Developmental Changes in Prostaglandin E₂ Receptor Subtypes in Porcine Ductus Arteriosus: Possible Contribution in Altered Responsiveness to Prostaglandin E₂

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Background—Prostaglandin E₂ (PGE₂) is important in ductus arteriosus (DA) patency, but the types of functional PGE₂ receptors (EP) in the developing DA are not known. We postulated that age-dependent alterations in EP and/or their subtypes may possibly contribute to the reduced responsiveness of the newborn DA to PGE₂.

Methods and Results—We determined PGE₂ receptor subtypes by competition binding and immunoblot studies on the DA of fetal (75% and 90% gestation) and newborn (<45 minutes old) pigs. We studied the effects of EP receptor stimulation on cAMP signaling in vitro and on term newborn (<3 hours old) DA patency in vivo. Fetal pig DA expressed EP₂, EP₃, and EP₄ receptors equivalently, but not EP₁. In neonatal DA, EP₁, EP₃, and EP₄ were undetectable, whereas EP₂ density was similar in fetus and newborn. Prostaglandin-induced changes in cAMP mirrored binding data. 16,16-Dimethyl PGE₂ and 11-deoxy PGE₁ (EP₂/EP₃/EP₄ agonist) produced more cAMP in fetus than newborn, but butaprost (selective EP₂ agonist) caused similar cAMP increases in both; EP₃ and EP₄ ligands (M&B28767 and AH23848B, respectively) affected cAMP production only in fetus. After birth, administration of butaprost alone was as effective as 11-deoxy PGE₁ and 16,16-dimethyl PGE₂ in dilating DA in vivo.

Conclusions—The data reveal fewer PGE₂ receptors in the DA of the newborn than in that of the fetus; this may contribute to the decreased responsiveness of the DA to PGE₂ in newborn. Because EP₂ receptors seem to mediate the effects of PGE₂ in the newborn DA, one may propose that a selective EP₂ agonist may be preferred as a pharmacological agent to maintain DA patency in infants with certain congenital heart diseases. (Circulation. 1999;100:1751-1756.)

Key Words: ductus arteriosus ■ receptors ■ prostaglandins ■ pediatrics

Prostaglandins play a major role in maintaining patency of the fetal ductus arteriosus (DA).³ The marked sensitivity of the DA to prostaglandin E₂ (PGE₂) suggests that this is the most important prostanoïd regulating vessel patency.³ PGE₂ exerts its effects through a diverse group of receptors, classified as EP₁, EP₂, EP₃, and EP₄.⁴ Activation of EP₁ increases inositol 1,4,5-triphosphate (IP₃) formation and elicits vasoconstriction,⁵ stimulation of EP₂ and EP₃ increases cAMP and leads to vasodilation,⁴,⁶ and stimulation of EP₃ decreases cAMP and opposes vasodilation.⁴

At present, the relative types and importance of EP receptors in the DA are uncertain. Pharmacological evidence suggests that the EP₁ receptor is the predominant PGE₂ receptor in the fetal rabbit DA.⁷ Conversely, genetic disruption of the EP₂ receptor does not diminish DA patency in the fetal and neonatal mouse.⁸ However, the type of EP receptors expressed in higher species, and especially in the newborn, is not known. This is of particular relevance because responsiveness of the DA to PGE₂ in the newborn is significantly less than that in the fetus.⁹,¹⁰ Because PGE₂ acts on several distinct receptor subtypes, each coupled to different second messengers, we hypothesized that differences in the relative density and/or proportion of EP receptors could explain, at least in part, the differences in the responsiveness of the fetal and neonatal DA to PGE₂.⁹,¹⁰

We therefore studied the expression of EP receptor subtypes and EP receptor signaling factors in the DA of fetal and newborn pigs. We also assessed the role of these receptors in the newborn in vivo. Our findings reveal that the DA of the newborn expresses negligible amounts of the EP₁ receptor, but does express the other 3 EP subtypes (EP₂, EP₃, EP₄) in equivalent proportions. In the DA of the newborn pig, the density of PGE₂ receptors is significantly reduced compared with that in the fetus because of complete loss of EP₃ and EP₄.
receptors; however, the number of EP receptors remains unchanged, such that EP2 seems to mediate all PGE2-dependent relaxation in the newborn DA.

