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Evidence for a Role of Phosphodiesterase 4 in Lipopolysaccharide-Stimulated Prostaglandin E2 Production and Matrix Metalloproteinase-9 Activity in Human Amniochorionic Membranes

Stéphanie Oger, Céline Méhats, Emmanuelle Dallot, Dominique Cabrol, and Marie-Josèphe Leroy

Chorioamniotic infection is a leading cause of preterm premature rupture of fetal membranes (amnion and chorion). Bacterial infection induces an inflammatory response characterized by elevated production of proinflammatory cytokines; the latter activate the production of both PGs that stimulate uterine contractions, and matrix metalloproteinases (MMPs) that degrade the extracellular matrix of the chorioamniotic membranes. The inflammatory response is under the control of cAMP content, which is partly regulated by phosphodiesterases (PDE). In this study, we investigated the role of the PDE4 family in the inflammatory process triggered by LPS in a model of amniotic chorioamniotic explants. We found that PDE4 family is the major cAMP-PDE expressed in human fetal membranes and that PDE4 activity is increased by LPS treatment. Selective inhibition of PDE4 activity affected LPS signaling, because PDE4 inhibitors (rolipram and/or cilomilast) reduced the release of the proinflammatory cytokine TNF-α and increased the release of the anti-inflammatory cytokine IL-10. PDE4 inhibition reduced cyclooxygenase-2 protein expression and PGE2 production and also modulated MMP-9, a key mediator of the membrane rupture process, by inhibiting pro-MMP-9 mRNA expression and pro-MMP-9 activity. These results demonstrate that the PDE4 family participates in the regulation of the inflammatory response associated with fetal membrane rupture during infection. The PDE4 family may be an appropriate pharmacological target for the management of infection-induced preterm delivery.


Physiological rupture of fetal membranes, the amnion and chorion, occurs just before or during labor, and is associated with an inflammatory process (1). Intrauterine infection amplifies the inflammatory response and therefore may lead to preterm premature rupture of the fetal membranes (PPROM), a leading cause of preterm birth (2). Bacterial invasion of uteroplacental tissues induces massive production of proinflammatory cytokines (IL-1β and TNF-α) by both immune and nonimmune cells. This culminates in amniotic, chorionic, and decidual cell synthesis of mediators involved in uterine contraction, including PGs. PGE2 and PGF2α levels are elevated in amniotic fluid of women with preterm labor associated with intra-amniotic infection. These increases result mainly from induced expression of the cyclooxygenase 2 isoenzyme (COX-2) (3). Cytokine release is also involved in fetal membrane rupture, through the activation of matrix metalloproteinases (MMPs). MMPs are secreted enzymes responsible for degrading and remodeling the components of the extraacellular matrix. They participate in many normal biological processes (embryonic development, angiogenesis, cervical ripening, etc.) and also in pathological states (arthritis, cardiovascular diseases, fibrotic lung disease, etc.). Among the MMPs expressed in fetal membranes, MMP-2 and MMP-9 (gelatinases A and B) degrade collagen IV, a major basement membrane component. MMP-9 is a key mediator of the fetal membrane rupture process during intrauterine infection. Microbial invasion of the amniotic cavity increases MMP-9 levels in amniotic fluid (4). Moreover, the bacterial endotoxin LPS stimulates TNF-α and IL-1β production by the amnion and chorion, leading to MMP-9 secretion and activation (5).

In addition to proinflammatory cytokines, gestational membranes generate a second wave of cytokines, the anti-inflammatory cytokines (6). IL-10, an anti-inflammatory cytokine, inhibits the transcription of proinflammatory cytokine genes such as IL-1β, TNF-α, IL-6, and IL-8 (7). IL-10 levels are elevated in amniotic fluid of women with infection-associated preterm labor (8). In the presence of LPS, IL-10 down-regulates TNF-α release, PGE2 production, and MMP-2 and MMP-9 mRNA and protein expression by cultured human amniochorionic membranes (9, 10).

