Characterization of PGE$_2$ receptors in fetal and newborn lamb ductus arteriosus

ASMÄA BOUAYAD, HIROKI KAJINO, NAHID WALEH, JEAN-CLAUDE FOURON, GREGOR ANDELFINGER, DAYA R. VARMA, AMANDA SKOLL, ALEJANDRO VAZQUEZ, FERNAND GOBEIL JR, RONALD I. CLYMAN, AND SYLVAIN CHEMTOB. Characterization of PGE$_2$ receptors in fetal and newborn lamb ductus arteriosus. Am J Physiol Heart Circ Physiol 280: H2342–H2349, 2001.—Although the role of PGE$_2$ in maintaining ductus arteriosus (DA) patency is well established, the specific PGE$_2$ receptor subtype(s) (EP) involved have not been clearly identified. We used late gestation fetal and neonatal lambs to study developmental regulation of EP receptors. In the fetal DA, radioligand binding and RT-PCR assays virtually failed to detect EP$_1$ but detected EP$_2$, EP$_3$, and EP$_4$ receptors in equivalent proportions. In the newborn lamb, DA total density was one-third of that found in the fetus and only EP$_2$ was detected. Stimulation of EP$_2$ and EP$_4$ receptors increased cAMP formation and was associated with DA relaxation. Though stimulation of EP$_3$ inhibited cAMP formation, it surprisingly relaxed the fetal DA both in vitro and in vivo. This EP$_3$-induced relaxation was specifically diminished by the ATP-sensitive K$^+$ (K$_{ATP}$) channel blocker glibenclamide. In conclusion, PGE$_2$ dilates the late gestation fetal DA through pathways that involve either cAMP (EP$_2$ and EP$_4$) or K$_{ATP}$ channels (EP$_3$). The loss of EP$_3$ and EP$_4$ receptors in the newborn DA is consistent with its decreased responsiveness to PGE$_2$.

THE DUCTUS ARTERIOSUS (DA) performs two major functions: to remain patent during fetal life and to close rapidly after birth to separate the pulmonary and systemic circulations (20). Prostaglandins, particularly PGE$_2$, play a major role in maintaining the patency of the fetal DA (12, 14, 15). The relaxant effects of PGE$_2$ have been attributed to its ability to increase intracellular cAMP concentrations (17, 43). Immediately after birth, the response of DA to PGE$_2$ is markedly reduced (2, 11) thereby promoting DA closure. The mechanisms for this decreased responsiveness to PGE$_2$ are not well understood.

PGE$_2$ exerts its effects through a diverse group of receptors classified as EP$_1$, EP$_2$, EP$_3$, and EP$_4$ (16). Although pharmacological evidence suggests that EP$_4$ may be the main functional PGE$_2$ receptor in fetal rabbit DA (40), genetic disruption of this receptor does not induce DA closure in either fetal or newborn mice (34). Thus at present, the types of PGE$_2$ receptors that govern DA tone are uncertain. We (5) have recently found that the DA of the fetal pig expresses three EP receptor subtypes that would appear to have different effects on ductus contractile tone. We identified two cAMP-stimulating EP receptors (EP$_2$ and EP$_4$) and one cAMP inhibiting receptor (EP$_3$). In contrast, we detected only EP$_2$ in the newborn pig (5). However, it remains to be explained how loss of a cAMP-inhibiting (EP$_3$) and a cAMP-stimulating (EP$_4$) EP receptor can result in decreased responsiveness of the newborn DA to PGE$_2$.

Therefore we proceeded to determine the developmental profile of EP receptor expression in another species, the ovine, and to examine the effects of EP receptor stimulation on DA signaling events and contractile responses. Our findings reveal that the fetal lamb expresses, in equal proportions, the same three EP receptor subtypes detected in the fetal pig (5). Similarly, EP$_2$ is the only EP receptor identified by binding studies in the newborn ovine DA. Although stimulation of cAMP-generating EP$_2$ and EP$_4$ receptors resulted in the expected DA relaxation, surprisingly stimulation of cAMP-inhibiting EP$_3$ receptors also produced relaxation. This effect was mediated via a previously undescribed cAMP-independent pathway for EP$_3$ involving activation of ATP-sensitive K$^+$ (K$_{ATP}$) channels. The loss of a relaxant EP$_3$ receptor in the newborn DA is consistent with decreased responsiveness of the DA to PGE$_2$ in the immediate neonatal period.

