release of cyclooxygenase metabolites reflected by measurements in lung lymph. Permeability appears to increase only after those metabolites have decreased. Also, inhibitors of arachidonic acid cyclooxygenase (indomethacin and meclofenamate) do not prevent the late phase increase in flow of protein rich lymph, even though they inhibit the increase in lymph concentrations of cyclooxygenase metabolites.  

We have also studied the effects of exogenous infusion of a number of cyclooxygenase products of arachidonate and have not been able to demonstrate a convincing increase in vascular permeability caused by any of them.  

What about lipoxigenase products? Large doses of corticosteroids given before infusing endotoxin in sheep will prevent the late phase increase in lymph flow.  

We have measured lung lymph concentrations of 5-hydroxyeicosatetraenoic acid (5-HETE), a lipoxigenase product of arachidonate, in lung lymph during endotoxemia, demonstrating large increases in 5-HETE concentrations peaking later than cyclooxygenase products, about coincident with the onset of increased permeability.  

Granulocytes are one potential source of lipoxigenase products. When sheep are depleted of granulocytes by hydroxyurea, the increase in permeability occurring late after endotoxin is significantly attenuated.  

These data are inconclusive still, but there seems to be mounting evidence that lipoxigenase products of arachidonate may be involved either directly or indirectly in mediating the increase in lung vascular permeability after endotoxin.

REFERENCES

1 Snapper JR, Ogletree ML, Hutchison AA, Brigham KL. Meclofenamate prevents increases of lung (R1) following endotoxemia in unanesthetized sheep. Am Rev Respir Dis 1981; 123:200


6 Ogletree ML, Brigham KL. Indomethacin augments endotoxin induced increased lung vascular permeability in sheep. Am Rev Respir Dis 1979 (abstract); 119:383


Pulmonary Vascular Responses to Thromboxane A2, as Unmasked by OKY-1581*  
A Novel Inhibitor of Thromboxane Synthesis


In most organ systems studied so far, including the lung, fatty acid precursor arachidonic acid, which is derived from the breakdown of phospholipids in cell membranes, is converted into the cyclic endoperoxide intermediates, PGs, and PGI2, by a microsomal cyclooxygenase.  

The endoperoxide intermediates (PGs and PGI2) are then converted by specific terminal enzymes into primary prostaglandins (PG), thromboxane A2 (TXA2), or prostacyclin (PGI2). The distribution and activity of terminal enzymes determine the pattern and relative amounts of vasoactive and bronchoactive products formed from endoperoxide metabolism in an organ system.  

Many reports indicate that the endoperoxide intermediate PGI2, endoperoxide analogs, PGE2, PGI2, and PGD2, all increase pulmonary vascular resistance in a variety of species.  

In contrast, PGE2 has dilator activity in the pulmonary circulation of fetal and neonatal animals.  

The pulmonary vascular effects of TXA2 are uncertain, but this labile substance has potent smooth muscle-stimulating and platelet-aggregating activity and its breakdown product, TXB2, has modest pressor activity in the pulmonary vascular bed.  

In contrast to the effects of all of the above mentioned

*From the Departments of Pharmacology and Surgery, Tulane University School of Medicine, New Orleans.  
Reprint requests: Dr. Hyman, Tulane Medical School, 1430 Tulane Avenue, New Orleans 70112

728

Lung Defense, Injury and Repair
substances, the newly discovered product of arachidonic acid metabolism, PGI₄, has pulmonary vasodilator activity. In addition, the recently discovered metabolite of PGI₄, 6-keto-PGE₃, has potent vasodilator activity in the pulmonary vascular bed. The purpose of the present report was to determine the actions of TXA₄ on the feline pulmonary vascular bed as unmasked by OKY-1581, a novel inhibitor of thromboxane synthesis.

