ET$_A$-receptor blockade and ET$_B$-receptor stimulation in experimental congenital diaphragmatic hernia

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THE FETAL PULMONARY CIRCULATION is characterized by high pulmonary vascular resistance (PVR) and low blood flow. At birth, PVR drops dramatically, and pulmonary blood flow increases 8- to 10-fold. Some newborns, however, fail to successfully achieve and sustain this decline in PVR. The resulting syndrome, known as persistent pulmonary hypertension of the newborn (PPHN), contributes significantly to neonatal morbidity and mortality (19). To date, the mechanisms that maintain high PVR in the fetus and contribute to the transition of the pulmonary circulation remain incompletely understood.

Congenital diaphragmatic hernia (CDH) is a complex disease that occurs in 1 in 2,000 live births (28). Its mortality remains high, reaching 50% despite recent advances in neonatal intensive care (39). The pathophysiology of CDH includes pulmonary hypoplasia (1), surfactant deficiency (11), and anomalies of the pulmonary vascular bed, resulting in PPHN (29).

Various endothelium-derived vasoactive factors play a critical role in the modulation of pulmonary vascular tone. Endothelin (ET)-1 is a vasoactive peptide released by the endothelium with both constrictor and dilator activities. ET-1 acts on at least two receptors, ETA and ETB (34), which mediate vasoconstriction and vasodilatation, respectively, in the developing fetal lamb circulation (16). In contrast, ETA-receptor blockade and ETB-receptor stimulation significantly differed in CDH animals compared with control animals. Imbalance of ET-1-receptor activation favoring pulmonary vasoconstriction rather than altered NO-mediated pulmonary vasodilatation is likely to account for persistent pulmonary hypertension of the newborn in fetal lambs with a surgically created CDH.

Inhaled NO is also been shown to play an important role in the modulation of pulmonary vascular tone in the perinatal period (10, 13, 42). NO stimulates soluble guanylate cyclase (sGC) (27), thereby increasing intracellular cGMP levels and causing vasodilatation. cGMP, in turn, is rapidly hydrolyzed and inactivated by cGMP-specific phosphodiesterase (PDE type 5) enzymes, thus limiting the vasodilating response to NO.

Inhaled NO is a selective pulmonary vasodilator that significantly improves oxygenation in newborns with...
PPHN associated with various causes of severe respiratory diseases (35, 40). However, inhaled NO is often ineffective in CDH (26, 41). The altered response to inhaled NO observed in CDH may reflect either an inability of smooth muscle cells to relax as a result of decreased sGC activity or, alternatively, an increased degradation of cGMP due to increased PDE5 activity. On the other hand, anomalies in the ET-1 pathway such as increased ET\textsubscript{A} receptor-mediated vasoconstriction or decreased endothelial ET\textsubscript{B} receptor-mediated vasodilation may contribute to PPHN in CDH.

Although there are possibly other causes of pulmonary hypertension in CDH, little is known about the role of NO and ET-1 in the pathophysiology of PPHN in CDH. We hypothesized that the imbalance between the NO-cGMP and ET-1 pathways may, in part, account for the hemodynamic effects of inhaled NO in CDH. To test this hypothesis, we performed pharmacological studies to examine the hemodynamic effects of 1) endothelium-dependent [acetylcholine (ACh)] and -independent [sodium nitroprusside (SNP)] vasodilators, 2) specific cGMP PDE5 inhibitors [zaprinast (Zap) and dipyridamole (Dip)], and 3) the ET\textsubscript{A}-receptor antagonist BQ-123 and the ET\textsubscript{B}-receptor agonist sarafotoxin 6c (SF6c) on the pulmonary circulation in the near-term chronically prepared fetal CDH lamb model. This experimental model, when created surgically at 80 days of gestation, during the glandular stage of lung development, accurately mimics the human condition with respect to the morphological and physiological changes in the lung parenchyma and vascular bed (20).

