Pulmonary vasoconstriction induced by mitral valve obstruction in sheep

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Herme-Weiler, Casilda I., Tomonobu Koizumi, Richard Parker, and John H. Newman. Pulmonary vasoconstriction induced by mitral valve obstruction in sheep. J. Appl. Physiol. 85(5): 1655–1660, 1998.—We hypothesized that left atrial hypertension results in pulmonary vasoconstriction, which is obscured by the expected passive decrease in pulmonary vascular resistance. The objectives of this study were to demonstrate and quantify the vasoconstrictive changes that occur in the pulmonary circulation during experimental left atrial hypertension, to determine the site of vasoconstriction, and to explore its mechanism. Sheep were instrumented for measurement of pulmonary arterial (Ppa), left atrial (Pla), and systemic arterial pressures (Psa) with a Foley balloon catheter to variably obstruct the mitral valve. Distal pulmonary venous hypertension (16). In addition, obliterative arteriolar constriction triggered somehow by pulmonary venous hypertension (16). In the presence of chronic left heart failure or valvular disease, pulmonary hypertension is postulated to result from both passive backward transmission of the elevated left atrial pressure (Pla) and pulmonary arteriolar constriction triggered somehow by pulmonary venous hypertension (16). In addition, obliterative changes can occur in the pulmonary vascular bed, especially in mitral stenosis as a complication of longstanding edema and microvascular hemorrhage (10, 11). Since the early years of open-heart surgery, it has been observed that pulmonary vascular resistance (PVR) may decrease rapidly after mitral valve repair (16). This phenomenon has been noted in patients after heart transplantation as well (4, 21). This hypothesized form of reactive pulmonary vasoconstriction has not been re-created in an animal model, and its mechanism is not understood.

MATERIALS AND METHODS

Fifteen unanesthetized yearling sheep (20–30 kg) of either sex were studied. The sheep were prepared during a single operation (23, 32). Sheep were anesthetized with 25 mg/kg iv of pentobarbital sodium and ventilated with the use of an Ohmeda 7000 ventilator. Anesthesia was maintained with halothane in 60% O2. Through a left thoracotomy, Silastic catheters were sutured directly into the main pulmonary artery and the left atrium. An 18-Fr Teflon-coated, balloon-tipped Foley catheter was placed through a left atrial incision and positioned in the mitral valve orifice so that inflation of the balloon would cause partial obstruction of flow through the mitral valve. Through a neck incision, a Silastic catheter was placed in the carotid artery, and the jugular vein was cannulated with a no. 8 Cordis introducer for later passage of a Swan-Ganz thermodilution catheter. The sheep were allowed to recover for a minimum of 5–7 days before experiments were begun. Free access to food and water was allowed during this period and during all experiments.

Physiological measurements. All measurements were made with the sheep awake and standing quietly in a cage. Pulmonary arterial pressure (Ppa), Pla, and systemic arterial pressures (Psa) were recorded by using pressure transducers (Hewlett-Packard, Andover, MA), signal conditioners (Valdyne, Northridge, CA), and an electronic recorder (AstroMed, W. Warwick, RI). Because the difference between Ppa and Pla was small during NO inhalation, we tested Ppa and Pla profiles against each transducer to ensure that they gave the exact readings. Cardiac output (CO) was measured by thermodilution with the use of an Edwards model 9520A thermodilution computer (Edwards Laboratory, Santa Ana, CA). A Swan-Ganz catheter was advanced into the pulmonary arterial bed through the Cordis introducer guided by pressure deflections. CO was taken as the mean of four to six determinations by using boluses of 5 ml of iced saline.

Pulmonary arterial wedge pressure (Ppaw) was determined by using a 5-Fr Swan-Ganz catheter that was advanced into the pulmonary arterial bed and wedged with the balloon...
deflated (23). As in previous studies, the distal wedge was ascertainment by 1) a wedge pressure greater than Pla, 2) a Po$_2$ higher than and Pco$_2$ lower than arterial blood drawn retrograde through the catheter, and 3) waveforms consistent with downstream deflections (23). Although the position of the catheter tip relative to the left atrium is not certain, wedge pressure was always higher than Pla, suggesting appropriate positioning. Arterial blood pressure and gas tensions (ABG) were measured on a blood-gas analyzer (Corning, Medfield, MA). PVR was calculated as mean (Ppa - Pla)/CO: upstream PVR = (Ppa - Ppaw)/CO PVR; and downstream PVR = (Ppaw - Pla)/CO. The true anatomic location of upstream and downstream segments is unknown but is probably divided at veins the size of the diameter of the catheter.

