Estradiol improves pulmonary hemodynamics and vascular remodeling in perinatal pulmonary hypertension

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Parker, Thomas A., D. Dunbar Ivy, Henry L. Galan, Theresa R. Grover, John P. Kinsella, and Steven H. Abman. Estradiol improves pulmonary hemodynamics and vascular remodeling in perinatal pulmonary hypertension. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L374–L381, 2000.—Partial ligation of the ductus arteriosus (DA) in the fetal lamb causes sustained elevation of pulmonary vascular resistance (PVR) and hypertensive structural changes in small pulmonary arteries, providing an animal model for persistent pulmonary hypertension of the newborn. Based on its vasodilator and antimitogenic properties in other experimental studies, we hypothesized that estradiol (E2) would attenuate the pulmonary vascular structural and hemodynamic changes caused by pulmonary hypertension in utero. To test our hypothesis, we treated chronically instrumented fetal lambs (128 days, term = 147 days) with daily infusions of E2 (10 μg; E2 group, n = 6) or saline (control group, n = 5) after partial ligation of the DA. We measured intrarterial pulmonary and systemic artery pressures in both groups throughout the study period. After 8 days, we delivered the study animals by cesarean section to measure their hemodynamic responses to birth-related stimuli. Although pulmonary and systemic arterial pressures were not different in utero, fetal PVR immediately before ventilation was reduced in the E2-treated group (2.43 ± 0.79 vs. 1.48 ± 0.26 mmHg·ml−1·min, control vs. E2, P < 0.05). During the subsequent delivery study, PVR was lower in the E2-treated group in response to ventilation with hypoxic gas but was not different between groups with ventilation with 100% O2. During mechanical ventilation after delivery, arterial partial O2 pressure was higher in E2 animals than controls (41 ± 11 vs. 80 ± 35 Torr, control vs. E2, P < 0.05). Morphometric studies of hypertensive vascular changes revealed that E2 treatment decreased wall thickness of small pulmonary arteries (59 ± 1 vs. 48 ± 1%, control vs. E2, P < 0.01). We conclude that chronic E2 treatment in utero attenuates the pulmonary hemodynamic and histological changes caused by DA ligation in fetal lambs.

IN THE FETUS, pulmonary vascular resistance (PVR) is high, with <10% of the combined ventricular output entering the lungs (39). At birth, PVR falls, and the lungs assume their normal postnatal role in gas exchange. Factors that mediate the fall in PVR are incompletely understood but include rhythmic distention of the lungs, increased oxygenation, shear stress, and the release of several vasoactive mediators, including nitric oxide (NO) and prostacyclin (PGI2) (1, 7, 45, 52). Failure to achieve the normal fall in PVR results in persistent pulmonary hypertension of the newborn (PPHN), a syndrome characterized by right-to-left shunting of blood with severe hypoxemia, loss of pulmonary vasoreactivity, and muscularization of small pulmonary arteries (24). Although in utero ligation of the ductus arteriosus (DA) in fetal lambs provides an animal model for PPHN (2), strategies for improving the hemodynamic and morphometric changes associated with perinatal pulmonary hypertension remain limited.

Estradiol (E2) is a sex steroid hormone with potent effects on vascular tone and structure in diverse nonhypertensive adult circulations (13, 50). E2 causes vasodilation of the uterine vasculature of nonpregnant ewes and of the systemic and coronary circulations of women (23, 28, 32, 47). Additionally, E2 decreases the proliferation of smooth muscle cells in response to growth factors in vitro and in studies of atherogenesis in vivo (10, 17, 31). Mechanisms through which E2 mediates its vasoactive effects include increased release of NO (9, 11) and PGI2 (30, 33), decreased release of or response to endothelin (ET)-1 (43), and activation of Ca2+-dependent K+ channels (16, 34).

Recent studies suggest that E2 may also have vasoactive properties in the pulmonary circulation of the fetus. In isolated fetal pulmonary artery (PA) endothelial cells, E2 upregulates endothelial NO synthase (eNOS; see Ref. 29), the enzyme primarily responsible for NO generation, and acutely stimulates NO release (27). Additionally, preliminary studies from our laboratory suggest that exogenous E2 causes marked pulmonary vasodilation in normal late-gestation fetal lambs (35). However, whether E2 alters vascular tone or structure in the hypertensive fetal pulmonary circulation is unknown.