**METHODS**

The study was approved by the Animal Care and Use Committee of the Ecole de Chirurgie, Assistance Publique-Hôpitaux de Paris (Paris, France).

**Surgical Preparation**

Creation of the diaphragmatic hernia. Pregnant ewes between 80 and 85 days of gestation (term 147 days) were fasted for 24 h before surgery. The ewes were sedated with intravenous pentobarbital sodium (250 mg) administered through an external jugular vein line and anesthetized with a lumbar intrathecal dose of 2 ml of 1% xylocaine. Intramuscular amoxicillin (1 g) was given to the ewe. Pentobarbital sodium dosing was adjusted so that the ewes were sedated but breathed spontaneously throughout surgery. Under sterile conditions, the uterus was exposed through a midline abdominal approach. The fetal lamb's left forelimb was delivered through a uterine incision. After local infiltration of 1 ml of 1% xylocaine, an incision in the left hemithorax at the level of the ninth intercostal space was made to expose the fetal diaphragm. After a short incision in the left hemidiaphragm, the stomach was pulled manually into the thorax. After fetal chest closure, the fetus was placed back into the uterus. Amoxicillin (500 mg) was given in the amniotic cavity, and the hysterotomy was closed. The abdominal incision of the ewe was closed in two layers.

Chronic preparation. Between 125 and 128 days of gestation, the ewes were reoperated on to insert polyvinyl catheters and flow transducers. The first surgical steps were the same as described in Creation of the diaphragmatic hernia. Once the fetal skin incision was made under the left forelimb, catheters were inserted into the axillary vein and artery and directed into the superior vena cava and ascending aorta, respectively. A left thoracotomy exposed the heart and great vessels. Catheters were inserted into the left pulmonary artery (LPA), main pulmonary artery (MPA), and left atrium by direct puncture through purse-string sutures. A 6-mm ultrasonic flow transducer (Transonic, Ithaca, NY) was placed round the LPA to measure LPA blood flow. After the catheters and flow transducer were secured, the fetus was replaced in the uterus. A catheter was left in the amniotic cavity, amoxicillin (500 mg) was given, and the hysterotomy and abdominal incision were closed as described in Creation of the diaphragmatic hernia. The catheters and flow transducer cable were tunneled subcutaneously to an external flank pouch on the ewe. The ewe and the fetus were given 48 h to recover from surgery before studies were initiated. Prophylactic amoxicillin (250 mg) and gentamicin (80 mg) were infused into the amniotic cavity for 3 days after surgery. Catheter patency was ensured by daily flushes of 2 ml of heparinized saline (1 ml = 100 IU).

Age-matched control animals underwent only the second surgery, i.e., the chronic preparation.

**Pulmonary Hemodynamic Measurements in Awake Ewes**

LPA blood flow was measured continuously with an ultrasonic flow transducer connected to an internally calibrated flowmeter (Transonic) with a digital display. An end-diastolic correction factor was added to the mean LPA blood flow. The aortic (Ao), MPA, and left atrial (LA) pressures were connected to a pressure transducer (Baxter, Bentley Labatories, Uden, The Netherlands), with mean and phasic pressure measurements continuously recorded. Ao, MPA, and LA were freshly prepared on the day of the study. Zap (Sigma-Aldrich, St. Quentin-Fallavier, France) and Dip (4 mg/ml; Boehringer Ingelheim, Germany) were initially dissolved in 50 mM NaOH, further diluted to a final concentration of 2.2 mg/ml, and frozen until use. BQ-123 (Neosystem, Strasbourg, France) and SF6c were diluted in 0.9% saline and frozen until use. On the day of study, aliquots were diluted to final concentrations of 0.01 mg/ml and 0.25 µg/ml for BQ-123 and SF6c, respectively.

