Biochemical Pharmacology, Vol. 38, No. 1, pp. 9–15, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
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CORRELATION BETWEEN SELECTIVE INHIBITION OF THE CYCLIC NUCLEOTIDE PHOSPHODIESTERASES AND THE CONTRACTILE ACTIVITY IN HUMAN PREGNANT MYOMETRIUM NEAR TERM

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(*Received* 5 *November* 1987; *accepted* 28 *June* 1988)

Abstract—The present study was carried out to determine the ability of various pharmacological agents to selectively inhibit each cytosolic form of phosphodiesterase isolated from the longitudinal layer of human myometria near term. Among the drugs tested, zaprinast specifically inhibits the first form of PDE which hydrolyses both substrates (cAMP and cGMP) and is stimulated by the Ca^{2+}-calmodulin complex. A second form of PDE specific for cAMP hydrolysis and Ca^{2+}-calmodulin insensitive is only present during pregnancy. Rolipram is the most potent and selective inhibitor of this second form. It is also the most efficient compound to inhibit *in vitro* the spontaneous contractions of near term myometria. The double effect of rolipram suggests an important role of the second form of PDE in the mechanisms of contractility during the pregnancy. In addition rolipram or other derivatives might be of a therapeutic interest in the prevention of prematurity in so far as they are devoid of undesirable maternal and fetal side effects.

In humans, the maintenance of pregnancy as well as the onset of labor appear to depend on a multifactorial system. Premature birth, which constitutes the major cause of neonatal morbidity and mortality, remains one of the most obscure problems in obstetrics. Several therapies including various relaxant drugs have been successively used and at present β-mimetics are the most extensively administered. Little information is available concerning the biochemical mechanisms whereby the interaction of β-adrenergic agonists with their specific receptors promotes relaxation. Nevertheless it appears that the cAMP‡ messenger system plays a determinant role. Intracellular levels of cAMP result from two enzymatic activities: synthesis by adenylate cyclase and hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). During the third trimester of pregnancy, the period when tocolytic therapeutics are generally administered, we have demonstrated in human myometrium the presence, in a low density, of high affinity β-adrenergic receptors, positively coupled to adenylate cyclase [1–3]. However, although β-agonists were relatively efficient in inhibiting uterine contractions, various reports have shown that continuous exposure of human myometrium to β-agonists results in desensitization and a loss of permanent relaxation [4]. In addition, β-mimetic therapies are not devoid of undesirable maternal and fetal side effects. For these different reasons, another approach was to prescribe a PDE inhibitor such as aminophylline alone [5] or associated to a β-mimetic [6] for the treatment of premature labor.

PDE constitutes a complex enzymatic system and different molecular forms have been described in various tissues [7, 8]. In human myometrium, we had previously found that most of the cyclic nucleotide PDE activity was in the cytosolic fraction [9]. Using the current DEAE–cellulose chromatographic method, we have demonstrated the presence in myometrium of non-pregnant women of a single form of PDE, characterized by its lack of substrate specificity and its calcium–calmodulin dependence [10]. A second form, specific of cAMP hydrolysis and calcium–calmodulin insensitive was found during the third trimester of pregnancy. This latter is predominant near term [9]. It seems that these two forms are independently controlled.

The present work was undertaken to determine the physiological responsibilities of each form of PDE in the control of uterine motility during pregnancy. We examined the selective inhibitory effect of various compounds on the two enzymatic forms isolated from near term myometrium. In other tissues, these agents have previously been classified either as non-specific (e.g. IBMX, papaverine, etc.) or as selective inhibitors of one of the forms (e.g. rolipram, zaprinast, cilostamide, etc.) [11]. The effect of several of these selective inhibitory drugs on the spontaneous contractile activity of myometrial strips has been tested *in vitro*.

Previous studies have described myometrium as a

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‡ Abbreviations used: cAMP, adenosine 3′:5′-cyclic monophosphate; cGMP, guanosine 3′:5′-cyclic monophosphate; PDE, 3′:5′-cyclic nucleotide phosphodiesterase; EGTA, ethylene glycol bis (β-amino ethyl ether) *N,N,N′,N′* tetraacetic acid; IBMX 3-isobutylmethylxanthine.

heterogeneous tissue. It consists of several layers which differ in their embryological origin and histological contractile and pharmacological properties [1, 12, 13]. In the present study we have performed PDE investigations only in the longitudinal (outer) layer which exhibited near term several characteristics similar to those of the whole myometrium during labor [14].

