Pulmonary Venous Responses to Thromboxane A<sub>2</sub> Analogue and Atrial Natriuretic Peptide in Lambs

J. Usha Raj and J. Anderson

To characterize pulmonary venous vasoactivity and the factors that modulate it, we determined venous responses to a vasocostricter agent, thromboxane A<sub>2</sub> (TXA<sub>2</sub>) analogue U46619, and to a vasodilator agent, atrial natriuretic peptide (ANP), in 28 isolated blood-perfused lamb lungs under conditions of varying vascular tone and intraluminal pressures. TXA<sub>2</sub> was given in a 5 \( \mu g/kg \) bolus followed by a steady infusion of 1 \( \mu g/kg/min \) to three groups of lungs: group 1, \( n=4 \), with low vasomotor tone; group 2, \( n=8 \), with moderate vasomotor tone; and group 3, \( n=7 \), with moderate vasomotor tone and high venous intraluminal pressures. Group 3 lungs were reverse-perfused to obtain high venous pressures. ANP was given as two 10 \( \mu g/kg \) bolus injections, 5 minutes apart, to three groups of lungs: group 4, \( n=4 \), with low vasomotor tone; group 5, \( n=5 \), with moderate vasomotor tone; and group 6, \( n=8 \), with high vasomotor tone. Group 6 lungs were vasoconstricted with TXA<sub>2</sub> infusion. In all lungs, we measured blood flow and pressures in the pulmonary artery and left atrium and partitioned the venous segment by measuring pressures in 20–80-\( \mu m \) venules by micropuncture. We found that the venous constrictor response to TXA<sub>2</sub> was greatest in lungs with moderate basal vasomotor tone and low venous intraluminal pressures. In lungs with low vasomotor tone or high venous intraluminal pressures, the venous response to TXA<sub>2</sub> was attenuated. The vasodilator response to ANP was negligible in lungs with low vasomotor tone, probably because they were already maximally vasodilated, but similar in lungs with moderate and high vasomotor tone. We conclude that pulmonary venous response to TXA<sub>2</sub> is dependent on the degree of basal vasomotor tone and on venous intraluminal pressures but that the response to ANP is independent of the degree of vasomotor tone. (Circulation Research 1990;66:496–502)

In the lungs, veins exhibit considerable vaso-motion\(^1\)-\(^4\) and greatly influence microvascular pressures and fluid filtration.\(^3\)-\(^4\) A small change in venous resistance will significantly affect the hydrostatic pressures in filtering vessels upstream from the veins, even if there is no change in arterial resistance. Thus, microvascular pressures and fluid and protein flux are very sensitive to changes in venous resistance. We have previously reported that in newborn lambs pulmonary arteries and veins constrict during hypoxia\(^5\) and that thromboxane A<sub>2</sub> (TXA<sub>2</sub>) mediates the venous constriction during hypoxia.\(^5\) Also, there is some evidence that during hypoxia there is a greater release into the circulation of atrial natriuretic peptide (ANP), a powerful vasodilator,\(^6\) which may be a protective phenomenon. Because of these observations, we chose to study the response of the pulmonary veins to TXA<sub>2</sub> and ANP and to investigate some of the factors that modulate the venous response to these two vasoactive agents. We used isolated blood-perfused lungs of neonatal lambs for our studies and partitioned the venous segment by measuring pressures in 40–80-\( \mu m \) diameter venules by micropuncture.

**Materials and Methods**

**Isolated Perfused Lung Preparation**

We isolated and perfused lungs of 28 neonatal lambs, aged 6.5±1.7 days and weighing 4.7±1.4 kg, as described previously.\(^7\) Briefly, the lamb was anesthetized intramuscularly with ketamine (25 mg/kg body wt). Catheters were placed in the carotid artery and internal jugular vein, and an endotracheal tube was tied into the trachea. After intravenous infusion of 1,000 IU heparin sodium/kg body wt, we rapidly exsanguinated the lamb via the carotid artery cathe-
ter and collected the blood. Heart and lungs were removed, and fluid-filled cannulas were tied into the pulmonary artery and left atrium with care being taken that no air bubbles entered the pulmonary artery. The ductus arteriosus, aortic root, and both venae cavae were ligated, and a ligature was tied around the ventricles to occlude their lumen.

