Endothelin-receptor blockade does not alter closure of the ductus arteriosus

JEFFREY R. FINEMAN,1 YASUSHI TAKAHASHI,2
CHRISTINE ROMAN,1 AND RONALD I. CLYMAN1,2

1Department of Pediatrics and 2Cardiovascular Research Institute, University of California, San Francisco, California 94143-0544

THE DUCTUS ARTERIOSUS represents a persistence of the terminal portion of the sixth branchial arch that serves to divert blood away from the lungs toward the descending aorta and placenta during fetal life. At birth, rapid constriction of the ductus separates the right and left sides of the fetal heart and establishes the postnatal pattern of circulation. This rapid constriction is induced by the rise in blood oxygen tension at birth (5, 19). However, the biochemical basis for this oxygen response remains unknown.

Coceani and co-workers (9–12) hypothesized that oxygen-induced ductus constriction depends on the synthesis and release of endothelin-1 (ET-1). ET-1 is a 21-amino acid polypeptide produced by vascular endothelial cells that has potent vasoactive properties (28). Its vasoactive effects are complex and depend on a variety of factors including age, dose, vascular bed, and resting tone. They are mediated by at least two different receptors, ETₐ and ET₆. ETₐ receptors and a subpopulation of ET₆ receptors mediate vasoconstriction and are located on vascular smooth muscle cells (1, 25). A second subpopulation of ET₆ receptors mediate vasodilation and are located on vascular endothelial cells (26). An increasing amount of data suggests that ET-1 is an important mediator of normal vascular tone in many regional circulations, such as the cerebral, renal, and pulmonary circulations, and that aberrations in the ET-1 cascade participate in several pathophysiological disorders (20).

Coceani et al. (9) reported that exogenous ET-1 produces dose-dependent constriction of the ductus arteriosus in vitro; this occurs whether or not an intact endothelium is present. They also found that oxygen stimulates ET-1 release by the ductus arteriosus and that an ETₐ-receptor antagonist inhibits oxygen-induced constriction of the ductus arteriosus. These in vitro observations suggest an essential role for ET-1 in the closure of the ductus arteriosus after birth (10, 12). However, in vivo, when ET-1 or ET₆-receptor antagonists are administered to the fetus, they do not appear to affect the tone of the ductus arteriosus (2, 18). Although these in vivo studies do not support a role for ET-1 in ductus regulation in the low-oxygen environment of the fetus, they do not address its possible role in oxygen-induced ductus closure after birth.

In the present investigation, we used 13 late-gestation fetal lambs that were infused with a selective ETₐ-receptor antagonist (PD-156707) to study the role of endogenous ET-1 on ductus closure during the first 5 h after birth. In addition, we used nine fetal lambs to examine the effects of ETₐ-receptor blockade on oxygen-induced ductus arteriosus constriction in vitro. Our findings do not support a role for ET-1 in the initial constriction of the ductus arteriosus after birth.

METHODS

In Vivo Studies

Surgical preparation. Thirteen mixed-breed Western ewes (133.4 ± 0.8 days of gestation, term = 145 days) were operated on under sterile conditions with the use of local (2% lidocaine hydrochloride) and intravenous (0.001 mg·kg⁻¹·min⁻¹ diazepam and 0.24 mg·kg⁻¹·min⁻¹ ketamine hydrochloride) anesthesia. Fetal anesthesia consisted of local anesthesia with 2% lidocaine hydrochloride and ketamine hydrochloride (20 mg/kg im). Through a uterine incision, the fetal forelimb was exposed. Polyvinyl catheters were inserted into
the fetal pedal artery and vein and were advanced to the aorta and the superior vena cava, respectively. A left lateral thoracotomy was performed in the fourth intercostal space. Polyvinyl catheters were inserted into the internal thoracic artery and vein. The pericardium was incised along the main pulmonary trunk. A Teflon cannula attached to a polyvinyl catheter was inserted into the proximal main pulmonary trunk. Ultrasonic flow transducers (Transonic Systems, Ithaca, NY) were placed around the left pulmonary artery (no. 4 or 6) and ductus arteriosus (no. 6 or 8). The thoracotomy incision was closed in layers. Warm saline was instilled to replace the lost amniotic fluid, and the uterine incision was closed. A polyvinyl catheter was placed in the amniotic cavity. The catheters were filled with heparin sodium, plunged, and brought to the skin along with the transducer cables, where they were protected in a pouch secured to the ewe’s flank.