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MATERIALS AND METHODS

Materials. AH-6809, AH-23848B, and GR-63799X were generously provided by Dr. Simon Lister (Glaxo-Wellcome), butaprost by Dr. Harold Klünder (Bayer), M&B-28767 by Dr. Jean Hough (Rhone-Poulenc Rorer), and the PGI₂ analog (cicaprost) by Dr. Fiona McDonald (Schering, Berlin, Germany). 16,16-dimethyl-PGE₂ and carbaprostacyclin were purchased from Cayman Chemical (Ann Arbor, MI) and [³H]PGE₂ (165 Ci/mmol) from Amersham Pharmacia, Bio-tech (Mississauga, ON, Canada); all other chemicals were from Sigma (St. Louis, MO).

Tissue collection. Pregnant ewes were anesthetized with alternating intravenous injections of ketamine HCl (0.3 mg·kg⁻¹·min⁻¹; Ketaalar, Parke-Davies) and diazepam (0.01 mg·kg⁻¹·min⁻¹; Valium, Hoffman-LaRoche) and fetal lambs (mixed Western breed) delivered by cesarean section at 135 ± 3 days gestation (range, 125–140 days; term, 145 days). The fetus was given ketamine (30 mg/kg iv) before exsanguination to obtain DA. The same procedure was used to obtain DA from the newborn (<8 h after birth). Vessels were frozen immediately after removal with liquid N₂ and stored at −80°C. These procedures were approved by the Committee on Animal Research at the University of California, San Francisco.

Radioligand binding assays. [³H]PGE₂ binding and displacement studies were performed as described previously on DA membranes (5, 25). Frozen ductuses (with endothelium) were homogenized with a tissue grinder in 10 mM PBS buffer (pH 7.4) containing soybean trypsin inhibitor (1 mg/ml) and phenylmethylsulfonyl fluoride (PMSF, 5%). The homogenate was then centrifuged twice at 10,000 g for 15 min at 4°C to remove nuclei, undisrupted cells, and fibrous tissue. The combined supernatants were recentrifuged at 100,000 g for 60 min at 4°C. The membrane pellet was stored at −80°C (necessary to pool tissues) and used for receptor binding assay within 1 wk.

Aliquots of DA membranes (100–200 µg protein) were incubated at 37°C for 30 min in 100 µl of 10 mM PBS buffer (pH 7.4) containing soybean trypsin inhibitor (1 mg/ml) and PMSF, 5%. [³H]PGE₂ was added in the absence or presence of increasing doses of a nonselective EP agonist (16,16-dimethyl-PGE₂), an EP₁ antagonist (AH-6809), an EP₂ agonist (butaprost), an EP₃ agonist (M&B-28767), and an EP₄ antagonist (AH-23848B). Total EP receptor density (B_max) was determined using the equation B_max = B0 × (IC₅₀/Lo), where Bₐ is the specific binding of [³H]PGE₂ at 0% displacement and L the concentration of free-labeled ligand (18), with the use of the computer program PRISM (GraphPad) which also calculated IC₅₀ values. Because the affinity of PGE₂ for EP receptors (especially EP₂, EP₃, and EP₄) is comparable (1) one could determine their proportions (18, 28).

cAMP measurements. DA homogenates (100 µg protein) were incubated at 37°C for 10 min in an assay mixture (100 µl) containing: 10 mM Tris-HCl buffer (pH 8.0), 1 mM ATP, 7.5 mM MgCl₂, 15 mM creatine phosphate, 0.5 mM EGTA, 0.5 mM IBMX, 1 mM dithiothreitol, 1 mM benzamidine, 0.1 mM PMSF, and 185 U/ml creatine phosphokinase, 200 µg/ml aspirin, and 100 µg/ml soybean trypsin inhibitor (5, 25). The reaction was terminated with 200 µl of acidic ethanol. After centrifugation, cAMP was measured by radioimmunoassay as described by the manufacturer (Diagnostic Products). The assay is based on the competition between unlabeled cAMP and a fixed quantity of tritium-labeled compound ([³H]cAMP) for binding to an antiserum which has a high specificity for cAMP. The amount of [³H]cAMP bound to the antiserum is inversely related to the amount of cAMP present in the assay sample. Separation of the unbound nucleotide (including radiolabeled) is achieved by dextran-coated charcoal adsorption. The radioactivity of the supernatant (which contains the antibody-[³H]cAMP complex) is determined by liquid β-scintillation counting. The concentration of unlabeled cAMP in the sample is then determined from a standard curve.