METHODS AND MATERIALS

The pulmonary vascular effects of the prostaglandins, arachidonic acid and OKY-1581 were investigated in the feline pulmonary vascular bed under conditions of controlled pulmonary blood flow using recently described techniques. Adult cats, unselected as to sex were used in these studies and the animals were anesthetized with pentobarbital sodium, 30 mg/kg iv. For biochemical studies, adult cats, unselected as to sex, were anesthetized with sodium pentobarbital (30 mg/kg iv). The thorax was opened, and the pulmonary artery was perfused with ice-cold 0.1 M potassium phosphate buffer, pH 7.4, via the right ventricle, until the lungs were cleared of blood. The whole lungs were then quickly excised, rinsed with the ice-cold phosphate buffer, blotted dry and weighed. The following procedures were done at 0-4°C. The tissues were minced and homogenized in three volumes of the phosphate buffer with a Polytron PT-20 for 40 sec. The homogenate was centrifuged at 10,000 x g for 15 min. The supernatant was strained through cheese cloth and centrifuged at 90,000 x g for 70 min. The microsomal pellet thus obtained was washed and resuspended in the phosphate buffer, and stored at -45°C until use. Protein concentration was determined by standard techniques.

The preparation of radiolabelled PGH₄ from (L-¹⁴C) arachidonic acid and biochemical procedures have been described previously.

RESULTS

A typical radiochromatographic scan obtained following thin-layer chromatography of the products of incubation of (L-¹⁴C) PGH₄ with cat lung microsomes is shown in Figure 1A. The endoperoxide metabolites were identified by comigration with authentic PG standards. A characteristic pattern of three major peaks can be seen (Fig 1A). The two more polar peaks co-chromatographed with standard TXB₂ and 6-keto-PGF₁α, the stable breakdown products of TXA₄ and PGI₄, respectively. The fastest running peak, which moved slightly behind the compound DL-12-hydroxystearic acid, was presumably 12L-hydroxy-5,8,10-hepadecaetrieinioic acid (HHT), formed by chemical decomposition of PGH₄ and the action of thromboxane synthetase. TXB₂ and 6-keto-PGF₁α were not found in the incubation mixture of PGH₄ with heat-inactivated microsomes (Fig 1B). Instead, PGH₄ appeared to undergo decomposition to PGD₂, PGE₃ and PGF₂α. The TXB₂ peak was abolished and HHT formation was lowered to that observed in the presence of boiled enzyme in the presence of 20 mM imidazole (Fig 1C), a selective inhibitor of thromboxane synthetase. Addition of 10 mM tranylcypromine, an inhibitor of prostacyclin synthetase, to an incubation mixture resulted in the disappearance of 6-keto-PGF₁α as well as approximately equal decreases in the production of TXB₂ and HHT (Fig 1D). Unreacted PGH₄ was present, although its position was not labeled. In Figure 1B (boiled enzyme) it is the peak to the right of PGE₃ and to the left of HSA. The same peak was reduced in Figure 1A and 1C in which the conditions favor enzymatic metabolism of PGH₄.

When enzymatic metabolism was decreased by inhibition of prostacyclin synthetase and partial inhibition of thromboxane synthetase (10 mM tranylcypromine, Fig 1D), the unreacted PGH₄ peak increased. The results of these biochemical experiments indicate that the feline lung has marked thromboxane synthesizing capacity.

In hemodynamic studies, intralobar responses of arachidonic acid (AA) in doses of 3, 10 and 30 μg under baseline tone conditions increased lobar arterial pressure (Table I) in a dose-dependent manner without altering left atrial pressure. The increases in lobar arterial pressure in response to AA were greatly reduced after injection of OKY-1581, 2 mg/kg iv. In addition, at the higher (10 and 30 μg) doses of AA, a secondary vasodilator response was unmasked (Table I). All responses to arachidonic acid were abolished after administration of OKY-1581, 2.0 mg/kg iv, and indomethacin, 2.5 mg/kg iv (Table I).

FIGURE 1. Radiochromatogram of the products isolated from incubation of 5μM PGH₄ with 300 μg microsomal protein in 100 μl 0.1 M potassium phosphate buffer, pH 7.4, for 2 min at 37°C. (A) Cat lung microsomes; TXB₂ and 6-keto-PGF₁α conversion 23.4% and 17.2%, respectively. (B) Microsomes boiled for 10 min. (C) Microsomes + 20 mM imidazole. (D) Microsomes + 10 mM tranylcypromine. Migration of authentic PG standards are indicated. HSA denotes DL-12-hydroxystearic acid. The arrow indicates the origin.
Table 1—Influence of OKY 1581 and Indomethacin on Responses to Arachidonic Acid (AA) in the Pulmonary Vascular Bed

