Anti-Inflammatory and Utero-Relaxant Effects in Human Myometrium of New Generation Phosphodiesterase 4 Inhibitors

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ABSTRACT

The anti-inflammatory and uterine-relaxant effects of two potent phosphodiesterase 4 (PDE4) inhibitors of the latest generation: cilomilast (one of the most advanced PDE4 inhibitors in clinical development, reportedly more selective for PDE4D) and compound A (which displays 12-fold greater selectivity toward PDE4B and/or PDE4A than toward PDE4D) were evaluated in human uterine smooth muscle. We first established that these compounds exhibit greater efficacy in inhibiting total cAMP-PDE activity in pregnant versus nonpregnant myometrium (Emax = 78.0% ± 3.6% and 80.3% ± 2.2% in pregnant versus 57% ± 4.7% and 70.5% ± 5.9% in nonpregnant women for compound A and cilomilast, respectively; P < 0.05 for both compounds), confirming the prominent participation of PDE4 isoforms in cAMP hydrolysis in the near-term pregnant myometrium. Using pregnant myometrial explants, we have shown that both these drugs and also rolipram, the prototype PDE4 inhibitor, produce concentration-dependent inhibition of lipopolysaccharide (LPS) induced tumor necrosis factor alpha (TNFα) release with similar potency in each case (pD2 = 8.0 ± 0.5, 7.9 ± 0.2, and 7.6 ± 0.2 for compound A, cilomilast, and rolipram, respectively). The maximum inhibition produced is 65%. Pre-treatment with forskolin or 8-bromo-cAMP mimics the PDE4 inhibitor effect. Furthermore, compound A and cilomilast both produce concentration-dependent inhibition of the spontaneous contractions of myometrial strips and are more potent in pregnant than in nonpregnant myometrium (pD2 = 7.3 ± 0.7 and 8.1 ± 0.3 in pregnant versus 6.2 ± 0.9 and 6.6 ± 0.1 in non-pregnant myometrium for compound A and cilomilast, respectively; P < 0.05 for both compounds). This demonstrates that the PDE4 isoforms involved in the mechanism of contraction are different in the pregnant and nonpregnant myometrium. Our study highlights the importance of developing PDE4 inhibitors for the pharmacological management of infection-induced preterm labor.

INTRODUCTION

Preterm delivery remains the main cause of perinatal morbidity and mortality. Intrauterine infection is the most common pathological process for which a firm causal link with prematurity has been established, and for which a specific mechanism has been identified [1]. Microorganisms invade the chorionic decidua and activate the decidua, the fetal membranes, and the uterine smooth muscle or myometrium, leading to a local inflammation and the subsequent release of cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL1β). Modifications in the concentrations of these pro-inflammatory cytokines induce the massive synthesis and release of the uterotonic agents (e.g., prostaglandins, endotherhins) that stimulate uterine contractions and induce the onset of labor [2, 3].

The ubiquitous second messenger, cAMP, is involved in a large array of physiological processes. The onset and progression of the inflammatory response are sensitive to changes in the steady-state level of this cyclic nucleotide [4–6]. Cyclic AMP is also implicated in the mechanisms governing smooth muscle motility and in preventing the induction of contraction and its maintenance [7]. Methods of increasing the cAMP concentration in inflammatory and smooth muscle cells have been actively pursued with a view to developing new therapeutic agents.

Cyclic nucleotide phosphodiesterase (PDE) enzymes are responsible for cyclic nucleotide inactivation. PDEs form a superfamily, which comprises at least 11 distinct families [8]. The PDE4 family, which specifically hydrolyzes cAMP, is predominantly expressed in inflammatory cells and in smooth muscles such as those of the airways and myometrium [9, 10]. Interest in PDE4 as a molecular target for anti-inflammatory and myorelaxant drugs has increased greatly in recent years, particularly for the treatment of several inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), where the main goal is to down-regulate the production of cytokines by activated leukocytes [11, 12]. Numerous studies have demonstrated that selective PDE4 inhibitors [13–15] modulate inflammatory cell activation. However, the therapeutic promise of these compounds has been tempered by their significant side effects, particularly nausea and emesis. To improve their therapeutic index, one strategy currently being explored takes advantage of the fact that the PDE4 family is composed of four subtypes, PDE4A to PDE4D. Due to the presence of multiple promoters and to alternative splicing of the genes, multiple variants have been generated and classified into two main groups, the long and the short forms [8]. This multiplicity of PDE4 isoenzymes, which are expressed and regulated differently, suggests that if the functionally relevant subtype can be identified and selectively targeted, it might be possible to develop compounds with better side-effect profiles and with similar or better efficacy.

