Pregnancy Induces a Modulation of the cAMP Phosphodiesterase 4-Conformers Ratio in Human Myometrium: Consequences for the Utero-Relaxant Effect of PDE4-Selective Inhibitors

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ABSTRACT

The inhibitory impacts of RP 73401, a phosphodiesterase type 4 (PDE4) selective inhibitor of the second generation, versus rolipram, the prototypal PDE4 inhibitor, were evaluated and compared on cAMP phosphodiesterase (PDE) activity and contractility of the myometrium in nonpregnant and pregnant women. In enzymatic studies, RP 73401 and rolipram inhibited the cAMP PDE activity with significantly greater maximal efficiency in the myometrium of pregnant compared with nonpregnant women (75 versus 55%; P < .05). Although myometrial PDE4 presented a single class of interaction with RP 73401 [pD2 (−log IC50) = −8.2], it exhibited at least two classes of interaction with rolipram (pD2 = −8.2 and −5.6). In the myometrium of pregnant versus nonpregnant women, rolipram is significantly more efficacious in the concentration range >0.01 to 100 μM (P < .01), whereas no difference was observed for the concentration range <0.01 μM. In contractility studies, RP 73401 was equally effective in relaxing myometrial strips from both nonpregnant and pregnant women (pD2 = −8.8). Conversely, the ability of rolipram to inhibit contractions of the myometrium in pregnant women was significantly lower (pD2 = −7.2) compared with that in nonpregnant women (pD2 = −8.2; P < .01). Concomitantly, in the myometrium of pregnant women, a rise in immunoreactive PDE4B2 signal was detected, whereas the PDE4D3 signal was less intense. These results demonstrate that parallel to an accumulation of PDE4B2 isoform, a modification in the ratio of PDE4 conformers HPDE4 and LPDE4 (conformer that binds rolipram with high and low affinity, respectively) occurs in the myometrium of near-term pregnant women with an increase of LPDE4 functionally implicated in the contractile process. Such modifications provide a strong rationale to propose LPDE4 as potential pharmacologic targets for the design of new tocolytic treatments.

The last trimester of human pregnancy is crucial to the maturation of the fetus. The interruption of this process because of early delivery constitutes the major cause of perinatal morbidity and mortality. The underlying mechanism and causes of preterm labor are still poorly understood. However, an increasing body of data suggests that proinflammatory cytokines may constitute the final pathway toward term and preterm labor. This production of inflammatory mediators that trigger the cytokines/prostaglandins cascade leading to increased myometrial activity is implicated in the pathogenesis of infection induced-preterm labor (Radetsky, 1994).

Recently, the inhibitors of cAMP-specific phosphodiesterase type 4 (PDE4) have received much attention for their anti-inflammatory and myorelaxant properties (Teixeira et al., 1997). PDE4 belongs to the complex superfamily of phosphodiesterases, the sole enzymatic system responsible for the degradation of intracellular cyclic nucleotides. The PDE4 family comprises multiple enzymes derived from four distinct but related genes, 4A, 4B, 4C, and 4D, differentially expressed in various tissues and cell types (Conti et al., 1995; Houslay et al., 1998). PDE4 inhibitors are believed to provide therapeutic potential through selective elevation of intracellular cAMP. This ubiquitous second messenger is a key transducing molecule in numerous processes including modulation of proinflammatory mediators and muscle contraction. On the one hand, the accumulation of cAMP in immunocompetent cells, where PDE4 is the major cAMP-metabolizing enzyme, damp...
ens the production of proinflammatory cytokines, e.g., tumor necrosis factor α, interleukin-1, or interleukin-6 (Dent and Gi-embrycz, 1996). On the other hand, cAMP prevents the induction and maintenance of contraction through stimulation of cAMP-dependent protein kinase, which phosphorylates a range of proteins involved in the mechanism of smooth muscle contraction (Silver and Krafie, 1996). In human myometrium, we have found previously that among the PDE families, PDE4 is the predominant PDE isoenzyme class (Leroy et al., 1985, 1994). Moreover, myometrial PDE4 is involved in the relaxation/contraction process in that rolipram, the prototypical PDE4 inhibitor, exerts a potent relaxant effect in vitro on myometrial strips from pregnant women (Leroy et al., 1989).