At the cellular level, cAMP is a critical second messenger that mediates a broad array of physiological responses. It plays an important role in the immune system, exerting generally suppressive effects on inflammatory cell functions (11). The intensity and duration of the intracellular cAMP signal are partly regulated by the phosphodiesterase (PDE) enzyme superfamily, whose functions are to degrade the cyclic nucleotides cAMP and cGMP into their respective 5′-monophosphates. This process is catalyzed by another family of enzymes, the PDEs, whose inhibition can alter the cAMP content in various cell types (12). The PDE4 family includes four highly homologous isoenzymes and it is expressed in immune cells. PDE4 is a particularly attractive target for the treatment of human inflammatory diseases, fibrotic lung disease, etc.). Among the MMPs expressed in fetal membranes, MMP-2 and MMP-9 (gelatinases A and B) degrade collagen IV, a major basement membrane component. MMP-9 is a key mediator of the fetal membrane rupture process during intrauterine infection. Microbial invasion of the amniotic cavity increases MMP-9 levels in amniotic fluid (4). Moreover, the bacterial endotoxin LPS stimulates TNF-α and IL-1β production by the amnion and chorion, leading to MMP-9 secretion and activation (5).

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inactive metabolites 5’-AMP and 5’-GMP. Among the 11 distinct PDE families described to date, the PDE4 family is selective for cAMP hydrolysis (12, 13). Several studies have shown that the PDE4 family participates in regulating a wide range of inflammatory events (cytokine release, chemotaxis, and T-lymphocyte proliferation) (14–16). The PDE4 family is also involved in regulating extracellular matrix remodeling. It was recently reported that PDE4 is involved in tissue remodeling during acute lung injury in mice (17) and that PDE4 inhibitors modulate the capacity of fibroblasts cultured in collagen gels to degrade their surrounding matrix (18). Interestingly, although PGs play a pivotal role in inflammation and in contraction/relaxation processes, PDE4 family involvement in the PG synthesis pathway remains unexplored.

We have previously shown that the PDE4 family is involved in controlling uterine motility, because selective PDE4 inhibition abrogates spontaneous contractions of myometrial strips (19). Moreover, we recently demonstrated that the PDE4 family participates in regulating the inflammatory process in a model of near-term myometrium explants stimulated by LPS (20). Because the fetal membranes are a major source of inflammatory mediators involved in PPROM and in the activation of uterine contractions during infection, we examined PDE4 family participation in the inflammatory cascade that occurs in human amniochorionic explants exposed to LPS. We first established that the PDE4 family is responsible for the bulk of cAMP hydrolysis in both the amnion and the chorion, and that LPS enhances this PDE4 activity. We then investigated whether the PDE4 family is involved in pro- and anti-inflammatory cytokine release, PGE2 synthesis, and MMP-9 activity in infected fetal membranes.

Materials and Methods

Biological samples

Placentas were obtained from pregnant women delivered by elective cesarean section before onset of labor (38–40 wk of pregnancy) because of either cephalopelvic disproportion or a scarred uterus in an otherwise normal pregnancy. The fetal membranes were collected in sterile conditions. The amniochorionic membranes were dissected free of the placenta and washed with PBS to eliminate blood clots.

Fetal membrane tissue culture

Entire membranes were cut into 9-mm-diameter pieces by using a biopsy punch. Two pieces of tissue per well were placed in 24-well plates containing 2 ml of culture medium (DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin; Invitrogen Life Technologies). The explants were incubated at 37°C with 5% CO₂ for 48 h to allow them to stabilize (21). The membranes were then incubated with LPS (Escherichia coli 0127:B8; 1 ng/ml) for various times, with or without the PDE inhibitors, forskolin (10⁻⁵ M), or 8-bromo-cAMP (8-Br-cAMP; 10⁻⁵ M) or H89 (10⁻⁵ M). At the end of the incubation period, the supernatants and tissues were stored at −80°C. Cell viability, as assessed by trypan blue exclusion after trypsin and collagenase digestion of the membranes, was 96–99% at the end of the experiments.