**Drug Preparation**

ACh (16 mg/ml; Pharmacie Centrale des Hôpitaux, Paris, France), SNP (10 mg/ml; SERB Laboratories, L’Argueron Internationale) and Dip (4 mg/ml; Boehringer Ingelheim, Germany) were freshly prepared on the day of the study. Zap (Sigma-Aldrich, St. Quentin-Fallavier, France) was initially dissolved in 50 mM NaOH, further diluted to a final concentration of 2.2 mg/ml, and frozen until use. BQ-123 (Neosystem, Strasbourg, France) and SF6c were diluted in 0.9% saline and frozen until use. On the day of study, aliquots were diluted to final concentrations of 0.01 mg/ml and 0.25 µg/ml for BQ-123 and SF6c, respectively.

**Experimental Design**

Four different protocols are included in this study. For each protocol, n refers to the number of animals studied.

The NO-cGMP pathway was studied at different levels. ACh, an endothelium-dependent vasodilator, stimulates NO synthase (NOS) activity; SNP, an endothelium-independent vasodilator, directly activates sGC; Zap and Dip, two specific PDE5 inhibitors, prevent inactivation of cGMP by the PDE enzyme and sustain the vasodilating response of NO.

The ET-1 pathway can be explored by selective receptor antagonists and agonists. The selective ET\textsubscript{A}-receptor antagonist BQ-123 and the selective ET\textsubscript{B}-receptor agonist SF6c were used in our study.
The different protocols were performed in the same animal. Each animal received at most two drugs on the same day, with a recovery period of at least 3 h between the two drugs. This was set to recover baseline values before administration of the second study drug. ACh and SNP (mean gestational age 130 ± 3 days), Dip and Zap (131 ± 3 days), and BQ-123 and SF6c (132 ± 3 days) were given on the same day, respectively. The order of drugs was not randomized. The doses of the agents were determined according to previous studies with fetal lambs (16, 24, 42). The fetal body weights did not differ between the CDH (2,691 ± 421 g) and age-matched control animals (2,721 ± 434 g).

Protocol 1: Fetal pulmonary vascular response to prolonged intrapulmonary infusions of ACh in control and CDH animals. To investigate NOS activity, we studied the hemodynamic effects of ACh, an endothelium-dependent vasodilator. After 20 min of stable baseline measurements, ACh (16 µg/min) was infused into the LPA with a precalibrated syringe infusion pump (7 control and 4 CDH animals; mean gestational age 130 ± 3 days) for 120 min. AoP, MPAP, LAP, and LPA blood flow were recorded every 10 min during baseline measurements, for the 120-min perfusion period, and for 30 min during the recovery period.

Protocol 2: Fetal pulmonary vascular response to prolonged intrapulmonary infusions of SNP. To investigate the role of sGC, we used the same design for SNP (10 µg/min), an endothelium-independent vasodilator that directly stimulates sGC (n = 4 animals; mean gestational age 131 ± 3 days).

Protocol 3: Fetal pulmonary vascular response to prolonged (120-min) intrapulmonary infusions of Zap and Dip in control and CDH animals. To investigate the role of PDE5, we used two specific inhibitors of this enzyme, Zap (0.22 mg/min) and Dip (0.4 mg/min; mean gestational age 132 ± 3 days).

Protocol 4: Fetal pulmonary vascular response to prolonged (120-min) intrapulmonary infusions of BQ-123 and SF6c in control and CDH animals. To investigate the role of the ET-1-pathway, we used BQ-123 (1 µg/min), a specific blocker of the ETₐ receptor, and SF6c (0.4 mg/min), a specific ET₆ receptor agonist (mean gestational age 132 ± 3 days).

Data Analysis

Each data point represents the mean from a 1-min recording period. Data are expressed as means ± SE. Comparisons were made by two-way analysis of variance for repeated measures with Statview (Abacus Concepts, Berkeley, CA). When significant differences were identified, a post hoc analysis with Fisher’s protected least significant difference test was performed.