However, despite their attractive properties, rolipram and other archetypal PDE4 inhibitors elicit numerous side effects including nausea, emesis, and acid gastric secretion when administered to experimental animals or in the clinic (Texeira et al., 1997; Torphy, 1998). It becomes obvious to refine the potential targets of PDE4 inhibitors linked to a specific function. We began to address this issue in human myometrium by using a molecular approach. Analyses of the expression pattern of the four PDE4 genes revealed that little PDE4A and PDE4C mRNA was detected and did not evolve at the end of pregnancy, whereas PDE4B and PDE4D subtypes was the most abundant and the PDE4B2 mRNAs steady-state level increased in late pregnancy (Leroy et al., 1999). Interestingly, data exist for a role of the PDE4B2 isoform in inflammation; this isoform is possibly regulated by infection-induced mechanisms (Ma et al., 1999) and appears to be a major isoform in immunocompetent cells (Wang et al., 1999).

Other criteria to finely manipulate PDE4 activity arose from the evidence that PDE4 isoforms can adopt more than one active conformation. They are distinguished pharmacologically by their relative sensitivity toward rolipram; one conformational state interacts with rolipram with high affinity in the nanomolar range (HPDE4), and the other exhibits a much lower affinity in the micromolar range (LPDE4) (Ja-cobitz et al., 1996). A new generation of compounds such as RP 73401 emerged that discriminate between the PDE4 conformational states or inhibit both conformations with equal affinity (Ashton et al., 1994; Souness et al., 1995). These new tools have identified the correlation of certain side effects with inhibition of HPDE4, whereas potential therapeutic effects are related to LPDE4 interaction in inflammatory processes (Souness and Rao, 1997).

In view of these data, we conducted this work primarily to evaluate the PDE4B and PDE4D protein variants in the myometrium of both nonpregnant women and pregnant women. Additionally, by using a comparative study of the inhibitory impact of RP 73401 versus rolipram, we investigated the relative ratio of PDE4 conformers responsible for cAMP PDE activity and involved in spontaneous contraction of the myometrium.

**Experimental Procedures**

**Biological Samples.** Uterine samples were obtained from non-pregnant cycling women who were undergoing hysterectomy because of benign uterine tumors. Myometrial strips from normal muscle (myometrial longitudinal layer) were dissected free of serosa at a distance from macroscopic abnormalities. Pathologic examination of the samples was performed to exclude adenomyosis or malignant change.

Biopsies of the myometrium were obtained from pregnant women who presented with normal uncomplicated pregnancies but who were delivered by elective caesarian section performed before the onset of labor between the 38th and 40th weeks of pregnancy, for previously diagnosed cephalopelvic disproportion. Myometrial strips were excised in the uterine body at the antiplacement site from the longitudinal layer and were immediately collected on ice. Written informed consent was obtained from all donors. This study was approved by the Comité Consultatif de Protection des Personnes pour la Recherche Biomédicale (Paris-Cochin, France).

**Preparation of Myometrial Homogenates.** Myometrial tissue was homogenized (200 mg/ml), using an Ultra-Turrax apparatus (Bioblock, Illkirch, France), in ice-cold homogenization buffer containing 100 mM Tris-HCl (pH 7.4), 2 mM MgSO4, 2 mM EDTA, 10% glycerol, and 1 mM β-mercaptoethanol, supplemented with a protease inhibitor cocktail containing leupeptin (1 μM), aprotinin (10 μg/ml), pefabloc (25 μg/ml), benzamidine (130 μg/ml), and soybean trypsin inhibitor (50 μg/ml). Homogenate was centrifuged for 10 min (10000g at 4°C) to remove the tissue debris and stored immediately at −80°C until use.

**Immunodetection of PDE4 Isozymes.** Immunoblotting was performed using K118 rabbit polyclonal antibodies raised against PDE4B subtype (kindly donated by Dr. M. Conti, Stanford University, Stanford, CA) (Iona et al., 1998) and 61D10E murine monoclonal antibodies designed to be specific for PDE4D isoforms (kindly donated by Dr. S. Wolda, ICOS Corp., Seattle, WA).

