Influence of a Prostaglandin Endoperoxide Analogue on the Canine Pulmonary Vascular Bed

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SUMMARY We evaluated the effects of an analogue of the prostaglandin endoperoxide, PGH₂, in the canine pulmonary vascular bed. The analogue increased pulmonary arterial pressure whereas cardiac output and left atrial pressure were unchanged. Although pulmonary vascular resistance was increased markedly, only small increases in systemic vascular resistance were observed. In experiments in which blood flow to a lobe was maintained constant, the analogue produced dose-related increases in lobar arterial and small vein pressure but little change in left atrial pressure. These data suggest that the analogue increased resistance to flow by constricting intrapulmonary veins and upstream vessels presumed to be small arteries. The increase in resistance was similar when the lung was perfused with dextran or with blood. In addition, the analogue increased inflation pressure; however, similar increments in vascular resistance were obtained in ventilated and nonventilated lung lobes. Indomethacin, in doses which abolished responses to arachidonic acid, did not attenuate the response to the analogue. These results suggest that interaction with formed elements, increases in airway tone, or stimulation of prostaglandin synthesis contribute little if anything to the pressor response to the analogue. These data show that the analogue is far more potent than the biseenoic prostaglandins in the pulmonary vascular bed and suggest that endoperoxides may represent an active form of the prostaglandins in the lung.

PROSTAGLANDINS E₂ and F₂₀ (PGE₂ and PGF₂₀) are formed in the lung from the precursor, arachidonic acid, and their effects on the pulmonary vascular bed have been investigated in a variety of species. In dog, cat, sheep, swine, and calf PGF₂₀ was found to be a potent pulmonary presor substance, whereas in dog, swine, and lamb, PGE₂ elicited small increases in pulmonary vascular resistance. The prostaglandin precursor, arachidonic acid, has been reported to have pressor activity in the canine pulmonary vascular bed and its effects are blocked by inhibitors of prostaglandin synthesis.


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thesis of endogenous prostaglandins on the response to this analogue.

Methods

The cardiopulmonary effects of the analogue were studied in 70 mongrel dogs of either sex weighing 14–23 kg. Dogs were anesthetized with pentobarbital sodium (30 mg/kg, iv) and strapped to a Philips heart table in the supine position. In 23 dogs in which the effects of the analogue on pulmonary and systemic vascular resistance were evaluated, a 6F Edslab thermal dilution catheter was passed into the main pulmonary artery from the external jugular vein under fluoroscopic guidance (Philips image intensifier). Pulmonary arterial pressure was measured from the distal port on the Edslab catheter. A 7F Teflon catheter was passed into the left atrium transeptally and large bore catheters were positioned in the aorta from a femoral artery and in a femoral vein. Cardiac output was determined with an Edwards thermal dilution computer, model 9500, after injection of 5 ml of 5% dextrose solution (cooled to 0°C) into the superior vena cava (proximal port on the Edslab catheter). Values for cardiac output averaged 120 ml/kg per min and compared favorably with cardiac outputs determined by the indicator-dilution technique in this laboratory. The dogs breathed room air or room air enriched with O₂ spontaneously through a cuffed endotracheal tube.

In 47 dogs in which constant flow perfusion of the left lower lobe was employed, a specially designed 20F double-lumen balloon catheter was introduced through a jugular vein into the arterial branch of the left lower lung lobe under fluoroscopic guidance. A 1.5-mm Teflon catheter with its tip positioned about 2 cm distal to the tip of the perfusion catheter was used to monitor perfusion pressure in the lobar artery. Catheters with side holes near the tip were passed into the main pulmonary artery and femoral artery and transeptally into a small intrapulmonary vein and the left atrium. Precautions were taken to ensure that pressure measurements were made without wedging in veins 2–3 mm in diameter. Briefly, a 0.9-mm Teflon catheter with two side holes near the tip was passed through a 3-mm Teflon catheter that previously had been wedged in a small intrapulmonary vein. The 0.9-mm catheter was then withdrawn 1–3 cm from the wedge position until pressure dropped abruptly. The 0.9-mm catheter was fixed in place with a Cope adaptor after the larger catheter had been withdrawn to the left atrium. These methods have been described in detail previously.Ä All vascular pressures were measured with Statham P23D transducers zeroed at the level of the middle of the right atrium, and mean pressures were recorded on an oscilloscopic recorder (model DR-12, Electronics for Medicine). After all catheters had been positioned and the dogs heparinized (500 U/kg, iv) the balloon on the perfusion catheter was distended with 2–4 ml of 50% sodium diatrizoate (Hypaque Winthrop) until pressure in the lobar artery and small vein decreased to near left atrial pressure. The vascularly isolated left lower lobe then was perfused with a Sarns roller pump (model 3500) with blood withdrawn from the right atrium. The pumping rate was adjusted so that mean pressure in the perfused lobar artery approximated mean pressure in the main pulmonary artery and thereafter was not changed during the experiment. The pumping rate averaged 260 ml/min. These dogs spontaneously breathed room air or room air enriched with O₂ through a cuffed endotracheal tube. In experiments in which the lobe was perfused with dextran solution a 14F withdrawal catheter was placed transeptally in the vein draining the left lower lobe. In the intact spontaneously breathing dog, the lung was perfused at a rate of 160–330 ml/min with a 10% solution of low molecular weight dextran (Sigma) in saline warmed to 37°C. The perfused dextran along with small amounts of blood were removed from the withdrawal catheter using a siphon system. In experiments in which the effects of the analogue on airway pressure were evaluated, the dogs intubated with a Carlen's endobronchial divider (no. 39) and the left lower lobe and right lungs were ventilated separately with a Harvard dual-cylinder respiratory (model 618) at a rate of 20 cycles/min with stroke volumes of 110 ml/min for the left lower lobe and 280 ml/min for the right lungs. The dogs received succinylcholine chloride (Anectine, Burroughs Wellcome), 2.5 mg/kg, iv, to paralyze ventilation. Translobar airway pressure was measured with a Statham differential transducer (PM5) bridged between the left side of the endobronchial divider and the pleural space and was recorded on the Electronics for Medicine recorder. Under conditions of constant volume respiration, changes in peak translobar airway pressure reflect changes in airway resistance and dynamic compliance. In experiments in which ventilation to the left lower lobe was arrested by clamping the left side of the divider during expiration, the lobe was perfused with arterialized blood to prevent the effects of hypoxia on the lung.