Methods

Animals

DAs were removed from fetal pigs (78 to 90 and 100 to 105 days of gestation [term, 114 days]) and term newborn piglets (1.25 to 1.7 kg, killed within 45 minutes of vaginal birth with pentobarbital [120 mg/kg intracardiac] under halothane anesthesia). Tissues were immediately rinsed in ice-cold Krebs buffer (pH 7.4) of the following composition (mmol/L): NaCl 120, KCl 4.5, CaCl2 2.5, MgSO4 1.0, NaHCO3 27, KH2PO4 1.0, and glucose 10 and then frozen in liquid N2; we have shown that freezing does not affect PGE2 binding.13 A group of newborn piglets (<3 hours old) was used to study the effects of prostaglandin analogues on DA diameter in vivo.

EP Receptor Characterization

Tissues were prepared for prostaglandin binding as described.11–13 Aliquots of DA homogenate (200 μg protein) were incubated at 37°C for 30 minutes with various concentrations of [3H]PGE2 (Amersham) in the presence or absence of 25 μmol/L 16,16-dimethyl PGE2. The reaction was terminated with ice-cold 5 mmol/L Tris-HCl buffer (pH 7.4), the homogenate was filtered through Whatman GF/C glass filter disks, and the radioactivity was counted in a liquid-scintillation counter (Beckman LS 7500). Subtypes of PGE2 receptors were studied by displacement of bound [3H]PGE2 with 16,16-dimethyl PGE2, AH6809 (Glaxo-Wellcome), butaprost (Bayer), M&B28,767 (Rhone-Poulenc Rorer), and sulprostone, 11-deoxydimethyl PGE2. The reaction was terminated with ice-cold 5 mmol/L Tris-HCl buffer (pH 7.4), the homogenate was filtered through Whatman GF/C glass filter disks, and the radioactivity was counted with a β-counter (Beckman LS 7500). Subtypes of PGE2 receptors were studied by displacement of bound [3H]PGE2 with 16,16-dimethyl PGE2, AH6809 (Glaxo-Wellcome), butaprost (Bayer), M&B28,767 (Rhone-Poulenc Rorer), and sulprostone, 11-deoxydimethyl PGE2, and AH23848B (Glaxo-Wellcome).14 Receptor densities (Bmax), affinity constants (Ka), and concentrations of ligands that displace 50% of bound [3H]PGE2 (IC50) were determined from Scatchard plots and displacement curves with the computer programs Prism (GraphPad) and Ligand, respectively.14

Immunoblotting of EP Receptors

Western blotting of EP1, EP3, and EP4 receptors was conducted on DA membranes prepared as described13 and on cell lysates from human embryonic kidney (HEK) 293 cells (Invitrogen), which overexpress each of these receptors16 and hence were used as positive controls. After immunoblotting with EP1, EP3, or EP4 specific polyclonal rabbit antibodies17 (1:1000; EP1 antibodies are not available), immunoreactive bands were visualized by chemiluminescence (Amersham) according to the manufacturer’s instructions.

cAMP Assay

The effects of PGE2 analogues on cAMP were determined.12,13 Briefly, DA homogenates (100 μg protein) were incubated in an assay mixture (100 μL) containing 10 mmol/L Tris-HCl buffer (pH 8.0), 1 mmol/L ATP, 7.5 mmol/L MgCl2, 15 mmol/L creatine phosphate, 185 μmol/L creatine phosphokinase, 200 μmol/L aspartic acid, 0.5 mmol/L EGTA, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 1 mmol/L dithiothreitol, 0.1 mmol/L benzamidine, 0.1 mmol/L phenylmethylsulfonyl fluoride, and 100 μg/mL soybean trypsin inhibitor in the presence or absence of test agents at 37°C for 10 minutes. The reaction was terminated with 200 μL acidic ethanol. After centrifugation, cAMP was measured by radioimmunoassay (Diagnostic Products).