Previous studies suggest that E2 may have beneficial effects on vascular structure and function in adult models of chronic pulmonary hypertension. E2 attenuates the development of myointimal proliferation and...
right ventricular hypertrophy (RVH) in adult rats after injection with monocrotaline (12). Based on the vasoactive and antiproliferative effects of E₂ in other experimental settings, we hypothesized that prolonged E₂ treatment would improve pulmonary hemodynamics and attenuate hypertensive structural remodeling of small pulmonary arteries in fetal lambs after intraterine DA ligation. To test our hypothesis, we compared the hemodynamic response to birth-related stimuli of E₂-treated and control lambs. We also compared the severity of structural changes of small pulmonary arteries and the degree of RVH induced by pulmonary hypertension. We report that chronic E₂ treatment attenuates muscularization of small pulmonary arteries and improves hemodynamics and gas exchange at birth in fetal lambs after DA ligation.

MATERIALS AND METHODS

Pregnant, mixed-breed (Columbia-Rambouillet) ewes were used in this study. All procedures and protocols were reviewed and approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center and followed the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996.

Fetal surgical preparation. The surgical preparation for this model has been described in detail previously (1). Surgery was performed at 124–127 days gestation (term = 147 days) after ewes had fasted for 2 days. Animals were sedated with intravenous pentobarbital sodium and intrathetal tetra-caine hydrochloride (1%, 3 ml) and were given intramuscular penicillin G (600,000 units) and gentamycin (80 mg) immediately before surgery. Under sterile conditions, a midline abdominal incision was made, and the uterus was externalized. A hysterotomy was made, and the left fetal forelimb was exposed. Polyvinyl catheters (20 gauge) were placed in the left axillary artery and vein and advanced into the ascending aorta and superior vena cava, respectively. A left thoracotomy and pericardial incision were made, and the heart and great vessels were exposed. With the use of a 14-gauge intravenous placement unit (Angiocath; Travenol, Deerfield, IL), a 20-gauge catheter was placed through the superior vena cava into the main PA (MPA). The DA was exposed using blunt dissection. A piece of umbilical tape was passed around the DA and cinched partially ligated using umbilical tape. A catheter was placed in the amniotic cavity to serve as a pressure referent. The uterus was sutured, and the left fetal forelimb was exposed. Polyvinyl catheters (20 gauge) were placed in the left axillary artery and vein and advanced into the ascending aorta and superior vena cava, respectively. A left thoracotomy and pericardial incision were made, and the heart and great vessels were exposed. With the use of a 14-gauge intravenous placement unit (Angiocath; Travenol, Deerfield, IL), a 20-gauge catheter was placed through the superior vena cava into the main PA (MPA). The DA was exposed using blunt dissection. A piece of umbilical tape was passed around the DA and cinched partially ligated using umbilical tape. A catheter was placed in the amniotic cavity to serve as a pressure referent. The uterus was sutured, and a dose of ampicillin (500 mg) was given in the amniotic cavity. The catheters were externalized to a flank pouch on the ewe after the abdominal wall was closed. Postoperatively, ewes were allowed to eat and drink ad libitum and were generally standing within 12 h. Fetuses were treated with ampicillin (500 mg) infused into the venous catheter for the first two postoperative days. All catheters were gently flushed daily with 1–2 ml of heparinized normal (0.9%) saline to maintain catheter patency.

During physiological studies, PA and aortic pressure measurements were made by connecting the externalized catheters to computer-driven pressure transducers (MP100A; Biopac Systems, Santa Barbara, CA). Pressure transducers were calibrated using a mercury column manometer before each study. Pressure measurements were referenced to simultaneously recorded amniotic pressure. Heart rate was determined from phasic pressure tracings. Arterial blood gas measurements included pH, Pco₂, Po₂ (ABL 500; Radiometer, Copenhagen, Denmark), O₂ saturation, and hemoglobin (OSM3 Hemoximeter; Radiometer).