RESULTS

Baseline values of all measured hemodynamic parameters in control and CDH lambs are summarized in Table 1. MPAP and PVR were higher in the CDH group compared with those in the control animals at all baseline time points (Table 1).

Protocol 1: Fetal Pulmonary Vascular Response to Prolonged Intrapulmonary Infusions of ACh in Control and CDH Animals

Figure 1 summarizes the responses of AoP, MPAP, LPA blood flow, and PVR in control and CDH animals during the 2-h perfusion of ACh. As shown, ACh induced an increase in LPA blood flow and a decrease in PVR in both groups. MPAP was higher in CDH animals than in control animals throughout the study. There was no difference between the two groups with regard to AoP, LPA blood flow, and %PVR.

Protocol 2: Fetal Pulmonary Vascular Response to Prolonged Intrapulmonary Infusions of SNP in Control and CDH Animals

Figure 2 summarizes the responses of AoP, MPAP, LPA blood flow, and PVR in control and CDH animals during the 2-h perfusion of SNP. As shown, SNP caused a prolonged increase in LPA blood flow and a decrease in PVR in both groups. MPAP was higher in CDH animals than in control animals throughout the study. There was no difference between the two groups with regard to AoP, LPA blood flow, and %PVR.

Table 1. Baseline values in control and CDH lambs

<table>
<thead>
<tr>
<th>Drug</th>
<th>MPAP, mmHg</th>
<th>AoP, mmHg</th>
<th>LAP, mmHg</th>
<th>LPA Blood Flow, ml/min</th>
<th>PVR, mmHg·ml⁻¹·min</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Control</td>
<td>46 ± 1</td>
<td>42 ± 1</td>
<td>2 ± 1</td>
<td>61 ± 2</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CDH</td>
<td>51 ± 1*</td>
<td>43 ± 1</td>
<td>1 ± 1</td>
<td>62 ± 1</td>
<td>0.83 ± 0.03*</td>
</tr>
<tr>
<td>SNP</td>
<td>Control</td>
<td>46 ± 1</td>
<td>45 ± 1</td>
<td>2 ± 1</td>
<td>60 ± 1</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CDH</td>
<td>51 ± 1*</td>
<td>47 ± 1</td>
<td>1 ± 1</td>
<td>59 ± 2</td>
<td>0.84 ± 0.03*</td>
</tr>
<tr>
<td>Zap</td>
<td>Control</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
<td>2 ± 1</td>
<td>60 ± 2</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>CDH</td>
<td>52 ± 1*</td>
<td>47 ± 1</td>
<td>0 ± 1</td>
<td>60 ± 1</td>
<td>0.84 ± 0.03*</td>
</tr>
<tr>
<td>Dip</td>
<td>Control</td>
<td>48 ± 1</td>
<td>46 ± 1</td>
<td>2 ± 1</td>
<td>60 ± 2</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CDH</td>
<td>52 ± 1*</td>
<td>47 ± 1</td>
<td>1 ± 1</td>
<td>62 ± 1</td>
<td>0.83 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>BQ-123</td>
<td>49 ± 1</td>
<td>47 ± 1</td>
<td>2 ± 1</td>
<td>62 ± 1</td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>52 ± 1*</td>
<td>48 ± 1</td>
<td>0 ± 1</td>
<td>62 ± 1</td>
<td>0.83 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>CDH</td>
<td>52 ± 1*</td>
<td>48 ± 1</td>
<td>0 ± 1</td>
<td>66 ± 1</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>SF6c</td>
<td>47 ± 1</td>
<td>45 ± 1</td>
<td>2 ± 1</td>
<td>65 ± 2</td>
<td>0.81 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE. ACh, acetylcholine; SNP, sodium nitroprusside; Zap, zaprinast; Dip, dipyridamole; SF6c, sarafotoxin 6c; CDH, congenital diaphragmatic hernia; MPAP, main pulmonary arterial pressure; AoP, aortic pressure; LAP, left atrial pressure; PVR, pulmonary vascular resistance; HR, heart rate.*P < 0.05 vs. control animals.
Protocol 3: Fetal Pulmonary Vascular Response to Prolonged Intrapulmonary Infusions of Zap and Dip in Control and CDH Animals