Surgical Preparation and Echocardiography

Newborn pigs (between 1.5 and 2 hours after delivery; 1.3 to 1.7 kg) were prepared to test the effects of specific EP receptor agonists on DA patency; in the piglet, the DA closes completely by 4 to 6 hours of age.18 We were not able to perform in vivo studies on fetal pigs because facilities were not available to operate on sows. The newborn piglets were anesthetized with halothane (2%) for 15 minutes during tracheostomy and catheterization of the umbilical vein and artery, and anesthesia was discontinued after surgery. Animals were ventilated with air with a Harvard small-animal respirator, maintained on α-chloralose (50 mg/kg bolus followed by 10 mg · kg−1 · h−1 infusion), and paralyzed with pancuronium (0.1 mg/kg); body temperature was maintained at 38°C. The surgical procedure was completed within 15 minutes, and piglets were allowed to stabilize for an additional 30 minutes.

Echocardiographic measurements were performed with an Acuson 128 XP10C real-time ultrasound imaging system using 7.5- and 5-MHz transducers duplexed with a range-gated Doppler as previously described19,20; Doppler signals were filtered by 100-Hz high-pass filter. The DA was visualized through a left second intercostal parasternal approach. Measurements of the DA diameter were repeated 3 times. The smallest diameter of the DA lumen was monitored until it reached 0.6 to 0.8 mm in diameter. Experimental drugs were then injected, and the smallest diameter was measured every minute for the next 10 to 25 minutes.

Animals (3 to 4 per treatment) were randomly assigned to receive 10-minute infusions of saline (1 mL, controls) or 0.083 μg · kg−1 · min−1 of EP receptor agonists; the doses used have been shown previously to be effective in vivo.21 The effect of EP receptor agonists on DA patency was also tested in animals pretreated with indomethacin (3 mg/kg IV for 5 minutes), once the DA diameter reached 0.6 to 0.9 mm (within 20 minutes).

Statistical Analysis

Data were analyzed by Student’s t test and by 2-way ANOVA factoring for time and treatment; means tests were compared by the Tukey-Kramer method. Statistical significance was set at P<0.05. Data are expressed as mean±SEM.

Results

PGE2 Receptors in Fetal and Newborn DA

[3H]PGE2 bound specifically to fetal and newborn DA membranes. Maximum binding of [3H]PGE2 to the fetal DA was similar at both gestational ages studied (78 to 90 days: 34.9±2.5 fmol/mg protein, n=3; 100 to 105 days: 35.1±3.1 fmol/mg protein, n=3). Maximum specific binding of [3H]PGE2 was 3-fold greater in fetal than newborn DA (Figure 1A and 1B and Table 1); the Kd did not differ between the fetus and newborn.

In fetal DA membrane preparations (Figure 1C), butaprost (EP2 agonist), M&B28,767 (EP3 agonist), sulprostone (EP1 agonist), and AH23848B (EP3 antagonist) caused an equivalent displacement of [3H]PGE2 (Figure 1C and 1E and Table 2); 11-deoxy PGE1 (EP2/EP3 agonist) displaced virtually all [3H]PGE2, but the EP1 antagonist AH6809 was ineffective (Figure 1C and 1E and Table 2). In contrast to fetal DA, [3H]PGE2 bound to newborn DA membranes was no longer displaced by M&B28,767, sulprostone, or AH23848B (Figure 1D). Butaprost and 11-deoxy PGE1 were the only PGE analogues (along with 16,16-dimethyl PGE2) capable of fully displacing [3H]PGE2 from the newborn DA (Figure 1D and Table 2). IC50 values of ligands tested were comparable to those reported in other porcine tissues.11,12 Results indicate the presence of EP2, EP3, and EP4 in fetal DA.