The lungs were perfused with the blood drained from the lamb. If the volume of blood was insufficient, a solution of 5% dextran 70 in Ringer’s lactate was added to the blood. Blood was warmed (38°–39°C), filtered (40-µm filters), and degassed before entering the lungs. A calibrated roller pump (Integral Variable Drive, Cole-Parmer Instrument, Chicago, Illinois) circulated the blood through the lungs. We continuously measured pulmonary artery and left atrial pressures with Statham pressure transducers (model P23, Gould, Cleveland, Ohio). Zero reference level for vascular pressures was the top surface of the lung (site of all micropunctures). Pulmonary artery pressure was varied by altering blood flow, and left atrial pressure was varied by adjusting the height of the venous reservoir. All lungs were perfused in zone 3. Airway pressure was kept constant at 7 cm H2O with a gas mixture of 30% O2, 6% CO2, and 74% N2; left atrial pressure was 8 cm H2O, relative to the top of the lung. At the beginning of the experiment, blood flow was adjusted so that pulmonary artery pressure was about 30 cm H2O, and thereafter, blood flow was kept constant until the end of the experiment.

Blood pH, O2, and CO2 tensions were measured at 10-minute intervals (Radiometer BMS 3M1C2, Copenhagen, Denmark). Hematocrit and blood glucose concentrations were monitored every 20 minutes.

**Venous Pressure Measurement**

We partitioned the pulmonary veins by measuring venular and left atrial pressures and by determining the pressure drop across the venous segment. We measured pressures in 20–80-µm diameter subpleural venules with glass micropipettes and the servomonitor pressure-measuring system.7,8 Glass micropipettes with a tip diameter of 2–4 µm were filled with 1.2 M NaCl colored with green dye and were connected electrically and hydraulically to a servomonitor pressure-measuring system (model 4A, Instruments for Physiology and Medicine, San Diego, California). The lung surface was stabilized with a metal ring that also held a pool of normal saline for obtaining the zero reference pressure. Subpleural vessels were viewed through a stereomicroscope (Carl Zeiss, Thornwood, New York) at ×80 and ×120 magnification with illumination from a cold light source (Intralux 5000, Volpi, Auburn, New Jersey). Venules were identified by observing the direction of blood flow from small to larger vessels. We accepted microvascular pressure measurements that fulfilled the following criteria: 1) reproducible zero reference pressure obtained both before and after the pressure measurement, 2) an immediate response in the microvascular pressure tracing when either the pulmonary artery or left atrial pressure was perturbed, 3) immediate washout of injected dye from the pipette by the flowing blood, indicating that the pipette tip was lying freely in the vessel lumen, and 4) a pressure measurement that was independent of small changes in the optimal servomotor gain setting, indicating that the pipette tip was in liquid.

**Experimental Protocols**

The response of the entire pulmonary circulation and the pulmonary veins to a vasoconstrictor agent, TXA2 analogue U46619, was studied under three conditions: 1) low vasomotor tone, 2) moderate vasoconstrictor tone, and 3) moderate tone and high venous intraluminal pressures.

Group 1 consisted of lungs with low vasomotor tone (n=4). At the time of collection of blood, indomethacin, an inhibitor of cyclooxygenase enzyme, and Piriprost U60257, a 5’-lipxygenase inhibitor (gift of Dr. M. Bach, Upjohn, Kalamazoo, Michigan) was added to the blood to stop the production of vasoconstrictor prostaglandins and leukotrienes. Indomethacin solution was added to achieve a blood concentration of 20 µg/ml9 and U60257 was added to achieve a concentration of 200–500 µg/ml.5,10 We have previously shown that lungs treated in this manner have lower basal vasomotor tone than untreated lungs.9

To test that the drugs indomethacin and U60257 did not affect the responsiveness of the pulmonary vasculature to other vasoactive agents, we tested the response of these lungs to a bolus injection of 200 µg angiotensin. In untreated lungs, a bolus injection of 200 µg angiotensin results in a transient rise in pulmonary artery pressure of 5–10 cm H2O. The treated lungs demonstrated a similar vascular response to angiotensin; this occurrence indicated that the vasoactivity of the vasculature was unaffected by the presence of indomethacin and U60257.

Group 2 consisted of lungs with moderate vasomotor tone (n=8). These lungs were perfused with untreated blood. Basal pulmonary vasomotor tone was generally higher in these lungs than in group 1 lungs.

Group 3 consisted of lungs with moderate vasomotor tone and high venous intraluminal pressure (n=7). These lungs were perfused with untreated blood as in group 2 lungs. To obtain high distending pressures in the veins, we reverse-perfused the lungs. Blood entered the lungs via the left atrium and drained from the pulmonary artery. At the start of perfusion, blood flow was adjusted to maintain left atrial pressure around 30 cm H2O; the pulmonary artery pressure was 8 cm H2O.