Isometric tension in vitro. The freshly collected DA was divided into 1-mm thick rings placed in separate 10-ml organ baths in a darkroom as previously described (12, 13). The rings were suspended between two stainless steel hooks at 38°C in a modified Krebs buffer (pH 7.4) of the following composition (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 0.9 MgSO₄, 1 K,H₂PO₄, 11.1 glucose, and 23 NaHCO₃ (pH 7.4). The buffer was equilibrated with 5% CO₂-30% O₂-65% N₂. In some experiments the luminal endothelium was removed by scraping the surface with a fine wire as previously described (15). The bath solution was changed every 20 min. Isometric tension was measured by Grass PT03C force transducers (Quincy, MA). The tissues were equilibrated with 30% O₂, 5% N₂, and 65% CO₂ until the tension reached a plateau (~100–120 min). Indomethacin at 5.6 µM [a dose that was previously reported to cause maximal inhibition of PGE₂ or 6-keto-PGF₁α production in the ductus (10,13)] was then added to the bath solution, and the rings were allowed to reach a new steady-state tension over the next 60–90 min. The nitric oxide (NO) synthase inhibitor, 10⁻⁶-nitro-1-arginine methyl ester (L-NAME) (0.1 mM) was then added to a dose previously found to cause the maximal inhibition of NO synthesis in the ductus (10, 13). The rings were exposed to indomethacin and L-NAME for the remainder of the study protocol. Maximal contraction was determined from the response to a 100 nM KCl that gave the maximal contraction of the ductus (13).

Sensitivity of the DA to relaxing agents was determined from vessels precontracted with indomethacin and L-NAME (precontraction tension). Cumulative dose-response curves were constructed for PGE₂, butaprost, M&B-28767, and GR-63799X (also an EP₃ agonist), cicaprost and carbaprostacyclin (PGI₂ analogs), as well as cAMP stimulants and mimetics [ forskolin and 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP)]. The concentrations that produced 50% of maximal response (EC₅₀ values) were determined from each dose-response curve. In some experiments, a cumulative dose-response curve was performed to a relaxing agent after the tissue had been equilibrated with either an EP₁ receptor antagonist (AH-23848B) or one of the following K⁺ channel inhibitors 4-aminopyridine (4AP), iberiotoxin, or glibenclamide (33). In all experiments, we allowed the tension in the rings to reach a new steady-state plateau after addition of the drug and before another concentration or experimental agent was added to the bath. After the addition of all prorelaxant drugs, sodium nitroprusside (0.1 mM) was added to each ring to determine its minimal tension. The difference between the maximal contraction and minimal tension was considered the maximal active tension (20 ± 3 g, n = 120). The difference in tensions between the precontraction tension (with indomethacin and L-NAME) and the minimal tension (with sodium nitroprusside) was considered the net tension (15 ± 3 g, n = 120). The tension was expressed as a percentage of the net tension. Tissues were removed from the baths after the experiments and wet weights recorded.

Effects of different agents on DA tone in vivo. Pregnant ewes (120–140 days of gestation) were anesthetized with ketamine (30 mg/kg iv). A cesarean section was performed and the fetus with intact placental circulation was exteriorized and its head submerged in warm saline to prevent...
breathing. Body temperature was maintained at 38.5°C by an overhead lamp. The femoral artery was cannulated for blood pressure recording using a pressure transducer (Gould, Valley View, OH), and the jugular vein was cannulated for the administration of drugs. Arterial blood gas was measured with an ABL300 blood gas analyzer (Radiometer, Copenhagen, Denmark) before and after each drug infusion.

