Pulmonary vasodilator effects of norepinephrine during the development of chronic pulmonary hypertension in neonatal lambs

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Background. This experimental study was performed to determine the effects of norepinephrine on: (i) the pulmonary vascular tone during the development of pulmonary hypertension (PH) in the fetus and (ii) the circulatory adaptation at birth after chronic intrauterine PH.

Methods. Chronically instrumented fetal lambs were randomized into two groups: (i) a group with PH obtained by antenatal partial ligation of the ductus arteriosus (DA) (n=9) and (ii) a control group without DA ligation (n=6). Pulmonary vascular responses to norepinephrine (1.5 μg min⁻¹) were measured in utero 7 days after surgery. At day 8 post-surgery, after delivery, animals were ventilated for 3 h with oxygen 100%. The group with PH was randomly assigned to receive norepinephrine or saline.

Results. Mean pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) were higher in the PH group (P<0.01). Norepinephrine-induced decrease in PVR was more pronounced in the PH group than in the control group (63 vs 35%, respectively; P<0.01). In the PH group, the decrease in PVR during mechanical ventilation was greater in the animals receiving norepinephrine than in the animal receiving saline (from 1.05 (0.12) to 0.1 (0.02) vs from 1.04 (0.1) to 0.2 (0.04) mm Hg ml⁻¹ min⁻¹, respectively; P<0.01). After 3 h of ventilation, mean PVR in the PH lambs treated by norepinephrine was similar to those measured in the control lambs. Aortic pressure was higher in the group treated with norepinephrine.

Conclusion. The data suggest that norepinephrine may improve post-natal pulmonary adaptation in the newborn with persistent PH both by increasing systemic vascular pressure and by increasing pulmonary blood flow.

Br J Anaesth 2004; 93: 818–24

Keywords: arteries, small pulmonary arteries; complications, pulmonary hypertension; pharmacology, nitric oxide; sympathetic nervous system, norepinephrine

Accepted for publication: August 4, 2004

Persistent pulmonary hypertension of the newborn (PPHN) results from the failure of the pulmonary circulation to dilate at birth. This syndrome is characterized by sustained elevation of pulmonary vascular resistance (PVR),¹ causing extra-pulmonary right-to-left shunting of blood across the ductus arteriosus (DA) and foramen ovale and severe hypoxaemia.¹ PPHN is also usually associated with low systemic pressure and low cardiac output because of increased right ventricular afterload and myocardial dysfunction.²³

An experimental model of chronic intrauterine PH can be obtained by partial compression or ligation of the DA in the late gestation ovine fetus.⁴⁵ In this model, chronic DA compression causes sustained intrauterine pulmonary hypertension (PH) without chronically increased pulmonary blood flow or hypoxaemia.⁴⁵ Past studies have also demonstrated increased smooth muscle thickness in small pulmonary arteries,⁶ and blunted endothelium-dependent vasodilation after 9–12 days of DA compression.⁷⁻⁹

An increase in plasma norepinephrine concentration has been observed at the end of gestation. Birth induces a marked surge in catecholamine secretion. We have reported previously that norepinephrine infusion induces a potent nitric oxide (NO)-dependent pulmonary vasodilation in fetal lambs.¹⁰¹¹
Therefore, we hypothesize that norepinephrine may alter pulmonary vascular tone during fetal life and may improve circulatory adaptation at birth in a model of PPH. To test this hypothesis, we compared pulmonary vascular effects of norepinephrine in chronically prepared fetal lambs with and without PPH obtained by antenatal partial ligation of the DA for 8 days.