We have previously shown that PDE4 isoforms are involved in the contraction/relaxation process of human myo-

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metrium. This conclusion was based on the observation that rolipram, the prototype PDE4 selective inhibitor, completely relaxes the spontaneous contraction of myometrial strips [16, 17]. The four PDE4 subtypes are expressed in human pregnant and nonpregnant myometrium and, interestingly, the level of PDE4B2, a short-form product of the PDE4B gene, is greater in near-term pregnant myometrium than in nonpregnant tissue [18, 19]. We have recently shown that this PDE4B2 isoform is up-regulated in human myometrial cells by pro-inflammatory cytokines [20], suggesting that this isoform is involved in the inflammatory response that occurs in human myometrium during the early stages of labor or during infection-induced preterm deliveries.

In view of these data, the present study was conducted using a model involving myometrial explants from pregnant and nonpregnant women in order to evaluate the anti-inflammatory and relaxant properties of two PDE4 inhibitors: compound A, which displays 12-fold greater selectivity for PDE4B and/or PDE4A than for PDE4D [21], and cilomilast (SB 207499), one of the most advanced PDE4 inhibitors currently undergoing clinical development [22], which is 10 times more selective for PDE4D than for PDE4A, B, or C. The anti-inflammatory and relaxant properties of these compounds were compared with their ability to inhibit cAMP PDE activity.

MATERIALS AND METHODS

Biological Samples

Myometrium biopsies were obtained from pregnant women delivered by elective caesarian section prior to the onset of labor (38th and 40th weeks of pregnancy) because of a diagnosed cephalo-pelvic disproportion by elective caesarian section prior to the onset of labor (38th and 40th weeks of pregnancy) due to in-utero fetal distress. Written informed consent was obtained from all donors. This study was approved by the CCPPRB Ethics Committee (Paris-Cochin, France).

Cyclic AMP Phosphodiesterase Assays

Myometrial tissue was homogenized (100 mg/ml), using an Ultra-Turrax apparatus (Ika-Werke, Staufen, Germany), in ice-cold homogenization buffer: 100 mM Tris/HCl (pH 7.4), 2 mM MgSO4, 2 mM EDTA, 10% glycerol, and 1 mM β-mercaptoethanol. This was supplemented with a protease inhibitor cocktail: leupeptin (1 μM), aprotinin (10 μg/ml), Pefabloc (25 μg/ml), benzamidine (130 μg/ml), and soybean trypsin inhibitor (50 μg/ml). The PDE assay was then performed. Cyclic AMP PDE activity was determined using the Kincaid and Manganiello method [23]. Specific activities were measured in high-affinity conditions with 1 μM [3H]cAMP as the substrate (Amersham, Aylesbury, UK) in the absence or presence of increasing concentrations of selective PDE4 inhibitors added 10 min before the beginning of the reaction. We dissolved the inhibitors in dimethylsulfoxide (DMSO) as a 10-2 M stock solution, which was further diluted to provide a range of concentrations used in the assays. The basal cytokine level detected in the supernatants of the myometrial explants was 0.4 ± 0.1 pg/ml and 0.17 ± 0.04 pg/mg wet weight in pregnant and nonpregnant myometrium, respectively.

Measurement of the Concentration of TNFα

We determined the levels of TNFα in the supernatants of myometrial explants by enzyme-immunoassay (ELISA) according to the manufacturer’s instructions (Cayman Chemicals, Ann Arbor, MI). The sensitivity of the assay was less than 1.5 pg/ml, and both the interassay and intraassay coefficients of variation were less than 10%. The basal cytokine level detected in the supernatants of the myometrial explants was 0.4 ± 0.1 pg/ml and 0.17 ± 0.04 pg/mg wet weight in pregnant and nonpregnant myometrium, respectively.