Samples (40 μg of protein/lane) were dissolved v/v in Laemmli buffer and boiled for 5 min before undergoing 8% polyacrylamide gel electrophoresis (PAGE) in the presence of SDS. After electrophoretic separation, proteins were transferred to a nitrocellulose membrane (Amersham, Aylesbury, UK) using a Bio-Rad Blottransblot apparatus (Bio-Rad, Richmond, CA). Blots were dried and then blocked for 1 h in 10% nonfat dried milk powder in Tris-buffered saline/Tween 20 (TBST; 10 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.6) at room temperature. Blocked membranes were washed three times with TBST. The blots then were incubated for 2 h at room temperature with the primary antibody (1:500 dilution of K118 or 1:10,000 dilution of 61D10E in TBST containing 1% nonfat dried milk powder). After three washes with TBST, blots then were incubated for 45 min with the appropriate horseradish peroxidase-linked secondary antibody and washed five times with TBST. Immunoreactive proteins were detected by chemiluminescence (ECL reagents; Amersham).

**cAMP-Phosphodiesterase Assay.** cAMP PDE activity was determined using the method of Kincaid and Manganiello (1988). Activities were measured in high-affinity conditions with 1 μM [3H]cAMP as substrate, in the absence or presence of an increasing dose of the selective inhibitors (10−10 to 10−4 M) added 10 min before the beginning of the reaction. The compounds were dissolved in 100% dimethyl sulfoxide (DMSO) as a 10−3 M stock and diluted in 1% DMSO to provide a range of concentrations for use in the assays; diluted DMSO was shown not to affect cAMP PDE activity in the concentrations used in this study. Specific activity was expressed in picomoles per minute per milligram of protein. Results were expressed as a percentage of control cAMP PDE activity. All assays were carried out in the linearity conditions with respect to time and protein concentration. Protein concentrations were determined using the Bio-Rad modified Bradford protein assay with BSA as a standard.

**In Vitro Contractile Studies.** Segments (8–12 × 2–3 mm) were suspended in parallel for isometric tension recordings using Bio-science UF1 tension transducers (Phymep, Paris, France), in 6 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 Na2HPO4, 30 mM NaHCO3) maintained at 35°C. An optimum resting tension of 600 mg was applied to each segment, and a spontaneous tone was allowed to develop. The myometrial strips,
after equilibration for 2 h in Kreb's solution with washing every 15 min, presented spontaneous contractile activity with regular frequency and intensity. Measurements were processed by Maclab/8e software package (ADInstruments Ltd, Hastings, UK). Concentration-relaxation curves were constructed with cumulative addition of selective PDE4 inhibitors (rolipram or RP 73401, 10⁻¹⁰ to 10⁻⁴ M, final concentration) at an interval of two periods (10 min). Only one concentration-response curve was recorded for each strip. Strips that showed unstable responses or that did not recover, i.e., did not present regular contractions after several washings at the end of experiments, were discarded. Areas under the tension curve were measured for a given time. Results are expressed as a percentage of maximum relaxation, basal contractions corresponding to 0% relaxation.

Comparison of the Inhibitory Effects of RP 73401 and Rolipram on cAMP PDE Activity. We investigated the effects of RP 73401, a second-generation PDE4 inhibitor, versus rolipram on the cAMP-PDE activity of myometrial tissues obtained from nonpregnant and pregnant women.

As presented in Fig. 2A, inhibition with RP 73401 of cAMP PDE activity from homogenates of either pregnant or nonpregnant myometrial tissues gave a typical sigmoid dose-response curve with a Hill coefficient close to unity (Table 1). Thus, RP 73401 appeared to obey simple competitive inhibition. Potency of the drug was equivalent in both tissues in the nanomolar range. However efficiency of the drug was significantly different according to the sample origin: the maximal effect observed on cAMP PDE activity from pregnant tissue was higher than that for cAMP PDE activity of tissue originating from nonpregnant women (Table 1). Assuming that RP 73401 is an equally potent competitive inhibitor of all PDE4 conformers (Souness et al., 1995), this indicates that the proportion of PDE4 activity is greater in pregnant than nonpregnant myometrium.