MATERIALS AND METHODS

Chemicals. 8-[^{3}H]cAMP (sp. act. 26 Ci/mmol), 8-[^{3}H]cGMP (sp. act. 18 Ci/mmol), [^{14}C]adenosine (sp. act. 500 Ci/mol) and [^{14}C]guanosine (sp. act. 525 Ci/mol) were supplied by the Radiochemical Center (Amersham, U.K.). Cyclic nucleotides (cAMP, cGMP), 5′-nucleotides (5′-AMP, 5′-GMP), snake venom (*Crotalus atrox*), DEAE–cellulose (0.88 mEq/g coarse mesh), EGTA, bovine serum albumin (fraction V), and calmodulin were obtained from Sigma (St Louis, MO) and anion exchange resin AG1-X2 (200–400 mesh) from Bio-Rad (Richmond, VA). PDE inhibitors: IBMX (3-isobutyl-methylxanthine), papaverine, aminophylline and dipyridamole were purchased from Sigma. Rolipram (ZK 62711) was a gift from Schering and zaprinast (MB 22948) from May & Baker. Cilostamide and valeramide (AAL 05) were generously donated by Cl. Lugnier and J. C. Stoclet (U-243 INSERM, ERA 787 CNRS, Strasbourg, France). The other reagents used were of the highest grade commercially available.

Biological samples. Myometrial tissue was obtained from women who presented normal uncomplicated pregnancy but were delivered by elective cesarean section performed prior to the onset of labor, between the 38th and 40th weeks of pregnancy, for previously diagnosed cephalopelvic disproportion. Tissue samples were excised in the uterine body at the anti-placental site from the longitudinal layer and were immediately collected on ice. They were dissected free of serosa and quickly frozen at −80° for PDE investigations or immediately placed in Krebs solution for tension measurements. This procedure received the approval of the ethical committee of the "Institut National de la Santé et de la Recherche Médicale" (INSERM).

PDE preparations. Three pools of myometria were used for these experiments. Each one contained five myometria obtained from five different pregnant women. Myometrial tissues were homogenized (60 mg wet wt/ml) in ice-cold medium (2×10^{-3} M $MgSO_4$, 2×10^{-2} M Tris–HCl, pH 7.5) using a ground-glass Potter–Elvehjem apparatus. The homogenate was centrifuged at 105,000 *g* for 2 hr at 4° and the supernatant (cytosolic fraction) was applied onto a DEAE–cellulose column previously equilibrated with the homogenization buffer (2×10^{-3} M $MgSO_4$, 2×10^{-2} M Tris–HCl, pH 7.5). The column was then washed with about 50 ml of the same medium and eluted with 400 ml of 0–0.5 M $(NH_4)_2SO_4$ exponential gradient, pH 7.5, at a flow rate of 0.3 ml/min. Fractions of 6.5 ml were collected in tubes containing 0.165 ml of bovine serum albumin (40 mg/ml) and assayed for PDE activities. In order to eliminate $(NH_4)_2SO_4$ the two different peaks collected were dialyzed for 8 hr with three changes of buffer (2×10^{-3} M $MgSO_4$, 2×10^{-2} M Tris–HCl, pH 7.5), fractionated into aliquots and stored at −80°. No detectable loss of enzymatic activity of the different peaks was observed after 2 months at −80°.

PDE assay. Cyclic nucleotide PDE activities were measured by the two-step isotopic method [15] as previously described for cAMP hydrolysis [16] and modified for cGMP hydrolysis [17]. The reaction mixture contained 5×10^{-3} M $MgSO_4$, 8×10^{-2} M Tris–HCl (pH 8.0), 0.3 μCi of 8-[^{3}H]cAMP or 8-[^{3}H]cGMP, unlabeled cyclic nucleotides at concentrations previously defined as high affinity conditions (1×10^{-6} M cAMP, 1×10^{-7} M cGMP) [9], and enzymatic preparation in a final volume of 0.2 ml. All assays were conducted in triplicate and were carried out in conditions of linearity with respect to time and protein concentration. Blank values were determined by adding a heat-inactivated enzymatic preparation to the assay mixture and were subtracted from the experimental values. Results were expressed as picomoles of cyclic nucleotide hydrolyzed per 30 min per 0.1 ml of eluted fraction.

