Redox Status and Pulmonary Vascular Reactivity*

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Oxygen radicals are produced during oxidative metabolism in proportion to the tissue oxygen tension. The studies reported here have shown that oxygen radicals or the sulphydryl oxidant, diamide, caused pulmonary vasodilatation in the isolated perfused rat lung. Could oxygen radicals play a role in the physiologic control of pulmonary vascular smooth muscle tone?

Hyperoxia increases oxygen radical production in the lungs above the levels found during normoxia. In many biologic reactions the rate of production of such radicals is directly proportional to oxygen tension. Sulphydryl oxidation of enzymes and membranes by oxygen radicals is an important factor in oxygen toxicity.

The oxidation of sulphydryl groups is known to be involved in the relaxation of systemic arterial strips by vasodilators, and oxygen radicals, generated by the addition of xanthine oxidase, cause relaxation in small pial and cerebral arteries. However, the concept that the oxidation of sulphydryl groups, incuded by oxygen radicals, might play a part in the physiologic control of pulmonary vascular smooth muscle tone has not been studied. The sulphydryl oxidizing agents diamide, t-butyl hydroperoxide and 2-butanone peroxide inhibit hypoxic pulmonary vasoconstriction in the anesthetized dog. This paper reports that diamide, or the production of oxygen radicals by the addition of xanthine and xanthine oxidase, causes a reduction in the pulmonary pressor response to hypoxia of the isolated perfused rat lung.

METHODS

Diamide

The lungs of seven Sprague-Dawley male rats (300–380 g) were perfused with heparinized rat blood (0.04 ml/g body weight/min) and ventilated at 70 breaths/min with an inspiratory pressure of 9 cmH2O and an end-expiratory pressure of 2.5 cmH2O using a humidified gas mixture of 20 percent O2, 5 percent CO2, balance N2. The entire perfusion system was kept at 38 ± 0.5°C.

Seven hypoxic challenges (2.5 percent O2, 5 percent CO2, balance N2) lasting 10 minutes each were run sequentially, with ten-minute normoxic control periods separating them. Mean PA pressure, pH, PC02, and PCO2 measurements were made during the control periods just before the hypoxic challenges and after eight and ten minutes of hypoxia. During the fifth hypoxic challenge, diamide, 1 mg/0.1 ml saline (Sigma), was injected into the PA cannula port, after the eight-minute measurement.

Xanthine/Xanthine Oxidase: Allopurinol

The lungs of 21 Sprague-Dawley male rats were ventilated as before and perfused with a physiologic salt solution containing bovine serum albumin (4.0 percent) and meclofenamate (6 µg/ml). The lungs were subjected to nine cycles of pressor challenges, each consisting of angiotensin II (1 µg) in the pulmonary arterial line followed, after a recontrol period of eight minutes, by hypoxia (10 minutes). After the sixth cycle fresh perfusate was substituted by switching to a second bath. In seven control lungs no additional intervention was made. In a second group of seven lungs the same procedure was followed except that xanthine (0.1 mM) and xanthine oxidase (0.6 mg) were added to the perfusate bath (30 ml) after the third cycle. In the third group of seven lungs the same procedure was followed as in the second group, except that allopurinol (2 mg) dissolved in 0.2 ml (250 mg) dimethyl sulfoxide (DMSO) was added to the bath after the first cycle. To examine the possibility that allopurinol, or the solvent DMSO, might have an effect independent of the inhibition of the added xanthine oxidase, we performed an additional control experiment in the lungs of 15 pathogen-free Sprague-Dawley rats. Lungs from five control rats were perfused and challenged with nine cycles of angiotensin II followed by hypoxia.

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without additional intervention. In five, DMSO (0.2 ml) was added after the first cycle, and in five allopurinol (5 mg in 0.2 ml) was added after the first cycle.