Figures 3 and 4 summarize the responses of AoP, MPAP, LPA blood flow, and PVR in control and CDH animals during the 2-h perfusion of Zap and Dip, respectively. As shown, Zap and Dip induced a prolonged increase in LPA blood flow and a decrease in PVR in both groups. MPAP was higher in CDH animals than in control animals throughout the study. There was no difference between the two groups with regard to AoP, LPA blood flow, and %PVR.

Protocol 4: Fetal Pulmonary Vascular Response to Prolonged Intrapulmonary Infusions of BQ-123 and SF6c in Control and CDH Animals

Figure 5 summarizes the responses of AoP, MPAP, LPA blood flow, and PVR in control and CDH animals during the 2-h perfusion of BQ-123. BQ-123 induced a prolonged increase in LPA blood flow and a decrease in PVR in both groups after 20 min of infusion. These changes lasted for 30 min after the end of infusion period. However, pulmonary vasodilatation was significantly more pronounced in CDH animals than in control animals.

Figure 6 depicts the responses of AoP, MPAP, LPA blood flow, and PVR in control and CDH animals during the 2-h perfusion of SF6c. As shown, SF6c induced a biphasic response characterized by a transient increase in LPA blood flow and a decrease in PVR followed by a sustained decrease in LPA blood flow and an increase in PVR in control animals. In CDH animals, SF6c-induced vasodilatation was absent, and there was no increase in PVR above baseline (Fig. 6C).

DISCUSSION

In this study, we investigated both the L-arginine-NO-cGMP and the ET-1 pathways. Stimulation of the L-arginine-NO-cGMP pathway elicited the same pulmonary vascular response in CDH and control animals, suggesting that neither NO deficiency nor the effect of NO on cGMP production is impaired in fetal herniated lambs. This study also suggests no increase in PDE5 activity because the hemodynamic effects of Dip and Zap did not significantly differ between CDH and control animals. In contrast, the vasodilatory effect of the selective ETA-receptor antagonist BQ-123 was significantly greater in herniated animals compared with that in control animals. Furthermore, the transient decrease in PVR induced by the selective ETB-receptor agonist SF6c, which was normally seen in control animals, was blunted in the CDH group. These findings suggest that, unlike the L-arginine-NO pathway, blockade of ETA or activation of ETB receptors elicited...
Fig. 2. Response to prolonged (120-min) intrapulmonary infusions of sodium nitroprusside (SNP) on AoP (A), MPAP (B), LPA blood flow (C), and %PVR (D) in control and CDH animals. Values are means ± SE; n, no. of animals. SNP caused prolonged increase in LPA blood flow and a decrease in PVR in both groups. MPAP was higher in CDH animals than in control animals. There was no difference between the 2 groups with regard to AoP, LPA blood flow, and %PVR. *P < 0.05 vs. control animals. *P < 0.05 vs. baseline.

Fig. 3. Response to prolonged (120-min) intrapulmonary infusions of zaprinast (Zap) on AoP (A), MPAP (B), LPA blood flow (C), and %PVR (D) in control and CDH animals. Values are means ± SE; n, no. of animals. Zap induced a prolonged increase in LPA blood flow and a decrease in PVR in both groups. MPAP was higher in CDH animals than in control animals. There was no difference between the 2 groups with regard to AoP, LPA blood flow, and %PVR. *P < 0.05 vs. control animals. *P < 0.05 vs. baseline.
different responses in CDH compared with those in control animals.

