Potential Role of Arachidonic Acid Metabolites in
Hypoxic Pulmonary Vasoconstriction*

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Hypoxic vasoconstriction is important for lung ventilation/perfusion matching. The mechanism of hypoxic vasoconstriction remains elusive. Arachidonic acid is released from hypoxic tissues; possibly vasoconstricting arachidonate metabolites are involved in hypoxic pulmonary vasoconstriction. Data are presented that consider (a) lipoxygenase product(s) as "local," which could be involved in the hypoxia induced pulmonary vasoconstriction.

The lung is a major manufacturing site of arachidonic acid products. As diverse stimuli as hyperinflation, increase in vascular shear stress, endotoxin, and embolization lead to arachidonic acid release and synthesis of active metabolites. Prostacyclin, a major lung arachidonate metabolite and a potent pulmonary vasodilator, modifies the hypoxic pressor response. Because arachidonic acid is released from hypoxic tissues, we wondered whether metabolites with pulmonary vasoconstricting properties were released from the lung and whether release of such metabolites was temporally related to hypoxic pulmonary vasoconstriction.

Because the lung produces thromboxane and leukotrienes, both known pulmonary vasoconstrictors, we wished to investigate whether the isolated lung generated thromboxane and leukotrienes and whether either would be a likely mediator of hypoxic vasoconstriction.

If one considers the possibility that a mediator is produced by some lung cell on stimulation by airway hypoxia, it is appropriate to apply certain criteria which must be fulfilled by a mediator candidate to qualify. Such criteria have recently been established by Fishman. In first experiments using the isolated rat lung perfused with a cell- and plasma-free physiologic salt solution, we measured lung vascular effluent thromboxane B2 production (by radioimmunoassay) during baseline perfusion and shortly after hypoxic vasoconstriction and found that thromboxane concentrations were not different before and after hypoxic vasoconstriction. Because effluent thromboxane concentrations must not necessarily reflect lung parenchyma events, we wished clearly to increase lung thromboxane generation by adding exogenous arachidonate acid to the lung (criterion 4 in Table 1) expecting pulmonary vasoconstriction and increased hypoxic vasoconstriction. When arachidonate acid was injected as a pulmonary arterial bolus, thromboxane increased in the lung effluent, but prostacyclin increased much more, and thus subsequent hypoxic vasoconstriction was transiently blunted. Finally, because an inhibitor of thromboxane synthesis was available to us, we wished to see whether hypoxic vasoconstriction

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Table 1—Criteria Characterizing a Potential Mediator of Hypoxic Vasoconstriction

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<th>Mediator Criteria</th>
<th>Thromboxane Products</th>
<th>Lipoygenase Products</th>
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<tr>
<td>&quot;The mediator or its precursor must exist in the lung&quot;</td>
<td>+</td>
<td>Alveolar macrophages (24 mast cells, Ca⁺⁺-ionophore (19) PAF release LTC₄ from lungs (25,18)</td>
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<tr>
<td>&quot;The mediator must cause pulmonary vasoconstriction&quot;</td>
<td>+</td>
<td>(11,13,14,16)</td>
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<tr>
<td>&quot;The source of the mediator must be strategically located near resistance vessels&quot;</td>
<td>?</td>
<td></td>
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<tr>
<td>&quot;A mechanism must be present to turn on and to inactivate the mediator&quot;</td>
<td>+</td>
<td>Plasma binding (11), oxidation (25)</td>
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<td>&quot;Inhibition and depletion of mediator should inhibit hypoxic vasoconstriction&quot;</td>
<td>—Dazamgrel does not inhibit</td>
<td>+ Diethyldicarbazine, FLP 55712, U60257</td>
</tr>
<tr>
<td>&quot;Agents which modify hypoxic vasoconstriction should modify the mediator production/action&quot;</td>
<td>+ Ca⁺⁺-Antagonists (26,27,28) agents which increase cAMP</td>
<td></td>
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could be uncoupled from lung thromboxane synthesis (criterion 5, Table 1). When lungs were treated with dazamgrel (10⁻⁶ M), an inhibitor of thromboxane synthetase, lung effluent thromboxane levels approached the limits of detectability, yet hypoxic vasoconstriction was maintained (Fig 1). Therefore, we concluded that thromboxane A₄ was an unlikely mediator of hypoxic vasoconstriction.

Next, we wished to examine whether 5-lipoxygenase products were involved in hypoxic pulmonary vasoconstriction. Table 1 presents the available evidence which may make lipoxygenase products potential candidates. First, leukotrienes increase pulmonary artery pressure and cause constriction of isolated pulmonary arteries. Second, leukotrienes are produced by the lung, and on stimulation with Ca⁺⁺-ionophore A²37718 pulmonary arteries generate considerable amounts of leukotriene-like material. Diethyldicarbazine (DEC) and U60257, both inhibitors of leukotriene synthesis, block hypoxic vasoconstriction in isolated perfused rat lungs; nordihydroguaiaretic acid (NDGA; an inhibitor of lipoxygenase in some systems) was recently shown to inhibit hypoxic vasoconstriction in the ferret lung. Morganroth et al. recently showed reversible inhibition of hypoxic vasoconstriction in awake, instrumented rats after IP injection of DEC. A leukotriene receptor blocker, FPL 55712, which is structurally unrelated to the above-mentioned synthesis blockers, also inhibited hypoxic vasoconstriction in isolated rat lungs.