After physiological studies were completed, fetuses were killed with a large dose of pentobarbital sodium. A midline thoracotomy was rapidly performed, and the heart and lungs were removed en bloc. After placement of a large-bore cannula into the MPA, the pulmonary vessels were perfused with 1% paraformaldehyde in 0.1 M borate buffer (pH 7.4) at a pressure of 30 cmH₂O. A tracheal catheter was placed, and the airway was simultaneously perfused with warm agarose delivered by a peristaltic pump (15). The lungs were then immersed and stored in paraformaldehyde-borate buffer for later processing.

Drug preparation. A stock solution of E₂ (Sigma Pharmaceuticals) was made in 100% ethanol at a concentration of 1 mg/ml and was stored at 4°C. The stock solution was diluted to a final concentration of 10 μg/ml with sterile water immediately before administration (final concentration of ethanol, 1%).

Protocol 1: Effects of daily E₂ treatment on fetal pulmonary hemodynamics after DA ligation. The purpose of this protocol was to determine whether E₂ treatment would attenuate the development of pulmonary hypertension caused by partial ligation of the DA. Before surgery, animals were randomized to either control n = 7) or E₂ (n = 6) treatment groups. Treatment was begun immediately after DA ligation. Five control animals were given saline and two were given 1% ethanol vehicle (1 ml in the MPA over 1 min; control). E₂ animals were given 10 μg of E₂ (1 ml) in the MPA over 1 min. Dosing was repeated every 24 h for the duration of the study (8 days). PA and aortic pressures and arterial blood gas measurements were recorded daily.

Protocol 2: Effects of daily E₂ treatment on the hemodynamic response to birth-related stimuli after DA ligation. The purpose of this protocol was to determine whether E₂ treatment would increase pulmonary vasodilation to birth-related stimuli during delivery studies by cesarean section. Eight days after the initial surgery, delivery studies were performed on control n = 7) and E₂ (n = 6) fetuses. Ewes were sedated with intravenous pentobarbital sodium and intrathetal tetra-caine hydrochloride (1%, 3 ml), and a hysterotomy was performed. The left fetal vessels were again exposed by repeat left thoracotomy. With the use of careful blunt dissection, the left PA (LPA) was exposed, and a 6-Fr ultrasonic flow transducer (Transconsysmics, Ithaca, NY) was placed around it. A 20-gauge polyvinyl catheter was placed in the left atrial appendage through a loop of suture and secured. The flow transducer was connected to an internally calibrated flowmeter (Transconsysmics) to measure left pulmonary blood flow (QLPA). Aortic, MPA, and LA catheters were connected to pressure transducers. Intravenous panceuronium bromide (0.3 mg) was administered to the fetus to prevent spontaneous fetal breathing. Hemodynamic variables, which included PA pressure (PAP), aortic pressure, left atrial pressure (LAP), and Q̇LPA, were measured continuously thereafter for the duration of the delivery study. Left lung PVR was calculated using the formula (PAP – LAP)/QLPA.

Four sequential delivery study time points were defined. The baseline (“Fetal”) measurements were made after the flow transducer and LA catheter had been placed and hemodynamic parameters had stabilized for 10–15 min. To measure the serial responses to ventilation and high O₂ exposure, a tracheotomy was then performed, and the fetus was intubated with a 3.0-mm endotracheal tube and treated with a natural surfactant (Infasurf, 6 ml; generously given by Dr. E. A. Eagan). Animals were placed on a time-cycled, pressure-limited neonatal ventilator (Infant Star 950; Infrasonics, San Diego, CA) at the initial settings defined below and with a fraction of inspired O₂ (FIO₂) of <0.10 (“Low O₂” time point).
The low $F_{IO2}$ was used to maintain a constant fetal arterial partial $O_2$ pressure ($PaO_2$) $<20$ mmHg to assess the response to rhythmic lung distension independent of changes in oxygenation. The $F_{IO2}$ was then increased to 1.0 ("high $O_2"$ time point) to assess the response to increased $O_2$ tension. Finally, the umbilical cord was ligated while ventilation with 100% $O_2$ continued ("cord tied" time point). At each time point, hemodynamic parameters were allowed to stabilize for 15 min before recording measurements and proceeding to the next time point. Arterial blood gas measurements were recorded at each time point.