**Ultrasonography of DA.** Real-time and Doppler echographic studies were performed with an Acuson 128 XP/10c real-time ultrasonographic imaging system that used 7.5- and 5-MHz transducers as previously described (6). Two-dimensional imaging of the DA was obtained through a left parasternal approach (second intercostal space), and the anulation of the transducer was such that the ultrasonographic beam was always parallel to or within 20 degrees of the orientation of the blood flow. The DA was preconstricted with indomethacin (0.75 mg/kg) to <50% of the original diameter. Vasorelaxant responses to sulprostone (EP3 agonist) were determined by measuring the smallest diameter of the vessel on a two-dimensional representation of the echocardiogram before and every 5–10 min after the drug injections. Each measurement of the DA diameter was repeated twice and expressed in mm.

**Preparation of total RNA, reverse transcription, and polymerase chain reaction.** Total RNA was isolated from fetal DA tissue using the QIAGEN RNeasy mini kit (QIAGEN; Valencia, CA) according to manufacturer instructions. Total RNA (3 μg) was reverse transcribed with 100 units of Moloney murine leukemia virus Rnase H reverse transcriptase (Life Technologies) in the presence of random hexanucleotide primers as described elsewhere (34, 45). The cDNAs were used for the amplification of specific fragments of EP2, EP3, and EP4 receptors by PCR following standard procedures (35 amplification cycles) (34, 45). To amplify a fragment composed of the transmembrane domain I and the second intracellular loop of the EP2 receptor, the forward primer 5′-ATCTTGGGGTGGGCAA-3′ and the reverse primer 5′-CGCTTGTCACGGTAGTGGCT-3′ were used. To amplify an EP3 receptor fragment composed of the transmembrane domains IV and V, the forward 5′-GGCTACGCTTGCCCTGTT-3′ and reverse 5′-GCTTGGCCCTGACGGGA-3′ primers were used. To amplify COOH-termini of the EPAA,RR,C isomers the forward primer 5′-ATAATGATGGTAGAAATGAT-3′ was used with the reverse primers R3 5′-CTACTGATGCTCAAGTGTATG-3′ and R4 5′-GCCCCCTTCCTCTCCCTTGTT-3′. Primer R5 5′-ATTTCAATTG-GATAGTAGATGTC-3′ was used to amplify the EP3D COOH-terminal. The forward and reverse primers used to amplify the EP4 receptor were 5′-AAGTCCGCGCAAGGAGGACGAA-3′ and 5′-CTTGCTCCAGTGATGGCTGT-3′, respectively. The PCR products were subcloned into the pPCR-Script Amp SK(+) plasmid (Stratagene, La Jolla, CA) and sequenced with the use of an ABI prism 310 Genetic Analyzer sequencer (PE Applied Biosystems, Foster City, CA).

**Statistical analysis.** Data were analyzed by Student’s t-test and by two-way ANOVA factoring for time or concentration and treatment. The Bonferroni correction was used for comparison among means. Statistical significance was set at P < 0.05. Data are expressed as means ± SE.

**RESULTS**

**Competitive displacement of [3H]PGE2 in the fetal DA.** EP2, EP3, and EP4 receptor ligands caused comparable displacement of specifically bound [3H]PGE2 from fetal DA membrane preparations. Accordingly, the estimated density of EP2, EP3, and EP4 (product of the proportion of each EP receptor deduced from competitive binding studies and Bmax of EP receptors (total [3H]PGE2 binding)) on fetal DA membranes was similar (Fig. 1B). The EP1 receptor antagonist AH-6809 was virtually ineffective (Fig. 1, A and B, Table 1). IC50

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**Fig. 1.** Representative displacement curves of specifically bound [3H]PGE2 from lamb ductus arteriosus (DA) membrane preparations from fetal (A and B) and newborn (C and D) lambs. Estimated density of each EP receptor subtype (Bmax) (B and D) was calculated as total maximum binding to all EP receptor subtypes times the proportion of displacement caused by ligands selective to each EP receptor subtype. Values are means ± SE of 3–4 experiments. 16,16-dimethyl (DM)-PGE2, nonselective EP receptor agonist; AH-6809, EP1 selective antagonist; butaprost, EP2 selective agonist; M&B-28767, EP2 selective agonist; AH-23848B, EP4 antagonist.
Table 1. Competitive inhibition of [3H]PGE2 binding to ductus arteriosus membranes from fetal and newborn lambs by different agents