IBMX, papaverine and aminophylline were dissolved in distilled water; rolipram, cilostamide and valeramide were dissolved in 1% dimethylsulfoxide, zaprinast and dipyridamole in 0.04 N NaOH and 0.04 N HCl, then neutralized in distilled water. Appropriate concentrations of solvents were used for controls. The IC_{50} values (concentration which produced 50% inhibition of substrate hydrolysis) for the various agents examined were determined from the concentration–response curves (1×10^{-7} to 1×10^{-3} M).

Tension measurements. Myometrial tissues were immediately placed in Krebs solution (see composition below) previously bubbled with 95% O_2–5% CO_2 and maintained at 4° until used. In general six myometrial strips (8–12 mm long by 2–3 mm in cross-section) from each muscle piece were suspended in a 20 ml organ bath containing Krebs solution pH 7.4, at 37°, continuously gassed with 95% O_2–5% CO_2. The composition of the Krebs solution is as follows (mM): NaCl 114.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7 and ascorbic acid 1.1. Isotonic contractions were measured using a Grass FTO3 transducer connected to a Grass 79B polygraph [18, 19]. The preparations were allowed to equilibrate for 2 hr, during which time passive tension was set at 2 g by repeated adjustments. After this period, when spontaneous contractions became regular in frequency and intensity, inhibitory cumulative concentration–response curves were determined with each compound studied: papaverine, aminophylline, rolipram, and zaprinast. Drugs were added directly into the bath. All concentrations given are final bath concentrations. Since in general 3–4 hr were necessary for the construction of a cumulative dose–response curve, only one complete curve was obtained for each strip. Each inhibitor was tested on three different myometrial preparations. The maximal initial contraction obtained in each individual experiment was taken as 100% and all contractions were calculated as a function of this value. The

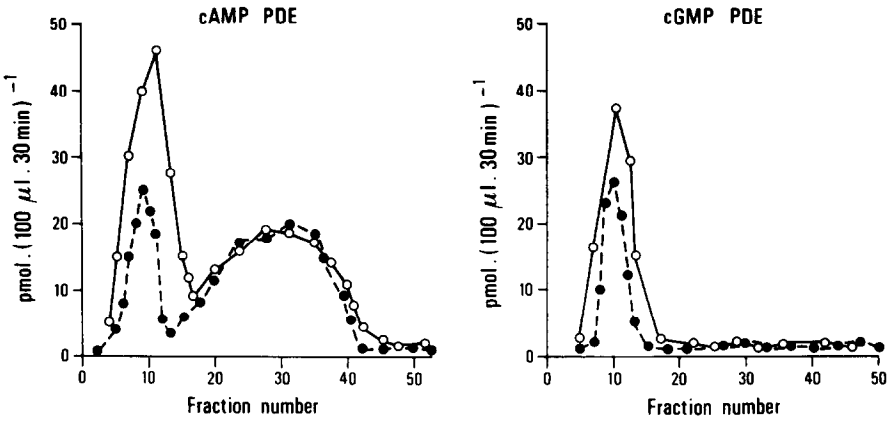

Fig. 1. DEAE–cellulose chromatography of the soluble fraction (105,000 *g* supernatant fraction) from a pool of myometria obtained at the end of pregnancy. 130 mg of protein were applied onto a 15 × 0.9 cm column DEAE–cellulose previously equilibrated with the homogenization medium (2×10^{-3} M $MgSO_4$, 2×10^{-2} M Tris–HCl, pH 7.5). The column was developed with the same medium containing an exponential gradient (0–0.5 M) of $(NH_4)_2SO_4$. PDE activities were assayed with 1×10^{-6} M cAMP and 1×10^{-7} cGMP in the prosence of either 1×10^{-3} M EGTA (●) or 1×10^{-5} M $CaCl_2$ and 0.5 µg calmodulin (○). Each point was the mean of triplicate determinations. Activities recovered from the columns were approximately 78% for cAMP and cGMP PDE in the presence of EGTA and 106% in the presence of Ca^{2+}–calmodulin. The same elution profiles were reproduced with three enzymatic preparations originating from three different pooled myometria.

inhibitory potency was determined at the level of IC_{50} values. Controls were made with each solvent used for the dilution of the four inhibitors.

