1975). Such nonspecificity might have been responsible for the differences in the magnesium and time courses of the pressor responses to the five inhibitors. On the other hand, some of the differences also could have been due to differences in the degree and rate of inhibition of ATP production. In summary, although we believe that our results provide further indirect support for the idea that depression of oxidative ATP production in some lung cell causes pulmonary vasoconstriction, we also emphasize that chemical inhibitors often have actions in addition to those for which they are best known.

There are several similarities between the pulmonary vasoconstrictor responses to airway hypoxia and to chemical inhibitors of oxidative ATP production. Lloyd (1964) found that hypoxia, repeatedly caused vasoconstriction when injected into the arterial line of an isolated lung perfused with blood from a donor dog instead of with blood in a recirculating system. Lloyd (1964, 1965, 1966a, 1966b) also observed in isolated dog lungs that pressor responses to hypoxia and to intraarterial cyanide, but not to serotonin or epinephrine, were blunted by prolonged perfusion, cooling, alkalosis, or perfusion with physiological salt solution instead of with blood or plasma. We found a correlation between the magnitudes of the pressor responses to inhibitors and to hypoxia, but pressor responses to inhibitors and to angiotensin II were not correlated. Verapamil and an increased level of calcium chloride blunted the pressor responses to hypoxia and to chemical inhibitors of oxidative ATP production more than they did the pressor response to angiotensin II, suggesting that the mechanisms of the hypoxic and inhibitor responses were similarly dependent on calcium influx. These similarities suggest that hypoxia and chemical inhibitors of oxidative ATP production cause pulmonary vasoconstriction by the same mechanism.

In our studies, there were also differences between the pulmonary vascular effects of hypoxia and the chemical inhibitors. First, pressor responses to inhibitors were not sustained as long as were those to hypoxia, and, second, the inhibitors, but not hypoxia, led to severe blunting of reactivity to other pressor stimuli. Although these differences might indicate that hypoxia and chemical inhibitors had substantially different actions, it also should be considered that the differences were more quantitative than qualitative. For example, pulmonary pressor responses to severe hypoxia also are not sustained as well as are those to moderate hypoxia (Lloyd, 1964; Tucker and Reeves, 1975; Sylvester et al., 1979). In addition, Lloyd (1964, 1965) found in dog lungs that concentrations of cyanide and dinitrophenol lower than those used in our study caused well-sustained pressor responses and did not blunt reactivity to other stimuli. Thus, the evidence suggests that airway hypoxia and chemical inhibitors of oxidative ATP production have similar, dose-dependent effects on the pulmonary vasculature. Assuming that the one action shared by airway hypoxia, cyanide, azide, antimycin A, rotenone, and dinitrophenol is depression of oxidative ATP production, we believe it possible that, whereas mod-
rate depression of ATP production causes pulmonary vasoconstriction, severe inhibition leads to pulmonary vasodilation. However, the possibility remains that hypoxia and the chemical inhibitors of oxidative ATP production are acting through different mechanisms.

There are two major conceptual problems with the idea that hypoxic pulmonary vasoconstriction is elicited by a depression of oxidative ATP production. One problem is how a decrease in ATP production causes an energy-dependent process, i.e., constriction of vascular smooth muscle. There are at least two speculative explanations. In one case, the decrease in ATP production might occur in some tissue other than vascular smooth muscle and be linked to constriction through release of a chemical mediator. Alternatively, the supply of energy to processes which maintain membrane potential or calcium permeability of the vascular smooth muscle might be more sensitive than the contractile apparatus to a decreased rate of oxidative ATP production. The other problem is that, whereas hypoxic pulmonary vasoconstriction is detectable at an airway Po2 of 70 mm Hg or above (Barer, 1976) the Po2 of isolated lungs (Bassett et al., 1974; Fisher et al., 1976) and of isolated mitochondria (Chance et al., 1974) must fall to extremely low levels before ATP production is reduced measurably. Again, there are some speculative answers to this problem.

In the first case, measurement of the effect of hypoxia on the heterogeneous whole lung might not reflect a decrease in the energy state of a small population of cells with an increased sensitivity to Po2. In the second case, there is evidence that the metabolism of mitochondria in vitro is not as sensitive to Po2 as that of mitochondria in vivo (Jobbiss, 1977) and, it might also be that, as has been suggested for the carotid body (Mills and Jobbiss, 1972), the oxygen-sensing cells in the lung contain a cytochrome oxidase with a low affinity for oxygen.

Finally, since a moderate decrease in rate of oxidative phosphorylation is not necessarily accompanied by a fall in level of ATP (Honig, 1968), it should be considered that the hypoxic response might be mediated by changes in the levels of metabolites other than ATP. Substantiation of the possibility that depression of oxidative ATP production is the signal for hypoxic pulmonary vasoconstriction will require identification of the oxygen-sensing cells and direct measurement of oxidative metabolism.

It is interesting that, like the pulmonary circulation, the carotid chemoreceptor is stimulated both by hypoxemia and by chemicals which inhibit oxidative ATP production (Krylov and Anichkov, 1968; Joels and Neil, 1968). In addition, the response of the carotid chemoreceptor to chemical inhibitors is augmented by hypoxemia (Mulligan and Lahiri, 1978). Thus, it has also been suggested that hypoxia is sensed in the carotid chemoreceptor by decreased oxidative ATP production (Joel and Neil, 1968; Krylov and Anichkov, 1968; Mulligan and Lahiri, 1978).

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Effects of Anesthesia on the Canine Carotid Chemoreceptor Reflex

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SUMMARY We studied the effects of a-chloralose (100 mg/kg, iv), Na pentobarbital (25 mg/kg, iv) and halothane (1 vol% and 2 vol%) on the response to carotid chemoreceptor stimulation (CCRS) in eight chronically instrumented dogs. CCRS was accomplished by means of intracarotid injections of nicotine while ventilation was held constant in the unanesthetized state and following administration of one of three different anesthetics. In the conscious state, CCRS elicited intense bradycardia and peripheral vasoconstriction as reflected by a 173 ± 14% increase in initial cardiac cycle length and a 216 ± 12% increase in mean iliac vascular resistance. Each anesthetic, studied on separate days, attenuated these responses to CCRS strikingly (P < 0.01). For instance, after a-chloralose, CCRS increased iliac resistance by only 55 ± 14% and cardiac cycle length by only 27 ± 13%. After Na pentobarbital, CCRS increased iliac resistance by 12 ± 4% and cardiac cycle length by 8 ± 5%. After inhalation of halothane (1 vol%), CCRS increased iliac resistance by 28 ± 7% and cardiac cycle length by 11 ± 5%, whereas halothane (2 vol%) abolished these responses to CCRS. Thus, general anesthesia interferes severely with carotid chemoreceptor control of the circulation. Whereas halothane and Na pentobarbital altered responses to CCRS the most, we found that even a-chloralose, which has been thought to maintain or augment reflex responses, was able to depress the response to CCRS strikingly. 

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