In Vitro Contractile Studies

Segments of myometrium (8–12 mm × 2–3 mm) were suspended in parallel for isometric tension recordings using Bioscience UF1 tension transducers (Marty Technologie, Marcilly sur Eure, France) in 5-ml organ baths containing aerated (95% O2:5% CO2) Krebs buffer (11.1 mM glucose, 6.2 mM KCl, 144 mM NaCl, 2.5 mM CaCl2, 0.5 mM MgCl2, 1 mM NaH2PO4, 30 mM NaHCO3) maintained at 37°C. We applied an optimum resting tension of 1500 mg to each segment, and spontaneous muscle tone was allowed to develop. The myometrial strips, after equilibrating for 2 h in Krebs solution, were washed every 1 min, exhibiting spontaneous contractile activity with regular frequency and intensity. Concentration–relaxation curves were constructed for the cumulative addition of selective PDE4 inhibitors (final concentration of 10-10 to 3.10-5 M) at intervals of two periods (currently 10 min). Only one concentration–response curve was recorded for each strip. Strips showing unstable responses or which did not recover (i.e., did not display regular contractions after washing several times at the end of experiments) were discarded. The data were processed by the Powerlab/8e Software package (ADInstruments Ltd., Hastings, UK). Areas under the tension curve were measured for a given time. Results are expressed as a percentage of total relaxation, the baseline contractions corresponding to 0% relaxation. Total abolition of contractions (100% relaxation) was produced by 10-4 M of rolipram, as previously reported [16]. Controls that were treated with DMSO at the concentrations used to test cumulative additions of PDE4 inhibitors showed that this had no effect on contractility.

Materials

The drugs used were rolipram, a gift from Schering Health Care Ltd. (Burgess Hill, West Sussex, UK); cilomilast and compound A (trans-4-[2-amino-4-oxo-5-phenyl-1,3-thiazolidin-4-yl]-4-[3-(cyclopentoxy)–4-methoxyphenyl]-ethylylidene-1-amine) [21], a gift from Glaxo-Wellcome (King of Prussia, PA). We purchased all other reagents from Sigma (St. Louis, MO), except for Pefabloc, which was supplied by Interchim (Montluçon, France).

Data Analysis

We performed concentration-response curve analysis using Prism v. 3.00 (GraphPad Software, Inc., San Diego, CA). PD2 values correspond to −log [EC50], where EC50 is the concentration of drug that produces 50% of the maximum effect, and Emax is the maximum response that can be produced by the highest concentration of the drug used. Statistical comparisons between pregnant and nonpregnant groups were carried out using a one-way ANOVA. Differences in potencies and maximum effects were determined by post hoc tests using the Bonferroni correction. The t-test was applied for paired and unpaired data as appropriate. All differences were considered significant when P < 0.05.

RESULTS

Effects of Compound A or Cilomilast on cAMP PDE Activity

We evaluated the effects of compound A and cilomilast on the cAMP PDE activity of myometrial tissue from pregan-
FIG. 1. Inhibitory effects of compound A and cilomilast on cAMP-PDE activity in myometrial tissue from pregnant and nonpregnant women. Aliquots of myometrial homogenates from pregnant and nonpregnant women were assayed in the absence and presence of increasing concentrations of compound A (A) or cilomilast (B). Results are reported as the percentage of cAMP PDE activity (specific activity in nonpregnant tissue homogenates, 130.2 ± 3.7 pmol min⁻¹ mg⁻¹; specific activity in pregnant tissue homogenates, 86 ± 15.5 pmol min⁻¹ mg⁻¹). Aliquots from the same samples were assayed in parallel with both drugs. Data show the mean ± SEM of four experiments with homogenates from four pregnant and four nonpregnant women.

The inhibition curves of compound A fitted a sigmoid monophasic model in pregnant and nonpregnant women. Both drugs produced a concentration-dependent inhibition of the cAMP-PDE activity in both pregnant and nonpregnant myometrial homogenates (Fig. 1).