As demonstrated in Fig. 2B, the inhibition dose-curve for rolipram gave a shallow inhibition plot with a Hill coefficient of approximately 0.5 (Table 1) for both tissues. The dose-response curves appeared to be biphasic, and showed clearly at least two classes of interaction with rolipram, one with a high affinity in the nanomolar range (pD₂₀) and a second with a much lesser affinity in the micromolar range (pD₅₀). These observations indicate that the PDE4 family in human myometrium exhibits the two conformational states, HPDE4 and LPDE4. The maximal efficiency observed with rolipram...
was significantly greater in pregnant than in nonpregnant myometrium (Table 1), confirming the increase in PDE4 proportion responsible for the degradation of cAMP in the pregnant state. However, for inhibition at rolipram concentrations lower than 10 nM, which reflects inhibition of the PDE4 class with nanomolar sensitivity to rolipram, no significant difference was seen in either curve. The difference between the two plots was significantly greater for the higher concentrations of rolipram; the dose-curve of the pregnant myometrium was more pronounced in the micromolar range as compared with nonpregnant tissue. These findings suggest that a specific increase in the proportion of the PDE4 class with micromolar sensitivity to rolipram, i.e., LPDE4 conformers, occurs in the myometrium of pregnant women.

Comparison of the Relaxant Effects of RP 73401 and Rolipram on Spontaneous Contractions of Myometrial Strips. To further establish the role of PDE4 conformers in the regulation of myometrial contractility, the effects of RP 73401 versus rolipram were examined on contractions of myometrial strips from nonpregnant and pregnant women. RP 73401 or rolipram was added to the muscle baths in increments of 0.5 or 1 log units to achieve concentrations ranging between $10^{-10}$ and $10^{-4}$ M.

In isolated strips of myometrium from nonpregnant women, the inhibition was dose-dependent, reaching 100% inhibition at $10^{-6}$ M for both drugs (Fig. 3A). The potency of RP 73401 to relax the strips was correlated with its potency to inhibit myometrial cAMP PDE activity, in nanomolar concentration, suggesting that it produced its relaxant effect through inhibition of PDE4 (Table 2). Interestingly, no difference between pD2 values for RP 73401 and rolipram was observed. The potency of this latter compound was better correlated to a high-affinity interaction with PDE4 (nanomolar range), suggesting that rolipram induced relaxation through selective inhibition of HPDE4 in nonpregnant myometrium.

In isolated strips of myometrium from pregnant women, the relaxant effect of RP 73401 remained unchanged compared with nonpregnant strips (Fig. 3B). The potency of that compound was equivalent to that observed in nonpregnant strips and was correlated with its potency to inhibit cAMP PDE activity (Tables 1 and 2). Conversely, the rolipram concentration-response curve was significantly shifted to the right compared with the curve constructed with nonpregnant tissues (Fig. 3A). The ability of rolipram to induce relaxation in pregnant myometrial strips was significantly less (in the micromolar range) than the potency observed in nonpregnant strips. This difference reflects that rolipram-induced relaxation in pregnant myometrium correlates more with an interaction with LPDE4 than with HPDE4 conformers.

Discussion

In this study, we have identified a dramatic change in the expression of two PDE4 isoforms in the late-pregnant myometrium. The PDE4B2 protein is accumulated in this tissue, whereas PDE4D3 protein is reduced. Along with these data, we detected from the enzymatic studies a modification in the participation of the PDE4 isoforms in cAMP hydrolysis at the end of pregnancy. This modulation account for an increase in LPDE4 conformers, involved in the cAMP degradation, i.e., PDE4 conformers that bind rolipram with an affinity in the

Fig. 2. Inhibitory effects of RP 73401 and rolipram on cAMP PDE activity of myometrial homogenates. Aliquots of myometrial homogenates from either nonpregnant women (■) or pregnant women (□) were assayed in the absence or presence of increasing concentrations of RP 73401 (A) or rolipram (B). PDE activity and protein concentration were measured as detailed under Experimental Procedures. Results are given as percentages of control cAMP PDE activity (specific activity of nonpregnant homogenates: 128.2 ± 19.8 pmol/min/mg; specific activity of pregnant homogenates: 70.4 ± 18.8 pmol/min/mg). Aliquots from the same samples were assayed in parallel with both drugs. Curve-fitting was obtained with the Inplot computer software, which favors a one-site curve-fitting for inhibition with RP 73401 and a two-site curve-fitting over a one-site curve-fitting for inhibition with rolipram. Data represent the means ± S.E. of seven experiments with homogenates from different nonpregnant women and six experiments with homogenates from different pregnant patients. *P < .05 and **P < .01 significantly different from values obtained with nonpregnant samples.
micromolar range. Moreover, one of our more intriguing findings was the switch observed in the PDE4 conformers involved in the contractile process of the myometrium during pregnancy. We demonstrated that relaxation of nonpregnant myometrial strips induced by PDE4 inhibitors was rather linked to inhibition of HPDE4, whereas, surprisingly, the LPDE4 would be involved in the contractility of near-term myometrium.