After recovery from anesthesia, the ewe was returned to the cage. Antibiotics (2 × 106 U penicillin G procaine and 100 mg gentamicin sulfate) were administered intravenously to the ewe and into the amniotic cavity during surgery and daily thereafter. All protocols were approved by the Committee on Animal Research of the University of California, San Francisco.

Measurements. Arterial and venous pressures were measured by Statham P23 Db pressure transducers (Statham Instruments, Hato Rey, PR). Mean pressures were obtained by electrical integration. All pressures obtained in utero were zeroed against the amniotic cavity pressure. Left pulmonary and ductus arteriosus blood flows were measured on an ultrasonic flowmeter (Transonic Systems). All hemodynamic variables were continuously recorded on a Gould multichannel electrostatic recorder (Gould, Cleveland, OH). Systemic arterial blood gases and pH were measured on a Corning 15C pH/blood gas analyzer (Corning Medical and Scientific, Medfield, MA). Ductus arteriosus resistance was calculated as (mean pulmonary arterial pressure – mean systolic arterial pressure)/ductus arteriosus blood flow per kilogram. The fetal weight before the infusions began was estimated using standardized fetal sheep growth charts established in our laboratory.

Experimental protocol. After a 48-h recovery period, an intravenous infusion of PD-156707 (10 mg/kg bolus over 10 min followed by an infusion of 10 mg·kg⁻¹·h⁻¹) or its vehicle (sterile water) was begun in the fetus; the infusion was begun 60 min before the ventilation study began and continued throughout the study period. Preliminary pharmacokinetic studies found that this infusion rate produced plasma concentrations of 5.4 µg/ml (10⁻⁵ M) of PD-156707. PD-156707 concentrations in this range are five times greater than those needed to block ETA receptors in vivo (17). In addition, our preliminary studies also showed that the vasoconstricting response of exogenous ET-1 (250 ng/kg) was blocked by this infusion rate. Before the ventilation study began, the pregnant ewe was given epidural anesthesia (4 ml of 1% tetracaine hydrochloride) and intravenous sedation (100 mg ketamine hydrochloride) and intravenous saline (3 ml/kg; Ony, Amherst, NY) was instilled into the airway to preclude the possibility of surfactant deficiency. The fetal lamb was then mechanically ventilated with a time-cycled pressure-limited ventilator (Sechrist, Anaheim, CA) while still connected to the placenta. The ventilator settings were: peak inspiratory pressure 28 cmH₂O, positive end-expiratory pressure 5 cmH₂O, inspiratory time 0.4 s, respiratory rate 50 breaths/ min, and fractional inspired O₂ concentration = 1.0. Peak inspiratory pressures and ventilatory rates were reduced to maintain an arterial Pco₂ between 30 and 35 Torr; other ventilator settings remained constant. The beginning of mechanical ventilation was considered the time of delivery (0 h). After 30 min of ventilation, the umbilical cord was clamped, the lamb was delivered through the hysterotomy into a 37–38°C water bath, and mechanical ventilation was continued. The initial 30-min period of ventilation, with the lamb still attached to the placenta, was used to ensure adequate lung expansion without the need for high inspiratory pressures.

The hemodynamic variables (mean pulmonary and systemic arterial pressure, systemic venous pressure, and left pulmonary and ductus arteriosus blood flows) were monitored continuously throughout the 5-h study period. Systemic arterial blood gases were obtained every 5–15 min over the study period. The lamb was anesthetized with intermittent doses of ketamine hydrochloride (–20 mg/kg im). Sodium bicarbonate was given intermittently to maintain a systemic arterial pH > 7.35. Blood loss caused by sampling was replaced with 0.9% saline and 5% dextrose. Rectal temperature was maintained at 37–38°C. After 5 h, the lamb was given a lethal dose of pentobarbital sodium followed by bilateral thoracotomy. At autopsy, the fetus was weighed and catheter placement and closure of the ductus arteriosus were confirmed.