The inhibition curves of compound A fitted a sigmoid monophasic model in pregnant and nonpregnant tissue (Fig. 1A), with equivalent potency values found for both tissues (Table 1). The concentration-response curves obtained with cilomilast in pregnant and nonpregnant tissue fitted a two-phase model better (Fig. 1B). This reflects the ability of this compound to inhibit at least two different entities: the first entity was inhibited in the nanomolar range (pD₂ = 8.7 in pregnant and 8.6 in nonpregnant myometrium), and the second, which has much less affinity, in the micromolar range (pD₂ = 6.4 in pregnant and 6.2 in nonpregnant myometrium; Table 1). These two potencies were equivalent in pregnant and nonpregnant tissue.

The compound A and cilomilast concentration curves were significantly different in pregnant and nonpregnant tissues (ANOVA, P < 0.01 for compound A and P < 0.02 for cilomilast). The efficacy of the drugs depended on the origin of the sample: the maximum effects induced by compound A and cilomilast in pregnant tissues were significantly higher than the effects induced in myometrium taken from nonpregnant women (Table 1).

LPS-Induced TNFα Secretion in Myometrial Explants

In order to determine the optimum concentration of LPS required to obtain the greatest release of TNFα, we constructed a concentration-response curve up to 20 μg/ml LPS for myometrium tissues obtained from pregnant and nonpregnant women. As shown in Figure 2, LPS induced the release of TNFα in the supernatant of the explant in a

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Compound A</th>
<th>Cilomilast</th>
<th>Cilomilast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eₘₐₓ</td>
<td>pD₂</td>
<td>Eₘₐₓ</td>
</tr>
<tr>
<td>Pregnant</td>
<td>78.0 ± 3.6</td>
<td>8.7 ± 0.1</td>
<td>80.3 ± 2.2</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>57.0 ± 4.7b</td>
<td>8.6 ± 0.2</td>
<td>70.5 ± 5.9b</td>
</tr>
</tbody>
</table>

* Aliquots of myometrial homogenates were assayed for cAMP-PDE activity in the presence or absence of increasing concentrations of either compound A or cilomilast. Data show the mean ± SEM of four experiments for compound A and for cilomilast concentration response curves with myometrial homogenates from four pregnant and four nonpregnant women.

b P < 0.05, significantly different from values obtained with pregnant samples.
concentration-dependent manner, peaking at 10 μg/ml after 6 h of treatment. At all the concentrations tested, the release of TNFα induced by LPS was significantly greater in the pregnant than nonpregnant explants. Measurements in the supernatant of explants incubated for 24 h with 10 μg/ml LPS demonstrated that both pregnant and nonpregnant tissues showed a significant reduction in the concentration of TNFα as compared with the values found after incubating for 6 h (Table 2). Under the optimum condition of LPS treatment (incubation for 6 h at 10 μg/ml), TNFα release was at least 2.5 times higher in the supernatant of pregnant explants than those of nonpregnant tissues.

**Effects of Compound A, Cilomilast, and Rolipram on the LPS-Induced Production of TNFα in Myometrial Explants**

To investigate the anti-inflammatory properties of the PDE4 inhibitors on human myometrium, we evaluated the effects of increasing concentrations (10^{-9} to 10^{-5} M) of compound A, cilomilast, and rolipram on LPS-induced TNFα release in pregnant myometrial explants.

All three drugs reduced the production of TNFα in the supernatant of myometrial explants incubated with LPS; this reached a maximum inhibition of about 65% at 10^{-5} M, the highest concentration tested (Fig. 3). They all induced comparable concentration-dependent inhibition, and there was no statistically significant difference between the pD2 values obtained for the three inhibitors (Table 3).

The effects of cyclic AMP elevating agents, forskolin and 8-Br-cAMP, were investigated under the same experimental conditions in an attempt to clarify the involvement of a cAMP-dependent mechanism in the inhibition of LPS-induced TNFα. As shown in Figure 4, forskolin and 8-Br-cAMP markedly decreased the LPS-stimulated elevation of TNFα measured after 6 h of treatment.