Methods

Animal preparation

All animal procedures and protocols used in this study were reviewed and approved by the French ‘Ministère de l’Agriculture, de la Pêche et de l’Alimentation’ before the studies were conducted. Fifteen mixed-breed pregnant ewes between 130 and 133 days gestation (term = 147 days) were fasted for 48 h before surgery. Ewes were sedated with i.v. pentobarbital sodium (total dose: 2–4 g) and anaesthetized with bupivacaine 1% hydrochloride (4 mg) by lumbar puncture. Under sterile conditions, the fetal lamb’s left forelimb was delivered through uterine incision. I.M. injection of 6 μg of Sufentanil™ was performed. A skin incision was made after local infiltration with lidocaine. Polyvinyl catheters (20 gauge) were advanced into the aorta and the vena cava through the axillary vessels. A left thoracotomy exposed the heart and great vessels. Catheters were inserted into the left pulmonary artery (LPA) (22 gauge), main pulmonary artery (20 gauge), and left atrium (20 gauge). An ultrasonic flow transducer, size 6 (Transonic Systems, Ithaca, NY), was placed around the LPA to measure blood flow. In nine animals, the DA was partially ligated (Fig. 1). The fetus was gently replaced into the uterus. Catheters were exteriorized through a s.c. tunnel to an external flank pouch. Estimated weight of the fetal lambs was 3000 g.

Eight days after the initial surgery, ewes were sedated with i.v. pentobarbital sodium (total dose: 2–4 g) and anaesthetized with bupivacaine 1% hydrochloride (4 mg) by lumbar puncture. Then, the fetal lambs were carefully delivered through an abdominal incision and hysterotomy. After an injection followed by a continuous infusion of nalbuphine (0.6 mg then 0.15 mg h⁻¹) and an i.v. injection of pancuronium bromide (200 μg), the animals were dried and warmed (heating table) throughout the study. Temperature was continuously monitored (target temperature=39.5°C). Haemodynamic variables were measured continuously thereafter for the duration of the delivery study. The baseline measurements were recorded after haemodynamic parameters had stabilized for 30 min. Saline (6 ml h⁻¹) was infused via the venous catheter. A tracheostomy was then performed after local infiltration with lidocaine. The animals were intubated with a 3.5-mm tracheal tube and ventilated for 3 h with a time-cycled, pressure-limited neonatal ventilator (Babylog 8000, Draeger, Germany). Initial settings were: peak inspiratory pressure=32 cm H₂O; positive end-expiratory pressure=5 cm H₂O; ventilator rate=40 cycles min⁻¹; inspiratory time=0.6 s; and fraction of inspired oxygen concentration=1.00. After blood gas stabilization, the umbilical cord was ligated. Subsequent ventilator adjustments were made based on arterial blood gas values. Target blood gas parameters were pH=7.35–7.45 and Paco₂ = 35–45 mmHg. Blood gas analyses were performed at 15 min intervals.

At the end of the experiments, the animals were killed with i.v. pentobarbital (1 g) and a thoracotomy was rapidly performed to remove, in bloc, the heart and lungs.

Physiologic measurements

The flow transducer cable was connected to an internally calibrated flowmeter (T201, Transonic Systems, Ithaca, NY), for continuous measurements of LPA blood flow. Catheters were connected to an arterial pressure transducer (Merlin monitor, Hewlett-Packard, USA). Pressures were referenced to the amniotic cavity pressure. PVR in the left lung was calculated as the difference between mean pulmonary artery and left atrial pressures divided by mean left pulmonary blood flow. Blood samples from the main pulmonary artery catheter were used for blood gas analysis and oxygen saturation measurements.

Experimental design

Animals were assigned to: (i) a group with PH obtained by antenatal partial ligation of the DA (n=9) and (ii) a control group without DA ligation (n=6).

Four different experimental protocols were performed.

Protocol 1. Fetal haemodynamic effects of chronic partial ligation of the DA. To investigate the effects of DA ligation
on the fetal pulmonary circulation, we studied haemodynamic parameters at days 2, 4, 7, and 8 after surgery in both control and PH groups. Haemodynamic measurements included mean pulmonary artery pressure (PAP), mean aortic pressure (AoP), LAP, amniotic pressure, LPA blood flow (Qp), and heart rate (HR). PVR in the left lung was calculated and daily blood gas analysis was performed. Data were compared between the two groups.

Protocol 2. Fetal pulmonary haemodynamic responses to norepinephrine after partial DA ligation. To determine whether chronic intrauterine PH alters the pulmonary vasodilator response to norepinephrine, we used an infusion protocol described previously.\textsuperscript{12} We studied the haemodynamic response to norepinephrine infusion 7 days after surgery in both control and PH groups. Duration of each experiment was at least 240 min and drugs were infused into the venous catheter. Saline (6 ml h\textsuperscript{-1}) was first infused for at least 30 min. After 30 min of stable baseline measurements, norepinephrine was infused at a rate of 1.5 \(\mu\)g min\textsuperscript{-1} (=0.5 \(\mu\)g kg\textsuperscript{-1} min\textsuperscript{-1}) (6 ml h\textsuperscript{-1}) for 120 min (from 30 to 150 min). The catheter was then flushed with saline (6 ml h\textsuperscript{-1}) for 30 min (from 150 to 180 min). Mean PAP, LAP, AoP, Qp, and HR were recorded at 5-min intervals and PVR in the left lung was calculated.