In Vitro Studies

Nine fetal lambs (130.4 ± 4.7 days gestation) were delivered by cesarean section. The ewe was anesthetized with a constant intravenous infusion of ketamine HCl and diazepam throughout the procedure. The fetus was given ketamine HCl (30 mg/kg im) before rapid exsanguination. These procedures were approved by the Committee on Animal Research at the University of California, San Francisco. The ductus arteriosus was dissected free of loose adventitial tissue and divided into 1-mm-thick rings (50 ± 17 mg) that were placed in separate 10-ml organ baths and kept in a dark room, as described previously (7). Throughout the experiment, the rings were suspended between two stainless steel hooks at 38°C in a modified Krebs solution ([in mM]: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 0.9 MgSO₄, 1 KH₂PO₄, 11.1 glucose, and 23 NaHCO₃) equilibrated with 5% CO₂ (pH 7.4); balance either 95% N₂ or 30% O₂–65% N₂. The bath solution was changed every 20 min. Isometric responses of circumferential tension were measured by Grass FT03C force transducers (Quincy, MA). Each of the rings was stretched to an initial length, which results in a maximal contractile response to increases in oxygen tension. Initially the rings were stretched during a 30-min interval in medium equilibrated with low fetal Po₂ (20–34 mmHg, 0.15–0.26 kPa starting tension). During this period PD-156707 (10⁻⁴ M; n = 7) or its vehicle (sterile water; n = 7) was added to the bath solution. After 30 min of gas bubbling the bath solution was changed to 30% O₂–65% N₂–5% CO₂ (Po₂ 175–200 mmHg, 1.31–1.50 kPa), and the tension was allowed to reach a new plateau (~90–120 min). Indomethacin (5.6 × 10⁻⁶ M, an inhibitor of prostaglandin production) then was added to the bath solution, and the rings were allowed to reach a new steady-state tension over the next 60–90 min. We previously showed (6, 7) that this concentration of indomethacin maximally inhibits prostaglandin E₂ and I₂ production in the ductus. After indomethacin, 6-anilino-5,8-quinindione (LY-83583, an inhibitor of soluble guanylate cyclase, 10⁻⁵ M) and ET-1 (10⁻⁸ and 10⁻⁷ M) were added to
the bath solution. These concentrations of ET-1 were chosen because they produce 15 and 80%, respectively, of the maximal contractile response of the ductus to ET-1 (data not shown). In all experiments, we allowed the tension in the rings to reach a new steady-state plateau after a drug addition before another experimental agent was added to the bath. After the addition of all contractile drugs, potassium Krebs solution (containing 100 mM KCl substituted for an equimolar amount of NaCl) was used to measure the maximal amount of tension that could be developed by the ductus (maximal contraction). The maximal relaxation of each ductus ring was then determined by the response to sodium nitroprusside (SNP; 10⁻³ M).

The difference in tensions between the maximal contraction and the maximal relaxation was considered the net active tension developed by the ring. Changes in tension for each experimental condition were expressed as a percentage of net active tension. The net active tension was always greater than the difference in tension between the maximal contraction and the starting tension by 12 ± 5% (P < 0.05, n = 9 rings). This indicates that the ductus rings were actively contracting even at the time of their initial mounting in the organ bath. After the experiment the rings were removed from the baths and blotted dry, and their wet weights were determined.

Drug preparation. PD-156707 and PD-14505 were synthesized by the Medicinal Chemistry Department of Parke-Davis Pharmaceutical Research (Ann Arbor, MI). ET-1 (Sigma, St. Louis, MO) was resuspended in 10 ml of sterile water and stored at 20°C. Immediately before administration, each dose of ET-1 was measured and diluted to 1 ml with 0.9% saline.

The following compounds were added to the bath solution: indomethacin (Sigma), LY-83583 (Biomol, Plymouth Meeting, PA), SNP (Sigma), ET-1, and PD-156707. Daily stock solutions of indomethacin (4.2 × 10⁻² M) and LY-83583 (2 × 10⁻² M) were prepared in ethanol. The final concentration of ethanol in the organ bath did not affect tissue contractility. All other compounds were dissolved directly in Krebs solution.

Statistical Analysis

In vivo studies. Hemodynamic variables, arterial blood gases, and pH were compared over time within each study group by ANOVA for repeated measures. Comparisons of these variables between study groups were made by the unpaired t-test. Comparisons of ductus arteriosus resistance were made by the Mann-Whitney U-test. Values are expressed as means ± SD. P < 0.05 was considered significant.

In vitro studies. Statistical analysis was performed by the paired Student's t-test. When more than one comparison was made, Bonferroni correction was used. Values are expressed as means ± SD. Drug doses refer to final molar concentration in the bath.