Gas delivery system. NO gas [50 parts/million (ppm) in N$_2$] was mixed with O$_2$ by using a Bennett 50-psi gas blender and was delivered by a high-flow regulator through low-resistance ventilator tubing and one-way valves to prevent rebreathing (19). A plastic anesthesia mask, fitted with a rubber adapter to provide a good seal, was placed around the sheep's snout. The fraction of inspired O$_2$ was set at 0.21 on the blender. The final concentration of NO at the mouth was ~36 ppm. From other studies in sheep, it has been shown that NO has a pulmonary vasodilator effect at concentrations as low as 2 ppm, and maximal effects occur at ~40 ppm (19). Control gas (21% O$_2$-79% N$_2$) was administered through the same apparatus to control for any effects of the gas delivery system.

Experimental protocols. Experimental protocols were designed to measure the response to NO at baseline conditions and at two levels of left atrial hypertension. After stable baseline values were obtained for 20 min, control gas (79% N$_2$:21% O$_2$) was administered by face mask for 10 min, during which time pressures were recorded continuously and CO and ABG were obtained. The inspired gas was then changed to the NO mixture to maintain a concentration of ~36 ppm of NO and 21% O$_2$. Ppa, Pla, and Psa were continuously monitored, and CO and ABG were obtained after 10 min of NO exposure. After NO inhalation, the sheep were allowed to rest for more than 10 min. Pla was then raised by 10 cmH$_2$O by graded inflation of the left atrial balloon. After 10 min of stable hemodynamics at the new Pla, control gas was administered for 10 min at the end of which CO and ABG were measured. Throughout the experiment, mean values for Ppa, Pla, and Psa were recorded continuously. NO was then administered for 10 min, and CO and ABG were obtained at the end of the interval. After NO inhalation, values were allowed to rest to control levels over several minutes before the atrial balloon was deflated. Before the next intervention, stable baseline values were recorded for at least 20 min. The atrial balloon was then inflated to increase the Pla by 20 cmH$_2$O and was maintained at this level for 20 min until stable. Control gas and NO were separately administered for 10 min each with measurement of hemodynamics and ABG at each step. After the atrial balloon was deflated, animals were monitored until hemodynamic variables returned to baseline. They were then returned to the pen and allowed to rest for at least 24 h before the next experiment.

Pulmonary vascular segmental resistances. In five sheep, Ppaw was obtained as part of the hemodynamic measurements, to better determine the size of the pulmonary vasodilation that occurs with left atrial hypertension. As outlined above, measurements were obtained at baseline and at Pla of 10 and 20 cmH$_2$O. Control gas and NO were administered by face mask for 10 min each, by using the same protocol outlined above.

Mechanism of reactive vasoconstriction. In an attempt to explore the mechanism of reactive vasoconstriction, we performed receptor blockade experiments in four yearling sheep using each animal as its own control. Animals were pretreated either with atropine to block cholinergic muscarinic receptors or with phentolamine to block α-adrenergic stimulation during 20-cmH$_2$O left atrial hypertension. To determine whether a vasoconstrictor eosinophil-derived from arachidonic acid is active during acute left atrial hypertension, we tested cyclooxygenase blockade with intravenous ibuprofen, although ibuprofen would block dilated prostaglandins as well. We did not test for lipoxygenase products in this study.

The protocol was slightly modified from that outlined above. First, a baseline response to NO was measured at 20-cmH$_2$O left atrial hypertension. The balloon was then deflated, and variables were allowed to return to baseline. The animal was pretreated with either phentolamine (5-mg bolus via the pulmonary artery catheter; Ref. 18), or ~2 mg iv of atropine, with the dose titrated to increase the heart rate by 25–30 beats/min, or 10 mg/kg iv ibuprofen. The left atrial balloon was then inflated to increase the Pla by 20 cmH$_2$O. Control gas and NO were administered for 10 min, with measurements of CO, vascular pressures, and ABG taken during this time. The balloon was deflated, and the sheep were allowed to rest. Drugs were given on different days to avoid cross-reactivity and were administered via the pulmonary artery catheter. The doses chosen were based on those known to cause blockade, on the basis of published studies from our laboratory (17–19, 22).

Statistical evaluation. Data were entered on Quattro Pro (Borland) and transferred to Fig P (Statistical Software Systems) and Minitab (State College, PA) for graphical representation and analysis. Data are presented as means ± SE. We compared data before and after NO by paired t-test or the Wilcoxon rank sum test. We used the Kruskal-Wallis nonparametric ANOVA test to establish differences among the three groups and Mann-Whitney test for intergroup comparisons (see Fig. 3). Differences were considered significant at P < 0.05.