The immunoblot analysis allowed the detection of a modification in the expression of at least two discrete PDE4 immunoreactive species, PDE4D3 and PDE4B2, the former with an apparent decreased expression and the latter with an increased expression in the late-pregnancy myometrium. This result is in agreement with our previous data where, by using a semiquantitative reverse transcription-polymerase chain reaction approach, we showed an increase in the mRNAs steady-state level of PDE4B2 in the late-pregnancy myometrium, whereas no significant change in the expression of the other PDE4 genes was detected (Leroy et al., 1999). Additionally, we have demonstrated in human myometrial cells in culture that the immunoreactive signal for the long-form PDE4D3 decreased on treatments of these cells with cAMP analogs, whereas no change was observed in the PDE4D3 mRNAs steady-state level of PDE4B2 in the late-pregnancy myometrium, whereas no significant change in the expression of the other PDE4 genes was detected (Leroy et al., 1999). Additionally, we have demonstrated in human myometrial cells in culture that the immunoreactive signal for the long-form PDE4D3 decreased on treatments of these cells with cAMP analogs, whereas no change was observed in the PDE4D3 mRNAs steady-state level, suggesting that posttranslational mechanisms affect the PDE4D3 protein expression. Concomitantly, the short-form products of PDE4B and PDE4D genes, PDE4B2, PDE4D1, and PDE4D2, respectively, were shown to be inducible by cAMP-elevating agents in human myometrial cells (Méhats et al., 1999). These observations, in addition to our present results, suggest that in human myometrium PDE4B2 expression may be up-regulated by numerous factors that are known to act through modulation of the intracellular cAMP level, e.g., catecholamines and prostanoids. Furthermore, a putative function of PDE4B2 in the inflammatory process has been emphasized in human monocytes, where an induction of this variant by an interleukin-10-inhibitable signal transduction pathway was observed on endotoxin stimulation (Ma et al., 1999). These data highlight the potential participation of PDE4B2 in the adaptive process mechanisms that occur during the last trimester of pregnancy in human myometrium.

In the aim of determining whether modification of PDE4 expression pattern may have repercussions in cAMP degradation in the pregnant myometrium, we investigated the comparative effect of two PDE4 inhibitors, rolipram and RP 73401, on the cAMP PDE enzymatic activities. With both PDE4-selective inhibitors, we observed a rise in the participation of PDE4 isoforms in cAMP hydrolysis in the late-pregnant myometrium. Among the other PDE families identified in near-term myometrium, PDE4 isotypes gain importance, reaching almost 75% of cAMP PDE, although they represent only 55% in the nonpregnant state. These data are consistent with our previous results, which demonstrated a modification in the proportion of the different myometrial PDE isoforms during pregnancy (Leroy et al., 1987). Actually, initial DEAE-cellulose chromatography procedures had shown the presence of a peak of crude PDE in the nonpregnant state. In myometrium of pregnant women, an additional peak of PDE activity, which contained mostly cAMP-specific rolipram-sensitive PDE, has been isolated. These nonindividualized forms in this second peak were also recovered at the 32nd week of pregnancy, although not at the 16th week (Leroy et al., 1987; Leroy et al., 1994). This modification is of current interest and concern for clinical purpose because it occurs during the third trimester of pregnancy, a period when tocolytic therapeutics are needed.