RESULTS

In Vivo Studies

The baseline fetal hemodynamic variables and systemic arterial blood gases and pH were similar between the two groups and within the normal range for the laboratory. There were no differences in birth weight [PD-156707: 3.59 ± 0.7 kg (n = 6) vs. vehicle: 4.17 ± 0.7 kg (n = 7)], sex distribution, amount of sodium bicarbonate, saline, or 5% dextrose administered, or mean airway pressure used during the study. PD-156707 infusion had no effect on fetal hemodynamics or blood gas and pH variables (data not shown).

In the vehicle-treated lambs, ductus arteriosus resistance rapidly increased and ductus arteriosus blood flow rapidly decreased after ventilation was initiated (P < 0.05). Mean pulmonary arterial pressure decreased, whereas left pulmonary blood flow and arterial P O₂ (P a O₂) increased (P < 0.05; Table 1).

Similarly, in PD-156707-treated lambs, ductus arteriosus resistance, left pulmonary blood flow, and P a O₂ increased, whereas ductus arteriosus blood flow and mean pulmonary arterial pressure decreased after birth (P < 0.05; Table 2). There were no differences in ductus arteriosus resistance or the mean pulmonary-to-systemic arterial pressure gradient between the two groups throughout the 5-h study period (Fig. 1). Ductus arteriosus blood flow was lower in the PD-156707-treated lambs at 2, 3, and 4 h after birth (P < 0.05; Fig. 1B). At autopsy, the ductus was constricted in all lambs [narrowest diameter: PD-156707, 0.8 ± 0.5 mm (n = 6); control = 1.1 ± 0.9 mm, (n = 7)].

Table 1. Hemodynamic changes after birth in control lambs

<table>
<thead>
<tr>
<th>TimeAfter Delivery, h</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductus arteriosus blood flow, ml·kg⁻¹·min⁻¹</td>
<td>193.5 ± 48.0</td>
<td>25.9 ± 66.4*</td>
<td>77.4 ± 42.5*</td>
<td>66.7 ± 45.3*</td>
<td>41.1 ± 24.2*</td>
<td>30.2 ± 21.1*</td>
<td>19.3 ± 14.3*</td>
</tr>
<tr>
<td>Ductus arteriosus resistance, mmmHg·ml⁻¹·min⁻¹·kg⁻¹</td>
<td>0.007 ± 0.01</td>
<td>0.127 ± 0.05*</td>
<td>0.881 ± 1.46*</td>
<td>0.592 ± 0.64*</td>
<td>1.574 ± 2.31*</td>
<td>5.276 ± 10.57*</td>
<td>3.406 ± 4.15*</td>
</tr>
<tr>
<td>Left pulmonary blood flow, ml·kg⁻¹·min⁻¹</td>
<td>6.5 ± 5.2</td>
<td>96.8 ± 17.8*</td>
<td>67.9 ± 19.1*</td>
<td>52.7 ± 21.9*</td>
<td>50.4 ± 21.5*</td>
<td>37.1 ± 21.7*</td>
<td>41.0 ± 6.9*</td>
</tr>
<tr>
<td>Pulmonary arterial pressure, mmmHg</td>
<td>59.6 ± 6.5</td>
<td>55.5 ± 9.0</td>
<td>40.6 ± 6.7*</td>
<td>37.9 ± 8.3*</td>
<td>33.9 ± 6.7*</td>
<td>29.9 ± 7.6*</td>
<td>29.8 ± 7.3*</td>
</tr>
<tr>
<td>Systemic arterial pressure, mmmHg</td>
<td>58.1 ± 6.4</td>
<td>70.0 ± 9.2*</td>
<td>66.4 ± 5.9</td>
<td>60.4 ± 6.4</td>
<td>59.6 ± 7.5</td>
<td>56.1 ± 6.1</td>
<td>55.0 ± 8.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.06</td>
<td>7.36 ± 0.06</td>
<td>7.44 ± 0.10</td>
<td>7.45 ± 0.11</td>
<td>7.44 ± 0.06</td>
<td>7.46 ± 0.06*</td>
<td>7.49 ± 0.06*</td>
</tr>
<tr>
<td>P a O₂, Torr</td>
<td>49.5 ± 2.8</td>
<td>47.0 ± 5.3</td>
<td>36.6 ± 7.0</td>
<td>34.1 ± 7.2*</td>
<td>32.1 ± 6.7*</td>
<td>31.8 ± 7.4*</td>
<td>28.7 ± 3.1*</td>
</tr>
<tr>
<td>P a CO₂, Torr</td>
<td>18.3 ± 2.7</td>
<td>167.0 ± 85.7*</td>
<td>294.7 ± 91.9*</td>
<td>369.0 ± 138.5*</td>
<td>369.1 ± 106.9*</td>
<td>371.3 ± 113.5*</td>
<td>374.1 ± 159.1*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 lambs except for ductus arteriosus flow (n = 6). For ductus arteriosus blood flow, + = right-to-left shunt; − = left-to-right shunt. *P < 0.05 vs. 0 h (ANOVA).
Table 2. Hemodynamic changes after birth in PD-156707-treated lambs