RESULTS

Chromatographic analysis of cytosolic myometrial PDE activities

PDE activity of the cytosolic fraction is resolved by DEAE–cellulose chromatography into two peaks (Fig. 1).

The first peak (peak I) which eluted at low ionic strength hydrolyzes both substrates cAMP and cGMP. It is stimulated about two-fold in the presence of 1×10^{-5} M Ca^{2+} and 2.5 µg/ml of bovine heart calmodulin.

The second peak (peak II) which eluted at high ionic strength is highly selective for cAMP hydrolysis and is not stimulated by the Ca^{2+}-calmodulin complex. To characterize more thoroughly this second form, we have tested the effect of cGMP (1×10^{-8} to 1×10^{-3} M) on the cAMP PDE activity. Any significant inhibition of PDE is observed before 5×10^{-5} M cGMP, at 1×10^{-4} M only 20% of inhibition is obtained (data not shown).

Similar elution profiles had previously been obtained in studies of individual myometria [9]. These results legitimize the use of pools of myometria to test the inhibitory effect of pharmacological agents on PDE activity.

Inhibition of the PDE activity of chromatographically separated peaks

The effects of different PDE-inhibitors are evaluated on the two dialysed peaks of PDE activity present in human myometria near term. Table 1 summarizes the IC_{50} values determined for the different agents in these conditions.

The reference inhibitors IBMX and papaverine are found to exert relatively potent but non-selective inhibitory effects on all forms of PDE, although the effect of papaverine on peak I is less than on peak II. Aminophylline also exerts a weak non-selective effect particularly for cAMP PDE inhibition with an IC_{50} value greater than 3×10^{-4} M.

For the other drugs, claimed to be as "selective inhibitors", our system showed that cilostamide and valeramide do not have a clearly selective effect on the cAMP PDE activities of the two peaks but appears to exert preferential inhibition of cGMP PDE in peak I, with IC_{50} values in the 5×10^{-5} M range. In contrast, zaprinast exhibits the most potent effect on cAMP and cGMP hydrolysis of peak I, with IC_{50} values of about 1×10^{-5} M. For this peak, the two other compounds, cilostamide and valeramide, are only active in the 5×10^{-4} M range. For peak II, dipyridamole and rolipram are the most potent inhibitors of cAMP PDE. Rolipram is the more selective, with an IC_{50} value in the micromolar range, whereas it is the least potent on PDE of peak I, showing IC_{50} values greater than 10^{-3} M.

Figure 2 represents the concentration–response curves determined for papaverine, aminophylline, zaprinast and rolipram on cAMP and cGMP PDE activities of the two peaks. Here the low efficiency of aminophylline and the non-selective but effective inhibition of papaverine appear more clearly. Zaprinast and rolipram exhibit selective and potent inhibitory effects respectively on peak I (Figs 2A and 2B) and peak II (Fig. 2C). These four inhibitory drugs were chosen to test their ability to decrease spontaneous contractile activity of myometrial strips.

Table 1. Effects of PDE inhibitors on the two separated peaks of PDE activity isolated from a pool of myometria

	IC_{50} (μM)		
	Peak I		Peak II
Inhibitors/substrates	Cyclic AMP	Cyclic GMP	Cyclic AMP
IBMX	11.9	4.4	25.1
Papaverine	8.7	7.5	2.9
Aminophylline	320.0	30.2	630.4
Cilostamide	630.9	72.4	199.5
Valeramide	363.1	25.1	362.8
Zaprinast	13.5	3.3	316.2
Rolipram	>1000.0	>1000.0	7.6
Dipyridamole	158.0	79.2	47.8

The IC_{50} values were determined from the concentration–response curves, in which concentrations of inhibitors ranged from 1×10^{-7} to 1×10^{-3} M. Substrate concentrations were 1×10^{-6} M cAMP or 1×10^{-7} M cGMP. Results are representative of three determinations obtained on three different enzymatic preparations. The four underlined drugs are the inhibitors which were used in contraction experiments.