The pathophysiology of PPHN in CDH remains poorly understood. Structural changes in the pulmonary vascular bed in infants with CDH include 1) excessive muscularization of the preacinar arteries; 2) reduced external diameter or increased medial wall thickness of prealveolar and intra-alveolar arteries, obstructing the luminal area of these arteries; and 3) increased vasoconstriction (29). Increased muscularization of the pulmonary arterial wall has also been described in the CDH lamb model (33). Altered vasoreactivity in CDH stems from two major causes: 1) irreversible structural changes and 2) endothelial dysfunction.

Impaired endothelium-dependent vasodilatation might occur in various experimental conditions associated with pulmonary hypertension. For example, relaxation of pulmonary arterial rings in calves (37) and adult rats (2) with chronic hypoxic pulmonary hypertension in response to ACh is blunted. Similarly, loss of endothelium-dependent relaxation was also demonstrated in human pulmonary arterial rings from patients with chronic pulmonary hypertension associated with cystic fibrosis, α1-antitrypsin deficiency (5), and Eisenmenger's syndrome (6). In fetal sheep with PPHN induced by ligation of the ductus arteriosus, endothelium-dependent vasodilatation in response to prolonged infused ACh was also impaired, whereas pulmonary vasodilatation to direct stimulation of the membrane-bound guanylate cyclase by atrial natriuretic peptide or of sGC by NO was normal (25). Fike et al. (9) showed that chronic hypoxia decreases NO production and endothelial NOS expression in newborn pig lungs.

In experimental CDH, studies looking at NO activity have yielded conflicting results. In rats with nitrofen-induced CDH, decreased pulmonary NOS3 gene expression (30) and activity (21) have been documented. Conversely, NOS does not seem to be altered in the fetal lamb with CDH because NOS is evidenced by immuno-histochemistry in the MPA (19) and fourth-generation pulmonary arteries have identical basal and stimulated release of NO compared with control animals (15).

A dysfunction in the sGC-cGMP pathway has been proposed as a possible mechanism of PPHN. Basal and NO-induced cGMP production is decreased in the fetal lamb with ductus arteriosus occlusion, consistent with dysfunctional sGC activity (43). Alternatively, increased cGMP hydrolysis by PDE5 may account for impaired vasorelaxation as recently shown in animals with chronic intrauterine pulmonary hypertension induced by ductus arteriosus ligation (12).

These results are at odds with our results because we found that the marked and sustained vasodilatation in response to the PDE inhibitors Zap and Dip was similar in control and herniated animals in vivo. Other authors (15) have shown that the in vitro relaxation response of pulmonary arteries to Zap was not different between CDH and control animals. However, the relaxation
response of pulmonary veins to Zap was blunted compared with that in control animals (15).

Because exogenous, inhaled NO fails to improve oxygenation in infants with CDH, who already had optimal lung recruitment with exogenous surfactant and high-frequency oscillation (36, 41), and because the L-arginine-NO-cGMP pathway is preserved in our CDH model, one may speculate whether alterations in the ET-1 pathway alternatively contribute to PPHN in CDH. Although it is not clear whether ET-1 is a mere marker or a cause of PPHN, there is experimental evidence to suggest that ET-1 might play an important role in the pathophysiology of pulmonary hypertension (3). Plasma levels of ET in newborns with CDH are significantly higher compared with those in age-matched newborns (22). In our study, blockade of the ET\textsubscript{A} receptor by the specific antagonist BQ-123 caused a more pronounced pulmonary vasodilation in diseased animals compared with that in control animals, suggesting a higher pulmonary activity of the ET\textsubscript{A} receptor in the pulmonary circulation of CDH animals. Consistent with our findings are the results from Okazaki et al. (31) and Coppola et al. (4). In the CDH rat, Okazaki et al. (31) showed a 1.5-fold increase in ET-1 lung levels and a 2- to 4-fold increase in ET\textsubscript{A} mRNA levels compared with those on control animals. Coppola et al. (4) found that the contractile response to ET-1 was more pronounced in third-generation perfused pulmonary arteries of CDH rats compared with that in control animals.