To characterize pharmacologically this change in PDE4 proportion with the gestational state, we examined the potency of the two selective compounds to inhibit cAMP PDE activity. On the one hand, rolipram can inhibit differentially, at least, two subclasses of PDE4, one with a nanomolar sensitivity and the other with a 10- to 100-fold lower affinity, the so-called HPDE4 and LPDE4 conformers, respectively (Torphy et al., 1992; Jacobitz et al., 1996). On the other hand, RP 73401, a second generation inhibitor, does not discriminate between the two PDE4 conformers, and thus equally inhibits PDE4 in both conformations. The analyses of the dose effects for inhibition with rolipram of myometrial cAMP PDE activity suggested the presence of both PDE4 conformers. Indeed, nonsigmoid dose-response curves for rolipram that fit a model of two-states interaction were obtained in nonpregnant and pregnant myometrium. This is consistent with the existence, in this tissue, of two PDE4 subclasses that interact with rolipram with sensitivities that differ over 100-fold molar concentration. Moreover, the ratio of LPDE4 conformers increased in near-term myometrium, as suggested by the comparison of the shape of the dose-response curves in the micromolar range for rolipram.

So far, no data indicate that in whole cells a PDE4 isoform presents a defined conformational status. Studies in mammalian cell expression system have demonstrated that all PDE4 isoforms derived from the four genes can display the two types of sensitivity to inhibition by rolipram (Huston et al., 1996; Jacobitz et al., 1996; Bolger et al., 1997; Owens et al., 1997; Rocque et al., 1997). For a recombinant PDE4C isoform, differences in sensitivity to inhibition by rolipram have been observed depending on the cell system used (Owens et al., 1997). Furthermore, PDE4B2 has been shown to display both sensitivities to inhibition by rolipram when expressed in baculovirus (Rocque et al., 1997). Conversely, the relevant conformational state of the isoenzyme obviously
differ among various functions in tissues and cell types. For example, correlation has been demonstrated between inhibition of HPDE4 conformers and inhibition of acid secretion in rabbit gastric glands (Barnette et al., 1995), or between high affinity interaction with rolipram and emesis in dogs (Heaslip and Evans, 1995). In the central nervous system, PDE4 isoforms appear mostly in HPDE4 conformation and may be involved in psychotropic effects of rolipram (Schneider et al., 1997). Nevertheless, rolipram inhibition can serve as a detector of changes in PDE4 conformation, which provides a basis to design more selective compounds directed to a specific function.

In view of these data, we investigated the potency of rolipram to induce relaxation of myometrial strips compared with that of RP 73401. In nonpregnant strips, rolipram was as potent as RP 73401 to promote relaxation. This observation was consistent with results of other groups in airway smooth muscle, in which RP 73401 and rolipram were equipotent in relaxing tissues toned with contractile agents (Raeburn et al., 1994), and suggested an implication of HPDE4 conformers in the contractile process of nonpregnant myometrium. Surprisingly, in pregnant myometrium, the contractions were inhibited with a much higher dose of rolipram, whereas RP 73401 was still very potent to promote relaxation. These data indicate that, near-term, PDE4 are still potent in relaxing tissues toned with contractile agents (Raeburn et al., 1994), and suggested an implication of HPDE4 conformers in the contractile process of nonpregnant myometrium. However, the results obtained with RP 73401 were not significantly different from those obtained with nonpregnant strips.

FIG. 3. Inhibitory effects of RP 73401 and rolipram on contractile activity of myometrial strips. Increasing concentrations of RP 73401 (■) or rolipram (□) were added to myometrial strips from either nonpregnant women (A) or pregnant women (B) every two periods. Results are expressed as percentage of total relaxation. Strips from the same patients were subjected in parallel to both PDE4 inhibitors. For more details in the procedures, see Experimental Procedures. Data are the means ± S.E. for myometrial strips isolated from eight different nonpregnant women and six different pregnant patients. *P < .05 and **P < .01 significantly different from values obtained with RP 73401.

TABLE 2

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* Nonsignificantly different from values obtained with RP 73401.

** Nonsignificantly different from values obtained with nonpregnant samples.

† Significantly different (P < .01) from values obtained with nonpregnant samples.

‡ Significantly different (P < .01) from values obtained with RP 73401.

human monocytes (Souness et al., 1996) or in proliferation of T-cells (Essayan et al., 1994). Thus, the PDE4 conformational state is a characteristic of PDE4 isoforms that is derived more likely from a biological process and/or cell background than from an intrinsic property due to their amino acid sequences. This intriguing question remains to be answered. Nevertheless, rolipram inhibition can serve as a detector of changes in PDE4 conformation, which provides a basis to design more selective compounds directed to a specific function.

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References