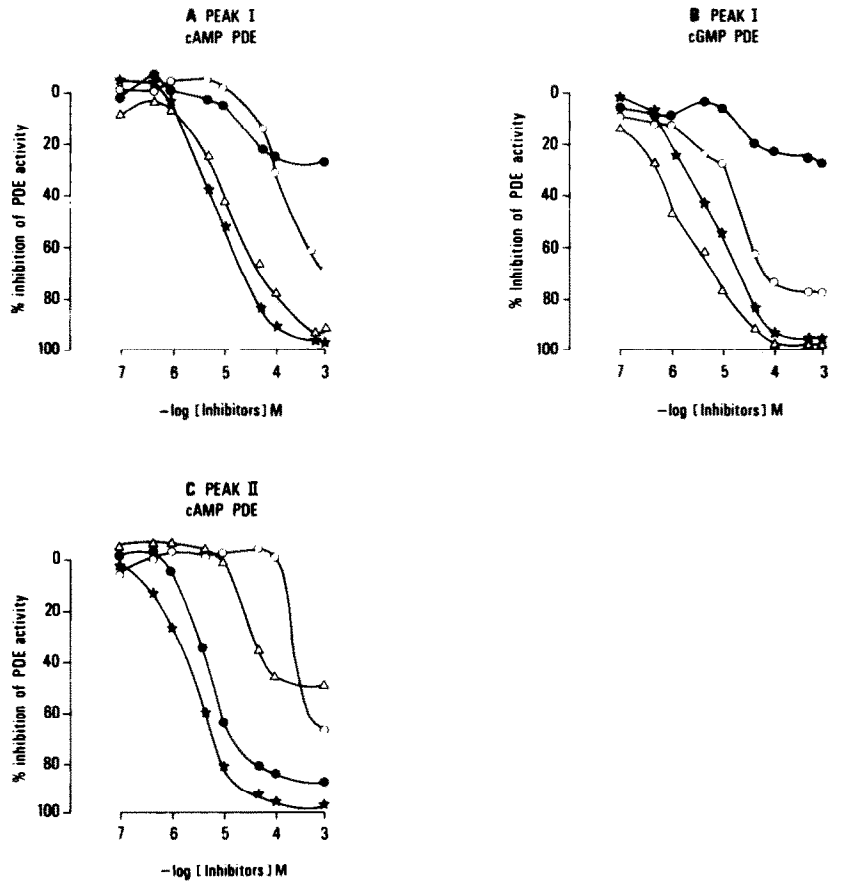

Fig. 2. Inhibition effects of papaverine (*), aminophylline (○), zaprinast (△) and rolipram (●) on cAMP PDE (A) and cGMP PDE (B) of peak I and cAMP PDE of peak II (C). The PDE activities were determined without EGTA or Ca^{2+}–calmodulin addition. Substrate concentrations for these experiments were 1×10^{-6} M cAMP and 1×10^{-7} M cGMP. Each point represents the mean of triplicate determinations of percent inhibition of PDE activity using a single enzymatic preparation. Similar concentration–response curves were obtained with two other preparations.

Fig. 3. Typical isotonic record of the spontaneous contractile activity of myometrium strips from pregnant women near term.

Contractile responses of myometrial strips to PDE inhibitors

Spontaneous contractile activity is observed after the stabilization period in approximately 80% of the myometrial strips studied. Figure 3 shows a typical isotonic record of the spontaneous contractions before addition of the inhibitors. Contractions are regular with respect to frequency, duration and amplitude.

A concentration-dependent inhibition of spontaneous contractions is obtained when papaverine (1×10^{-10} to 1×10^{-4} M), aminophylline (1×10^{-10} to 3×10^{-4} M), rolipram (1×10^{-10} to 3×10^{-5} M) and zaprinast (1×10^{-10} to 3×10^{-4} M) are administered to the preparations (Fig. 4). However, rolipram is the more potent inhibitor of spontaneous contractile activity with an IC_{50} value of about 1×10^{-7} M whereas IC_{50} values for papaverine, aminophylline and zaprinast are in the 1×10^{-4} M range. Rolipram also induces a 50% decrease in frequency of the contractions before the activity was abolished, whereas no consistent effect on basic tonus was found.