**RESULTS**

**Diamide**

The sequential hypoxic challenges reduced the oxygen tension of the blood leaving the lungs from about 185 mm Hg during normoxia to about 44 mm Hg during hypoxia. The pH was maintained between 7.35 and 7.46. The pulmonary arterial pressure which, given a constant flow and left atrial pressure, reflects pulmonary vascular resistance, reached a plateau of 16 to 17 mm Hg with repeated hypoxic challenges (Fig 1). Diamide reduced the pressure attained to 12 ± 0.5 mm Hg (p < .01 for comparison with response prior to diamide). The pressor response almost returned to prediamide levels with the next hypoxic challenge.

**Xanthine/Xanthine Oxidase and Allopurinol**

The hypoxic challenges reduced the oxygen tension of the perfusate leaving the lungs from about 142 mm Hg during normoxia to about 37 mm Hg during hypoxia. The pH stayed between 7.37 and 7.42. The pulmonary pressor response to hypoxia of the control group attained a plateau by the third challenge (Fig 1). The increase in pressure in response to the third challenge is shown as 100 percent. The addition of xanthine/xanthine oxidase reduced the response to the next four challenges (p < 0.1), but following the switch to fresh perfusate the reduction was no longer significant. Allopurinol prevented the inhibition of hypoxic vasoconstriction caused by xanthine/xanthine oxidase and tended to increase the pressor response (Fig 2). The pressor response to angiotensin II was significantly reduced in the xanthine/xanthine oxidase group only at the fifth challenge, in comparison to the control and allopurinol group. There was no difference in wet/dry lung weights between the three groups. There was no significant difference in the pressor responses to hypoxia between the control, DMSO alone and allopurinol in DMSO groups (Table 1).

**DISCUSSION**

The temporary inhibition of hypoxic pulmonary vasoconstriction by diamide in the isolated perfused rat lung complements the same observation in the anesthetized dog. It has been suggested that diamide specifically oxidizes the sulfhydryl groups in glutathione, but even if this is correct, other sulfhydryl groups would be rendered vulnerable to oxidation. The suggestion that it is the oxidation of sulfhydryl groups that causes pulmonary vasodilatation is strengthened by the observation that the alkylation of sulfhydryl groups by iodoacetamide causes pulmonary hypertension.

Oxygen radicals generated by the combination of xanthine oxidase and acetaldehyde or xanthine cause dilatation of systemic arteries. Recently the generation of hydrogen peroxide by the addition of β-D-glucose and glucose oxidase to the perfusate has been reported to prevent the pressor response to hypoxia in the isolated rat lung. In the present study the administration of xanthine oxidase and xanthine into the perfusate reduced hypoxic pulmonary vasoconstriction in the rat lung. The addition of the same agents has been described to produce pulmonary edema in the isolated rabbit lung within 35 minutes.

The absence of significant pulmonary edema in the current experiment, even after perfusion was continued for 160 min-

**Table 1—Pulmonary Arterial Pressures Attained During Repeated Hypoxic Challenges (mm Hg)**

<table>
<thead>
<tr>
<th>Group*</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.3±1.0</td>
<td>6.3±1.4</td>
<td>6.9±0.9</td>
<td>8.9±1.4</td>
<td>16.0±2.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>2.6±0.3</td>
<td>2.8±0.3</td>
<td>7.3±0.8</td>
<td>11.8±1.9</td>
<td>14.1±2.5</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>4.0±0.5</td>
<td>3.4±0.7</td>
<td>5.1±0.3</td>
<td>14.3±1.8</td>
<td>19.4±1.1</td>
</tr>
</tbody>
</table>

*DMSO and allopurinol in DMSO were added after the first hypoxic challenge. No significant difference between groups.
utes following the additions, could be secondary to the use of 20 times less xanthine. In the same way that normoxia causes pulmonary vasodilation but hyperoxia leads to oxygen toxicity, a small generation of oxygen radicals may produce vasodilation while an excess of radicals induces pulmonary edema. The addition of allopurinol in the dose used did not significantly increase the pressor responses to hyperoxia. The trend toward an increase in comparison to controls suggests that higher doses and the omission of the washout might warrant further study.