Prostaglandins E₂ and F₃₄, and the prostaglandin H₂ analogue, (15S)-hydroxyl-11α,9α-(epoxymethano)prosta-5Z-dienoic acid (Upjohn) were dissolved in ethyl alcohol and stored in a freezer. Stock solutions were prepared on a frequent basis in normal saline solution. Arachidonic acid, 99% pure (Sigma or NuCheck) and indomethacin were prepared daily as sodium salts in 1% sodium carbonate in normal saline.

All data were analyzed by the methods described for paired and group comparison.¹⁹ Pulmonary vascular resistance was calculated by dividing pulmonary arterial pressure minus left atrial pressure by the cardiac output. Systemic vascular resistance was calculated by dividing aortic pressure by the cardiac output after having determined that right atrial pressure was not altered by the analogue in four experiments. All values were expressed as mean ± SEM and a P value of less than 0.05 was considered significant.

Results

CARDIOPULMONARY ACTIONS OF THE ANALOGUE

The cardiopulmonary effects of the PGH₂ analogue are illustrated in Figure 1 and data from 14 experiments are summarized in Table 1. Injection of the analogue into the superior vena cava in doses of 1, 3, and 10 μg resulted in a dose-related increase in pulmonary arterial pressure but
no change in left atrial pressure or cardiac output. The increase in calculated pulmonary vascular resistance was 34%, 86%, and 158% at 1, 3, and 10 μg of the analogue, respectively. Although the analogue elicited large increases in pulmonary arterial pressure, only small increments in aortic pressure were observed (Table 1). The increase in systemic vascular resistance was significant only at the 10-μg dose, at which a 21% rise occurred (Table 1).

To determine whether the analogue was inactivated by the lung, the effects of injection of this substance into the right and left side of the circulation were compared in a second group of dogs. Injection of the analogue, 10 μg, into the left atrium resulted in a significant increase in aortic pressure and, after a delay of approximately 10-18 seconds, an increase in pulmonary arterial pressure (Table 2). Pressure in the left atrium and the cardiac output were unchanged (Table 2). The increase in pulmonary vascular resistance was significantly greater when the analogue was injected into the superior vena cava (30%) than when injected into the left atrium (21%).

**TABLE 1 Cardiopulmonary Effects of the Endoperoxide Analogue in the Anesthetized Dog**

<table>
<thead>
<tr>
<th>Amount injected</th>
<th>Pressure (mm Hg)</th>
<th>Cardiac output (liters/min)</th>
<th>Pulmonary vascular resistance (mm Hg/liter/min)</th>
<th>Systemic vascular resistance (mm Hg/liter/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 8)</td>
<td>17.5 ± 1.7</td>
<td>2.4 ± 0.5</td>
<td>2.27 ± 0.21</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>1 μg (n = 10)</td>
<td>22.8 ± 2.2*</td>
<td>2.4 ± 0.5</td>
<td>2.26 ± 0.18</td>
<td>9.0 ± 1.6*</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>17.9 ± 1.2</td>
<td>3.0 ± 0.6</td>
<td>1.93 ± 0.17</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>3 μg (n = 14)</td>
<td>31.5 ± 2.2*</td>
<td>3.3 ± 0.7</td>
<td>1.97 ± 0.19</td>
<td>14.3 ± 2.1*</td>
</tr>
<tr>
<td>Control (n = 14)</td>
<td>17.5 ± 1.0</td>
<td>3.8 ± 0.6</td>
<td>1.86 ± 0.14</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>10 μg (n = 14)</td>
<td>38.2 ± 2.0*</td>
<td>4.3 ± 0.7</td>
<td>1.78 ± 0.15</td>
<td>19.0 ± 2.8*</td>
</tr>
</tbody>
</table>

*P* < 0.05 when compared to control, paired comparison.