A treatment protocol for ventilator manipulations and administration of intravenous fluid was carefully followed during the delivery study to ensure consistent treatment of each animal. Initial ventilator settings included a rate of 30 breaths/min, a peak inspiratory pressure (PIP) of 35 cmH$_2$O, a positive end-expiratory pressure of 6 cmH$_2$O, and an inspiratory time (IT) of 1.0 s. Subsequent ventilator adjustments were made based on arterial blood gas values and chest wall excursion. Target blood gas parameters were pH = 7.35–7.45 and arterial partial $CO_2$ pressure ($PaCO_2$) = 35–45 Torr. If $PaCO_2$ fell below 35 Torr, PIP was progressively decreased to a minimum of 22 cmH$_2$O. Ventilator rate and IT were gradually decreased thereafter if $PaCO_2$ remained below the target value after reaching a PIP of 22 cmH$_2$O. Alternatively, if $PaCO_2$ increased above 45 Torr, ventilator rate was increased by 5 breaths/min, and IT was decreased to maintain the inspiratory-to-expiratory ratio at 1:1. The $F_{IO2}$ was maintained at $<0.10$ during the low $O_2$ time point and increased to 1.00 thereafter. Hypotension, defined as mean arterial pressure $<30$ mmHg, was treated with a rapid infusion of normal (0.9%) saline (estimated 10 ml/kg over 5 min). If hypotension persisted after two infusions of saline, an infusion of normal (0.9%) saline (estimated 10 ml/kg over 5 min). If hypotension persisted after two infusions of saline, an infusion of normal (0.9%) saline (estimated 10 ml/kg over 5 min) was used to maintain a constant fetal arterial pressure ($PaCO_2$) = 35–45 Torr.

Protocol 3: Effects of daily E2 treatment on the development of RVH after DA ligation. The purpose of this protocol was to determine whether E2 attenuates the development of RVH associated with pulmonary hypertension after DA ligation. After completion of the delivery study, the MPA was ligated immediately distal to the pulmonary valve, and the heart was dissected from the lungs. The atri a were removed, and the free right ventricular wall and papillary muscle were separated from the left ventricle and septum. RVH is expressed as the proportion of weights of the right ventricle and the left ventricle plus septum.

Protocol 4: Effects of daily E2 treatment on muscularization of small pulmonary arteries (<100 µm) after DA ligation. The purpose of this protocol was to determine whether E2 treatment attenuates the muscularization of small pulmonary arteries induced by pulmonary hypertension after DA ligation. Histological sections of distal left lung were stained with hematoxylin and eosin. Sections of lung were then examined microscopically under $\times 40$ magnification, and small pulmonary arterioles were scored in a blinded fashion using a Zeiss Interactive Digital Analyzer System (ZIDAS; Zeiss, Thornwood, NY). Vessels were grouped according to their associated airway structure (terminal bronchiole, respiratory bronchiole, and alveolar duct). Degree of arterial muscularization is expressed as percent wall thickness (%WT) and was determined using the formula %WT = (2WT)/ED, where ED is external diameter.

Statistical analysis. Statistical analysis was performed using the SuperANOVA statistical software package (Abacus Concepts, Berkeley, CA). Comparisons were made between groups from protocols 1 and 2 using univariate repeated-measures ANOVA by linear contrast analysis. Comparisons within groups over time were made by repeated-measures ANOVA with Student-Newman-Keuls post hoc testing. Comparisons were made between groups in protocols 3 and 4 using an unpaired t-test. Data are presented as means ± SE. Significance is set at $P < 0.05$.

RESULTS

Protocol 1: Effects of daily E2 treatment on fetal pulmonary hemodynamics after DA ligation. Hemodynamic and morphometric data from saline- and ethanol-infused animals were similar and therefore combined as control data for this and subsequent protocols. DA ligation initially increased mean PAP in both study groups (68 ± 3 and 65 ± 3 mmHg, control and E2, day 1). Mean PAP increased progressively in control animals during the study period (68 ± 3 vs. 80 ± 2 mmHg, day 1 vs. day 8, $P < 0.01$) but not in the E2-treated animals (65 ± 3 vs. 73 ± 7 mmHg, day 1 vs. day 8, $P = $ not significant; Fig. 1). PAP did not differ between groups at any study time point. Aortic pressures were similar between groups and did not change during the study period. Arterial $O_2$ tension was higher in E2-treated animals on day 8. Arterial $CO_2$ tension, pH, and saturation did not differ between groups (Table 1).