<table>
<thead>
<tr>
<th>Time After Delivery, h</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductus arteriosus flow, ml·kg⁻¹·min⁻¹</td>
<td>+211.6 ± 35.9</td>
<td>-61.5 ± 68.0*</td>
<td>-19.2 ± 52.0*</td>
<td>-10.1 ± 19.2*</td>
<td>-10.0 ± 7.4*</td>
<td>-3.3 ± 2.0*</td>
<td>-1.0 ± 8.0*</td>
</tr>
<tr>
<td>Ductus arteriosus resistance, mmHg·m⁻¹·kg⁻¹</td>
<td>0.011 ± 0.01</td>
<td>0.214 ± 0.27</td>
<td>1.309 ± 1.13*</td>
<td>2.369 ± 2.25*</td>
<td>3.508 ± 1.37*</td>
<td>15.451 ± 23.86†</td>
<td>15.948 ± 24.52†</td>
</tr>
<tr>
<td>Left pulmonary blood flow, ml·kg⁻¹·min⁻¹</td>
<td>4.3 ± 6.8</td>
<td>89.5 ± 19.3*</td>
<td>87.2 ± 25.1*</td>
<td>71.2 ± 28.0*</td>
<td>64.7 ± 31.6*</td>
<td>59.4 ± 30.2*</td>
<td>68.3 ± 31.7*</td>
</tr>
<tr>
<td>Pulmonary arterial pressure, mmHg</td>
<td>59.0 ± 7.1</td>
<td>54.3 ± 10.1</td>
<td>42.7 ± 8.5*</td>
<td>38.1 ± 7.9*</td>
<td>33.5 ± 5.3*</td>
<td>34.3 ± 5.9*</td>
<td>29.8 ± 4.5*</td>
</tr>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>58.0 ± 6.6</td>
<td>630.8 ± 8.7</td>
<td>60.8 ± 13.3</td>
<td>56.8 ± 11.7</td>
<td>61.7 ± 14.9</td>
<td>52.4 ± 13.3</td>
<td>52.6 ± 10.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.32 ± 0.06</td>
<td>7.31 ± 0.19</td>
<td>7.38 ± 0.17</td>
<td>7.40 ± 0.16</td>
<td>7.42 ± 0.09</td>
<td>7.39 ± 0.11</td>
<td>7.48 ± 0.08*</td>
</tr>
<tr>
<td>(P_{\text{aO}_2}), Torr</td>
<td>61.0 ± 9.3</td>
<td>50.6 ± 23.8</td>
<td>44.3 ± 24.7</td>
<td>39.5 ± 23.4</td>
<td>32.8 ± 7.7*</td>
<td>34.0 ± 9.7*</td>
<td>30.6 ± 7.3*</td>
</tr>
<tr>
<td>(P_{\text{aO}_2}), Torr</td>
<td>17.4 ± 2.3</td>
<td>290.3 ± 176.3*</td>
<td>317.7 ± 242.0*</td>
<td>330.2 ± 223.1*</td>
<td>359.7 ± 152.7*</td>
<td>334.8 ± 126.8*</td>
<td>371.0 ± 126.7*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 lambs except for ductus arteriosus flow (n = 5 (hours 0–3), n = 4 (hours 4–5)). *P < 0.05 vs. 0 h (ANOVA); †P = 0.06 vs. 0 h (n = 4).