Table 1. Transpulmonary hemodynamic changes with left atrial hypertension and NO inhalation

<table>
<thead>
<tr>
<th></th>
<th>Ppa, cmH$_2$O</th>
<th>Pla, cmH$_2$O</th>
<th>Psa, mmHg</th>
<th>CO, l/min</th>
<th>PVR, Wood units</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL + CG</td>
<td>17.1 ± 0.6</td>
<td>3.5 ± 0.5</td>
<td>86.1 ± 3.1</td>
<td>4.8 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>BL + NO</td>
<td>15.4 ± 0.5*</td>
<td>3.2 ± 0.4</td>
<td>85.2 ± 2.7</td>
<td>4.7 ± 0.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Pla10 + CG</td>
<td>24.4 ± 0.8</td>
<td>13.1 ± 0.5</td>
<td>83.9 ± 2.7</td>
<td>4.3 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Pla10 + NO</td>
<td>18.4 ± 0.6*</td>
<td>13.0 ± 0.5</td>
<td>83.8 ± 3.0</td>
<td>4.3 ± 0.2</td>
<td>1.3 ± 0.1*</td>
</tr>
<tr>
<td>Pla20 + CG</td>
<td>33.3 ± 0.5</td>
<td>23.5 ± 0.6</td>
<td>81.6 ± 2.3</td>
<td>4.4 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Pla20 + NO</td>
<td>25.9 ± 0.5*</td>
<td>22.8 ± 0.5</td>
<td>81.3 ± 1.9</td>
<td>4.4 ± 0.2</td>
<td>0.7 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 14 sheep. BL, baseline; CG, control gas; NO, nitric oxide; Ppa, mean pulmonary artery pressure; Pla10 and Pla20, mean left atrial pressure at 10 and 20 cmH$_2$O, respectively; Psa, mean systemic arterial pressure; CO, cardiac output; PVR, pulmonary vascular resistance. *P < 0.05 vs. same value without NO.
RESULTS

As expected, left atrial hypertension resulted in elevation of Pla. Increasing Pla by 20 cmH2O caused a mild reduction in PVR, probably secondary to pulmonary vascular recruitment and passive distension (Table 1 and Figs. 1–3). Increasing Pla by 20 cmH2O caused a small but significant decrease in the Psa. CO decreased mildly but significantly at both 10 and 20 cmH2O of Pla. ABG values did not change with left atrial hypertension or during NO inhalation (Table 2).

NO inhalation caused a small but significant decrease in Ppa during baseline conditions and significantly reduced the pulmonary hypertension caused by acute increases in Pla (Fig. 4). The Ppa-to-Pla difference during NO inhalation at 20 cmH2O of left atrial hypertension was as low as 2 cmH2O, yielding PVR values as low as 0.2 Wood units. Inhaled NO caused a significant decrease in PVR at both levels of left atrial hypertension but did not significantly change the PVR at baseline. NO inhalation alone did not affect CO or Psa (Table 1 and Figs. 1–3). The change in PVR induced by NO increased with increasing levels of Pla (Fig. 3). Because lung vascular distension and recruitment should be greatest at the highest level of Pla, it is unlikely that the effect of NO represented dilation of unperfused segments.

Pulmonary vascular segmental resistances. Distal wedge pressure decreased with inhalation of NO at baseline and at both levels of left atrial hypertension (Table 3). Upstream and downstream PVR were significantly decreased with NO inhalation during left atrial hypertension, and this effect was especially striking in downstream vessels, where PVR decreased to levels as low as 0.1 ± 0.1 Wood units with a CO of 4.6 ± 0.4 l/min and a driving pressure of ~0.6 cmH2O. Looking at the effect of left atrial hypertension combined with NO inhalation, we saw that the downstream PVR decreased by 50% at a Pla of 10 cmH2O and by 83% at a Pla of 20 cmH2O. The absolute change at Pla of 20 cmH2O was about equal in upstream and downstream vessels: 0.5 resistance units in each.

Mechanism of reactive vasoconstriction. Phentol-amine had the expected hemodynamic effects at baseline, a mild reduction in Pla coupled with an increase in PVR (control). Inhaled NO caused a significant decrease in PVR at both levels of left atrial hypertension.