Protocol 3. Effects of norepinephrine on the postnatal circulatory adaptation at birth after partial ligation of the DA. To determine whether norepinephrine would increase pulmonary vasodilatation at birth in lambs with chronic intrauterine PH, the group with antenatal partial DA ligation (\(n=9\)) was randomly assigned to receive norepinephrine (1.5 \(\mu\)g min\textsuperscript{-1} started at the beginning of the ventilation) or saline. The norepinephrine infusion rate was chosen for consistency with earlier studies on norepinephrine effects in the late-gestation fetal lamb. In particular, we found that a rate of 0.5 \(\mu\)g kg\textsuperscript{-1} min\textsuperscript{-1} increases pulmonary blood flow in the ovine fetus.\textsuperscript{10} The control group received only saline. Norepinephrine was infused for 3 h until the end of the experiment. Mean PAP, LAP, AoP, Qp, and HR were recorded at 5-min intervals and PVR in the left lung was calculated.

Protocol 4. Effects of partial antenatal DA ligation on muscle of small pulmonary arteries, and on the development of right ventricular hypertrophy. To determine the degree of change in the muscle of small pulmonary arteries and right ventricular hypertrophy after 8 days of chronic antenatal PH, 5 \(\mu\)m contiguous sections of distal left lung were stained with haematoxylin and eosin. In each microscopic specimen, small pulmonary arteries land-marked by their association with small terminal bronchioles were measured. Two blinded observers performed morphometric analysis of at least 20 consecutive pulmonary arteries per animal. External diameter (ED) and wall thickness (WT) were measured and per cent wall thickness (2WT \times 100/ED) was calculated.

To assess development of right ventricular hypertrophy, the free wall of the right ventricle (RV) was removed from the fetal heart and weighed. Then, the left ventricle plus septum (LV+S) was weighed separately. The ratio (RV)/(LV+S) was calculated.

Data analysis
Results
Protocol 1. Fetal haemodynamic effects of chronic partial ligation of the DA. From day 2 to day 8 following DA ligation, mean PAP increased by 35% (from 52 (3) to 67 (4) mm Hg; \(P<0.05\)) and mean PVR increased by 25% (from 0.85 (0.06) to 1.1 (0.1) mm Hg ml\textsuperscript{-1} min\textsuperscript{-1}; \(P<0.05\)). In this group, mean AoP increased from 41 (4) to 48 (9) mm Hg (\(P<0.05\)). Conversely, mean PAP and PVR did not change from day 2 to day 8 after the initial surgery in the control group. Mean PAP and PVR were higher and mean left pulmonary blood flow (Qp) was lower in the DA ligation group than in the control group (\(P<0.05\)) (Fig. 2). Mean left atrial pressure before infusion was 2 (1) mm Hg and did not change during the study period. HR did not change during the study.

Protocol 2. Fetal pulmonary hemodynamic responses to norepinephrine after partial ligation of the DA. In the two groups, norepinephrine infusion induced a similar increase in AoP (by 12% for the DA ligated vs 13% in controls). In the DA ligation group, norepinephrine infusion increased PAP by 10% (\(P<0.05\)), and progressively increased Qp by 220% after 80 min of drug infusion (\(P<0.05\)). Mean PVR progressively decreased by 65% after 80 min of drug infusion (\(P<0.05\)). In the control group, norepinephrine infusion increased PAP by 15% after 20 min of drug infusion (\(P<0.05\)), and progressively increased Qp by 60% after 60 min of drug infusion (\(P<0.05\)). Mean PVR progressively decreased by 35% after 40 min of drug infusion (\(P<0.01\)).