<table>
<thead>
<tr>
<th>Competing Agents</th>
<th>Fetus</th>
<th>Newborn</th>
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<tr>
<td></td>
<td>% Inhibition</td>
<td>IC50, nM</td>
</tr>
<tr>
<td>16,16-dimethyl-PGE2</td>
<td>100 ± 10</td>
<td>4.3 ± 2.7</td>
</tr>
<tr>
<td>AH-6809</td>
<td>4 ± 1</td>
<td>ND</td>
</tr>
<tr>
<td>Butaprost</td>
<td>26 ± 5</td>
<td>1,608 ± 248</td>
</tr>
<tr>
<td>M&amp;B-28767</td>
<td>38 ± 4</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>AH-23848B</td>
<td>37 ± 2</td>
<td>3,340 ± 1,307</td>
</tr>
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Values are means ± SE of 3–4 experiments. ND, not determined because percent inhibition of [3H]PGE2 binding is too low for accurate determinations. Ductus arteriosus were prepared as described in the MATERIALS AND METHODS. Percent inhibition (proportion of displacement) and IC50 values were determined from curves of displacement of bound [3H]PGE2. [3H]PGE2 was 10 nM and 8.6 nM for the fetus and the newborn, respectively. % Inhibition refers to that of bound [3H]PGE2.

Changes in values were consistent with those previously reported in the literature (1, 16, 32).

Effects of different agents on cAMP production in the fetal DA. Both the nonselective EP receptor agonist 16,16-dimethyl-PGE2 and the selective EP receptor agonist butaprost increased cAMP production in the fetal lamb DA (Table 2). In contrast, the selective EP3 receptor agonist GR-63799X had no effect on cAMP production by itself but inhibited forskolin-stimulated cAMP production (Table 2). In the absence of currently available EP4 agonists, the role of EP4 was tested with the use of the EP4 antagonist AH-23848B in the presence of 16,16-DM PGE2. AH-23848B alone did not alter cAMP production but decreased 16,16-DM PGE2-induced stimulation of cAMP formation (Table 2), which suggests that stimulation of EP4 receptors accounts for some of the increased cAMP production induced by the nonselective EP receptor agonist.

Effects of different agents on cAMP production in the fetal DA in vitro. The fetal DA contracted spontaneously (77 ± 8% maximal active tension) after addition of indomethacin and L-NAME (in the presence of 30% oxygen). As anticipated, agents that led to elevations in cAMP levels, namely forskolin and 8-Br-cAMP, relaxed the fetal DA (Fig. 2, A and B) with relatively low potency as previously described (37, 41). Analogs of PGI2, the effects of which are also coupled to an increase in cAMP (16), relaxed the DA albeit with a potency <1,000 times less than PGE2 (Fig. 2C, Table 3). Similarly, stimulation of EP receptors that increase cAMP relaxed the DA; the nonselective EP agonist PGE2 and the selective EP2 agonist butaprost relaxed the DA (Fig. 2, C and D), and inhibition of the EP4 receptor with AH-23848B inhibited the relaxant effects of PGE2 (Fig. 2E, Table 3). In contrast, although EP3 stimulation decreased cAMP production (Table 3), activation of EP3 receptors with M&B-28767 or GR-63799X caused relaxation of the (preconstricted) fetal DA by >70% (Fig. 2F). To assess whether effects of EP3 were G protein-dependent, tissues were treated with pertussis toxin, which diminished the inhibitory effect of M&B-28767 on cAMP formation but did not alter its relaxant actions.

Effects of PGE2 analogs on the diameter of the fetal DA in vivo. We also tested the effects of EP3 stimulation on DA tone in vivo. Infusion of the EP3 receptor agonist sulprostone relaxed the indomethacin-induced contraction of the fetal DA in vivo (Fig. 3) consistent with our in vitro results (Fig. 2F); a similar effect was seen with 16,16-dimethyl-PGE2 (data not shown).