**Effects of Compound A or Cilomilast on the Spontaneous Contractions of Myometrial Strips**

We investigated the effects of compound A and cilomilast on the spontaneous contractions of myometrial strips obtained from pregnant and nonpregnant women. The cumulative addition of either compound A (Fig. 5A) or cilomilast (Fig. 5B) ranging from 10^{-10} to 3.10^{-5} M, induced concentration-dependent inhibition of the spontaneous contractions of myometrial strips obtained from either pregnant or nonpregnant women.

For both compounds, the curves for the pregnant tissue were significantly shifted to the left (ANOVA, P < 0.05). Compound A and cilomilast were more potent in pregnant than in nonpregnant myometrial tissue (Table 4).

**DISCUSSION**

The present study demonstrates that two PDE4 inhibitors of the latest generation have potent anti-inflammatory properties in human myometrium, in addition to fully inhibiting spontaneous contractions of myometrial strips. Moreover, because these drugs discriminate between distinct PDE4 isoforms, our results indicate that the PDE4s involved in the myometrial contraction during late pregnancy differ from those involved in the nonpregnant uterus.

We first established the pharmacological properties of compound A and cilomilast on cAMP-PDE activity in pregnant and nonpregnant myometrium. Both of these selective inhibitors of PDE4 exhibit greater efficacy on the pregnant than nonpregnant myometrium. These data are consistent with the findings of our earlier study of rolipram and RP 73401, two archetypal PDE4 inhibitors, which demonstrate-

**TABLE 2.** Time-response analysis of LPS-induced TNFα release using myometrial tissues obtained from either pregnant or nonpregnant women.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pregnant</th>
<th>Nonpregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.4 ± 0.10</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>LPS 10 μg/ml</td>
<td>7.0 ± 0.80⁹</td>
<td>2.6 ± 0.60⁶,⁷</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.06</td>
<td>0.1 ± 0.02</td>
</tr>
</tbody>
</table>

⁹ Explants of pregnant or nonpregnant myometrium were incubated with 10 μg/ml of LPS for 6 h and 24 h. Data (pg/mg wet weight) are the mean ± SEM of four separate experiments using myometrial explants from four pregnant and four nonpregnant women.

⁶ P < 0.001 vs. basal.

⁷ P < 0.01 vs. pregnant myometrium.

⁸ P < 0.05 vs. 6 h.

**FIG. 3.** Inhibitory effects of compound A, cilomilast, and rolipram on LPS-induced TNFα release from myometrial tissue obtained from pregnant women. Explants of pregnant myometrium were incubated with 10 μg/ml LPS for 6 h in the absence or presence of increasing concentrations of each of the PDE4 inhibitors. Results are expressed as a percentage of the basal TNFα production (0.4 ± 0.1 pg/mg wet weight). All values represent the mean ± SEM of five separate experiments from myometrial explants from five different women.

**TABLE 3.** Maximum effect and potency values of compound A, cilomilast, or rolipram on LPS-induced TNFα release from myometrial explants obtained from pregnant women.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>E_max (pg/mg)</th>
<th>pD2 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound A</td>
<td>65.8 ± 5.2</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>59.0 ± 4.3</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Rolipram</td>
<td>68.2 ± 6.0</td>
<td>7.6 ± 0.2</td>
</tr>
</tbody>
</table>

⁸ Explants of pregnant myometrium were incubated with 10 μg/ml LPS for 6 h in the absence or presence of increasing concentrations of each PDE4 inhibitor. All values represent the mean ± SEM of five separate experiments using explants of myometrium from five different pregnant women.
ed an increase in the participation of PDE4s in cAMP hydrolysis in the near-term myometrium [19, 26].