Table 2. Arterial blood-gas changes with left atrial hypertension and NO inhalation

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PCO2, Torr</th>
<th>PO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL + CG</td>
<td>7.49 ± 0.01</td>
<td>32.2 ± 2.1</td>
<td>98.1 ± 3.3</td>
</tr>
<tr>
<td>BL + NO</td>
<td>7.48 ± 0.01</td>
<td>29.0 ± 1.6</td>
<td>100.2 ± 3.4</td>
</tr>
<tr>
<td>Pla10 + CG</td>
<td>7.47 ± 0.01</td>
<td>31.1 ± 1.9</td>
<td>102.4 ± 3.7</td>
</tr>
<tr>
<td>Pla10 + NO</td>
<td>7.48 ± 0.01</td>
<td>29.5 ± 1.8</td>
<td>106.1 ± 2.9</td>
</tr>
<tr>
<td>Pla20 + CG</td>
<td>7.46 ± 0.01</td>
<td>30.0 ± 1.9</td>
<td>102.1 ± 4.5</td>
</tr>
<tr>
<td>Pla20 + NO</td>
<td>7.47 ± 0.01</td>
<td>29.7 ± 1.7</td>
<td>109.9 ± 3.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 14 sheep.
CO (18). During left atrial hypertension, phentolamine raised CO, resulting in increased pulmonary vascular pressures, making the maintenance of a stable Pla impossible. Because of this uncontrolled response, we were unable to make conclusions about the role of \( \alpha \)-receptor-mediated vasoconstriction. Neither atropine (n = 4) nor ibuprofen (n = 2) had any observed effects on Ppa or PVR during stable hemodynamic at 20 cmH\(_2\)O of Pla.

**DISCUSSION**

We have attempted to describe the magnitude and circumstances of a new observation: that passive back pressure in the pulmonary circulation results in substantial vasoconstriction that occurs in parallel with mechanically induced dilation of the pulmonary vascular bed.

Passive dilation of the pulmonary vascular bed is a well-described phenomenon in which the PVR decreases with increasing pressure transmitted from a hypertensive left atrium or obstructed pulmonary veins. This phenomenon has been best studied in vitro where flow and transpulmonary airway pressures can be tightly controlled and varied (3, 29). Elevations in Pla cause a graded decrease in PVR (3). The partitioning of this reduction in PVR across the pulmonary circulation is less clear. Some of the reduction is likely due to dilation of large pulmonary veins and some due to recruitment of pulmonary capillaries, and the degree to which the pulmonary arterioles dilate is unclear. Nonetheless, the net response of the pulmonary circulation to back pressure is dilation.

In contradistinction to the dilating effect of back pressure, pulmonary vasoconstriction has been postulated to occur clinically in conditions of passive congestion of the lung. The earliest observations were in patients receiving mitral commissurotomy or mitral valve replacement, whose pulmonary vascular pressures and resistances were noted to decrease rapidly over hours to days after the procedure, followed by a slower decrement over the following months (16). A similar observation has been made in patients with chronic congestive heart failure from left ventricular disease, especially in the hours to days after cardiac transplantation (4, 21, 24). Hemodynamic data to support this observation include a large pulmonary arterial diastolic pressure-to-Ppaw gradient seen in many cases of “passive” pulmonary hypertension. Pulmonary vasoconstriction has also been hypothesized to account for the redistribution of flow away from the lung bases in response to left atrial hypertension (7). Thus indirect evidence exists for a reactive component to acute and chronic passive congestion of the pulmonary vascular bed, but this phenomenon is unexplained, and its magnitude is unknown.

The development of NO inhalation has provided a useful tool for understanding pulmonary hemodynamics, primarily because exogenous NO is a potent but selective pulmonary vasodilator, with no effects on systemic tone and no effect on CO unless the right ventricle is compromised (1, 5, 8, 9). NO also has a rapid on-off transient. In this study, we used these character-

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**Table 3. Upstream and downstream hemodynamic changes with left atrial hypertension and NO inhalation**