Role of K+ channels on EP receptor-induced fetal DA relaxation. Because EP3 receptor stimulation caused DA relaxation, despite reducing cAMP generation, we examined whether EP receptor stimulation might relax the DA through other signaling pathways. K+ channels have previously been shown to play a role in DA tone (30, 42). We performed PGE2 dose-response curves in the presence and absence of specific K+ channel inhibitors. The relaxant effects of PGE2 were not affected by the voltage-gated potassium- and calcium-activated potassium-channel blockers 4-aminopyridine and iberiotoxin, respectively (Table 3). However, the KATP channel blocker glibenclamide significantly inhibited the relaxation caused by the EP3 agonist M&B-28767. Glibenclamide did not affect relaxation caused by agents that increase cAMP (butaprost, forskolin, and 8-Br-cAMP) (Table 3). Removal of luminal endothelial cells neither altered the PGE2- or M&B-28767-induced relaxation of the DA nor did it modify the inhibitory effects of glibenclamide on PGE2 and M&B-28767-induced relaxation (Table 4).

Detection of EP receptor subtypes and isoforms in the fetal DA by PCR. We amplified an EP3 receptor fragment from the ovine DA that encompassed transmembrane domains IV and V of the receptor. Because its sequence was 100% homologous with the corresponding bovine sequence (31), we designed oligonucleotide primers on the basis of the bovine sequence to identify which of the different EP3 receptor carboxyterminal isoforms might be present in the fetal DA (31). Only the EP3D isoform fragment was successfully amplified from the ovine fetal DA. The PCR product sequence

Table 2. Net cAMP synthesis by ductus arteriosus from near-term fetal lamb

<table>
<thead>
<tr>
<th>Agents</th>
<th>Net cAMP synthesis, pmol·mg protein⁻¹·min⁻¹</th>
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<tr>
<td>16,16-dimethyl-PGE2 (1 μM)</td>
<td>2.54 ± 0.46</td>
</tr>
<tr>
<td>Butaprost (1 μM)</td>
<td>1.56 ± 0.28*</td>
</tr>
<tr>
<td>GR-63799X (0.5 μM)</td>
<td>0.31 ± 0.14</td>
</tr>
<tr>
<td>Forskolin (1 μM)</td>
<td>25.61 ± 1.01</td>
</tr>
<tr>
<td>Forskolin (0.1 μM) + GR-63799X</td>
<td>20.77 ± 0.14●</td>
</tr>
<tr>
<td>AH-23848B (10 μM)</td>
<td>0.60 ± 0.23●</td>
</tr>
<tr>
<td>16,16-DM-PGE2 (1 μM) + AH-23848B (10 μM)</td>
<td>1.31 ± 0.05*</td>
</tr>
</tbody>
</table>

Data are means ± SE of three experiments. Ductus homogenates (100 μg) were incubated with indicated agents for 10 min at 37°C and cAMP measured at different times. Net cAMP synthesis was calculated as the difference in cAMP formation between the basal (unstimulated) state (3.82 ± 0.7 pmol·mg protein⁻¹·min⁻¹) and after addition of stimulant. *P < 0.05 vs. 16,16-dimethyl-PGE2, †P < 0.05 vs. forskolin alone.
identified was 100% homologous with that of the bovine EP3D (for relevant bases 895–1145 from the cDNA sequence) (31).

Using EP2-specific primers, we amplified an EP2 receptor fragment from the ovine fetal DA that had 90, 87, and 85% homology with the corresponding sequences from the human, mouse, and rat EP2 receptors, respectively (4, 21, 35). Using EP4 specific primers, we also amplified an EP4 fragment, which was virtually identical to the reported ovine EP4 receptor sequence (GenBank accession AF035418) and had 94% homology with the corresponding sequence of the human EP4 receptor (29). The presence of EP2, EP3D, and EP4 receptor mRNAs in the fetal ovine DA is consistent with our displacement binding data (Fig. 1 and Table 1).

