Pulmonary Vascular Effects of Prostaglandin D₂, but Not Its Systemic Vascular or Airway Effects, Are Mediated Through Thromboxane Receptor Activation

Landon S. King, Masao Fukushima, Mukul Banerjee, Kyung Ho Kang, John H. Newman, and Italo Biaggioni

Prostaglandin D₂ (PGD₂), a product of the cyclooxygenase pathway of arachidonic acid metabolism, is a major secretory product of activated mast cells¹ and also is released from lung macrophages.² It is, therefore, potentially involved in a number of processes associated with activation of these cells, such as asthma,³ lung inflammation,⁴ and systemic mastocytosis.⁵ The mechanisms of vascular and airway effects of PGD₂ are not completely understood, partly because PGD₂ has vascular effects that vary with species, site, and age. PGD₂ produces pulmonary arterial dilation in fetal goats⁶ and lambs,⁷ whereas it produces pulmonary vasoconstriction in adult goats,⁶ dogs,⁸ and sheep.⁹ There is a transition from dilation to constriction after birth,⁶,¹⁰ but the mechanism for this effect is unknown. PGD₂ increases systemic arterial pressure in sheep but decreases blood pressure in humans¹¹ and dogs.¹² Finally, PGD₂ administered by infusion or inhalation produces bronchoconstriction in humans¹² and dogs,¹³ as does its principal metabolite, 9α,11β-prostaglandin F₂.¹⁴ This is of particular interest because
of the possible role mast cells are suspected to play in allergic asthma.

PGD$_2$ appears to activate several prostaglandin receptors, including a PGD$_2$ receptor (DP-receptor) and the thromboxane A$_2$/endoperoxide receptor (TX/E). The constrictor effects of PGD$_2$ in vascular smooth muscle in vitro are postulated to be mediated through the TX/E receptor, whereas the prostaglandin receptor by which PGD$_2$ induces bronchoconstriction is uncertain. In cats, SQ-29,548, a selective TX/E receptor antagonist, does not block PGD$_2$-induced bronchoconstriction.

In this study, we examined the effect of PGD$_2$ on respiratory mechanics and on the pulmonary and systemic vasculature in adult sheep. Our aim was to determine if the effects of PGD$_2$ were mediated through activation of TX/E receptors. Our results indicate that PGD$_2$ produces pulmonary vasoconstriction through activation of TX/E receptors. On the other hand, PGD$_2$ produces systemic vasoconstriction and bronchoconstriction independent of TX/E receptor activation. Finally, in the preconstricted pulmonary circulation, PGD$_2$ causes vasodilation when TX/E receptors are blocked.

**Materials and Methods**

**Animal Preparation**

Adult sheep (25–35 kg) were instrumented for measurement of vascular pressures and lung mechanics as previously described. A total of 17 sheep were studied. After induction with 300–500 mg i.v. thiamylal sodium, sheep were intubated and mechanically ventilated with a mixture of oxygen and halothane. Silastic envelopes (0.01-inch thickness) were placed in the right pleural space through a right thoracotomy for measurement of pleural pressure. Silastic catheters (0.062-in. i.d., 0.125-in. o.d.) were placed in the pulmonary artery and left atrium from a pleural thoracotomy for pressure measurements. A 16-mm Transonic flow probe (TI101 Ultrasound Bloodflow Meter, Transonic Systems, Inc., Ithaca, N.Y.) was placed around the main trunk of the pulmonary artery. Through a neck incision, a Silastic catheter was passed into the carotid artery for measurement of systemic pressure and sampling of blood for gas tensions and pH. An 8F Cordis introducer (Cordis Corp., Miami) was inserted into the external jugular vein for later placement of a 7F Swan-Ganz thermodilution catheter. On a different occasion, a tracheostomy was performed. Animals then were allowed to recover for at least four days before experimentation. Sheep were kept in metabolic cages with free access to food and water at all times.