Table 1. Blood gas measurements on day 1 and day 8 from control and E2-treated fetal lambs after DA ligation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>E2</th>
<th>Control</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.35 ± 0.02</td>
<td>7.34 ± 0.02</td>
<td>7.33 ± 0.01</td>
<td>7.32 ± 0.01</td>
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<td>$PaCO_2$, Torr</td>
<td>52 ± 2</td>
<td>52 ± 1</td>
<td>53 ± 2</td>
<td>54 ± 2</td>
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<tr>
<td>$PaO_2$, Torr</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>16 ± 0</td>
<td>18 ± 2*</td>
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<tr>
<td>Saturation, %</td>
<td>52 ± 5</td>
<td>51 ± 3</td>
<td>43 ± 3</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. $PaCO_2$ and $PaO_2$, arterial partial pressure of $CO_2$ and $O_2$, respectively; E2, estradiol; DA, ductus arteriosus. *$P < 0.05$, E2 vs. control.
Table 2. Hemodynamic and blood gas variables during delivery study of E2 and control lambs after DA ligation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>E2</th>
<th>Control</th>
<th>E2</th>
<th>Control</th>
<th>E2</th>
<th>Control</th>
<th>E2</th>
<th>Control</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA flow, ml/min</td>
<td>37 ± 6</td>
<td>49 ± 8</td>
<td>41 ± 7</td>
<td>67 ± 11</td>
<td>82 ± 16</td>
<td>146 ± 36</td>
<td>95 ± 16</td>
<td>139 ± 29</td>
<td></td>
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<tr>
<td>PAP, mmHg</td>
<td>69 ± 3</td>
<td>67 ± 4</td>
<td>64 ± 3</td>
<td>56 ± 4*</td>
<td>59 ± 4</td>
<td>47 ± 4*</td>
<td>56 ± 4</td>
<td>47 ± 4*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoP, mmHg</td>
<td>49 ± 3</td>
<td>45 ± 2</td>
<td>42 ± 4</td>
<td>39 ± 2</td>
<td>47 ± 5</td>
<td>42 ± 3*</td>
<td>46 ± 6</td>
<td>44 ± 4</td>
<td></td>
<td></td>
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<tr>
<td>LAP, mmHg</td>
<td>2 ± 1</td>
<td>2 ± 0</td>
<td>2 ± 0</td>
<td>3 ± 1</td>
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<td>3 ± 0</td>
<td>2 ± 0</td>
<td>2 ± 1</td>
<td></td>
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<tr>
<td>PVR, mmHg·ml⁻¹·min</td>
<td>2.43 ± 0.79</td>
<td>1.48 ± 0.26*</td>
<td>1.84 ± 0.43</td>
<td>0.95 ± 0.26*</td>
<td>0.85 ± 0.19</td>
<td>0.50 ± 0.21</td>
<td>0.65 ± 0.10</td>
<td>0.39 ± 0.10</td>
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<tr>
<td>MAP, cmH2O</td>
<td>NA</td>
<td>NA</td>
<td>16.9 ± 0.6</td>
<td>16.2 ± 0.2</td>
<td>17.6 ± 0.6</td>
<td>14.9 ± 0.2*</td>
<td>15.2 ± 0.8</td>
<td>12.8 ± 0.7*</td>
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<tr>
<td>pH</td>
<td>7.18 ± 0.06</td>
<td>7.26 ± 0.02*</td>
<td>7.18 ± 0.05</td>
<td>7.31 ± 0.02*</td>
<td>7.29 ± 0.02</td>
<td>7.38 ± 0.02*</td>
<td>7.26 ± 0.04</td>
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<tr>
<td>PaCO₂, Torr</td>
<td>60 ± 3</td>
<td>56 ± 1</td>
<td>59 ± 4</td>
<td>46 ± 3</td>
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<td>38 ± 6</td>
<td>53 ± 7</td>
<td>36 ± 2</td>
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<tr>
<td>PaO₂, Torr</td>
<td>17 ± 2</td>
<td>19 ± 0</td>
<td>17 ± 1</td>
<td>15 ± 1</td>
<td>30 ± 3</td>
<td>47 ± 10</td>
<td>42 ± 11</td>
<td>80 ± 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂ Saturation, %</td>
<td>37 ± 8</td>
<td>46 ± 3</td>
<td>36 ± 5</td>
<td>33 ± 4</td>
<td>66 ± 10</td>
<td>87 ± 5*</td>
<td>77 ± 14</td>
<td>88 ± 5</td>
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<td>Hemoglobin</td>
<td>6.9 ± 0</td>
<td>7.2 ± 0</td>
<td>7.0 ± 0</td>
<td>7.1 ± 0</td>
<td>6.7 ± 0</td>
<td>7.0 ± 0</td>
<td>7.0 ± 0</td>
<td>7.2 ± 0</td>
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<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. LPA, left pulmonary artery; PAP, pulmonary artery pressure; AoP, aortic pressure; LAP, left atrial pressure; PVR, pulmonary vascular resistance (left lung); MAP, mean airway pressure; NA, not available. *P < 0.05 E2 vs. control.

