

Up-regulation of rat myometrial phospholipases C β 1 and C β 3 correlates with increased term sensitivity to carbachol and oxytocin

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Abstract

In the present study, we compared rat uterine contractility and myometrial inositol phosphate (InsP) production in response to activation of muscarinic and oxytocin receptors during pregnancy and at term. The level of myometrial phospholipase (PL) C β was also determined by Western blotting at different stages of pregnancy and following administration of oestradiol, progesterone or vehicle. The results showed an increased potency of carbachol (CCh), a cholinergic muscarinic agonist, and oxytocin (OT) to enhance myometrial InsP production at term. This correlated with an increased potency of both agonists to induce contraction of the circular but not the longitudinal muscle. For both InsP production and contractile activity, the maximal response of CCh was unaltered, while that of OT was significantly increased. Interestingly, the increased

responsiveness to CCh and OT was associated with an up-regulation of PLC β 1 and PLC β 3 enzymes. Such regulation is under the control of oestradiol since administration of this steroid to pregnant rats increased the amount of both enzymes by 200–260%. In contrast, progesterone administration was without effect. The present study presents the first evidence that the expression of rat myometrial PLC β 1 and PLC β 3 is under the positive control of oestradiol. This could participate in the enhancement of myometrial InsP accumulation and uterine contraction at term in response to CCh and OT. Based on contraction studies, we also propose that the longitudinal and circular uterine muscles differ in the regulation of the PLC pathway during pregnancy.

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Introduction

Phospholipase (PL) C, by hydrolyzing the membrane phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-triphosphate (InsP₃), increase InsP₃-dependent liberation of Ca²⁺ from intracellular stores. The subsequent activation of myosin light chain kinase promotes the interaction between actin and myosin, thereby resulting in contraction. Among the large PLC family (11 genes), the four members of the PLC β subfamily (PLC β 1, PLC β 2, PLC β 3 and PLC β 4) are activated by G protein-coupled receptors. PLC β 1 and PLC β 4 are only activated by the Gq α subunit whereas PLC β 2 and PLC β 3 are also sensitive to the G $\beta\gamma$ complex (Rebecchi & Pentylala 2000).

The PLC signalling pathway plays an important role in the regulation of uterine contractility. Indeed, contractant factors like oxytocin (OT), prostaglandins or norepinephrine utilize PLC-coupled receptors (OT receptors (OTR), prostaglandin F₂ α receptors (FP) and α 1-adrenergic receptors (AR) respectively) to increase intracellular concentrations of Ca²⁺.

During pregnancy, these signalling pathways are silent due to the negative control exerted by progesterone. For instance, progesterone inhibits the expression of OTR and FP (Larcher *et al.* 1995, Ou *et al.* 2000). Progesterone can also indirectly modulate the PLC signalling pathway by enhancing the expression of β -AR and their cognate Gs α protein (Vivat *et al.* 1992, Elwardy-Mérézak *et al.* 1994). It has been shown that the subsequently produced cAMP reduces myometrial inositol phosphate (InsP) production through phosphorylation of PLC β by protein kinase A in the rat (Dodge *et al.* 1999). Activation of rat myometrial β -AR was also reported to reduce basal and agonist-stimulated InsP production during pregnancy through a cAMP-independent mechanism involving K⁺ channels (Khac *et al.* 1996, Mhaouty-Kodja *et al.* 2004). At term, the concomitant progesterone withdrawal and increase of oestradiol concentrations are thought to promote transcriptional activation of OTR and FP (Larcher *et al.* 1995, Ou *et al.* 2000). This results in enhanced phosphoinositide hydrolysis and uterine responsiveness to OT and prostaglandins (Goureau *et al.* 1992, Dodge *et al.* 1999).