In Vitro Studies

The presence of 30% oxygen \(\left(\text{PO}_2\right) 175–200 \text{ mmHg}, \text{1.31–1.50 kPa}\) caused the vehicle-treated ductus arteriosus rings to contract to a tension that was 23 ± 14% of the net active tension (Fig. 2). Previously, we (8) and Coceani et al. (9) showed that this contraction is independent of the presence of a luminal endothelium. Indomethacin (22–5% net active tension) caused additional increases in tension. When ET-1 was added to the oxygen plus indomethacin plus LY-83583-contracted ductus rings, there were additional increases in tension (10⁻⁸ M ET-1, 4.0 ± 4%; 10⁻⁷ M ET-1, 19 ± 4% net active tension). The responses of the ductus arteriosus to 30% oxygen, indomethacin, and LY-83583 were similar in rings pretreated with PD-156707 (10⁻⁵ M ET-1, 10.2 ± 3.4 on July 1, 2017 http://ajpheart.physiology.org/ Downloaded from

DISCUSSION

Relative patency of the ductus arteriosus is regulated by both dilating and contracting factors. Extensive in vivo and in vitro data suggest that oxygen is the primary stimulus for the initial constriction of the ductus arteriosus after birth (reviewed in Ref. 5). However, exposure of the ductus arteriosus to oxygen is associated not only with vasoconstriction but also with the release of vasodilators like prostaglandin E₂, nitric oxide, and cGMP (4, 7, 8, 12, 15).

Recent in vitro studies by Coceani and co-workers (9–11) implicate a role for ET-1 in oxygen-induced constriction of the ductus arteriosus. Exogenous ET-1 produces dose-dependent constriction of isolated ductus arteriosus rings, and endogenous ET-1 is released from the ductus arteriosus with oxygen exposure (9–11). In addition, both the synthesis of ET-1 and its contractile response in the ductus appear to be dependent on a cytochrome P-450 pathway that has been implicated in oxygen-mediated ductus constriction (10, 11, 13). Finally, both phosphoramidon, which blocks the conversion of proendothelin-1 into its functional form, and BQ-123, which selectively blocks the ETA receptor, attenuate oxygen-induced ductus constriction in vitro (11).

The predominant vasoconstricting effects of ET-1 are mediated by ETA receptors, which are located on vascular smooth muscle cells. Therefore, to investigate the role of endogenous ET-1 on ductus closure in vivo, we studied the effects of an ETA-receptor antagonist, PD-156707, on ductus closure during the first 5 h after birth. This was assessed by ductus arteriosus blood flow, the pressure gradient generated across the ductus arteriosus, the calculated ductus resistance, and postmortem lumen measurements. PD-156707 is highly selective for the ETA receptor and inhibits the binding of [¹²⁵I]-labeled ET-1 to cloned human ETA receptor and ETB receptor with inhibitory constant values of 0.17 and 133.8 nM, respectively (23). In rabbits, PD-156707 infusion rates of 0.03 mg·kg⁻¹·h⁻¹ completely blocked the vasoconstricting effects of exogenous ET-1, with corresponding plasma concentrations that were <0.05 µg/ml (10⁻⁷ M; Refs. 17, 24). In newborn lambs, we have demonstrated that PD-156707 infusion rates of 1.0 mg·kg⁻¹·h⁻¹ completely and selectively block the vasoconstricting effects of exogenous ET-1 (250 ng/kg) with corresponding plasma concentrations of 1.4 µg/ml (2.5 × 10⁻⁶ M; data not shown). To ensure adequate ETA receptor blockade we used a 10-fold higher infusion rate in the present study (10 mg·kg⁻¹·h⁻¹), which resulted in a stable plasma concentration of 5.4 µg/ml (10⁻⁵ M) and blocked the vasoconstricting effects of exogenous ET-1 in our animals. We found that the infusion of PD-156707 did not alter ductus arteriosus closure during the first 5 h after birth, suggesting that endogenous ET-1 does not play a significant role in closure of the ductus arteriosus after birth.

We also investigated the effects of ETA-receptor blockade on oxygen-induced ductus constriction in vitro. We examined the effects of oxygen alone, oxygen plus
indomethacin (an inhibitor of prostaglandin production), and oxygen plus indomethacin and LY-83583 (a soluble guanylate cyclase inhibitor, the secondary messenger for nitric oxide production). We found that ETA-receptor blockade altered neither the constriction produced by oxygen alone nor the constriction produced by oxygen in association with prostaglandin and soluble guanylate cyclase blockade. We used concentrations of the ETA-receptor blocker PD-156707 that were 10-fold higher than those needed to inhibit near-maximal concentrations of exogenous ET-1 (10^{-7} M). Although data from Coceani’s laboratory (11) suggest that oxygen-induced ET-1 release by the ductus is quite low, higher concentrations of endogenously produced ET-1 at the receptor site during oxygen-induced ductus constriction cannot be excluded.