Determination of cAMP-PDE activities

For pharmacological identification of the different cAMP-PDE families in fetal membranes, the amnion and chorion were first manually separated. They were then homogenized (100 mg/ml) with an Ultra-Turrax apparatus and tissues were stored at −80°C after trypsin and collagenase digestion of the membranes, was 96–99% at the end of the experiments. was inhibited by the relevant selective PDE inhibitor, 8-methoxy-3-isobutyl-1-methylxantine (IBMX) (10⁻⁵ M) for PDE1, cilostamide (10⁻³ M) for PDE3, and rolipram (10⁻⁵ M) for PDE4. cGMP (10⁻⁵ M) was used to inhibit PDE3 and to activate PDE2.

Reverse transcription and PCR amplification

Total RNA was extracted from entire fetal membranes using the TRIzol reagent method, reverse transcription was obtained with M-MLV reverse transcriptase, and PCR were performed with TaqDNA polymerase, as recommended by the manufacturer (Invitrogen Life Technologies). The amplification profile consisted of denaturation at 94°C for 1 min, annealing for 1 min at the specific temperature of 62°C, and extension at 72°C for 1 min with a final extension step at 72°C for 10 min. Primers for pro-MMP-9 were designed according to Fortunato et al. (24) (antisense: GCA CTC CGA GAT GTG ATC; sense: CCT TCT ACG GCC ACT ACT). After 33 cycles of denaturation and extension, a 15-μl aliquot from each reaction mix was resolved by electrophoresis on 3% NuSieve agarose gel and visualized by ethidium bromide staining under UV light. The DNA molecular mass standard was a 123-bp ladder (Invitrogen Life Technologies). DNA contamination was ruled out by conducting PCR control reactions with mRNA but without reverse transcriptase. GAPDH, a housekeeping gene, was used as internal control (antisense, GAT GCC ATG GAC TGT GG; sense, GGA GAA GGC TGG GCC).

Zymography

The proteolytic activity of pro-MMP-9 secreted into the culture medium was assessed by zymography on 8% SDS gel incorporating 1 mg/ml gelatin. Culture medium corresponding to 0.5 mg of tissue was loaded and subjected to electrophoresis in nonreducing conditions. The gels were then washed twice in 2.5% Triton X-100 for 30 min and incubated for 18 h at 37°C in 50 mM Tris-HCl, 5 mM CaCl₂, 200 mM NaCl, and 0.02% sodium azide (pH 7.6). The gels were stained with Coomassie blue in 30% ethanol and 10% acetic acid for 1 h at room temperature and then destained in 25% ethanol and 10% acetic acid. Clear bands on a blue background indicate the presence of gelatin-degrading proteinases. The gelatinolytic activity of pro-MMP-9 (92 kDa) was estimated by scanning the wet gels with a densitometer and analyzing the data with the NIH Image 1.60 software package (National Institutes of Health, Bethesda, MD). The results were expressed in arbitrary densitometric units.

Western blot analysis

Homogenized membranes (10 mg of protein/lane) were diluted (v/v) in Laemmli buffer and boiled for 5 min before 10% SDS-PAGE. Proteins were then transferred to a nitrocellulose membrane (Amersham Biosciences) using a Transblot apparatus (Bio-Rad Laboratories). Blots were blocked for 1 h in 10% nonfat-dried milk in 10 mM Tris, 150 mM NaCl, and 0.1% Tween 20 (pH 7.6) (TBS-T), at room temperature. Blocked membranes were washed three times with TBS-T. The blots were then incubated for 90 min at room temperature with anti-human COX-2 polyclonal IgG (Santa Cruz Biotechnologies) at a dilution of 1/1000 in TBS-T containing 1% nonfat dried milk. After three washes in TBS-T, the blots were incubated for 45 min at room temperature with HRP-linked donkey anti-goat secondary IgG Ab (Santa Cruz Biotechnologies) diluted 1/2000 in TBS-T containing 1% nonfat dried milk. Immunoreactive proteins were detected by chemiluminescence (Amersham Biosciences ECL reagents) following the manufacturer’s instructions.