**Hemodynamics**

Pulmonary arterial (P$_{PA}$), left atrial (P$_{LA}$), and systemic arterial (P$_{SA}$) pressures were measured continuously during the experiments (MP-15D transducers, Micron Instruments, Los Angeles; 7754B system strip-chart recorder, Hewlett-Packard Co., Palo Alto, Calif.). The level of the left atrium was used as the reference for transducer placement. Cardiac output (CO) was measured continuously with the Transonic flow probe and recorded on the strip-chart recorder. CO measurements by flow probe were calibrated to thermodilution CO values (model 9520A cardiac output computer, Edwards Laboratories, Santa Ana, Calif.) by linear regression analysis, using isoprotene-nol infusion to produce stepwise increases in CO. Pulmonary vascular resistance (PVR) was calculated as (mean P$_{PA}$−mean P$_{LA}$/CO. Mean arterial blood pressure (MABP) was calculated as $\frac{1}{3}$ systolic+$\frac{2}{3}$ diastolic pressure. Total systemic resistance was calculated as MABP/CO. Heart rate was determined from the systemic arterial pressure tracing.

**Lung Mechanics**

During experiments, a number 10 cuffed Shiley tracheal tube was inserted into the tracheostomy. Airway opening pressure was measured through a multiple-sidehole catheter positioned 0.5 cm beyond the end of the tracheal tube. Pleural pressure was measured directly from the Silastic envelope placed in the pleural space. Transpulmonary pressure was calculated as the difference between airway opening pressure and pleural pressure. All pressure signals were measured using Validyne differential pressure transducers and amplifiers (Validyne Engineering Corp., Northridge, Calif.). Airflow was measured with a Fleisch No. 1 pneumotachograph connected to the tracheal tube. Signals were electronically integrated with a pulmonary mechanics computer (Buxco Electronics, Sharon, Conn.). Dynamic compliance was calculated as tidal volume divided by transpulmonary pressure at points of zero flow and expressed in milliliters per centimeter of water. Airflow, tidal volume, transpulmonary pressure, and dynamic compliance were recorded breath-by-breath (recorder 8818, Gould, Cleveland, Ohio) throughout the experiment. Before each set of measurements of lung mechanics, the animals' lungs were inflated to 40 cm H$_2$O airway opening pressure using a tube in water connected to the expiratory port.

**Materials**

PGD$_2$ (Cayman Chemical Co. Inc., Ann Arbor, Mich.) was initially dissolved to a concentration of 10 mg/ml in 100% ethanol and stored at $-20^\circ$C. PGD$_2$ then was diluted in normal saline the day of the study and kept on ice until use. The TX/E receptor antagonist SQ-29,548, a generous gift of E.R. Squibb and Sons, Princeton, N.J., was dissolved in 100% ethanol with Trizma Base; approximately 90% of the ethanol was evaporated under nitrogen gas before dilution in normal saline to a concentration of 1 mg/ml. SQ-29,548 was prepared fresh for each experiment. In vitro studies have shown that SQ-29,548 is a selective TX/E receptor antagonist. In particular, SQ-29,548 has no effect on platelet PGD$_2$ receptors even at concentrations 100-fold higher than those required to block TX/E receptors. In vivo, higher doses than
the ones used in the present study had no effect on PGD$_2$-induced bronchoconstriction in cats. The TX/E receptor antagonist AH-23848, a gift of Clinical Supplies Unit, Glaxo Group Research,Ware, Herts, UK, was dissolved in 100% ethanol and 10% sodium carbonate and then diluted in normal saline before administration. OKY-046, an inhibitor of thromboxane synthase, a gift of Kissei Pharmaceutical Co., Matsumoto, Japan, was dissolved in normal saline and stored at $-20^\circ$C. Ibuprofen, a cyclooxygenase inhibitor, was a gift of Upjohn Co., Kalamazoo, Mich. The thromboxane mimetic U46619, a gift from the Upjohn Co., was dissolved to 10 mg/ml in 100% ethanol and stored at $-20^\circ$C and was diluted to a concentration of 25 $\mu$g/ml in normal saline before use.

**Protocols**

Hemodynamic and airway functions were stable for a 45–60-minute baseline period before experiments. PGD$_2$, 0.1–25 $\mu$g/kg, was infused as a slow bolus injection over 2 minutes in random order into the superior vena cava through the proximal port of a Swan-Ganz catheter or into the left atrium. Infusions were made at 20–30-minute intervals, allowing time for hemodynamic values to return to baseline. After the initial set of infusions, sheep received one of the following drugs intravenously: SQ-29,548, 0.2 mg/kg loading dose followed by a 0.2 mg/kg/hr infusion; AH-23848, 1 mg/kg given over 10 minutes; OKY-046, 10 mg/kg given over 10 minutes; ibuprofen, 10 mg/kg given over 5 minutes. PGD$_2$ doses then were repeated approximately 15 minutes after each inhibitor was infused. Only one drug was tested each experimental day. In ancillary studies we showed that the hemodynamic responses to PGD$_2$ were reproducible over time.