Protocol 2: Effects of daily E2 treatment on the hemodynamic response to birth-related stimuli after DA ligation. Hemodynamic and arterial blood gas measurements for each of the four delivery study time points are shown in Table 2 and Fig. 2. One of the ethanol vehicle control animals developed refractory acidemia during placement of the flow transducer and died before delivery. Therefore, data from six control animals were available for analysis. At the fetal time point (baseline value, before ventilation), Q_LPA and PAP were not different between groups. PVR in the left lung was lower in E2-treated animals at the fetal and hypoxic ventilation time points but was not different between groups during ventilation with 100% O₂ (Table 2). In response to tracheotomy and mechanical ventilation, PAP fell more markedly in E2-treated lambs (6 ± 1 vs. 12 ± 2 mmHg, control vs. E2, P < 0.05) and was lower than controls at each study time point after the baseline time point. Similarly, with ventilation, Q_LPA was higher in the E2-treated animals. PaCO₂ was lower and PaO₂ was higher at the final study time point (after ligation of the umbilical cord) in the E2 group (Table 2). Control animals required higher mean airway pressures during mechanical ventilation than E2 animals (Table 2). Four of six control animals and two of six E2 animals were treated with bicarbonate during the study (15 ± 6 and 10.5 ± 4.5 meq bicarbonate/animal treated, control and E2).

Protocol 3: Effects of daily E2 treatment on the development of RVH after DA ligation. E2 treatment did not change right ventricular weight (12.1 ± 1.2 vs. 11.1 ± 1.1 g, control vs. E2), left ventricular plus septal weight (14.9 ± 1.3 vs. 13.7 ± 1.4 g, control vs. E2), or the right ventricle-to-left ventricle plus septum ratio (0.80 ± 0.02 vs. 0.81 ± 0.04, control vs. E2).

Protocol 4: Effects of daily E2 treatment on muscularization of small pulmonary arteries (<100 µm) after DA ligation. E2 treatment attenuated the degree of muscularization of small pulmonary arteries, as assessed by measurements of percent wall thickness (59 ± 1 vs. 48 ± 1%, control vs. E2, P < 0.01). The differences were evident regardless of the associated airway structure (Table 3 and Figs. 3 and 4).

DISCUSSION

We found that daily E2 treatment of fetal lambs with pulmonary hypertension caused by ligation of the DA improved pulmonary hemodynamics and gas exchange at birth. Although E2 did not lower fetal PAP, our preparation did not allow us to measure pulmonary blood flow in utero. After placement of the flow probe on the LPA, PVR before mechanical ventilation was lower in E2-treated lambs than in controls, suggesting that E2 attenuated the progressive increase in fetal PVR after...
DA ligation, which characterizes this model (2). During mechanical ventilation after delivery, E2-treated lambs had higher LPA blood flow and lower PAP at each time point during the study. E2 also attenuated the degree of muscularization of small pulmonary arteries but did not decrease the development of RVH.