In addition, the initial vasodilator response to the specific agonist of ET\textsubscript{B} receptors, SF6c, was reduced in CDH animals. ET\textsubscript{B} receptors are located mainly on endothelial vascular cells, and it is thought that their activation causes vasodilatation through NO release from pulmonary endothelial cells. Thus the activation of ET\textsubscript{B} receptors often results in stimulation of endothelial NOS. Because we have demonstrated normal relaxation to the endothelium-dependent vasodilator ACh, impaired NOS activity is unlikely in this study. We submit that the reduced vasodilator response to SF6c is due to impaired transduction of ET\textsubscript{B} endothelial receptors rather than to impaired NOS activity in CDH animals. There are, however, other mechanisms such as impaired prostacyclin release (7) or potassium-channel activation (14) that may account for the lack of ET\textsubscript{B}-mediated vasodilation. Alternatively, more direct damage to the vascular endothelium may also alter ET\textsubscript{B}-receptor expression or function. No significant increase in PVR was observed during SF6c infusion, suggesting that there was no significant change in ET\textsubscript{B}-mediated vasoconstriction. This could result from a lack of ET\textsubscript{B}-receptor expression on pulmonary vascular smooth muscle cells in the fetal lamb lung (16). A decreased number of functional ET\textsubscript{B} receptors may also account for decreased clearance of ET-1.
which, therefore, becomes more readily available for
ETA-receptor activation. This, in turn, may explain the
increased dilator response to the ETA-receptor antago-
nist BQ-123 and the decreased response to the ET B-
receptor agonist SF6c. However, ET-1 levels were not
measured in this study.

The structural changes in the pulmonary vascular
bed in infants with CDH are well characterized and
have also been described in the CDH lamb model (33).
In addition to its effect on pulmonary vascular tone,
increased ET-1 activity also stimulates smooth muscle
cell proliferation, which, in turn, causes a further
increase in PVR (8). ET-1 hyperactivity may account for
functional and structural abnormalities in the pulmo-
nary vasculature of CDH. These anatomic changes may
lead to a “fixed” and nonreversible component of the
pulmonary vascular bed, thus explaining, in part, why
inhaled NO is ineffective in CDH. However, the ob-
served vasodilatation was not present in CDH animals. *P < 0.05 vs. control animals.

In summary, because the responses to the endothe-
lium-dependent (ACh) and -independent (SNP) vasodi-
lators and to cGMP PDE inhibitors (Zap and Dip) were
similar in control and CDH animals, we suggest that
the L-arginine-NO-cGMP pathway is not altered in the
animals with surgically induced CDH. The selective
ETA-receptor antagonist BQ-123 caused a greater de-
crease and the selective ET B-receptor agonist SF6c
caused a lesser decrease in PVR in herniated animals
compared with those in the control animals. This
suggests that the ET-1 pathway is altered, resulting in
an excessive response to ETA-receptor blockade and a
reduced vasodilator response to ETB-receptor stimula-
tion in CDH.

Whether hyperreactivity of the ET-1-pathway ac-
counts for the histological (pulmonary vascular remod-
eling) and functional (pulmonary vessel hyperreactiv-
it) anomalies seen in the pulmonary vascular bed in
CDH remains to be established. On the basis of this
study, we speculate that therapeutic use of an ETa-

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Fig. 6. Response to prolonged (120-min) intrapulmonary infusions of sarafotoxin 6c on AoP (A), MPAP (B), LPA
blood flow (C), and %PVR (D) in control and CDH animals. Values are means ± SE; n, no. of animals. Sarafotoxin 6c
induced a biphasic response characterized by a transient increase in LPA blood flow and a decrease in PVR followed
by a sustained decrease in LPA blood flow and an increase in PVR in control animals. Transient period of
vasodilatation was not present in CDH animals. *P < 0.05 vs. control animals.
receptor antagonist might prove more useful than inhaled NO in CDH.

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