Compound A and cilomilast both inhibit myometrial PDE activity in a concentration-dependent manner and display a mean potency in the rank of order of their potencies in inhibiting human PDE4 recombinant variants [21]. Although the nonlinear regression analysis favors a one-site interaction model, rather than a two-site model for compound A, the curve is shallow, reflecting the ability of this compound to discriminate between different PDE4s in a weakly selective manner. The cilomilast curve fits a two-site model better, with the curve exhibiting a composite slope. It is worth noting that this compound, one of the most advanced PDE4 inhibitors currently undergoing clinical trials [22], appears to interact in a complex manner with PDE4s in vitro. The extensive study of Hatzelmann and Schudt [27] comparing the abilities of cilomilast and other PDE4 inhibitors to inhibit the function of various types of leukocytes revealed the wide range of potency of cilomilast with active concentrations ranging from 40 to 6000 nM. The authors proposed that this difference could result from the pharmacokinetics of this compound, and particularly from its strong interaction with plasma-binding proteins. However, in our enzyme assay, the samples were diluted, minimizing this effect. In addition, the complex behavior of cilomilast cannot be explained by the presence of the two conformations of PDE4, with a low and high affinity rolipram-binding site, respectively (LPDE4 and HPDE4), in the myometrium [19] because cilomilast inhibits these two conformations with equal potency [28]. Further studies are needed to characterize in detail the interactions between cilomilast and different PDE4 variants. Nonetheless, no difference in apparent potency was detected between pregnant and nonpregnant tissues for these two compounds, suggesting that the inhibitors have the same targets in both tissues. However, because of the small difference in potency between PDE4A/4B and PDE4D, more selective compounds are needed for a quantitative study.

In order to evaluate the anti-inflammatory properties of the PDE4 inhibitors, we used a model of myometrial explants exposed to LPS. LPS is an active component of the gram-negative bacteria wall and induces preterm parturition in mice [29, 30]. It has also been isolated from the amniotic fluid of women during preterm labor [31]. Using our model, we observed that more TNFa was produced in response to LPS by the pregnant than nonpregnant myometrium, indicating that pregnant tissue was more sensitive to this stimulating agent. This finding is consistent with the fact that gestational tissues are potential sources of pro-inflammatory cytokines [32] and highlights the ability of the myo-

FIG. 4. Effects of forskolin and 8-Br-cAMP on LPS-induced TNFa release on myometrium obtained from pregnant women. Explants were incubated with the vehicle (basal), forskolin (10^{-5} M), or 8-Br-cAMP (10^{-5} M) in the absence or presence of 10 µg/ml LPS for 6 h. Data (pg/mg wet weight) are the mean ± SEM of three separate experiments using myometrial explants from three different pregnant women. *P < 0.01 versus LPS 10 µg/ml.

FIG. 5. Inhibitory effects of compound A and cilomilast on the contractile activity of myometrial strips from pregnant and nonpregnant women. Increasing concentrations of compound A (A) or cilomilast (B) were added to myometrial strips from pregnant and nonpregnant women. The results are expressed as the percentage of total relaxation. Strips from the same patients were subjected in parallel to both PDE4 inhibitors. Data are the mean ± SEM of eight experiments using myometrial strips from eight nonpregnant and eight pregnant women.

TABLE 4. Maximum effect and potency values of compound A and cilomilast on the spontaneous contractile activity of myometrial strips.a

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Compound A</th>
<th>Cilomilast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E_{max}</td>
<td>pD_{2}</td>
</tr>
<tr>
<td>Pregnant</td>
<td>100.0 ± 0.1</td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>92.5 ± 4.3</td>
<td>6.2 ± 0.9b</td>
</tr>
</tbody>
</table>

a The contractile activity of isolated myometrial strips was measured in the absence or presence of increasing concentrations of either compound A or cilomilast. Data are the mean ± SEM for myometrial strips isolated from eight nonpregnant and eight pregnant women.

b P < 0.05, significantly different from values obtained using pregnant samples.
metrium to contribute to the inflammatory process that occurs during parturition.

The three PDE4 inhibitors tested, compound A, cilomilast, and rolipram, all produced equipotent inhibition of LPS-induced TNFα that was comparable with their effects observed in human monocytes [14, 27, 28, 33]. Their order of potency is consistent with their efficacy in inhibiting cAMP-PDE activity, indicating that PDE4 is directly implicated in the inflammatory response of the infected myometrium.