Acetylcholine released from uterine parasympathetic nerves was also described to regulate rat uterine contractility through activation of muscarinic receptors (mRs) (Houdeau *et al.* 2003). However, its role in the initiation of labour still remains a controversial question. Indeed, while some studies reported a declined sensitivity and maximal response to the muscarinic agonist carbachol (CCh) (Riemer *et al.* 1986), others have described an enhanced CCh-mediated generation of myometrial InsP at the end of pregnancy (Lajat *et al.* 1996). Characterization of myometrial mR revealed the presence of M₂ and M₃ receptor subtypes that couple to adenylyl cyclase and PLC respectively (Lajat *et al.* 1996, Choppin *et al.* 1999). In contrast to the PLC-coupled receptors described above, no detectable changes in the density and properties of the M₃ receptor were observed during the course of pregnancy (Lajat *et al.* 1996). In correlation with these data, oestradiol treatment did not affect the expression of M₃ receptor in rat myometrial cells (Abdalla *et al.* 2004). However, we have previously shown that the expression of Gq α , the transducer protein that links mR and OTR to PLC β , increases at the end of pregnancy (Cohen-Tannoudji *et al.* 1995). Furthermore, we recently described an up-regulation of rat myometrial PLC β 1 and PLC β 3 at term (Mhaouty-Kodja *et al.* 2004).

In the light of these findings, the present study was designed to evaluate the effect of CCh on myometrial InsP production and uterine contractile activity in pregnant and term rats. Uterine responses to OT, which utilizes the same signal pathway as mR to induce uterine contraction, were also determined. Finally, the effects of oestradiol and progesterone on the expression of rat myometrial PLC β 1 and PLC β 3 were evaluated.

Materials and Methods

Animals and treatments

Sprague–Dawley rats were obtained from Janvier (Le Genest, France). The females were caged with males overnight and successful mating was determined by the presence of spermatozoa in the vaginal smear (day 1 of pregnancy). Animals were killed by cervical dislocation on days 12, 15, 16 and 21 of pregnancy or at term during the expulsion of fetoplacental units, in accordance with the guidelines for the care and use of laboratory animals (NIH Guide). The uterine horns were quickly isolated, cut open lengthwise and the fetoplacental units removed. The myometrium was then freed of adherent endometrium except for uterine tension studies.

Oestradiol-treated animals received subcutaneous injections of 0.5 ml olive oil containing 6 mg oestradiol (Sigma, L'isle d'Abeau, France) at 1000 and 1600 h on day 15 of pregnancy and were killed by cervical dislocation at 1000 h the following day (day 16). Progesterone-treated rats received subcutaneous injections of 0.5 ml olive oil

containing 5 mg progesterone (Sigma) on day 21 of pregnancy (1000 h) and were killed by cervical dislocation 30 h after injection (day 22). Control rats received the same volume of oil subcutaneously on the same schedule. The doses of progesterone and oestradiol administered significantly increased the myometrial levels of both steroids as we have determined in previous works (Elwardy-Mérézak *et al.* 1994, Cohen-Tannoudji *et al.* 1995).

Isometric tension measurement

Four millimetre long uterine strips were prepared from pregnant rats and mounted in organ baths containing 8 ml Krebs solution (144 mM NaCl, 6.2 mM KCl, 0.5 mM MgSO₄, 1 mM NaH₂PO₄, 30 mM NaHCO₃, 2.5 mM CaCl₂ and 2.8 mM glucose, pH 7.4) bubbled with 95% O₂/5% CO₂ and warmed to 30 °C. Depending on the orientation of the uterine strips in the organ bath, we measured isometric contractions of longitudinal muscle or circular muscle using a Bioscience UF1 tension transducer (Phymep, Paris, France) under 0.7 g resting force. A 30-min equilibration period was allowed before adding cumulative doses of CCh or OT (0.1 nM to 1 mM; Sigma). The concentration–response curves were recorded by computerized calculation of the integral under the tension/time curve for 3 min using Prism 4 software (Graph Pad, San Diego, CA, USA) for sigmoidal dose–response (non-linear regression fit). Isometric changes in tissue tension and maximal effects (E_{max}) are expressed as a percentage above the spontaneous activity in the absence of agonists, and potency as EC₅₀.

Myometrial InsP production

InsP production was measured as previously described (Mhaouty-Kodja *et al.* 2001). Myometrial strips were incubated at 37 °C for 4 h with 7 μ Ci myo-[³H]inositol (10–25 Ci/mmol; Perkin Elmer Life Sciences, Paris, France) in 1 ml Krebs bicarbonate buffer (117 mM NaCl, 4.7 mM KCl, 1.1 mM MgSO₄, 1.2 mM KH₂PO₄, 24 mM NaHCO₃, 0.8 mM CaCl₂ and 1 mM glucose, pH 7.4) in the presence of 95% O₂/5% CO₂. Increasing concentrations of CCh or OT (0.1 nM to 100 μ M) were added after 10-min incubation of myometrial strips with 10 mM LiCl (Sigma) in Krebs bicarbonate buffer. Assays were stopped 15 min later by freezing the strips in liquid N₂.