The mechanism by which diamide and the addition of xanthine/xanthine oxidae caused vasodilation was not examined. Endothelial or smooth muscle damage remain possibilities, although pulmonary vascular reactivity appeared to be returning to control levels after the washout. Oxygen radicals produced by the addition of xanthine/xanthine oxidase depress calcium transport by both cardiac and skeletal muscle sarcoplasmic reticulum. Loss of calcium from the smooth muscle cells as a result of disordered calcium transport might lead to a reduction in reactivity.

Changes in enzyme activity could also account for the change in smooth muscle tone. Oxidation of sulphydryl groups in the calcium-ATPase of plasma membranes, mitochondria and endoplasmic reticulum reduces activity. Again, depending on the differential inhibition of calcium-ATPases at various sites, calcium could be lost from the cell. Another plausible explanation invokes the observation that oxygen radicals stimulate the activity of guanylate cyclase, thus increasing intracellular cyclic GMP and causing relaxation.

Other possible mechanisms by which oxygen radicals might cause vasodilation include changes in membrane calcium permeability caused by lipid peroxidation, or changes in the activity of receptors to acetylcholine and other vasoactive substances. The depletion of glutathione may prevent the formation of constrictor leukotrienes which might help to mediate the pressor response to hypoxia. While the range of possible mechanisms involving oxygen radicals is wide, it is likely that diamide acts only through changes in sulphydryl redox status. Further study is likely to enhance our understanding of pulmonary vascular reactivity.

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DISCUSSION

The discussion began by considering the possible role of sulphydryl redox status in determining membrane potential. It was pointed out that the conductance of the calcium channels could be altered by changes in the sulphydryl groups in the proteins which constitute these channels. In addition, membrane fluidity is in part determined by the sulphydryl redox status of proteins in the membrane.

The specificity of oxidizing agents for the hypoxic pressor response was raised. In the anesthetized dog diamide causes a smaller reduction in the pulmonary vascular resistance re-

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Prostaglandins and Estradiol-Induced Attenuation of Hypoxic Pulmonary Vasoconstriction*

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Pretreatment with estradiol (20 mg IM) attenuated vaso-reactivity to decreases in inspired Pfo2, lowered baseline resistance measured under conditions of maximal vasodilation (Pio2 = 0 mm Hg), and appeared to increase prostaglandin release in isolated, blood-perfused lungs of juvenile female sheep. Indomethacin (40 μg/ml) inhibited prostaglandin release and restored hypoxic vasoactivity in estrogen-treated lungs, but did not alter the estrogen-induced decrease in baseline resistance. These results suggest that estradiol enhanced the production of prostanoids which secondarily attenuated hypoxic vasoactivity. The estradiol-induced decrease in baseline resistance, however, must have been mediated by some other mechanism.

We have previously found that isolated lungs from six-month-old male sheep had a greater vasoconstrictor response to acute hypoxia than females. Moreover, hypoxic responses measured in isolated lungs from six-month-old castrates within the first week of life, juvenile males, and juvenile females were not different from those measured in six-month-old, non-castrated males. These results suggested that the gender difference observed in isolated lungs of six-month-old sheep arose from attenuation of the hypoxic response in the female at the time of puberty, possibly because of enhanced release of female sex hormones. Consistent with this possibility, we found that estradiol pretreatment attenuated hypoxic pulmonary vasoconstriction in isolated lungs from juvenile female and six-month-old castrated male sheep. Estrogens enhance prostaglandin production in uterine tissue. In addition, Chang and colleagues recently demonstrated that estradiol stimulated release of prostacyclin by rat aortic smooth muscle. Since prostacyclin, a potent pulmonary vasodilator, may modulate pulmonary vasoconstrictor responses to hypoxia, the present study was performed to assess the role of prostaglandins in the attenuation of hypoxic pulmonary vasoconstriction induced by estradiol. The purpose of this communication is to report our preliminary findings.

METHODS

Twenty-three juvenile female sheep between 53 and 90 days of age weighing 10 to 31 kg were used in the experiments. Three to five days before the experiments, 12 sheep were given 20 mg of a long-acting...