DISCUSSION

The present study clearly indicates that the second form of PDE isolated from pregnant myometrium, which specifically hydrolysis cAMP, is selectively inhibited by rolipram. IBMX and papaverine inhibit the two myometrial PDE forms to comparable degrees as it has previously been described in vascular and cardiac smooth muscles [7, 20]. Aminophylline, another non-selective PDE inhibitor, well-known for its bronchodilatory properties, is weakly efficient in our system. The platelet anti-aggregatory agent cilostamide and its derivative, valeramide [7] classicaly reported in the literature as selective inhibitors of the cAMP PDE form, are not selective and are only active at high concentrations. On the other hand and as it was expected, the first form of Ca^{2+}–calmodulin sensitive PDE without substrate specificity is selectively inhibited by zaprinast. This agent was also described as a potent inhibitor of that form in the aorta, heart and platelets [20]. Dypiridamole, whose selectivity differs according to the tissue studied, is neither efficient nor selective on the two myometrial forms.

Thus rolipram is the most potent and selective inhibitor of the second form of PDE among the compounds tested, with an IC_{50} value in the micromolar range. The same molecular form, isolated from human, bovine and rat aorta exhibited similar sensitivity to this agent ($IC_{50} \simeq 5 \times 10^{-6}$ M) [7]. It is noteworthy that rolipram is also proving the most efficient drug to inhibit *in vitro* the spontaneous

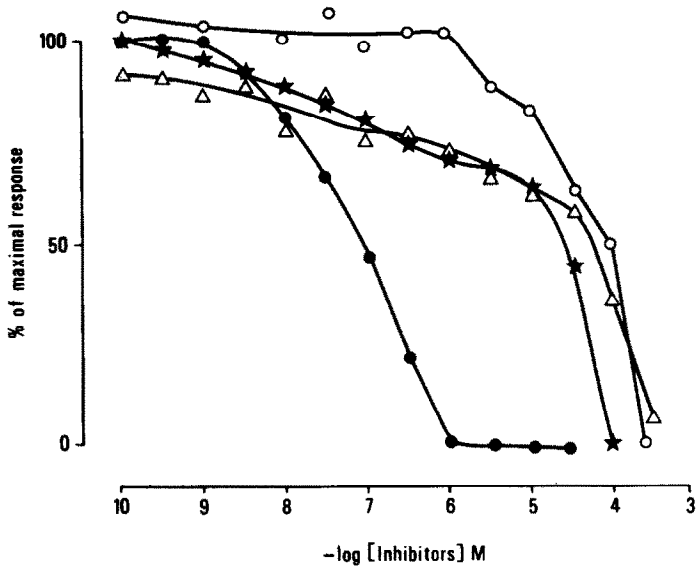

Fig. 4. Inhibitory concentration–response curves to papaverine (*), aminophylline (○), rolipram (●) and zaprinast (△) on the spontaneous contractions of the human isolated pregnant myometrium. These results are representative of three experiments performed on three different human myometria.

contractions of near term myometria. Papaverine, aminophylline and zaprinast are only active at high concentrations ($IC_{50} \simeq 1 \times 10^{-4}$ M) while IC_{50} value for rolipram is in the 1×10^{-7} M range. We must emphasize the ten-fold difference between IC_{50} values of rolipram inhibiting PDE activity and spontaneous contractions. It is not clear whether this discrepancy is due to the purification steps of the different PDE forms or to an amplification phenomenon during the signal transmission leading to the contractile response.