**PERFUSION EXPERIMENTS**

The above studies establish that the PGH₂ analogue increases pulmonary vascular resistance in the dog. To investigate the site of action in the pulmonary vascular bed, experiments were carried out in which blood flow was maintained constant with a pump, and pressures in small (2-3 mm) intrapulmonary veins were measured. In these experiments injection of the analogue as a bolus into the perfused lobar artery in the dose range of 0.03-10 μg resulted in a significant dose-related increase in lobar arterial perfusion pressure (Fig. 2). The increase in lobar arterial pressure was accompanied by a significant dose-related increase in pressure in the small intrapulmonary vein and in pressure gradient from lobar artery to small vein but no significant change in left atrial pressure (Fig. 2).

**EFFECTS IN VENTILATED AND NONVENTILATED LUNGS**

In experiments in which the left lower lobe and the right lungs were ventilated separately using a Carlen’s endobronchial divider and dual-cylinder Harvard respirator, injection of the analogue into the perfused lobar artery elicited a dose-related increase in peak translobar airway pressure (Fig. 3, upper panel). The increase in airway pressure reflects both an increase in airway resistance and a decrease in dynamic compliance. To determine whether the changes in airway and vascular pressure were related, pressor responses were compared in ventilated and nonventilated lung lobes. In these experiments responses were obtained when the left lower lobe was ventilated and when airflow to the lobe was stopped during expiration. The increase in lobar arterial pressure was similar when the lung was ventilated and when ventilation was arrested by occluding the left side of the endobronchial divider (Fig. 3, lower panel).

**INFLUENCE OF DEXTRAN PERFUSION**

To ascertain the contribution of interaction with formed elements, responses to the analogue were compared when the lung was perfused with blood and with a 10% dextran solution. In 10 dogs the increase in lobar arterial perfusion pressure in response to 3 μg of the analogue was 13.8 ± 1.9 mm Hg during lobar perfusion with blood and 12.7 ±
INFLUENCE OF INDOMETHACIN

The effects of indomethacin, an inhibitor of prostaglandin synthesis, on the response to the analogue were evaluated in the pulmonary vascular bed. The rise in lobar arterial pressure in response to the analogue was increased significantly after indomethacin, 2.5 mg/kg, iv (Fig. 4, left panel). In five of these nine dogs the effects of indomethacin on responses to arachidonic acid, the precursor for PGE₂ and PGF₂α, were investigated. The increase in lobar arterial pressure and the decrease in aortic pressure in response to injection of 3 mg of arachidonic acid into lobar artery were decreased significantly (Fig. 4, right panel).

RELATIVE POTENCY OF THE ANALOGUE

The structures of arachidonic acid, the fatty acid precursor, the endoperoxide intermediates and analogue, and the bisenoic prostaglandins are shown in Figure 5. The relative potency of the precursor, the endoperoxide analogue, and the bisenoic prostaglandins was compared in the pulmonary vascular bed. The PGH₂ analogue was approximately 10 times more potent than PGF₂α, 300 times more potent than PGE₂, and 3,000 times more potent than arachidonic acid in increasing lobar arterial perfusion pressure in the dog (Fig. 6).

Discussion

Results of the present study show that an analogue of PGH₂ increased pulmonary arterial pressure in the dog. Inasmuch as cardiac output and left atrial pressure were unchanged the increase in pulmonary arterial pressure suggests an increase in pulmonary vascular resistance. The effects of the analogue were dose-related and the substance also increased systemic vascular resistance; however, the effects on the pulmonary circulation were much greater. The effects of the analogue on the systemic vascular bed were similar when this substance was injected into the superior vena cava and the left atrium. These data suggest that the analogue is not inactivated to any great extent by the pulmonary vascular bed during perfusion with dextran. The increase in lobar pressure was not significantly different when the lung was perfused with blood or with dextran.