In the immediate postnatal newborn, EP3 and EP4 are absent, without significant change in the number of EP2 receptors (Figure 1E); this is reflected by the reduced [3H]PGE2 binding in the newborn DA (Figure 1A and 1B and Table 1).
Expression of EP<sub>3</sub>, EP<sub>4</sub>, and EP<sub>2</sub> Immunoreactive Protein in DA of Fetal and Newborn Pig

The DA of the fetal pig, but not of the newborn, expressed EP<sub>3</sub> (55-kDa band) and EP<sub>4</sub> (63-kDa band) immunoreactive protein consistent with binding data (Figure 2); EP<sub>2</sub> antibodies are unavailable.

PGE<sub>2</sub> Analogue–Induced Production of cAMP

PGE<sub>2</sub> receptor–coupled changes in cAMP generation were also consistent with the binding data. 16,16-Dimethyl PGE<sub>2</sub> and 11-deoxy PGE<sub>1</sub> (EP<sub>2</sub> agonist) produced a comparable dose-dependent increase in cAMP synthesis, which was greater in fetus than in newborn (Table 3).

In fetal DA, butaprost (EP<sub>2</sub> agonist) stimulated cAMP production in a concentration-dependent manner. The EP<sub>3</sub> agonist M&B28,767 and EP<sub>1</sub>/EP<sub>3</sub> agonist sulprostone had no effect on cAMP formation by themselves but reduced forskolin-induced cAMP synthesis; this suggests that EP<sub>3</sub> is coupled to inhibition of cAMP formation. Because no selective EP<sub>4</sub> agonist is currently available, we estimated the effects attributed to EP<sub>4</sub>, as we previously reported, by subtracting the increase in cAMP produced by the combination of 16,16-dimethyl PGE<sub>2</sub> and EP<sub>2</sub> antagonist AH23848B (which would stimulate all of the EP receptors except EP<sub>4</sub>) from the increase in cAMP produced by 16,16-dimethyl PGE<sub>2</sub> alone (which stimulates all EP receptors). In the fetal DA, AH23848B decreased the cAMP production induced by 16,16-dimethyl PGE<sub>2</sub> but not that stimulated by butaprost (Table 3).

Figure 1. Representative curves of specific binding and displacement of [H]PGE<sub>2</sub> on DA membranes of fetal and newborn pigs. A, Saturation isotherm of specifically bound [H]PGE<sub>2</sub>. Because maximum binding in fetuses at 78 to 90 and 100 to 105 days of gestation was similar, fetal tissues from both ages were combined in figures referring to fetus. B, Scatchard analysis of saturation isotherms in A, C and D, Displacement of specifically bound [H]PGE<sub>2</sub> (8 nmol/L) in DA of fetal (C) and neonatal (D) pigs by 16,16-dimethyl PGE<sub>2</sub> (●), EP<sub>1</sub> antagonist AH6809 (▲), EP<sub>2</sub> agonist butaprost (♦), EP<sub>3</sub> agonist M&B28,767 (▼), EP<sub>1</sub>/EP<sub>3</sub> agonist sulprostone (△), EP<sub>4</sub> antagonist AH23848B (○), and EP<sub>2</sub>/EP<sub>3</sub>/EP<sub>4</sub> agonist 11-deoxy PGE<sub>1</sub> (◊). E, Estimated B<sub>max</sub> of specific EP receptors was calculated as maximum binding of PGE<sub>2</sub>×proportion of EP subtypes derived from indicated ligands.

Table 1. Maximum Binding and Affinity Constant of [H]PGE<sub>2</sub> on DA Membranes From Fetal and Newborn Pigs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fetus</th>
<th>Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;max&lt;/sub&gt;, fmol/mg protein</td>
<td>34.9±2.5</td>
<td>12.3±1.6*</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;, nmol/L</td>
<td>10.8±3.0</td>
<td>6.1±2.3</td>
</tr>
</tbody>
</table>

Values are the mean±SEM of 3 or 4 experiments, each performed in duplicate. Because B<sub>max</sub> in fetus 78 to 90 and 100 to 105 days of gestation was similar, fetal tissue from both ages were combined for all tables.