Strips were homogenized in 7% trichloroacetic acid and the supernatant obtained was extracted with diethyl ether, neutralized with Tris–base and then chromatographed over anion–exchange resin (AG1-X8; Bio-Rad Laboratories, Marnes-la-Coquette, France). Total InsP eluted with 1 M ammonium formate/0.1 M formic acid was counted by liquid scintillation in a 1214 Rack-beta spectrometer (LKB, Turku, Finland) for tritium.

Western blot analysis

Rat myometrium was homogenized in 0.5 mM EDTA and 10 mM Tris pH 7.4 supplemented with a cocktail of protease inhibitors (Sigma). After centrifugation at 4 °C, supernatants were collected and submitted to 100 000 g centrifugation at 4 °C for 1 h to separate plasma membranes from cytosol. Protein concentration of cytosolic fraction was determined according to Bradford (1976) with bovine serum albumin as standard. Samples were stored at -80 °C until use.

Twenty micrograms of proteins were subjected to SDS-PAGE in 7.5% gels and transferred to polyvinylidene difluoride membranes (Perkin Elmer Life Sciences). The blots were blocked overnight at 4 °C in Tris-buffered saline containing 5% non-fat dried milk, and incubated for 1 h at room temperature with polyclonal anti-PLC β (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:500. Incubation with peroxidase-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA, USA) diluted 1:10 000 was carried out for 45 min at room temperature. Immunoreactive bands were visualized by the chemiluminescence detection system (Amersham Pharmacia Biotech, Les Ulis, France) and quantified. The quantification of PLC β expression was determined by densitometric scanning followed by computer analysis using the NIH image 1.62 program.

Statistical analysis

Results are expressed as means \pm S.E.M. Statistical significance was assessed by Student's *t*-test for unpaired data. *P* values less than 0.05 were considered significant.

Results

Uterine contraction in response to CCh and OT in pregnant and term rats

We compared rat uterine activity in response to CCh and OT on day 15 of pregnancy and at term. For this, we measured isometric contractions of the longitudinal and circular muscles depending on the orientation of the uterine strips in the organ bath as described in Materials and Methods. All uterine strips exhibited spontaneous rhythmic contractions a few minutes after being mounted in the bath (data not shown). Addition of increasing concentrations of CCh or OT dose dependently enhanced the activity of the circular and longitudinal uterine muscles (Figs 1 and 2). This indicated the presence of functional mRs and OTRs in both myometrial layers during pregnancy and at term. In the circular muscle, EC₅₀ values for CCh and OT were respectively sevenfold and sixfold decreased at term when compared with day 15 of pregnancy, indicating that both agonists were more potent at inducing contraction at term (*P*<0.05; Fig. 2a and b and Table 1). The E_{max} to CCh was not significantly different

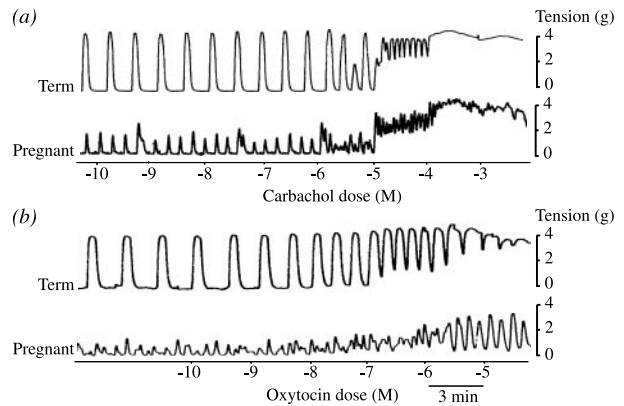


Figure 1 Representative recordings of uterine tension of the circular muscle in response to increasing doses of (a) CCh and (b) OT on day 15 of pregnancy and at term.