ELISA of TNF-α, IL-10, and PGE₂

TNF-α, IL-10, and PGE₂ concentrations were determined in the supernatants of amniochorionic explants by using commercial ELISA kits (Cayman Chemicals, for TNF-α and PGE₂; and BD Pharmingen, for IL-10). The detection limits of the assays was <1.5 pg/ml for TNF-α; 15 pg/ml for PGE₂; and 2 pg/ml for IL-10.

Materials

The following two PDE4 inhibitors were used: rolipram, a classical PDE4 inhibitor (a gift from Schering Health Care), and cilomilast, one of the most advanced PDE4 inhibitors currently undergoing clinical development for the treatment of bronchopulmonary disorders (a gift from GlaxoSmithKline). The PDE5 inhibitor, sildenafil, was a gift from Altana Pharma.

Anti-human IL-10 Ab was purchased from BD Pharmingen, and Pefabloc was purchased from Interchim. All other reagents were purchased from Sigma-Aldrich.
Statistical analysis

Statistical comparisons between different treatments were conducted using one-way ANOVA followed by post hoc tests, and p values of <0.05 were considered significant.

Results

Pharmacological characterization of cAMP-PDE families in amnion and chorion

The characterization of the cAMP-PDE families in amnion and chorion was established by a pharmacological approach using several PDE inhibitors. As shown in Fig. 1, the nonselective PDE inhibitor IBMX significantly suppressed cAMP hydrolytic activity in both membranes. However, a small percentage of activity was not blocked by IBMX, suggesting the presence of a cAMP-PDE family insensitive to IBMX (PDE8). The selective PDE1 inhibitor 8-methoxy-IBMX decreased cAMP-PDE activity by ~10% in both the amnion and the chorion. The selective PDE3 inhibitor cilostamide reduced cAMP-PDE activity by 12% in the amnion and 19% in the chorion. In the amnion, the addition of cGMP inhibited cAMP-PDE activity to a similar extent to that obtained with cilostamide, whereas cGMP slightly increased cAMP-PDE activity in the chorion, suggesting the presence of PDE2. In the presence of the selective PDE4 inhibitor rolipram, cAMP hydrolysis was inhibited by 80% in the amnion and 52% in the chorion. These results indicated that the PDE4 family is responsible for the bulk of cAMP hydrolysis in both the amnion and the chorion, and we therefore used entire fetal membranes in subsequent experiments.

![FIGURE 1](image1.png)

**FIGURE 1.** Pharmacological characterization of cAMP-PDE families in amnion and chorion. Amnion and chorion were separately assayed for cAMP-PDE activity. IBMX (5 × 10⁻⁴ M) was used as a nonspecific inhibitor, and rolipram (10⁻⁵ M), cilostamide (10⁻³ M), and 8-methoxy-IBMX (10⁻⁵ M) were used to inhibit PDE4, PDE3, and PDE1 families, respectively. cGMP (10⁻⁵ M) was used to inhibit PDE3 and to activate PDE2. Data are means ± SEM of six separate experiments performed in duplicate. Significance: *, p < 0.05; **, p < 0.01, compared with total cAMP-PDE activity.

Effect of LPS on cAMP-PDE4 and non-PDE4 activities

To determine whether PDE4 activity was affected in amniochorionic explants treated by LPS, the PDE4 activity was measured at different times of treatment. As shown in Fig. 2, PDE4 activity increased after 2 h of LPS exposure, reached a maximum after 4 h (near 2-fold increase), and then declined gradually to baseline after 24 h. The non-PDE4 activity (rolipram-resistant PDE activity) increased slightly but failed to be significant, suggesting that the effect of LPS on cAMP-PDE activity is limited to PDE4 activity.