The doses of receptor blockers and cyclooxygenase inhibitors used here were chosen because they have been shown previously to be effective in this animal model. In particular, we have shown that the dose of ibuprofen used effectively inhibits formation of cyclooxygenase products in sheep. Kubo and Kobayashi have shown that OKY-046 at the doses we used inhibits thromboxane formation under identical experimental conditions. Likewise, the dose of SQ-29,548 we used produces a 10-fold shift to the right of the dose–response curve of the thromboxane mimetic U46619 on pulmonary vasoconstriction in unanesthetized sheep.

To determine if the effects of PGF$_{2\alpha}$ were mediated through TX/E receptor activation, this eicosanoid was infused into the superior vena cava at 25–75 $\mu$g/kg before and during TX/E receptor blockade with SQ-29,548. Hemodynamic measurements were done as described above.

To determine the effects of PGD$_2$ on the preconstricted pulmonary vascular bed, hypoxic vasoconstriction was induced by connecting the tracheostomy tube to a humidified blended gas reservoir that delivered a gas mixture containing 12% O$_2$–88% N$_2$ until P$_{PA}$ increased to a stable value, usually after 7–10 minutes. An arterial blood gas was drawn to confirm a Pao$_2$ of less than 50 mm Hg, PGD$_2$ (10–25 $\mu$g/kg, n = 8) then was infused into the superior vena cava. Sheep were returned to breathing room air after the maximal effect of PGD$_2$ had been observed. This protocol then was repeated while the sheep received SQ-29,548.

**Statistics**

The effects of PGD$_2$ were evaluated by one-way analysis of variance. Two-way analysis of variance was used to determine the effects of the different interventions on PGD$_2$ actions. Analysis was done using the NCSS statistical software (NCSS, Kaysville, Utah). A value of $p < 0.05$ was significant. Results are reported as mean±SEM.

**Results**

**Effects of Prostaglandin D$_2$**

Infusion of PGD$_2$ into the superior vena cava (n = 6) resulted in a dose-dependent increase in P$_{PA}$, PVR, MABP, and heart rate. P$_{LA}$ and CO did not change significantly from baseline (Figure 1). Infusion of PGD$_2$ into the left atrium (n = 6) also increased P$_{PA}$, but this increase was of considerably less magnitude than that seen after infusion into the superior vena cava. Furthermore, PVR did not change significantly with left atrium infusions of PGD$_2$. P$_{SA}$ increased after infusion of PGD$_2$ into the left atrium. This increase was similar to that seen after superior vena cava administration (Table 1).

PGD$_2$ produced a dose-dependent decrease in dynamic compliance (n = 5); the duration of the response appeared to be dose-dependent as well. Airways were considerably more responsive to PGD$_2$ than the pulmonary vasculature. The effects of PGD$_2$ on dynamic compliance were already maximal at 5 $\mu$g/kg, a dose that had no significant effect on P$_{PA}$ (Figure 2B).

**Effect of Thromboxane A$_2$/Endoperoxide Receptor Antagonists and Enzyme Inhibitors**

To determine if the hemodynamic actions of PGD$_2$ were due to activation of TX/E receptors, two unrelated and selective thromboxane A$_2$ receptor antagonists were used. Both SQ-29,548 (n = 6) and AH-23848 (n = 5) produced significant blockade of the increase in P$_{PA}$ (Figure 3) but had no effect on the systemic vasoconstriction (Figure 2A) or on the tachycardia produced by PGD$_2$ (changes in heart rate produced by 25 $\mu$g/kg PGD$_2$ were 62±15 and 51±13 beats/min before and after SQ-29,548, respectively; $p > 0.05$). Likewise, PGD$_2$-induced dynamic compliance was not blocked by SQ-29,548 (n = 5, Figure 2B). In contrast, thromboxane receptor antagonism with SQ-29,548 blocked the fall in dynamic compliance ($-16±8\%$ versus $-61±4\%$) as well as the increase in P$_{PA}$ (5±1 versus 40±7 cm H$_2$O) produced by the thromboxane analogue U46619 (n = 4).

To rule out the possibility that PGD$_2$ would cause vasoconstriction through a secondary generation of
thromboxane in the lungs, the effects of PGD₂ were compared before and after cyclooxygenase inhibition or thromboxane synthase inhibition. Neither ibuprofen (n=4) nor OXY-046 (n=3) blocked PGD₂- induced pulmonary hypertension (Figure 3). They also had no effect on systemic pressure or heart rate changes produced by PGD₂.