This is the first study to demonstrate a protective effect of hormone therapy on the perinatal pulmonary circulation in an experimental model of chronic pulmonary hypertension. Despite exposure to similar PAP in utero, E2 attenuated the hypertensive structural changes of small pulmonary arteries. Previous studies suggest that high pressure exerts a direct proliferative stimulus on PA smooth muscle cells in pulmonary hypertension (26, 49). However, our results suggest that E2 can interrupt or modify this effect, perhaps by acting directly on the vascular smooth muscle cell (VSMC). Human VSMCs contain estrogen receptors (21), and E2 decreases proliferation of growth factor-stimulated venous smooth muscle cells (10) and decreases migration of VSMCs into Matrigel plugs both in vitro and in vivo (31). Alternatively, the protective effects of E2 may have resulted indirectly from E2-induced changes in pulmonary blood flow and shear stress. Because the flow probe was not placed until immediately before delivery in our study, we were unable to determine whether fetal pulmonary blood flow differed between groups during the course of treatment. However, the similar LPA flow between groups immediately before ventilation and umbilical cord ligation strongly suggests that in utero flow during the preceding 8 days was not different between groups.

Previous studies have examined the effects of E2 therapy in postnatal models of pulmonary hypertension. Gordon et al. (14) demonstrated that E2 pretreatment attenuates acute hypoxic vasoconstriction and increases release of PGI2 metabolites in isolated, perfused lungs from juvenile female lambs. Because indomethacin reduces the protective effects of E2 in that setting, the E2 effects are likely mediated, in part, through activation of the cyclooxygenase system. However, in that report, the acute effects of hypoxia on pulmonary hemodynamics were studied, in contrast with our study, which examined the effects of E2 on chronic, normoxic pulmonary hypertension. Farhat et al. (12) have reported that E2 reduces myointimal proliferation and attenuates the development of RVH in chronic pulmonary hypertension induced by monocrotaline injection in adult rats. In that study, however, the protective mechanism of E2 was not addressed.

In our study, the protective mechanism of E2 may have been through the NO-cGMP cascade. E2 causes vasodilation of several vascular beds in adult tissues (13, 50). For example, both local and systemic administration dramatically increase blood flow to the uterus of nonpregnant sheep (23, 38), and this effect is partially mediated by NO (37, 44). Several studies in human subjects have demonstrated changes in basal blood flow in response to E2 (36, 47). Additionally, E2 treatment can increase endothelium-dependent, NO-mediated vasodilation (5). E2 treatment of guinea pigs increases expression of eNOS (48), the enzyme responsible for NO generation. Data on the effects of E2 in the fetal pulmonary circulation are limited, but previous preliminary studies from our laboratory suggest that infusions of exogenous E2 dramatically increase pulmonary blood flow in late-gestation fetal lambs (35). Although we were unable to detect a similar increase in basal LPA flow between groups in the current study, our previous studies were undertaken in lambs without pulmonary hypertension. Given the degree of hemodynamic and structural alteration of the pulmonary circulation induced by DA ligation, it is not surprising that E2 might have different effects in the two models. In addition, in the current study, E2 was given as discrete, rapid boluses compared with prolonged, continuous infusions. It is unclear how this alternative dosing schedule might have affected the results. Other studies of isolated PA endothelial cells of nonhypertensive fetal lambs suggest that E2 increases eNOS mRNA expression (29). Additionally, we and others have recently shown that eNOS mRNA, protein and activity are reduced in experimental PPHN caused by DA ligation.