*P<0.01 vs corresponding value for fetus.
In the newborn DA, butaprost increased cAMP generation to values comparable to those in the fetus (Table 3); M&B28,767, sulprostone, and AH23848B had no effect on cAMP production.

**Effects of EP Receptor Agonists on DA Diameter In Vivo**

We examined the effects of EP receptor agonists (17-phenyl-trinor PGE$_2$ [EP$_1$]; butaprost [EP$_2$]; M&B28,767 [EP$_3$]; sulprostone [EP$_3$/EP$_4$]; and 11-deoxy PGE$_1$ [EP$_2$/EP$_3$/EP$_4$]) on patency of the newborn DA in vivo (Figure 3). 16,16-Dimethyl PGE$_2$ dilated both the untreated, spontaneously closing (Figure 3A) and the indomethacin-constricted DA (Figure 3B). Butaprost and 11-deoxy PGE$_1$ dilated the constricting DA as seen with 16,16-dimethyl PGE$_2$ (Figure 3); cessation of infusion of these PGE$_2$ analogues resulted in constriction of the DA. In contrast, 17-phenyl-trinor PGE$_2$, M&B28,767, and sulprostone did not affect DA diameter; 2- to 3-fold higher infusion rates of the EP$_1$ and EP$_3$ agonists also had no effects (data not shown). Hence, the vasodilatory effects of PGE$_2$ in the newborn seem to be accounted for by action on EP$_2$.

**Discussion**

Previous pharmacological studies have suggested that EP$_4$ is the dominant (relaxant) PGE$_2$ receptor in the fetal rabbit DA.\textsuperscript{7} In the mouse, however, disruption of the EP$_4$ gene did not affect ductal patency,\textsuperscript{8} suggesting possible species differences. We therefore set out to characterize the EP receptors in the DA of the fetus and newborn of a higher species, namely the pig.

Using binding and displacement, immunoblot, and stimulation of second-messenger cAMP, our studies revealed that the number and types of PGE$_2$ receptors differ between fetal and immediate postnatal newborn DA. There was a 3-fold higher density of PGE$_2$ receptors and a greater PGE$_2$-induced increase in cAMP in the fetus than in the newborn (Table 3); a rise in cAMP is usually associated with vasorelaxation.\textsuperscript{6} We identified EP$_2$, EP$_3$, and EP$_4$ receptors in the fetal pig DA; EP$_1$ receptor was undetectable (Figures 1 and 2). In the newborn DA, we observed a decrease in PGE$_2$ binding due to a loss of EP$_3$ and EP$_4$ receptors (Figures 1C through 1E and 2); the number of EP$_2$ receptors was essentially the same in the fetus and newborn. As a result, the EP$_2$ receptor appeared to mediate the vasorelaxant effects of PGE$_2$ on the full-term neonatal DA (Figure 3).

The mechanism(s) responsible for the birth-related decrease in PGE$_2$ receptors in the DA are currently unknown. We have previously observed that the loss of DA responsiveness to PGE$_2$ is directly related to the degree of postnatal DA constriction.\textsuperscript{9} However, a role for hypoxia to explain the selective loss of EP$_1$ and EP$_4$ is unlikely. Our data on [H]PGE$_2$ binding, immunoreactivity, and cAMP generation in newborn were obtained on DA of animals <45 minutes after birth. Although a 50% ductal constriction occurs over this time period,\textsuperscript{18} the partial pressure of oxygen also rises markedly immediately after birth; as a result, the DA tunica media may not develop significant hypoxia during this time.