![FIGURE 2](image2.png)

**FIGURE 2.** Time course of the LPS effect on PDE4 and non-PDE4 activities. Amniochorionic explants were incubated with LPS (1 ng/ml) for 2–24 h. PDE4 activity was gauged as the fraction of cAMP PDE activity that was inhibited by 10 μM rolipram, and non-PDE4 activity was estimated as the remaining cAMP PDE activity. All values represent the mean ± SEM of six separate experiments performed in duplicate. Significance: *, p < 0.05; ***, p < 0.001, compared with control (mean ± SEM of all controls measured at each time point; this value did not change during the experiment).
Effect of rolipram on LPS-induced PGE\textsubscript{2} production and COX-2 expression

To determine whether PDE4 inhibitors regulated PG synthesis, PGE\textsubscript{2} was measured in the supernatants of amniochorionic explants after LPS treatment. Incubation of fetal membranes with LPS caused a 6-fold increase in PGE\textsubscript{2} release, and this increase was significantly attenuated by rolipram (Fig. 5A). To examine whether the inhibitory effect of rolipram on LPS-induced PGE\textsubscript{2} synthesis was due to COX-2 induction, we performed immunoblot analysis with a COX-2-specific Ab. As illustrated in Fig. 5B, LPS increased the intensity of the 72-kDa band corresponding to COX-2 protein (25). In the presence of rolipram, the intensity of the band fell markedly, reflecting inhibition of LPS-induced COX-2 expression. Rolipram alone also induced COX-2 expression, but to a lesser extent than LPS.

Effect of PDE inhibition on LPS activation of pro-MMP-9

The ability of PDE3, PDE4, and PDE5 inhibitors to modulate LPS-treated fetal membrane extracellular matrix remodeling was examined by measuring the pro-MMP-9 activity by means of zymography in the supernatants of amniochorionic explants. The results shown in Fig. 6A show that incubation with LPS resulted in a 2-fold increase in pro-MMP-9 activity. The PDE4 inhibitors rolipram and cilomilast inhibited \( \approx 50\% \) of the pro-MMP-9 activity induced by LPS, whereas the other PDE inhibitors had no significant effect. Fig. 6B shows a representative zymography gel with a band at 92 kDa corresponding to pro-MMP-9 protein (5). The intensity of the 92-kDa band increased in the presence of LPS, and this increase was attenuated by the addition of rolipram.

**FIGURE 4.** Effect of 8-Br-cAMP and H89 on rolipram inhibition of LPS-induced TNF-\( \alpha \) release. Amniochorionic explants were incubated with vehicle or LPS (1 ng/ml) for 24 h in the presence or absence of rolipram (10\textsuperscript{-5} M) and/or H89 (10\textsuperscript{-5} M) or 8-Br-cAMP (10\textsuperscript{-5} M). Results are expressed as a percentage of basal TNF-\( \alpha \) production (0.12 \pm 0.03 pg/mg wet weight). All values represent the mean \pm SEM of three independent experiments performed in duplicate. Significance: * \( p < 0.05 \), compared with LPS alone (vehicle).

**FIGURE 5.** Effect of rolipram on LPS-induced PGE\textsubscript{2} production and COX-2 expression. Amniochorionic explants were incubated with vehicle or LPS (1 ng/ml) for 24 h in the presence or absence of rolipram (10\textsuperscript{-5} M) for 24 h. A, The PGE\textsubscript{2} concentration in supernatants was measured by enzyme immunoassay. All values represent the mean \pm SEM of four separate experiments performed in duplicate. Significance: * \( p < 0.05 \), compared with LPS alone (vehicle).
Effect of rolipram on IL-10 release induced by LPS

Because IL-10 has been described to modulate proinflammatory cytokine release, we examined whether rolipram enhanced IL-10 release in response to LPS. IL-10 release was measured in the supernatants of amniochorionic explants stimulated with LPS for various times in the presence or absence of rolipram. As shown in Fig. 7, IL-10 production increased after 4 h of LPS stimulation and continued to increase at 24 h. When rolipram was added, IL-10 release was significantly stronger than in the presence of LPS alone, for up to 12 h.