Our findings that rolipram, which is the more effective myorelaxant drug is also the selective inhibitor of the cAMP specific form of PDE, suggest an important role of this latter form in the mechanisms of uterine contractility. Furthermore, it is interesting to remind that this form appears during the pregnancy. Undetectable at 16 weeks (unpublished results), it has been identified as of the 32nd week of pregnancy and is largely represented near term [9, 10]. In previous reports, inhibitory studies on PDE activity from bovine coronary arteries or cardiac ventricles have demonstrated the presence of at least two subclasses of cAMP-specific PDE [21]. Although both enzymes have comparable affinities for cAMP as a substrate, they differ considerably in their response to the inhibitory effect of cGMP, as well as to various selective inhibitors. The fact that our cAMP-specific PDE form is non-inhibited by cGMP nor by amrinone, cilostamide or valeramide (data not shown) supports the hypothesis that peak II eluted by DEAE–cellulose chromatography is a single entity corresponding to a predominant rolipram-sensitive form. This idea was confirmed by our previous results [9] where peak II after a subsequent sucrose gradient centrifugation only generated one peak of cAMP PDE activity. Process involved in the ontogenesis of this cAMP PDE form remains to be elucidated. Conceivable mechanisms are proteolysis of the Ca^{2+}-calmodulin sensitive form or hormonal influences.

Whereas the relaxant effects of papaverine and aminophylline have been described on myometrial preparations [6, 22]; rolipram has never been tested *in vitro* on this tissue. In rat brain, rolipram is especially known for its antidepressant properties probably due to an enhancement of central noradrenergic transmission following upon the decrease of cAMP hydrolysis [23, 24]. A similar regulation might exist in myometrium.

In obstetrics, non-specific inhibitors of PDE have been proposed for the treatment of preterm labor. Comparative studies with aminophylline and a β-mimetic have concluded that the two drugs are similarly effective, while aminophylline has less cardiovascular side-effects [5, 25]. However, a high frequency of side-effects of this drug compared to the clinical tocolytic action has also been described [26]. Rolipram or other derivatives might be a good alternative for the prevention of premature labor in so far as they are devoid of contra-indications for pregnant women.

Acknowledgements—This study was supported in part by grants from CNAMTS-INSERM. We are very grateful to T. Keravis for helpful advice in purification steps of PDE and to C. Lugnier and J. C. Stoclet for providing PDE-inhibitors. We also acknowledge the excellent assistance of H. Blanchard and S. Langer of the Department of Biology, LERS, Paris, for contractile activity studies. We thank G. Butler-Browne for critical reading of the manuscript, and M. Verger for her secretarial assistance.

REFERENCES

1. Breuiller M, Rouot B, Leroy MJ, Blot PH, Kaplan L and Ferré F, Adrenergic receptors in inner and outer layers of human myometrium near term: characterization of beta-adrenergic receptor sites by ^{125}I-iodocyanopindolol binding. *Gynecol Obstet Invest* **24**: 28–37, 1987.
2. Litime MH, Pointis G and Ferré F, Adenylate cyclase activation by β-adrenergic agonists in longitudinal and circular layers from human pregnant myometrium: effects of Gpp(NH)p. *Med Sci Res* **15**: 919–920, 1987.
3. Litime MH, Leroy MJ, Breuiller M, Blanchard H, Hicks P, Uzan M and Ferré F, Le système messager AMPc dans le myomètre humain gravide. Sensibilité envers les agents β-adrenergiques. In: *Société Française pour l'Etude de la Fertilité*, Vol. 26 (Eds. Germain G, Roger M, Worcel M and Zorn JR), pp. 325–330. Masson, Paris, 1988.
4. Berg G, Andersson RGG and Ryden G, β-adrenergic receptors in human myometrium during pregnancy: changes in the number of receptors after β-mimetic treatment. *Am J Obster Gynecol* **151**: 392–396, 1985.
5. Barale E, Capriello P, Innocenti S, Cappeli N, Lanata L, Iponnoni C, Boldrini F and Fioretti P, Inhibition of uterine contractility by aminophylline in premature labor. In: *Uterine Contractility* (Eds. Bottari S, Thomas JP, Vokaer A and Vokaer R), pp. 436–440. Masson, New York, 1982.
6. Berg G, Andersson RGG and Ryden G, *In vitro* study of phosphodiesterase-inhibiting drugs: a complement to beta-sympathomimetic drug therapy in premature labor? *Am J Obstet Gynecol* **145**: 802–806, 1983.
7. Lugnier C, Schoeffter A, Lebec E, Trouthou E and Stoclet JC, Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta. *Biochem Pharmacol* **35**: 1743–1751, 1986.
8. Rossi P, Pezzotti R, Conti M and Geremia R, Cyclic nucleotide phosphodiesterase in somatic and germ cells of mouse seminiferous tubules. *J Reprod Fertil* **74**: 317–327, 1985.
9. Leroy MJ, Pichard AL, Cabrol D and Ferré F, Cyclic 3′:5′ nucleotide phosphodiesterase in human myometrium at the end of pregnancy: partial purification and characterization of the different soluble isoenzymes. *Gynecol Obstet Invest* **20**: 27–36, 1985.
10. Leroy MJ, Blot PH and Ferré F, Characterization by DEAE–cellulose chromatography of a single molecular form of cyclic-nucleotide phosphodiesterase in nonpregnant myometrium. *Gynecol Obstet Invest* **24**: 190–199, 1987.
11. Weishaar RE, Cain MH and Bristol JA, A new generation of phosphodiesterase inhibitors: multiple molecular forms of phosphodiesterase and the potential for drug selectivity. *J Med Chem* **28**: 537–545, 1985.
12. Pinto RM, Lerner U, Pontelli H and Rabow W, Effect of estradiol-17β on oxytocin-induced contraction of the three separate layers of human pregnant myometrium. *Am J Obstet Gynecol* **97**: 881–887, 1967.
13. Deals J, Uterine contractility patterns of the outer and inner zones of the myometrium. *Am J Obstet Gynecol* **44**: 315–326, 1974.
14. Lerner U, The uterine cervix and the initiation of labor: action of estradiol-17β. In: *Dilatation of the Uterine Cervix* (Eds. Naftolin F and Stubblefield PG), pp. 301–316. Raven Press, New York, 1980.