For lipoxygenase products to be involved in hypoxic vasoconstriction, one would require a close temporal relationship between activation of the 5-lipoxygenase pathway and vasoconstriction due to alveolar hypoxia. Indeed, 5-hydroxyeicosatetraenoic acid (5-HETE) increased in the lung effluent blood during hypoxic vasoconstriction. The measurement of this stable 5-lipoxygenase metabolite alone is not evidence for leukotriene synthesis, but does show that the 5-lipoxygenase pathway had been activated during hypoxia. Because current available techniques do not allow leukotriene measurements in blood, we measured lung leukotriene production in the lung lavage fluid before, during, and after hypoxic vasoconstriction. We found, using a combination of the guinea pig ileum bioassay, HPLC, and radioimmunoassay LTC₄ only during hypoxic vasoconstriction, but not before or after hypoxic vasoconstriction (Fig 2). DEC inhibited hypoxic vasoconstriction concomitant with LTC₄ production (Fig 2). Moreover, KCl-induced pulmonary vasoconstriction did not cause the appearance of LTC₄ in the lavage fluid (data not shown).

In recent studies we attempted to study the pulmonary vasoreactivity of arachidonic acid-deficient rats, since we postulated that this deficiency should impair lung leukotriene formation (although leukotrienes of the 3 and 5 series can still be made by the lung). We found that the lungs from rats raised on an essential fatty acid (EFA) free diet released less SRS (slow-reacting substance, since only bioassay criteria have been applied) when stimulated with the calcium ionophore A²33187 than their littermates, which were raised on a normal diet. The EFA-deficient rats had a lower plasma level of esterified arachidonic acid 36±5 μg/ml (n = 8) than the control rats 199±6 μg/ml (n = 4), and the isolated lungs from the EFA deficient rats showed decreased pressor responses to alveolar hypoxia and angiotensin II (Morganroth, unpublished observation).

Taken together, these investigations lead to the conclusion that 5-lipoxygenase products released from some lung cells...
may play a role in hypoxic pulmonary vasoconstriction. If so, these compounds are most likely acting as "local hormones" and their action is highly regulated by receptor binding, albumin binding, and metabolism.

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Questions were raised as to factors which might influence the production of the various arachidonic acid metabolites in the lung. Vascular responses to the exogenous arachidonic acid metabolites probably differ from those where the metabolites are generated endogenously. The responses vary between species, between individuals within a species, and between organs within an individual. The responses will also vary depending on gender and diet. Further, with isolated organs the handling of the tissue is important, \( eg \), the isolated perfused rat lung produces a large amount of prostacyclin if it is not handled gently. Baseline vascular tone is important, \( eg \), arachidonic acid infusion in the rat lung causes vasoconstriction when the tone is low and vasodilatation when given during hypoxic vasoconstriction. The consequences are that one must be careful in making comparisons of endogenous administration vs stimulation of endogenous production, as well as comparisons, say across species. Another consequence is that with regard to stimuli such as hypoxia, injury, or vasoconstrictor agonists, the different species may emphasize different metabolic pathways of arachidonic acid. In view of all of these variables, our present understanding of the function of the system must remain tentative.

The difficulties in concluding that leukotrienes mediate the hypoxic response were discussed. Although the work presented indicated that diethylcarbamazine (DEC) inhibited the hypoxic vasoconstriction in the rat lung and in the awake rat, a discussant indicated that it did not block hypoxic vasoconstriction in the ferret lung. DEC, particularly in large doses, is unlikely to be a specific inhibitor of the lipooxygenase pathway. Chronic administration in the rat showed some inhibition of the cyclooxygenase pathway. DEC also inhibited to a slight extent the pulmonary pressor response to angiotensin II. Although the possibility was raised that DEC had Ca\(^{2+}\) blocking properties, in the rat lung it was not as effective as was verapamil in blocking vasoconstriction induced by increased KCl.

Other aspects of the relationship between leukotrienes and hypoxic pulmonary vasoconstriction were discussed. In the isolated perfused rat lung leukotrienes have been shown to cause long-lasting pulmonary vasoconstriction which is not consistent with the rapid fall in pulmonary arterial pressure after hypoxia is relieved. They also produce edema which is not considered to be a part of the lung's hypoxic response. With regard to the dietary manipulations, lungs from the rats fed a diet deficient in essential fatty acids have a reduced pressor response both to hypoxia and to angiotensin II. It was considered that leukotrienes could act as amplifying substances for hypoxic pulmonary vasoconstriction rather than acting as obligatory mediators. In such a case they would be analogous to, but opposing, the moderating effect of another metabolite of arachidonate acid, prostacyclin. However, leukotrienes are not a part of all pulmonary vasoconstriction responses, because they were not identified in the lungs following vasoconstriction with potassium chloride. Although in the rat lung it was not certain which element of the lipooxygenase sequence was most active during hypoxic pulmonary vasoconstriction, the most likely candidate was LTC\(_4\).

The difficulties in attempting to identify and measure leukotrienes was stressed. They are not at present measurable in blood. Neither do they circulate as active compounds, probably because they are tightly bound to albumin. Thus, it is likely that they are locally acting substances. They can be identified in lung lavage, lung extract, and lung lymph.

Observations reported at the meeting emphasized the potential importance of several diverse mechanisms of hypoxic pulmonary vasoconstriction: membrane depolarization, oxidative phosphorylation, leukotrienes, and sulfhydryl redox status. A unifying hypothesis was sought but none was proposed.