To determine whether the effect of rolipram on LPS-induced IL-10 production is cAMP dependent, LPS-induced IL-10 release was measured in the presence of cAMP-elevating agents (forskolin or 8-Br-cAMP). As shown in Table I, both agents significantly increased LPS-induced IL-10 production.

Effect of anti-IL-10 Ab on rolipram inhibition of LPS-induced TNF-α release

To examine whether IL-10 is involved in the anti-inflammatory effect of rolipram, IL-10 in explant supernatants was neutralized with a specific Ab. As shown in Table II, the anti-IL-10 Ab increased LPS-induced TNF-α release in a concentration-dependent manner. This effect reflects the neutralization of the negative constraint exercised by IL-10 on TNF-α release in response to LPS. However, increasing concentrations of anti-IL-10 Ab did not affect the inhibitory effect of rolipram on TNF-α production, suggesting that IL-10 is not essential for rolipram inhibition of LPS-induced TNF-α release.

Discussion

During ascending intrauterine infection by Gram-negative bacteria, LPS triggers a local inflammatory response that participates in PPROM. Host recognition of the LPS motif requires interactions with specific receptors and downstream signaling pathways that involve several protagonists. In this study, we present evidence that the PDE4 family, specific for cAMP inactivation, is involved in regulating this inflammatory process.

We first detected cAMP-PDE activities in the amnion and chorion, in keeping with a study by Vesce et al. (26), who reported a similar level of total cAMP-PDE activity in the amnion. The pharmacological profile of the cAMP-PDE families present in the amnion and chorion, based on the effects of selective inhibitors on total cAMP hydrolysis, revealed the presence of at least four

Table I. Effect of 8-Br-cAMP and forskolin on LPS-induced IL-10 release

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>8-Br-cAMP</th>
<th>Forskolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.18 ± 0.01</td>
<td>0.43 ± 0.01</td>
<td>0.37 ± 0.10</td>
</tr>
<tr>
<td>LPS</td>
<td>0.54 ± 0.10</td>
<td>0.96 ± 0.30</td>
<td>1.30 ± 0.30</td>
</tr>
</tbody>
</table>

* Amniochorionic explants were incubated with vehicle or LPS (1 ng/ml) for 8 h in the presence or absence of 8-Br-cAMP (10⁻⁵ M) or forskolin (10⁻⁷ M). Data (picograms/milligram tissue) are means ± SEM of three separate experiments performed in duplicate.

### Significance:

* $p < 0.05$, compared to LPS alone.
cAMP-PDE families, with a marked predominance of the PDE4 family, in both tissues. To mimic chorioamnion infection, we used an amniochorionic explant culture model and found that LPS selectively enhanced PDE4 activity. This LPS-induced PDE4 activity, already reported in mouse macrophages (27), suggests the involvement of the PDE4 family in the LPS-induced inflammatory signaling pathway of fetal membranes.

Concurrently, our data revealed that the LPS signaling cascade was affected by selective inhibition of PDE4 activity. The first step of the inflammatory response triggered by LPS is the activation of inflammatory genes such as TNF-α. Interestingly, TNF-α release into amniochorionic supernatants in response to LPS was markedly inhibited by the selective PDE4 inhibitors rolipram and cilomilast to the same extent as the nonspecific PDE inhibitor IBMX, whereas PDE1, PDE3, and PDE5 inhibitors were ineffective. This anti-inflammatory effect of PDE4 inhibitors may be partly explained by the presence of inflammatory cells in the explants. Indeed, infection of fetal membranes is associated with recruitment of leukocytes such as neutrophils and monocytes (28, 29). These cells cooperate with amnion and chorion cells at the site of infection, amplifying the inflammatory response and increasing TNF-α release. PDE4 family accounts for most if not all cAMP-hydrolyzing activity in leukocytes, and PDE4 inhibitors suppress their activation (30). Thus, the effect of PDE4 inhibitors on LPS-induced TNF-α release by amniochorionic explants may be partly due to PDE4 inhibition in leukocytes. However, this does not exclude specific involvement of the PDE4 family in amnion and chorion cells in the regulation of LPS signaling. Such a hypothesis is currently under investigation in our laboratory.