**Effect of Prostaglandin D₂ in the Preconstricted Pulmonary Vascular Bed**

To determine if a vasodilatory effect of PGD₂ could be uncovered, the effects of PGD₂ on the pulmonary vasculature were determined in eight sheep during normoxia, during preconstriction of the pulmonary vasculature with hypoxia, and during combined hypoxia and thromboxane receptor blockade with SQ-29,548. During normoxia, PGD₂ increased P_PA by +19.5±4 cm H₂O. Sheep then were made to breathe 12% O₂-88% N₂. Hypoxia increased P_PA from 17±1 to 29±2 cm H₂O. From this higher baseline, PGD₂ produced a further increase in P_PA when the group was averaged (mean, +8±5 cm H₂O). However, PGD₂ decreased P_PA in three of these eight sheep. After a return to baseline on room air, the TX/E receptor antagonist SQ-29,548 was given, and sheep then were made hypoxic again. Pretreatment with SQ-29,548 did not prevent hypoxic vasoconstriction (16±2 to 28±2 cm H₂O). During combined hypoxia and TX/E blockade, PGD₂ decreased P_PA in all sheep (mean, −9±2 cm H₂O, Figure 4).

**Effect of Thromboxane A₂/Endoperoxide Receptor Antagonisms on the Actions of Prostaglandin F₂α**

In three sheep, PGF₂α produced a dose-dependent increase in P_PA, and also increased MABP (Table 2). TX/E receptor antagonism with SQ-29,548 antagonized the effect of PGF₂α on the pulmonary vasculature, while having no effect on its systemic actions (Table 2).

**Discussion**

In agreement with previous studies, PGD₂ produced pulmonary vasoconstriction in adult sheep. This effect, however, was blocked by two structurally unrelated and selective TX/E receptor antagonists,

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**TABLE 1. Cardiovascular Effects of Prostaglandin D₂ Infused Into the Superior Vena Cava or the Left Atrium**

<table>
<thead>
<tr>
<th>Prostaglandin D₂ (µg/kg)</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_PA (cm H₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SVC</td>
<td>11.3±2.2</td>
<td>27.2±8.4</td>
<td>40.5±8.6</td>
<td>&lt;0.05</td>
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<tr>
<td>LA</td>
<td>4.7±1.2</td>
<td>5.2±2.0</td>
<td>10.5±4.9</td>
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<tr>
<td>MABP (mm Hg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SVC</td>
<td>23±1</td>
<td>35±7</td>
<td>36±12</td>
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<tr>
<td>LA</td>
<td>25±5</td>
<td>29±10</td>
<td>35±10</td>
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</table>

Values are mean±SEM. P_PA, pulmonary arterial pressure; SVC, superior vena cava; LA, left atrium; MABP, mean arterial blood pressure; p values are those of two-factor analysis of variance between groups (SVC vs. LA).
SQ-29,548 and AH-23848, indicating that PGD2-induced pulmonary vasoconstriction is not mediated through activation of DP-receptors but rather through TX/E receptor activation. It could be argued that the TX/E antagonists used also may block DP-receptors. However, the selectivity of these antagonists for TX/E receptors and their lack of effect on DP-receptors is well established.18 Furthermore, these TX/E antagonists blocked only PGD2-induced pulmonary vasoconstriction, while having no effect on the other actions of PGD2. It seems unlikely, therefore, that the results could be explained by blockade of DP-receptors.

Neither cyclooxygenase inhibition with ibuprofen nor inhibition of thromboxane synthase with OKY-046 prevented the pulmonary vasoconstriction produced by PGD2. Therefore, the possibility that the pulmonary effects of PGD2 could be secondary to

**Figure 2.** Effects of prostaglandin D2 (PGD2) after pretreatment with vehicle (solid circles) or the thromboxane receptor antagonist SQ-29,548 (open circles) on pulmonary and systemic hemodynamics (panel A) and pulmonary dynamic compliance (panel B). PGD2 was infused as bolus injections into the superior vena cava in five adult sheep. The p value denotes significant differences between groups from two-factor analysis of variance. PPA, pulmonary arterial pressure; MABP, mean arterial blood pressure.