### Table 2. Competitive Inhibition by Different Agents of [H]PGE$_2$ Binding to DA Membranes From Fetal and Newborn Pigs

<table>
<thead>
<tr>
<th>Competing Agents</th>
<th>% Inhibition of Binding</th>
<th>IC$_{50}$, nmol/L</th>
<th>% Inhibition of Binding</th>
<th>IC$_{50}$, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>16,16-Dimethyl PGE$_2$</td>
<td>100±0</td>
<td>31±4</td>
<td>100±0</td>
<td>28.5±2.5</td>
</tr>
<tr>
<td>AH8809</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
<tr>
<td>11-Deoxy PGE$_1$</td>
<td>94±6</td>
<td>30±5.0</td>
<td>96±4</td>
<td>38±5.5</td>
</tr>
<tr>
<td>Butaprost</td>
<td>32±2$^*$</td>
<td>157±12</td>
<td>98±1</td>
<td>187±9.6</td>
</tr>
<tr>
<td>M&amp;B28,767</td>
<td>42±3</td>
<td>260±13</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>43±4</td>
<td>128±13</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
<tr>
<td>AH23848B</td>
<td>32±2</td>
<td>15050±323</td>
<td>&lt;1</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are mean±SEM of 3 to 5 experiments, each performed in duplicate. Concentration of [H]PGE$_2$ was 8 nmol/L. ND indicates not determined (inhibition of binding was too low for accurate calculation of IC$_{50}$).

$^*$P<0.01 vs corresponding value in newborn.

**Figure 2.** Immunoblot of EP$_1$, EP$_3$, and EP$_4$ receptor proteins (arrows) in DA of fetal (F) and newborn (N) pigs and in cell lysates from HEK 293 cells overexpressing EP$_1$, EP$_3$, and EP$_4$ receptors separately (positive controls). Molecular weight protein markers (kDa) are on right.
focused on ontogenic changes in PGE₂ receptors in the DA. Other mechanisms are also likely to participate in this age-dependent altered responsiveness of the DA to PGE₂. These include developmental changes in the rates of prostaglandin production, uptake, and degradation as well as in prostaglandin-coupled signal transduction and relaxant mechanisms, and the role of increasing oxygen tension on ductal

### Table 3. Effects of PGE₂ Analogues and Forskolin on Net cAMP Synthesis on DA From Fetal and Newborn Pigs

<table>
<thead>
<tr>
<th>Agent</th>
<th>Net cAMP synthesis, pmol · mg protein⁻¹ · min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fetus</td>
</tr>
<tr>
<td>16,16-Dimethyl PGE₂ 0.05 μmol/L</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>16,16-Dimethyl PGE₂ 0.1 μmol/L</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>16,16-Dimethyl PGE₂ 1 μmol/L</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>11-Deoxy PGE₂ 0.1 μmol/L</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>11-Deoxy PGE₂ 1 μmol/L</td>
<td>2.05±0.3</td>
</tr>
<tr>
<td>Butaprost 0.1 μmol/L</td>
<td>0.6±0.1†</td>
</tr>
<tr>
<td>Butaprost 1 μmol/L</td>
<td>1.25±0.4</td>
</tr>
<tr>
<td>Butaprost 1 μmol/L + AH23848B</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>10 μmol/L</td>
<td></td>
</tr>
<tr>
<td>Sulprostone 1 μmol/L</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>M&amp;B28,767 1 μmol/L</td>
<td>0.2±0.1†</td>
</tr>
<tr>
<td>Forskolin 0.1 μmol/L</td>
<td>19.0±0.6</td>
</tr>
<tr>
<td>Forskolin 0.1 μmol/L + M&amp;B28,767</td>
<td>15.9±1.1§</td>
</tr>
<tr>
<td>Forskolin 0.1 μmol/L + Sulprostone 1 μmol/L</td>
<td>15.7±1.0§</td>
</tr>
<tr>
<td>AH23848B 10 μmol/L</td>
<td>0.2±0.1†</td>
</tr>
<tr>
<td>AH23848B 10 μmol/L + 16,16-dimethyl PGE₂ 0.1 μmol/L</td>
<td>0.6±0.05†</td>
</tr>
<tr>
<td>11-deoxy PGE₁ 10 μmol/L</td>
<td>0.95±0.2†</td>
</tr>
</tbody>
</table>