15. Thompson WJ and Appleman MM, Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochem* **10**: 311–316, 1971.
16. Ferré F, Breuiller M, De Pariente D and Cédard L, Activité 3′,5′-AMP cyclique phosphodiesterase dans les couches externe et interne du myomètre humain en fin de grossesse. *C R Acad Sci* **285**: 73–76, 1971.
17. Boudreau RJ and Drummond GI, A modified assay of 3′,5′-cyclic AMP phosphodiesterase. *Anal Biochem* **63**: 388–399, 1975.
18. Schoemaker H, Hicks PE and Langer SZ, Calcium channel receptor binding studies for diltiazem and its major metabolites: functional correlation to inhibition of portal vein myogenic activity. *J Cardiovasc Pharmacol* **9**: 173–180, 1987.
19. Ballejo G, Calixto JB and Medeiro YS, *In vitro* effects of calcium entry blockers, chlorpromazine and fenoterol upon human pregnant myometrium contractility. *Br J Pharmacol* **89**: 515–523, 1986.
20. Weishaar RE, Burrows SD, Kobylarz DC, Quade MM and Evans DB, Multiple molecular forms of cyclic nucleotide phosphodiesterase in cardiac and smooth muscles and in platelets. *Biochem Pharmacol* **35**: 787–800, 1986.
21. Pang DC, Cyclic AMP and cyclic GMP phosphodiesterases: target for drug development. *Drug Dev Res* **12**: 85–92, 1988.
22. Singh M and Sharma PL, Effect of cyclic nucleotide phosphodiesterase regulators on isolated rat uterus: with observations on their mechanism of action. *Arch Int Pharmacodyn* **273**: 180–195, 1985.
23. Wachtel H, Potential antidepressant activity of rolipram and other selective cyclic adenosine 3′,5′-monophosphate phosphodiesterase inhibitors. *Neuropharmacol* **22**: 267–272, 1983.
24. Przegalinski E, Baran L, Siwanowicz J, Nowak G, Antkiewicz-Michaluk L and Vetaluni J, Chronic treatment with the potential antidepressant drug rolipram: the effect on the behavioural responses to adrenergic and dopaminergic receptor agonists with some biochemical correlates. *J Neural Transm* **64**: 211–226, 1985.
25. Liu DTY and Blackwell RJ, The value of a scoring system in predicting outcome of preterm labour and comparing efficacy of treatment with aminophylline and salbutamol. *Br J Obstet Gynecol* **85**: 418–424, 1978.
26. Lipshitz J, Uterine and cardiovascular effects of aminophylline. *Am J Obstet Gynecol* **131**: 716–720, 1978.