### Table 3. Percent wall thickness of small pulmonary arteries (<100 µm) from control and E2-treated lambs based on airway association

<table>
<thead>
<tr>
<th>Airway</th>
<th>Control</th>
<th>Estradiol</th>
</tr>
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<tbody>
<tr>
<td>Terminal bronchiole</td>
<td>58 ± 2</td>
<td>44 ± 2*</td>
</tr>
<tr>
<td>Respiratory bronchiole</td>
<td>58 ± 2</td>
<td>51 ± 2*</td>
</tr>
<tr>
<td>Alveolar duct</td>
<td>61 ± 3</td>
<td>52 ± 2*</td>
</tr>
<tr>
<td>All</td>
<td>59 ± 1</td>
<td>48 ± 1*</td>
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</tbody>
</table>

Values are means ± SE. Fifteen vessels were measured from control (n = 7) and E2-treated (n = 6) animals. Percent wall thickness was determined as (2·wall thickness)/external diameter. *P < 0.05 vs. control.
Whether E2 preserved the vasodilator response at birth and decreased PA muscularization by improving NO production in our study is unknown. Alternatively, the effects of E2 may result from increased NO bioavailability through decreased O2 radical-induced inactivation (4) or through other steps in the NO-cGMP cascade.

E2 may also affect other mediators that contribute to the pathogenesis of pulmonary hypertension in this model. ET-1 is a potent vasoconstrictor peptide with smooth muscle cell proliferative properties (6, 25, 51). Ivy et al. (18) have recently reported that DA ligation increases preproET-1 mRNA, the precursor peptide for ET-1. Pharmacological endothelin receptor A (ETa) blockade reduces pulmonary hypertension and vascular remodeling after DA ligation (19). E2 has been reported to decrease both ET-1 generation and its vasoconstrictive properties in some settings (3, 20, 43). It is possible that E2 attenuated the enhanced effects of ET-1 in our study, although E2 did not produce the same in utero reduction in PAP that ETa receptor blockade does (19). E2 also increases the release of and response to PGI2 in several vascular beds (30, 33). Although PGI2 is an important mediator of pulmonary vascular tone in the perinatal period (45) and has been used clinically to treat neonatal pulmonary hypertension (8), alterations in the cyclooxygenase-PGI2 axis and its contribution to the pathogenesis of perinatal pulmonary hypertension after DA ligation have not been investigated. Further studies are necessary to determine the interaction between E2, ET-1, and PGI2 in this model of pulmonary hypertension.

The optimal treatment dose of E2 in this model remains unclear. The E2 dose that we used in this study was based on previously published studies showing that a similar dose (6.8 µg) can increase flow in vascular beds with analogous basal blood flow to that in the LPA of fetal lambs (23). Our assumption was that we would achieve a local concentration similar to that in previous studies because the infusion was given directly into the LPA. Whether these results can be duplicated with systemic administration of hormone requires further study. The rationale for administration of the drug as a rapid infusion was to achieve saturation of estrogen receptors (assuming that the effect of E2 in this setting is “genomic,” or receptor mediated). Previous studies of the uterine circulation of nonpregnant ewes found that E2-induced vasodilation was achieved maximally after the dose was given as a bolus and that the effect could be blocked by prior administration of cycloheximide, an inhibitor of protein synthesis (23). Whether a continuous infusion of E2 in this model would have similar, or more marked, effects to bolus administration is not known.

Interpretation of our results is potentially limited by the differences in blood gas values between the two study groups during the delivery study. Despite liberal use of bicarbonate and ventilation at higher mean airway pressures, control lambs had lower arterial pH at delivery than did those animals treated with E2. At two of the four delivery study time points, they also had higher CO2 levels. Acidosis, and perhaps hypercarbia, can reduce pulmonary blood flow (40, 42), potentially confounding our results. However, we believe that the more pronounced hypercarbia and acidosis in the control group reflects more severe vascular disease with a corresponding inability to respond to ventilator and pharmacological manipulation. The more severe hypertensive histological changes in the control animals support the likelihood that hemodynamic differences at birth result from increased disease severity of a long-standing nature rather than acute differences in acid-base balance. A second potential confounding treatment effect may result from estrogen’s ability to stimulate surfactant production (22). We treated all of the animals with exogenous surfactant to attempt to minimize that effect, but differences in ventilator settings and gas exchange may have resulted in part from differences in endogenous surfactant production.

We conclude that E2 administration attenuates the hypertensive morphometric changes and improves gas exchange at delivery in perinatal pulmonary hypertension caused by DA ligation. E2 exerts this protective effect without lowering fetal PAP or decreasing the development of RVH. The mechanisms through which E2 acts in this model require further investigation. We
speculate that estrogen administration may have a therapeutic role in the treatment of perinatal pulmonary hypertension.

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