4.0 mm Hg during perfusion with dextran. The increase in lobar pressure was not significantly different when the lung was perfused with blood or with dextran.
extent by the canine lung. The site of vasoconstriction was investigated in experiments in which blood flow was controlled and pressure gradients across the left lower lobe were measured. Results of these experiments show that the analogue increased lobar arterial perfusion pressure in a dose-related manner over an extremely wide range of dose. Moreover, doses which establish concentrations of less than 2 ng/ml in lobar arterial blood increased lobar vascular resistance by more than 50%. The increase in lobar arterial pressure was associated with a dose-related increase in pressure in small intrapulmonary veins and an increase in the gradient of pressure from lobar artery to small vein but with little change in left atrial pressure. These data suggest that the increase in vascular resistance in the lobe is the result of vasoconstriction in intrapulmonary veins and upstream vessels presumed to be small arteries. The relative contribution of various segments of upstream vessels to the pressor response is unknown;

however, measurements of venous gradients suggest that veins 2–5 mm in diameter were constricted.

In addition to increasing vascular resistance in the lung, the PGH₂ analogue increased airway pressure under conditions of positive pressure ventilation. These data are in accord with the studies of Wasserman in which the analogue was shown to increase airway resistance and decrease dynamic compliance in the dog. Although the analogue increased airway pressure, the effects on airway and vascular smooth muscle did not seem to be related, since similar pressor responses were obtained in ventilated and nonventilated lung lobes.

The endoperoxides PGH₂ and PGG₂ and their analogues have been reported to stimulate platelet aggregation. In view of the platelet-aggregating potential of these substances and subsequent release of vasoactive substances, the effects of the analogue were compared in dextran- and blood-perfused lung lobes. Results of these studies show that the analogue elicited similar pressor responses when the lung lobe was perfused with dextran solution or with blood. These data suggest that obstruction of the vascular bed by platelet aggregates or the release of vasoactive substances from elements in blood contribute little if anything to the pressor response, since these elements were not present in the nonsanguineous perfusate. It has been reported that the endoperoxide analogues stimulate synthesis of endogenous PGH₂ and PGG₂ in some tissues. If the response of the pulmonary vascular bed to the analogue were dependent on synthesis of endogenous intermediates, then the response should be attenuated by inhibitors of prostaglandin synthesis. Indomethacin, a synthesis inhibitor in doses reported to decrease the formation of endoperoxides and primary prostaglandins, did not attenuate the pressor response to the analogue in the lobe. These data indicate that synthesis of endogenous endoperoxides or prostaglandins contributes little if anything to the pressor response. The extent of inhibition in these experiments was assessed by comparing responses to arachidonic acid, the precursor for the bisenoic prostaglandins, before and after indomethacin.

The increase in lobar arterial pressure and the decrease

![Figure 4](https://example.com/figure4.png) **Figure 4** Left panel: influence of indomethacin (2.5 mg/kg, iv) on the increase in lobar arterial pressure in response to injection of 0.3 and 1 μg of the endoperoxide analogue into the lobar artery. Right panel: influence of indomethacin (2.5 mg/kg, iv) on the increase in lobar arterial pressure and the decrease in aortic pressure in response to injection of arachidonic acid (3 mg) into the lobar artery.

![Figure 5](https://example.com/figure5.png) **Figure 5** Structures for arachidonic acid, the precursor for the bisenoic prostaglandins, the endoperoxides (PGH₂ and PGG₂), the endoperoxide analogue, and the bisenoic prostaglandins (PGE₂ and PGF₃α).

![Figure 6](https://example.com/figure6.png) **Figure 6** Dose-response curves comparing the relative potency of the endoperoxide analogue, PGF₃α, PGE₂, and arachidonic acid in increasing lobar arterial perfusion pressure in the intact dog. n indicates number of dogs an agent was tested in.
in aortic pressure in response to arachidonic acid were decreased by more than 95%. These data suggest that synthesis was decreased markedly in the lung by indomethacin. The explanation for the enhanced pressor response to the analogue after indomethacin is uncertain. Previous studies have shown, however, that the effects of PGF₆ₒ and PGE₂ on the pulmonary vascular bed were enhanced by this synthesis inhibitor.²⁴

Results of the present studies show that the endoperoxide analogue increased resistance to flow by constricting intrapulmonary veins and upstream vessels and, in terms of relative potency, was approximately 10 times more potent than PGF₆ₒ, 300 times more potent than PGE₂, and 3,000 times more potent than arachidonic acid. Studies with platelets and isolated smooth muscle indicate that the potencies of the PGH₂ analogue and PGH₂ are similar, whereas both substances are far more active than the bisenoic prosta glandins.¹⁴,¹⁵,²¹,²⁵ The present studies show that the endoperoxide analogue is a potent pressor substance in the canine pulmonary vascular bed. This observation, along with the finding that the major product of prostaglandin synthesis in the lung are metabolites of intermediates,² suggests that endoperoxides may represent an active form of the prostaglandins in the lung. It is possible therefore that pathophysiologic stimuli that release prostaglandins may release even larger quantities of these active intermediates which could have pronounced effects on the pulmonary vascular bed and airways.

References
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