Values are mean ±SEM of 4 or 5 experiments, each performed in duplicate. The net maximum stimulated production of cAMP was corrected for basal (unstimulated) synthesis of cAMP, which was 4.1±0.7 pmol·mg protein⁻¹·min⁻¹ for newborn and 2.9±0.6 pmol·mg protein⁻¹·min⁻¹ for fetus.

*P<0.05 vs values in fetus; †P<0.05 vs corresponding values with 16,16-dimethyl PGE₂ (1 μmol/L); ‡P<0.05 vs corresponding values with 16,16-dimethyl PGE₂ (0.1 μmol/L); §P<0.05 vs corresponding values with forskolin alone.

Figure 3. Effects of PGE₂ receptor agonists on DA diameter in newborn pigs. A, DA diameter in newborn pigs after infusion of saline ( ), 16,16-dimethyl PGE₂ ( ), EP₁/EP₃ agonist 11-deoxy PGE₁ ( ), EP₁ agonist 17-phenyl-trinor PGE₂ ( ), EP₂ agonist butaprost ( ), EP₂ agonist M&B28,767 ( ), or EP₁/EP₃ agonist sulprostone ( ). Diameter of aorta at isthmus was 5.8±0.9 mm. Effects of agents were studied when DA closed spontaneously to reach a diameter of 0.6 to 0.8 mm (at ~2.5 to 3 hours old). Shaded area along abscissa corresponds to period of PGE₂ analogue infusion (0.083 μg · kg⁻¹ · min⁻¹ IV). B, DA diameter of newborn pigs (1.5 hours old) first treated with indomethacin (INDO, 3 mg/kg IV) and subsequently infused with saline ( ), 16,16-dimethyl PGE₂ ( ), 17-phenyl-trinor PGE₂ ( ), butaprost ( ), or M&B28,767 ( ). Shaded area along abscissa corresponds to period of PGE₂ analogue infusion (0.083 μg · kg⁻¹ · min⁻¹ IV). Arrow points to time of administration of indomethacin. Effects of PGE₂ analogues were determined on DA when it reached a diameter similar to that in A. Ductal diameter was measured by echocardiogram as described in Methods section. Values are mean±SEM of 3 to 4 experiments for each agent. *P<0.05 vs corresponding values for saline, 17-phenyl-trinor PGE₂, M&B28,767, and sulprostone (ANOVA and Tukey-Kramer method).
function, which has been debated, these various aspects need to be examined separately.

In summary, the present study demonstrates a developmental alteration of EP receptors in the DA. However, changes in EP receptor profile in DA of the prematurely born newborn remain to be determined. The present study also provides a physiological basis for the potential use of more selective EP receptor ligands to control DA patency and potentially diminish the side effects associated with nonselective PGE therapy. For example, one could suggest specific therapies for maintaining ductal patency in infants with congenital heart disease, such as selective EP2 agonists, and possibly reduce PGE2-mediated fever due to EP2 stimulation. In the future, selective EP receptor antagonists targeted toward inflammation, fever, and pain in the pregnant subject will also need to be evaluated for their effects on the fetal DA.

Acknowledgments

This study was supported by grants from the Medical Research Council of Canada, the Heart and Stroke Foundation of Quebec, and the Fonds de la Recherche en Santé du Québec, and from the US Public Health Service, NIH, NHLBI HL-46691. The authors thank Hendrika Fernandez and Manon Lessard for their technical assistance. M. Bhattacharya and Dr. Hardy are recipients of Doctoral Research and Fellowship Awards, respectively, from the Medical Research Council of Canada.

References

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Circulation. 1999;100:1751-1756
doi: 10.1161/01.CIR.100.16.1751

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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