In all lungs, once blood flow and inflow pressure were stable, a venular pressure measurement was obtained. Then a 5 µg/kg body wt bolus of TXA2 was infused into the inflow cannula followed by a steady infusion of TXA2 at 1 µg/kg body wt/min. Once a steady-state pulmonary vascular response was
TABLE 1. Baseline Pulmonary Vascular Resistance in Three Groups of Isolated Lamb Lungs Before Infusion of Thromboxane A2 Analogue

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of lungs</th>
<th>Condition</th>
<th>Blood flow (ml/kg body wt/min)</th>
<th>Pulmonary vascular resistance (cm H2O · min/ml · kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Low vasomotor tone</td>
<td>96±19</td>
<td>0.226±0.5*</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Moderate vasomotor tone</td>
<td>50±7</td>
<td>0.440±0.07</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Moderate vasomotor tone with reverse perfusion</td>
<td>48±11</td>
<td>0.525±0.26</td>
</tr>
</tbody>
</table>

*p<0.05 vs. groups 2 and 3.

obtained, which was usually within 5–10 minutes, another venular pressure measurement was obtained.

The response of the pulmonary veins to vasodilator agent ANP was studied under three conditions: 1) low vasomotor tone, 2) moderate vasomotor tone, and 3) high vasomotor tone.

Group 4 consisted of lungs with low vasomotor tone (n=4). The lungs were treated as described for group 1 lungs.

Group 5 consisted of lungs with moderate vasomotor tone (n=5). The lungs were perfused with untreated blood.

Group 6 consisted of lungs with high vasomotor tone (n=8). These lungs are the same as in group 2. After obtaining a steady-state response to the vasoconstrictor agent TXA2, the response to ANP was studied in the presence of a vasoconstricted pulmonary vascular bed.

In all the lungs, the response to ANP was studied by observing the response to two 10 µg/kg body wt bolus injections of synthetic rat ANP (MW 3102; gift of Dr. David Shapiro, Merck Sharp & Dohme, West Point, Pennsylvania) given 5 minutes apart.

Terminally, in all lungs in groups 4–6, papaverine hydrochloride in a dose of 100 µg/ml perfusate was added to determine if there was any residual vasomotor tone.

Data Analysis

In each group of lungs, the response of the pulmonary vasculature to the vasoactive agent was statistically tested by a paired t test. Results shown are mean±SD for each group of lungs. Basal vascular resistance and basal vascular pressure drops among groups 1–3 and among groups 4–6 were compared by multiple unpaired t tests with the Bonferroni correction. The response to TXA2 analogue and ANP in the lungs was compared among groups by an analysis of variance for multiple comparisons. We accepted a value of p<0.05 as indicative of statistical significance.

Results

During the experiments, blood pH was 7.35–7.50, Pco2 was 30–45 mm Hg, and Po2 was 110–190 mm Hg. Mean hematocrit of blood was 20.8±3.8%.

Basal pulmonary vascular resistance in group 1 lungs with low vasomotor tone was significantly lower than in group 2 lungs with moderate vasomotor tone and in group 3 lungs that were reverse-perfused. The
basal vascular resistance in group 2 and 3 lungs was similar (Table 1).

The effects of TXA2 analogue infusion on pulmonary vascular pressures in the three groups of lungs are shown in Figure 1.

In all lungs in groups 1 and 2, pulmonary artery and venular pressures increased, and in group 3 lungs, left atrial and venular pressures increased after TXA2 infusion. The maximum effect was obtained within 5 minutes of steady infusion of the drug. Thus, with TXA2, the pressure drop in the venous segment as well as in the segment that contains the arteries and microvessels increased significantly, indicating that TXA2 constricts arteries and veins.

When we compared the response of the entire pulmonary vasculature to TXA2 among the three groups, we found that the percent increase in total vascular resistance was greater in the untreated lungs with moderate vasomotor tone (groups 2 and 3) than in the treated lungs with low vasomotor tone (group 1) (Figure 2). The venous constrictor response to TXA2 was greatest in the lungs with moderate basal vasomotor tone and low venous intraluminal pressures (group 2). In lungs with low basal vasomotor tone (group 1) and in lungs with high venous intraluminal pressures (group 3), the venous response to TXA2 was attenuated.

Basal pulmonary vascular resistances in the three groups of lungs, before infusion of ANP, were significantly different from one another (Table 2).