**Figure 3.** Effects of bolus injection of prostaglandin D2 (PGD2) (25 μg/kg i.v.) on pulmonary arterial pressure (Ppa) before (filled bars) and after (hatched bars) thromboxane receptor antagonism with SQ-29,548 (0.2 mg/kg followed by 0.2 mg/kg/hr, n=6) or AH-23848 (1 mg/kg, n=2); cyclooxygenase inhibition with ibuprofen (10 mg/kg, n=4); and thromboxane synthase inhibition with OKY-046 (10 mg/kg, n=3).
vasodilatory receptors for PGD₂. Whether this represents actual loss of PGD₂ receptors as a function of age remains to be determined. A similar age-related change has been shown for histamine H₁ and H₂ receptors in dogs.²⁷

PGD₂ caused systemic hypertension when infused into the left atrium or into the superior vena cava. The systemic response to PGD₂ was not blocked by TX/E receptor antagonists nor by cyclooxygenase or thromboxane synthase inhibitors. In the absence of specific DP-receptor antagonists, we can only speculate on the nature of the receptor that mediates this vasoconstrictive action. Because PGD₂ produces vasodilation in most other animal species,¹⁵ it is unclear if the vasoconstrictive actions observed in the present study are mediated through DP-receptors. It also is possible that the systemic vasoconstriction was due to conversion of PGD₂ into its vasoconstrictive metabolite 9α,11β-PGF₂α.²⁸ It must be noted that 9α,11β-PGF₂α is the principal in vivo metabolite of PGD₂ in humans, and its production increases markedly after systemic mast cell activation.²⁹

PGD₂ produced a dose-dependent decrease in dynamic compliance, in agreement with previous reports indicating PGD₂-induced bronchoconstriction.¹²,¹³ The mechanism of this action is not yet fully understood, because the putative DP-receptor is considered to mediate relaxation of smooth muscle.¹⁵ In the present study, the TX/E receptor antagonist SQ-29,548 had no effect on the PGD₂-induced fall in dynamic compliance, while effectively blocking the airways response of U46619, a thromboxane mimetic used as a positive control. This suggests that PGD₂ acts on different receptors in the airways than on the pulmonary vasculature. Of interest is the observation that the airways were considerably more sensitive to PGD₂-induced constriction than the pulmonary vasculature. This, coupled with the differential effects of TX/E receptor antagonism on the actions of PGD₂ on these two types of smooth muscle, further suggests that the receptor that mediates pulmonary vasoconstriction is different from the one that produces bronchoconstriction.

In summary, we have found that PGD₂ acts on at least two types of receptors in awake adult sheep. PGD₂ produces pulmonary vasoconstriction by activating thromboxane receptors. On the other hand, PGD₂ causes potent bronchoconstriction and systemic vasoconstriction through a different prostaglandin receptor, the identification of which will require the development of specific antagonists.³⁰,³¹ Finally, our results offer further evidence that the vasoconstrictive TX/E receptor is not “specific” for thromboxane, as it can be activated by PGD₂ and PGF₂α. This implies that TX/E receptor antagonists, although still useful pharmacological probes to determine the role of TX/E receptor activation in pathophysiological processes, should not be used to infer a unique role of endogenous thromboxane A₂, as has been previously suggested.²² By the same token, it is possible that PGD₂ participates in pulmo-

### Table 2. Hemodynamic Effects of Prostaglandin F₂α After Pretreatment With Vehicle or Thromboxane Receptor Antagonist SQ-29,548

<table>
<thead>
<tr>
<th>Prostaglandin F₂α (μg/kg)</th>
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<th>50</th>
<th>75</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA (cm H₂O)</td>
<td></td>
<td></td>
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<tr>
<td>Vehicle</td>
<td>6.7±2.0</td>
<td>15.2±4.5</td>
<td>21.2±16.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SQ-29,548</td>
<td>3.0±0.7</td>
<td>5.8±0.2</td>
<td>8.8±6.0</td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>20±15</td>
<td>25±21</td>
<td>23±12</td>
<td>NS</td>
</tr>
<tr>
<td>SQ-29,548</td>
<td>15±11</td>
<td>21±22</td>
<td>21±7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PPA, pulmonary arterial pressure; MABP, mean arterial blood pressure; p values are those of two-factor analysis of variance between groups (vehicle vs. SQ-29,548).
nary processes previously ascribed solely to thrombox-
ane A₂ on the basis of studies with receptor antagonists.

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boxane A₂ • pulmonary circulation • bronchial spasm • sheep