<table>
<thead>
<tr>
<th>Lobar Arterial Pressure (mm Hg)</th>
<th>OKY 1581</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12±1</td>
<td>12±1</td>
</tr>
<tr>
<td>AA, 3 μg</td>
<td>17±1*</td>
<td>11±0</td>
</tr>
<tr>
<td>Control</td>
<td>12±2</td>
<td>13±1</td>
</tr>
<tr>
<td>AA, 10 μg</td>
<td>22±1*</td>
<td>15±1*</td>
</tr>
<tr>
<td></td>
<td>11±0*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11±0</td>
<td>13±1</td>
</tr>
<tr>
<td>AA, 30 μg</td>
<td>31±2*</td>
<td>16±1*</td>
</tr>
<tr>
<td></td>
<td>10±1*</td>
<td></td>
</tr>
</tbody>
</table>

n = 15
*p<0.05 when compared to corresponding control
†secondary dilation

In the last series of experiments, the effects of OKY-1581 and indomethacin on responses to U-46619 were investigated in the feline pulmonary vascular bed and these data are summarized in Table 2. Intralobar injections of U-46619, 3, 10 and 30 ng, caused significant dose-related increases in lobar arterial pressure without altering left atrial pressure. The increases in lobar arterial pressure in response to U-46619, an agent which is thought to mimic the actions of TXA₄, were not altered after administration of OKY-1581, 2.0 mg/kg iv, or OKY-1581, 2.0 mg/kg iv and indomethacin, 2.5 mg/kg iv (Table 2).

Discussion

Results of the present biochemical studies demonstrate that cat lung microsomes synthesize predominantly 6-keto-PGF₆ and TXB₂, the stable metabolites of the vasoactive substances of PGH₂ and TXA₄, respectively. The formation of 6-keto-PGF₆ and TXB₂ from PGH₂ by the lung microsomes is inhibited by heat denaturation, indicating that these were enzymic reactions. Small amounts of PGD₂, PGE₂, and PGF₆ were formed in the incubation with microsomes, and became more apparent at high PGH₂ concentrations. We did not distinguish between enzymatic conversion and chemical decomposition of PGH₂ to these products in aqueous medium. Thromboxane synthetase, which catalyzes the conversions of PGH₂ to TXA₄ and to HHT, was selectively inhibited by imidazole in our study. Tranexamycromine markedly inhibited prostacyclin synthetase, but partially inhibited thromboxane synthetase as well. The results of biochemical studies suggest that the major endoperoxide metabolizing activities in the cat lung are prostacyclin and thromboxane synthetases.

The results of hemodynamic studies show that vasocoestrictor responses to AA are inhibited after administration of OKY-1581 suggesting that the pressor effects of AA are due in part to the formation of TXA₄. All responses to AA were blocked by indomethacin suggesting that they were due to formation of products in the cyclooxygenase pathway. U-46619 had marked vasocoestrictor activity in the feline pulmonary vascular bed. This agent was far more potent than PGF₆, PGD₂, PGE₂ or TXB₂ in increasing pulmonary lobar vascular resistance in the cat. It has recently been suggested that U-46619 may mimic the actions of TXA₄.* If U-46619 did mimic the actions of TXA₄ then thromboxane would be a powerful pressor agent in the pulmonary vascular bed. OKY-1581 had no significant effect on responses to U-46619 suggesting that it did not possess TXA₄ receptor blocking activity.

In summary, results of the present study suggest that: 1) TXA₄ is formed in the feline lung from exogenous AA; 2) TXA₄ has vasocoestrictor activity in the feline pulmonary vascular bed; 3) if U-46619 does actually mimic the effects of TXA₄, then TXA₄ would be the most potent pressor agent known in the pulmonary vascular bed.

Acknowledgment: We wish to thank the Ono Company for the OKY-1581 and The Upjohn Company for the prostaglandins used in this study. We also wish to thank Ms. Janice Igarro for help in preparing the manuscript. This work was supported in part by NIH grants HL11802 and HL15580.

References


748

Lung Defense, Injury and Repair