The effect of ANP on pulmonary vascular pressures under conditions of different basal vasomotor tone is shown in Figure 3. In lungs with low vasomotor tone (group 4), ANP infusion had a negligible effect on vascular pressures. In lungs with moderate and high vascular tone, ANP infusion resulted in a fall in both pulmonary artery and venular pressures. The pressure drop in both the venous segment and the segment containing microvessels and arteries decreased significantly, indicating that both arteries and veins relaxed with ANP. The percent decrease in total pulmonary vascular resistance was greater in lungs with moderate and high vasomotor tone (Figure 4). Addition of papaverine to group 4 lungs resulted in no decrease in pulmonary vascular pressures. However, in lungs of groups 5 and 6, papaverine resulted in a further decrease in pulmonary artery and venular pressures to 20±4 and 12±2 cm H2O, respectively.

### Table 2. Baseline Pulmonary Vascular Resistance in Three Groups of Isolated Lamb Lungs Before Infusion of Atrial Natriuretic Peptide

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of lungs</th>
<th>Condition</th>
<th>Blood flow (ml/kg body wt/min)</th>
<th>Pulmonary vascular resistance (cm H2O · min/ml · kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>Low vasomotor tone</td>
<td>92±12</td>
<td>0.233±0.043</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Moderate vasomotor tone</td>
<td>58±10</td>
<td>0.441±0.130*</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>High vasomotor tone</td>
<td>57±12</td>
<td>0.796±0.276†</td>
</tr>
</tbody>
</table>

*p<0.05 vs. groups 4 and 6.
†p<0.05 vs. groups 4 and 5.

The magnitude of lung microvascular pressure is important in the control of transmicrovascular liquid and protein flux as well as in the control of flow through the microcirculation. The pressure in the microvascular bed is influenced greatly by changes in vasomotor tone in the arterial and venous segments. Microvascular filtration is particularly sensitive to changes in venous resistance because venular constriction will increase not only microvascular pressures but also the surface area for fluid filtration upstream from the constriction. Recent data have provided new information that in the lung, arteries and veins can constrict independently. In adult lungs, it has been shown that during hypoxia arteries constrict predominantly,11,12 whereas histamine predominantly constricts veins.13,14 The significance of
venous constriction is apparent in the different response to hypoxia seen in neonatal and adult sheep. In the neonatal lamb lung, hypoxia causes constriction of arteries and veins with a concomitant decrease in the ratio of lymph to plasma protein concentration. In contrast, in adult sheep, hypoxia results primarily in arterial constriction with no change in lung lymph flow.

In this study, we wished to characterize the venous responses to two vasoactive agents, TXA$_3$ (a vasoconstrictor) and ANP (a vasodilator), and to determine some of the factors that modulate the venous responses to these agents. In previous experiments, we found that during hypoxia, TXA$_2$ mediated the venous constriction in lamb lungs; hence, we chose to study the effect of this agent on pulmonary veins. And the vasodilator that we chose to study was ANP because there is some evidence that during hypoxia the circulating levels of ANP are elevated, suggesting a possible role for ANP in modulating hypoxic vasoconstriction.

Our data show that the amount of venous constriction resulting from an infusion of TXA$_3$ analogue is in part dependent on the degree of basal vasomotor tone and on the magnitude of the intraluminal pressures. The concept that pulmonary vascular responses to vasoactive agents are dependent on the degree of resting vasomotor tone was first suggested by Rudolph and coworkers. In studying pulmonary vascular responses to acetylcholine in the dog, they found that when the underlying vasomotor tone was low, acetylcholine resulted in vasoconstriction whereas when tone was elevated, vasodilation resulted. A marked vasodilator effect of acetylcholine when resting vasomotor tone was high has also been shown in the fetal lamb lung and cat lung. However, other recent studies have shown that when tone is high, the vasopressor response to acetylcholine is augmented. This is similar to the effect of tone on the response of the pulmonary vasculature to TXA$_2$ in our studies.

The diminished vasoconstrictor response to TXA$_2$ when the lungs were reverse-perfused indicates that the high intravascular pressures in the veins directly opposed the ability of the veins to constrict. This is consistent with studies in lambs and sheep that
showed that in the presence of high pulmonary vascular pressures the hypoxic vasoconstrictor response was diminished. In adult sheep with elevated left atrial pressures, Bland et al.\textsuperscript{16} did not find any significant increase in pulmonary vascular resistance with hypoxia, indicating an absence of vasoconstriction. Similarly, Snapper et al.\textsuperscript{25} found that in adult sheep with a partially obstructed mitral valve and left atrial hypertension, hypoxia did not result in any change in pulmonary vascular resistance. In both these studies, when left atrial pressure was normal, hypoxia resulted in significant vasoconstriction. These results suggested that increased vascular intraluminal pressure opposed any direct vasoconstrictive effects of hypoxia.