Although an inhibitory effect of PDE4 inhibitors on LPS-induced TNF-α release by inflammatory cells has already been described (14, 16), their mechanism of action is largely unknown. In this study, we showed that the cell-permeable cAMP analog, 8-Br-cAMP, inhibited LPS-induced TNF-α release, whereas the PKA inhibitor, H89, did not affect the ability of rolipram to reduce TNF-α release. Such results strongly suggest that the effect of the PDE4 inhibitor, rolipram, on TNF-α release is dependent on a cAMP elevation but does not involve a PKA intervention. Such PKA-independent pathway had already been described by Jacob et al. (31), who demonstrated that PDE4 inhibitors exert their inhibitory effect on IL-1β-induced O2− production through MAPK activation in rat brachioalveolar lavages enriched in neutrophils. Also, we postulated that PDE4 inhibitors might exert their inhibitory effect on TNF-α release via the anti-inflammatory cytokine IL-10, well described for its anti-inflammatory properties in fetal membranes (9, 10). We found that rolipram potentiated LPS-induced IL-10 release. This up-regulation may be due to cAMP elevation following rolipram exposure, because cAMP-elevating agents such as forskolin and 8-Br-cAMP also increased IL-10 release by LPS-treated amniochorionic explants. However, IL-10 neutralization with a specific Ab did not reverse the effect of rolipram, indicating that IL-10 is not essential for this inhibitory effect. Our results contrast with those of a study performed in murine macrophages, in which an anti-IL-10 Ab blocked the inhibitory effect of rolipram on LPS-induced TNF-α release (14). However, in human monocytes, rolipram attenuates LPS-induced GM-CSF release through an IL-10-independent mechanism (32). The involvement of IL-10 in the inhibitory effect of rolipram may therefore differ according to the cell type studied.

Compelling data from clinical and ex vivo studies point to PGs and MMPs as key effectors in PPROM, and we therefore investigated the role of the PDE4 family in the regulation of these mediators after LPS activation. Bacterial products affect PG synthesis by fetal membranes (9, 33–35), and TNF-α produced by fetal membranes in response to LPS is a crucial modulator of PG production (36). In this study, we found that rolipram decreased LPS-induced PGE2 release and COX-2 expression, showing, for the first time, PDE4 inhibition of PG synthesis initiated by LPS. However, a very recent study showed that rolipram can block COX-2 induction and PG production in activated T cells (37). The authors reported that COX-2 inhibition by rolipram occurred mainly at the transcriptional level, through NFAT inhibition, and appeared to be cAMP/PKA independent. In our study, treatment of amniochorionic explants with rolipram alone induced slight COX-2 expression, which did not appear sufficient to enhance PGE2 production. Induction of COX-2 expression by cAMP-elevating agents has already been described (38, 39). This induction is mediated by cAMP/PKA-dependent phosphorylation of the transcription factor CREB, resulting in enhanced transcription of COX-2. In amniochorionic explants, the presence of LPS outweighed the effect of rolipram alone, suggesting either competition among transcription factors as seen with glucocorticosteroid receptors and CREB (40) or an effect of rolipram upstream of transcription in LPS signaling.