Percentage change in mean PVR during norepinephrine infusion was higher in the DA ligation group than in the control group (65 vs 35%, respectively; \(P<0.05\)) (Fig. 3). In the two groups, mean left atrial pressure before infusion was 2 (1) mm Hg and did not change during the study period.
Protocol 3. Effects of norepinephrine on the postnatal circulatory adaptation at birth after partial ligation of the DA. Baseline blood gas parameters (after birth and before mechanical ventilation) were similar in both groups (Table 1). PAP and PVR rapidly decreased and AoP and Qp increased in both groups after starting mechanical ventilation ($P<0.05$) (Fig. 4). After an immediate and similar increase of AoP in the two groups, the increase in AoP was higher in the hypertensive group treated by norepinephrine than in the hypertensive group infused with saline (by 40% in the norepinephrine group vs 10% in the saline group 2 h after the beginning of the ventilation ($P<0.001$)).

Within the DA ligation group, the decrease in PAP and PVR and increase in Qp during mechanical ventilation was similar in both groups (Fig. 2). *$P<0.05$ at 2 vs 8 days after DA ligation. AoP was similar in the two groups during the study period. Mean PAP and mean PVR were higher and mean left pulmonary flow was lower in the pulmonary hypertensive lambs. Values are expressed as mean (SEM).
Table 1 Blood gas parameters, mean plasma lactate concentration, mean AoP, mean PAP, mean left pulmonary blood flow (Qp), mean PVR, at baseline (before starting mechanical ventilation with oxygen 100%), in control group (without DA ligation), and in group with DA ligation and after 3 h of ventilation. *P<0.05 compared to control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline group n=6</th>
<th>Group with DA ligation n=9</th>
<th>3 h after starting ventilation</th>
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<tr>
<td></td>
<td>Control group n=5</td>
<td>Norepinephrine n=4</td>
<td>Control group n=5</td>
</tr>
<tr>
<td>PH</td>
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<td>7.39 (0.02)</td>
<td>7.36 (0.01)</td>
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<td>21 (2)</td>
<td>17 (4)</td>
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<tr>
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</tr>
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<td>HCO₃⁻, mEq litre⁻¹</td>
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<td>25 (2)</td>
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<td>2 (9)</td>
</tr>
<tr>
<td>Lactate, mmol litre⁻¹</td>
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<td>3.2 (1.6)</td>
<td>2.2 (0.5)</td>
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<tr>
<td>AoP, mm Hg</td>
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<td>51 (5)</td>
<td>46 (3)</td>
</tr>
<tr>
<td>PAP, mm Hg</td>
<td>49 (4)</td>
<td>74 (6)*</td>
<td>71 (3)*</td>
</tr>
<tr>
<td>Qp, ml min⁻¹</td>
<td>108 (15)</td>
<td>71 (6)*</td>
<td>69 (7)*</td>
</tr>
<tr>
<td>PVR, mm Hg ml⁻¹ min⁻¹</td>
<td>0.44 (0.06)</td>
<td>1.12 (0.16)*</td>
<td>1.06 (0.06)*</td>
</tr>
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</table>

Discussion

This study provides information regarding the potential beneficial haemodynamic effects of norepinephrine in PPHN. This syndrome is usually associated with low systemic pressure and low cardiac output because of increased right ventricular afterload and myocardial dysfunction. Cardiac failure further impairs tissue oxygen delivery. With inhaled NO—a selective pulmonary vasodilator—catecholamines, especially dopamine or dobutamine, are largely used in PPHN. However, evidence exists that dopamine may elevate pulmonary arterial pressure or PVR in the perinatal period. Furthermore, dobutamine has no or limited effects on systemic pressure although it increases cardiac output. Although norepinephrine induces a potent pulmonary vasodilation in the normal fetus, the effects of norepinephrine on the pulmonary circulatory adjustments at birth especially in PPHN remained to be studied. Our data suggest that norepinephrine may have two potentially beneficial effects in PPHN: (i) it increases AoP and (ii) it increases pulmonary blood flow.