<table>
<thead>
<tr>
<th></th>
<th>Ppa, cmH(_2)O</th>
<th>Pla, cmH(_2)O</th>
<th>Ppaw, cmH(_2)O</th>
<th>CO, l/min</th>
<th>Total PVR</th>
<th>Upstream PVR</th>
<th>Downstream PVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL + CG</td>
<td>16.6 ± 0.8</td>
<td>3.4 ± 0.5</td>
<td>8.4 ± 0.7</td>
<td>5.1 ± 0.5</td>
<td>2.6 ± 0.4</td>
<td>1.7 ± 0.1</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td>BL + NO</td>
<td>15.6 ± 0.5*</td>
<td>3.6 ± 0.5</td>
<td>7.6 ± 0.8*</td>
<td>4.8 ± 0.5</td>
<td>2.6 ± 0.3</td>
<td>1.7 ± 0.1</td>
<td>0.9 ± 0.3*</td>
</tr>
<tr>
<td>Pla10 + CG</td>
<td>24.2 ± 1.4</td>
<td>14 ± 0.3</td>
<td>16.8 ± 0.9</td>
<td>4.7 ± 0.4</td>
<td>2.2 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Pla10 + NO</td>
<td>19.8 ± 1*</td>
<td>13.6 ± 0.4</td>
<td>14.7 ± 0.4*</td>
<td>4.5 ± 0.4</td>
<td>1.4 ± 0.1*</td>
<td>1.1 ± 0.1*</td>
<td>0.3 ± 0.1*</td>
</tr>
<tr>
<td>Pla20 + CG</td>
<td>31 ± 0.7</td>
<td>24 ± 0.6</td>
<td>26.8 ± 0.7</td>
<td>4.6 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Pla20 + NO</td>
<td>27.2 ± 0.2*</td>
<td>23.8 ± 0.5</td>
<td>24.4 ± 0.4*</td>
<td>4.6 ± 0.4</td>
<td>0.8 ± 0.1*</td>
<td>0.6 ± 0.1*</td>
<td>0.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of n = 5 sheep. Ppaw, pulmonary arterial wedge pressure; total PVR = Ppa – Pla/CO; upstream PVR = Ppa – Ppaw/CO; downstream PVR = Ppaw – Pla/CO. *P < 0.05 vs. same value without NO.
istics of NO to delineate a vasoconstrictor effect of back pressure that occurs simultaneously with, and may be induced by, mechanical vasodilation. The effect is a rapidly occurring, acute vasoconstriction. It has a considerable magnitude, because its effect is to double PVR at a time when the bed is being mechanically dilated. There appears to be a dose response, because the vasoconstriction increased with increasing levels of left atrial hypertension. We did not design this study to ascertain the full-dose response of vasoconstriction in response to back pressure, so it is possible that the maximum vasoconstriction is greater than we have measured. Because pulmonary capillary pressure in vivo may exceed 30–40 mm Hg in some conditions of left heart and valvular dysfunction, it is conceivable that the degree of vasoconstriction is even greater in those conditions. Full delineation of the shape and magnitude of the vasoconstriction in response to back pressure is a future goal.

The site(s) of vasoconstriction in this model is unknown, but the evidence points indirectly to both arterioles and venules. NO is known to be both an arterial dilator and a venodilator. The absolute change in PVR was similar, ~0.5 Wood units, after NO at an elevation of Ppa of 20 cm Hg, but the percent change was much greater in downstream veins than in upstream vessels (Table 3). In vitro studies that use the double-occlusion method may be needed to accurately partition this effect.

The mechanism of this reactive vasoconstriction remains unknown. We attempted to seek potential causes or modifiers of the phenomenon. α-adrenergic vasoconstriction was sought by using the α-adrenergic receptor blocker phentolamine. We have used phentolamine successfully in previous studies of the sympathetic nervous system during exercise in sheep (18). Unfortunately, the systemic vasodilation and reactive increase in CO induced by phentolamine prevented maintenance of steady-state Pla and precluded any meaningful analysis, despite repeated efforts in four sheep. It seems unlikely that the α-adrenergic system is primarily responsible for the phenomenon, but the hypothesis remains untested. α-Blockade with a selective α2-receptor antagonist during constant flow will help test this possibility. Atropine had no discernible effect on the reactive vasoconstriction, which reduces the likelihood that a vasoconstrictor parasympathetic effect is occurring. We saw no discernible effect after cyclooxygenase blockade with intravenous ibuprofen (22), and it seems unlikely that such a rapid and reversible vasoconstrictor response would be mediated by a constrictor eicosanoid. Although we did not test endothelin-receptor antagonists, the time course of endothelin vasoconstriction is characterized by slow onset and prolonged toxicity, unlike the vasoconstriction that we have observed (20). It seems possible that the reactive vasoconstriction is mediated through stretch or mechanically induced receptors (14, 31). This hypothesis seems plausible because stretch of the pulmonary vessels should occur during passive back pressure. Pursuit of this possibility will be the focus of future studies.

Finally, how this acute reactive pulmonary vasoconstriction relates to chronic changes in the pulmonary vascular bed during prolonged passive pulmonary hypertension is unknown. Because remodeling of the vascular bed is known to occur as a consequence of back pressure in diverse conditions such as mitral stenosis, pulmonary veno-occlusive disease, and fibrosis mediastinitis of the pulmonary veins, it seems possible that this vasoconstriction might stimulate or augment the remodeling that occurs in vivo by increasing the hypertensive response. It may be analogous to acute hypoxic pulmonary vasoconstriction and chronic hypoxic pulmonary hypertension, two processes linked by hypoxia and partially related to the degree of the acute hypoxic vasoconstriction.

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