The mechanism by which PDE4 inhibitors suppress the release of TNFα is uncertain. Our data show that increasing cAMP levels, by adding 8-Br-cAMP or by stimulating the adenylyl cyclase by forskolin to LPS-stimulated myometrium, reduced TNFα production. This finding is consistent with the concept that the inhibition of TNFα production by PDE4 inhibitors is due to the maintenance of a cAMP constraint on LPS signaling [34]. It has been reported that 8-Br-cAMP or prostaglandin E1 reduces the amount of mRNA for TNFα, which indicates that cAMP or cAMP-elevating agents (including PDE4 inhibitors) regulate the production of this cytokine at the transcriptional level [14, 33, 35]. The presence of an AP1/CRE-like promoter sequence in the human TNFα gene suggests that protein kinase A-dependent regulation of cAMP response transcription factors may be involved [36]. The effect of PDE4 inhibitors on TNFα transcription may also be attributed to their ability to inhibit NFκB, a transcription factor implicated in LPS-induced stimulation of TNFα expression [37, 38]. Furthermore, PDE4 inhibitors could also inhibit TNFα production by increasing the level of the anti-inflammatory cytokine, IL-10 [13, 35].

It has now been demonstrated that first-generation PDE4 inhibitors such as rolipram and RP 73401 are potent and efficient inhibitors of spontaneous in vitro myometrial contractions [16, 19]. The maximum relaxation obtained with these PDE4 inhibitors is 2.5 times greater than that produced by salbutamol, a β2-adrenoceptor agonist commonly used in the clinical management of preterm labor [17]. In the present study, we have shown that compound A and cilomilast were both able to inhibit totally the spontaneous contraction of pregnant and nonpregnant myometrium. The observation that compound A and cilomilast are more potent in pregnant than in nonpregnant tissue suggests that there is a difference in the participation of various PDE4 isozymes in the mechanism of contraction in these two states of the myometrium. This difference may be explained by the massive influx of leukocytes, such as neutrophils or macrophages, which occurs in the uterine tissue around the time of parturition [39]. In leukocytes, which are important sources of utero-modulators such as pro-inflammatory cytokines, PDE4s are also very important for the hydrolysis of cAMP and are functionally involved in the production and/or secretion of these leukocyte products [10]. Thus the inflammatory state of pregnant myometrium may involve a specific PDE4 isoform, which is also implicated in the mechanism necessary for the transition of the uterine smooth muscle from the quiescent state to a contractile state that allows parturition to occur. One can speculate that this isoform is the PDE4B isoform, because the level of PDE4B2, a short-form product of the PDE4B gene detected in near-term pregnant myometrium, is higher in pregnant than in nonpregnant tissue [19]. It has now also been reported that PDE4B2 is a specific variant involved in regulating the inflammatory response [34, 40, 41]. Furthermore, we have recently shown that PDE4B2 is upregulated by IL-1β in human myometrial cells [20]. Further studies are underway to evaluate this hypothesis.

Pharmacological interventions in preterm labor attempt to target various protagonists involved in the signaling pathway leading to myometrial contractions. Beta-adrenergic agonists, oxytocin receptor antagonists, or cyclooxygenase-2 inhibitors are the most commonly used tocolytic agents, but none has clearly demonstrated any real clinical benefit and/or is often associated with severe maternal and fetal side effects [42]. Another strategy would be to target the cytokines, which play a central role in the biochemistry of preterm labor. By lowering their levels, it would be possible to dampen the inflammatory cascade leading to preterm contractions. The twofold ability of cAMP to mediate smooth muscle cell relaxation and to down-regulate inflammatory and immune cell activation has led to an increased interest in PDE4 as a molecular target for potential drugs.

Our findings regarding the anti-inflammatory and utero-relaxant effects of new PDE4 inhibitors on the human pregnant myometrium highlight the importance of developing such compounds for the pharmacological management of infection-induced preterm labor. Promising data from clinical trials with cilomilast, one of the most advanced PDE4 inhibitors currently undergoing clinical development for COPD, support the concept that the side effects of this class of drugs can be separated from their therapeutic benefits [43]. Furthermore, knowing that the PDE4B4 isoform is upregulated and may be implicated in the control of the uterine motility and inflammatory process at the end of pregnancy suggests that selective inhibitors of this isoform would be promising candidates.

ACKNOWLEDGMENTS

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REFERENCES