PPHN results from the failure of birth-related mechanisms that contribute to the normal decline in PVR at birth. PPHN may reflect a decreased production or responsiveness to vasodilator stimuli and/or increased production or responsiveness to vasoconstrictor stimuli. Structural change such as distal extension of smooth muscle and thickening of the media and adventitia in the pulmonary vessel walls have also been shown in PPHN. The relative role of abnormal pulmonary vascular reactivity and of pulmonary vascular remodelling in PPHN is unknown. Chronic partial compression of the DA in fetal lambs causes sustained intrauterine PH leading to structural changes in small pulmonary arteries and right ventricular hypertrophy, which show similarities to those described in human neonates. Vascular and cardiac remodelling has been observed as early as 5 days after DA ligation. Beside increased smooth muscle thickness in small pulmonary arteries, PH obtained by chronic DA compression, or ligation impairs pulmonary vasodilation to birth-related stimuli, including ventilation with oxygen 100%. Our results show that the norepinephrine-induced increase in pulmonary blood flow is preserved after 8 days of sustained PH despite attenuated responses to ventilation with oxygen 100% and despite vascular remodelling.

Previous experimental studies reported that right ventricular hypertension in the newborn may be associated with a significant decrease in AoP and cardiac output. Mechanisms may include leftward shift of the interventricular septum impairing the left ventricular filling, decreased left ventricular preload resulting from decreased pulmonary...
venous flow and/or decreased coronary artery perfusion resulting from increased right ventricular transmural pressure. Norepinephrine activates both \( \alpha \) (\( \alpha_1 \) and \( \alpha_2 \)) and \( \beta_1 \)-adrenoceptors. Alpha-adrenoceptors participate in sympathetically mediated vasoconstriction of human vessels. Activation of \( \beta_1 \)-adrenoceptors may increase cardiac contractility and cardiac output. Both \( \alpha_1 \)- and \( \beta_1 \)-adrenoceptor activation may explain the increase in AoP observed in our study. In adult dogs with acute right ventricular hypertension, elevation of AoP reversed right ventricular failure by restoring right coronary flow. Norepinephrine induces pulmonary vasodilation through NO release in pulmonary arteries in experimental models from fetal to adult. Furthermore, norepinephrine induces an NO-dependent pulmonary vasodilation in the ovine fetus. In vivo studies, preconstriction of the pulmonary vessels is clearly a prerequisite for norepinephrine to induce pulmonary vessels relaxation. We could speculate that, in vivo, the more elevated the basal pulmonary vascular tone, the greater would be the norepinephrine-induced pulmonary vasodilation. Our results support this hypothesis as the pulmonary vasodilator response to norepinephrine was enhanced in lambs with the highest basal PVR (DA ligation). Further evidence demonstrates that norepinephrine induces pulmonary vasodilation through NO release in pulmonary arteries in experimental models.
vasoconstriction and α₁β-adrenoceptor-mediated NO release and vasodilation. Thus, the degree of vascular response to norepinephrine may depend on the ratio of α₁ to α₂-adrenoceptors at the surface of the endothelium or of the smooth muscle cells. Previous studies have demonstrated that norepinephrine-induced vasodilation is related, at least in part, to NO release. Moreover, NO release was decreased in the DA compression model as shown by alteration of endothelium dependent vasodilation. Collectively, these results suggest that mechanisms other than NO release might also contribute to the observed norepinephrine-induced vasodilation.

Surprisingly high PaO₂ were found in the newborn lambs despite sustained PH at 3 h of mechanical ventilation with oxygen 100%. Lower PaO₂ was observed after 2 h of mechanical ventilation with oxygen 100% in fetal lambs whose DA was compressed for 9–12 days. Degree of vascular remodelling and altered reactivity depend on the duration the PH. Thus, it is likely that shorter duration of PH (8 vs 9 to 12 days) may explain this discrepancy. Whether or not norepinephrine-induced increase in pulmonary blood flow is impaired after more prolonged chronic antenatal PH is at present unclear.

In conclusion, norepinephrine-induced increase in pulmonary blood flow observed in the normal fetal lamb is preserved, before and at birth, in lambs with PH. It is possible that norepinephrine improves postnatal circulatory adaptation at birth in newborn infants with persistent PH both by increasing systemic pressure and by increasing pulmonary blood flow.

Acknowledgements
We thank Professor Steven H. Abman for help in developing the experimental ovine model. This work was supported by the cardiovascular committee of the ‘Fondation de France’, and by the ‘Centre Hospitalier Regional Universitaire’-CHR-U-de Lille.

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