ETB receptors occur predominantly on vascular endothelial cells and mediate vasodilation by generation of prostacyclin and nitric oxide (16). However, a subpopulation of ETB receptors has recently been identified on vascular smooth muscle cells that mediate vasoconstriction (3, 26). Although the overwhelming hemodynamic effect of ETB-receptor activation in vivo is vasodilation, we performed additional experiments using a nonselective ETA- and ETB-receptor antagonist, PD-14505, to exclude the possibility that our inability to demonstrate an inhibitory effect with PD-156707 was caused by ETB-receptor-mediated ductus constriction (14, 22). Both in vivo (n = 1 lamb, 50 µg·kg^{-1}·min^{-1}) and in vitro (n = 2 rings, 10^{-8} M), PD-14505 did not alter ductus constriction, whereas it completely blocked the hemodynamic effects of exogenous ET-1. This suggests that ductus constriction is not mediated by the ETB receptor.

Our in vitro finding that ET-1 produces dose-dependent constriction of the fetal lamb ductus arteriosus is consistent with previously reported data by Coceani et al. (9). However, in contrast to previous reports, we found that ETA-receptor blockade did not alter oxygen-induced ductus constriction (11). The reason for this discrepancy is unclear. Our study used a different selective ETA-receptor antagonist (PD-156707) than the study by Coceani and co-workers (BQ-123). Although side-by-side comparisons of BQ-123 and PD-156707 have not been performed in identical systems, pharmacological data suggest that PD-156707 is equally selective and perhaps slightly more potent an ETA-receptor antagonist than BQ-123 (23). In addition, Coceani et al. (9) induced significant ductus constriction with only 10^{-9} M ET-1, whereas 10^{-8} M ET-1 was required in our preparation. Therefore, inherent differences in the sensitivity of the ductus to ET-1 may explain some of the differences between our two labora-

**Fig. 1.** Changes in ductus arteriosus resistance (A), ductus arteriosus flow (B), and mean pulmonary arterial pressure (MPAP)-to-mean systemic arterial pressure (MSAP) gradient (C) after birth in vehicle and PD-156707-treated lambs. Ductus arteriosus flows: +, right-to-left shunt; −, left-to-right shunt. Values are means ± SD. Vehicle-treated lambs: n = 6 (A), 6 (B), and 7 (C). PD-156707-treated lambs: n = 5 (A), 5 (B), and 6 (C). *P < 0.05 vs. vehicle-treated lambs.
tories. This may be secondary, in part, to differences in sheep breeds, tissue bath preparation, and/or handling and storage of ET-1.

Although Kennedy and Clark (19) concluded over 50 years ago that oxygen was responsible for constriction of the ductus arteriosus after birth, its biochemical basis remains unclear. Several mechanisms have been implicated in mediating oxygen-induced constriction of the ductus arteriosus. These include stimulation of a cytochrome P-450 pathway and inhibition of voltage- and/or ATP-sensitive potassium channels (13, 21, 27). Recently, oxygen-induced release of ET-1 has been implicated as a mediator of ductus constriction at birth. However, the present in vivo and in vitro data suggest that ET-1 does not play a significant role in closure of the ductus arteriosus after birth.

The authors thank Dr. Joan Keiser (Medicinal Chemistry Dept., Parke-Davis Pharmaceutical Research, Ann Arbor, MI) for ongoing pharmaceutical expertise and for generously supplying PD-156707 and PD-14505. The authors also thank Dr. Hussein Hallak (Parke-Davis Pharmaceutical Research) for performing the plasma PD-156707 pharmacokinetics, Mario Trujillo for help in obtaining tissue specimens, and Françoise Mauray for help in the in vitro experiments.

This research was supported by National Heart, Lung, and Blood Institute Grants HL-46691 and HL-56061 (R. I. Clyman), Grant 9640010E from the American Heart Association (J. R. Fineman), and a grant from the Perinatal Associates Research Foundation.

Fig. 2. PD-156707, an endothelin type A (ET_{A})-receptor antagonist, blocks ET_{A}-induced ductus arteriosus contraction but has no effect on oxygen-, indomethacin (Indo)-, or LY-83583-induced ductus contraction. K\textsuperscript{+}, potassium Krebs solution; SNP, sodium nitroprusside. Vehicle-treated ductus, n = 7 rings; PD-156707-treated ductus, n = 6 rings.
REFERENCES