From our previous studies, we have found that in isolated lamb lungs hypoxia results in constriction of both arteries and veins. We also found that the venous constriction during hypoxia was probably mediated by TXA\textsubscript{2}. Furthermore, in studies with lambs, we have reported that there was a smaller increase in pulmonary vascular resistance with hypoxia in the presence of left atrial hypertension than when left atrial pressure was normal.\textsuperscript{24} These data obtained from lambs in vivo are consistent with our findings in isolated lamb lungs; in isolated lungs, the vasoconstrictor response to TXA\textsubscript{2} analogue was attenuated in the presence of elevated pulmonary venous pressures, and in lungs in vivo, the vasoconstrictor response to hypoxia was similarly attenuated when pulmonary venous pressures were high.

The newly described group of peptide hormones, atriopeptins or ANPs, have been shown to have several cardiovascular and renal effects, including natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion.\textsuperscript{26,27} Though ANP has been previously shown to be a potent vasodilator of large arteries in vitro,\textsuperscript{27} its effects in whole animal studies have been controversial.\textsuperscript{28} ANP has been shown to display regional vasorelaxant selectivity in vitro; for example, it has a greater effect on arteries than on veins and a greater effect on large vessels than on smaller ones.\textsuperscript{29} ANP elicits an endothelium-independent relaxation of blood vessels by activating membrane-associated particulate guanylate cyclase, cyclic GMP synthesis, and cyclic GMP-dependent protein kinase activation.\textsuperscript{30}

We have demonstrated that pulmonary veins in lambs are responsive to the vasodilatory effects of ANP. Other investigators\textsuperscript{31} have reported a vasodilatory effect of ANP in the pulmonary vascular bed of the intact-chest cat. However, in that study the authors did not determine if both pulmonary arteries and veins dilated in response to ANP. In the bovine lung, Ignarro et al.\textsuperscript{32} reported that intrapulmonary arteries, but not intrapulmonary veins, dilated in response to ANP. This response was not dependent on endothelium and was associated with an accumulation of cyclic GMP. Clearly, species differences exist. In isolated lamb lungs, pulmonary veins dilated in response to ANP. It is possible that pulmonary venous smooth muscle cells in lambs possess particulate guanylate cyclase that is responsive to ANP, which may not be true for the bovine species.

The response of pulmonary veins to ANP seems to be independent of the degree of basal vasomotor tone. In lungs with low vasomotor tone, there was no response to ANP, possibly because the pulmonary vasculature was already maximally vasodilated. We did not see any decrease in pulmonary vascular pressures with administration of papaverine in this group of lungs; this finding indicated that the smooth muscle in the pulmonary vasculature had negligible vasomotor tone. In lungs with moderate and high vasomotor tone, ANP resulted in a fixed amount of vasodilation. The dose of ANP that we used would have achieved a blood concentration far in excess of all reported physiological levels of ANP in blood. It is possible that we had fully saturated all ANP receptors in the pulmonary vasculature at these doses in both groups of lungs; this situation would explain the observation of a fixed amount of vasodilation.

In summary, we have demonstrated that TXA\textsubscript{2} is a powerful vasoconstrictor of the pulmonary vasculature including the veins and that ANP is a powerful vasodilator of pulmonary arteries and veins. The magnitude of the venous response to TXA\textsubscript{2} is modulated by basal vasomotor tone and by the magnitude of venous intravascular pressures. ANP effect in the pulmonary veins, when given in pharmacological doses, seems to be independent of basal vasomotor tone. One conclusion from our study is that the factors that determine the magnitude of pulmonary venous responses to a vasoconstrictor agent are quite different from those that modulate the response to a vasodilator agent. The amount of contraction of a smooth muscle cell to a vasoconstrictor, such as TXA\textsubscript{2}, may be, in part, dependent on the receptor-agonist interaction with its resultant intracellular events, as well as on the initial length-tension state of the smooth muscle cell. However, in the case of a vasodilator, such as ANP, the amount of relaxation of the smooth muscle cell is dependent on the interaction of the receptor-agonist alone and is independent of the initial tension-length state of the cell. Whether this is true for other vasoconstrictors and dilators has yet to be studied.

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