**DISCUSSION**

Results presented help to identify the types of PGE2 receptors that govern DA tone and how developmental changes in their expression may explain the decreased responsiveness of the DA to PGE2 in the immediate newborn period. We have previously reported the presence of EP3 receptors in the porcine fetal DA, and the loss of EP3 and EP4 receptors in the newborn (5). Stimulation of EP3 and EP4 led to opposite effects on...
were preconstricted with indomethacin (0.75 mg/kg iv) and once its diam-
cannulated. DA diameter was measured by echocardiogram. DA was
to prevent breathing. The femoral artery and jugular vein were
circulation was exteriorized and its head submerged in warm saline
diameter of fetal lambs in vivo. Animal preparation is described in
receptors coupled to relaxation (EP3 and EP 4)i nt h e
bust relaxation mediated by activation of K ATP chan-
vasomotor response to EP3 stimulation, namely, a ro-
tantly, the findings unveil a previously unreported
relaxation was not inhibited by removal of the luminal
endothelium. Possible explanations for this novel func-
relaxation of the DA to PGE2 seems to be at least partly
dependent on cAMP (EP2 and EP4) and KATP (EP3)
pathways.
The mechanisms responsible for the EP3-induced,
KATP-dependent relaxation of the DA are unclear. PGE2 has been reported to be coupled to KATP or calcium-activated potassium channels in different tis-
ues (3, 7); NO-mediated, cGMP-dependent kinase- (8,
19) and protein kinase A-mediated processes have been proposed (43). In our studies a role for protein kinase A appears to be unlikely because cAMP elevating agents
(butaprost, forskolin, 8-BrcAMP) did not relax the DA in a
glibenclamide-sensitive manner. Similarly, a role
for NO or endothelial derived prostanooids also appears
to be unlikely because the relaxant responses of the
EP3 agonists were observed in DA tissues pretreated
with NO synthase and cyclooxygenase inhibitors. Fur-
thermore, removal of luminal endothelium did not
modify either the EP3-induced relaxation or its de-
dence on the KATP channel. The EP3 receptor has
several isoforms that couple to different G proteins
(24, 31). These have the potential to elicit different
vasoactive responses. We identified an EP3 isoform in
the ovine DA identical to the bovine EP3D receptor (31)
that can couple to Gi, Gs, and Gq (31). It is unlikely that
the EP3D receptor stimulates the KATP channel
through a Gs-stimulated process: EP3 stimulation in-
hibited rather than stimulated cAMP in the DA and
cAMP did not relax the DA in a glibenclamide-sensitive
manner (Tables 2, 3). In most tissues, the EP3 receptor
activates with Gt (16, 24, 31, 32), which is responsible for
the inhibition of cAMP production; however, pertussis
toxin, an inhibitor of Gt, blocked the effects of EP3
stimulation on cAMP inhibition but did not affect the
EP3-induced relaxation. Hence, Gt may also be not
involved in the EP3-induced DA relaxation. Coupling of
the receptors to Gq could lead to relaxation if they were
present on the endothelium (9, 27, 39); however, this is
not consistent with our findings because EP3-induced
relaxation was not inhibited by removal of the luminal
endothelium. Possible explanations for this novel func-
onion of EP3 include activation of KATP by NO-indepen-
dent protein kinase G in response to EP3 stimulation
(44) or heterodimerization of EP3 with the KATP chan-
neurons (3, 7); NO-mediated, cGMP-dependent kinase-
dependent on cAMP (EP2 and EP4) and KATP (EP3)
pathways.

**Table 4. Effects of endothelial denudation of ductus
arteriosus on EC50 of relaxant response to PGE2 and
M&B-28767 in absence and presence of KATP channel blocker glibenclamide**

<table>
<thead>
<tr>
<th>Agents</th>
<th>EC50, nM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DA with intact endothelium</td>
</tr>
<tr>
<td>PGE2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>PGE2 + glibenclamide</td>
<td>2.8 ± 0.3*</td>
</tr>
<tr>
<td>M&amp;B-28767</td>
<td>180 ± 21</td>
</tr>
<tr>
<td>M&amp;B-28767 + glibenclamide</td>
<td>540 ± 76*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 2–3 experiments. DA, ductus arteriosus.
Ductal rings were prepared as described in legend to Fig. 2. *P <
0.01 compared with value immediately above.
nel as described with other G protein-coupled receptors (26). Extensive studies are in progress to address these issues.

Perspectives. In summary, total EP receptors in the lamb DA decrease after birth. The loss of both a vaso-dilating EP4 receptor and a previously undescribed vasodilating EP3 receptor could explain the loss of vasodilatory response of the DA to PGE2 in the early newborn period. Present data in the ovine DA support those in the porcine DA (5). They also suggest that a selective EP2 agonist may be more appropriate than the nonselective agonist PGE1 for maintaining ductal patency in newborn infants afflicted with certain congenital cardiac malformations.

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