In addition to regulating LPS-induced proinflammatory cytokine release and PG synthesis, PDE4 family members also participate in fetal membrane extracellular matrix remodeling. We found that PDE4 inhibitors inhibited pro-MMP-9 activity induced by LPS, whereas PDE1, PDE3, and PDE5 inhibitors had no effect. This emphasizes the selective involvement of the PDE4 family in LPS-induced pro-MMP-9 synthesis. Because rolipram inhibition of pro-MMP-9 release was associated with changes in pro-MMP-9 mRNA expression, we suspect that regulation occurs at a transcriptional level. Similar modulation of MMP-9 expression by PDE4 inhibitors has been described in bronchoalveolar lavage fluid of mice exposed to LPS (17) and also in human fetal lung fibroblasts cultured with TNF-α in three-dimensional collagen gels (18), but the mechanisms of their inhibitory effects are unclear. Previous studies have suggested the involvement of a PGE2/cAMP-dependent pathway in MMP-9 regulation (41, 42). COX-2 induction by LPS is involved in the signal transduction pathway leading to MMP-9 production in monocytes (43), and PGE2 stimulation increases MMP-9 secretion by fetal membrane explants (44). The ability of PDE4 inhibitors to block MMP-9 production may therefore be related to an inhibition of PGE2 due to suppression of

### Table II. Effect of anti-IL-10 Ab on rolipram inhibition of LPS-induced TNF-α release**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>LPS</th>
<th>LPS + Rolipram</th>
<th>Rolipram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.04 ± 0.01</td>
<td>16.33 ± 2.55</td>
<td>0.91 ± 0.06</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Anti-IL-10 (10 ng/ml)</td>
<td>0.05 ± 0.02</td>
<td>18.14 ± 1.90</td>
<td>2.65 ± 0.52</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Anti-IL-10 (100 ng/ml)</td>
<td>0.02 ± 0.01</td>
<td>19.60 ± 3.50</td>
<td>1.58 ± 0.35</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>Anti-IL-10 (1000 ng/ml)</td>
<td>0.63 ± 0.59</td>
<td>21.03 ± 3.00*</td>
<td>2.80 ± 0.44</td>
<td>0.05 ± 0.03</td>
</tr>
</tbody>
</table>

**Ammiochorionic explants were incubated with vehicle or LPS (1 ng/ml) for 24 h in the presence or absence of rolipram (10−5 M) and/or an anti-IL-10 antibody at the indicated concentrations. Data (pg/mg tissue) are means ± SEM of three separate experiments performed in duplicate.

* Significance: p < 0.05, compared to LPS alone.
COX-2. However, the participation of other signaling pathways cannot be excluded, because MAPK may also be involved in LPS induction of MMP-9 (41) and because cAMP elevation can disrupt the MAPK signaling pathway (45). PDE4 inhibitors, by increasing intracellular cAMP levels, might alter LPS-induced MMP-9 expression through a MAPK-dependent pathway, although this remains to be demonstrated.

Pharmacological interventions aimed at preventing preterm labor generally target molecules involved in myometrial contraction. However, clinical studies largely demonstrated that the current therapies remain disappointing, and infection-induced preterm labor is refractory to conventional treatments (46). Given the role of inflammation in infection-induced preterm labor, some new strategies focus on anti-inflammatory molecules. Nonsteroidal anti-inflammatory drugs that target COX activity may be effective, but therapeutic promises of COX-2 inhibitors have been tempered by their fetal adverse effects (47). Pharmaceutical companies are developing selective PDE4 inhibitors for their anti-inflammatory and myorelaxant properties, and a new generation of PDE4 inhibitors such as cilomilast and roflumilast are undergoing clinical development in bronchopulmonary disorders (48). In this study, we showed that the PDE4 family is strongly involved in regulating the inflammatory process induced by LPS treatment of fetal membranes. PDE4 inhibition led to a decrease in TNF-α release, concomitantly with an increase in IL-10 release. In addition, PDE4 inhibitors by decreasing PGE release would protect myometrium from its contractile effect and by inhibiting pro-MMP-9 expression would contribute to prevent PPROM. These findings, in addition to our previous work showing an evident role of the PDE4 family in the control of uterine motility (19, 20, 49) open up new therapeutic possibilities for the management of infection-induced preterm labor.

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Disclosures

The authors have no financial interest of interest.

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