between pregnant and term rats (Fig. 2a) whereas that of OT was increased by 100% at term (*P*<0.05; Fig. 2b). In the longitudinal muscle, no major changes in the potency and maximal response of CCh were observed between day 15 of pregnancy and term (Fig. 2c and Table 1). For OT, a significant increase in the E_{max} was seen only at 1 μ M

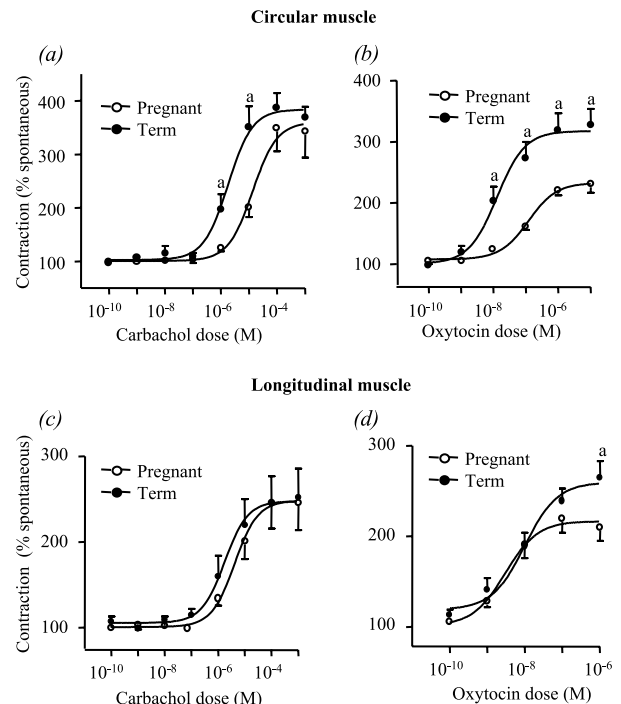


Figure 2 Contractile activity of the (a and b) circular and (c and d) longitudinal muscles of rat uterus on day 15 of pregnancy and at term in response to increasing concentrations of (a and c) CCh and (b and d) OT. Results are expressed as percent of spontaneous contraction and are means \pm S.E.M. of six to eight independent experiments. ^a*P*<0.05 versus the same concentration on day 15 of pregnancy.

Table 1 EC₅₀ values for contractile activity of the circular and longitudinal muscles and myometrial InsP production elicited by CCh and OT on day 15 of pregnancy and at term. Values are means \pm S.E.M. ($n=4-8$)

	CCh (μ M)		OT (nM)	
	Day 15	Term	Day 15	Term
Contraction				
Circular muscle	20 \pm 5	3 \pm 0.9 ^a	114 \pm 30	20 \pm 6 ^a
Longitudinal muscle	2.6 \pm 0.3	1.5 \pm 0.5	3 \pm 0.6	8 \pm 2 ^a
InsP production	2.3 \pm 0.9	0.004 \pm 0.001 ^a	3.6 \pm 0.9	1.5 \pm 0.25 ^a

^a $P<0.05$ compared with day 15 of pregnancy.

concentration ($P<0.05$; Fig. 2d) while a twofold decrease of the EC₅₀ occurred at term ($P<0.05$; Table 1).

The responsiveness of the circular muscle to CCh and OT was therefore increased at term. To assess whether this correlates with changes in mR and OTR signalling pathways, we compared myometrial InsP responses to CCh and OT between day 15 of pregnancy and term.

Basal and agonist-stimulated InsP production in pregnant and term rat myometrium

We incubated myometrial strips from pregnant and term rats in the absence or presence of increasing concentrations of CCh or OT. At term, basal InsP production was augmented by 72% (9325 \pm 351 c.p.m./100 mg tissue on day 15 of pregnancy versus 16 053 \pm 812 c.p.m./100 mg tissue at term; $P<0.0001$). In order to evaluate the net responses due to the specific activation of mRs or OTRs, agonist-stimulated InsP production was therefore expressed as percent of basal. Results illustrated in Fig. 3 show that CCh and OT increased InsP production dose dependently on both day 15 of pregnancy and at term. The EC₅₀ values for both agonists were significantly lower at term when compared with day 15 ($P<0.05$; Table 1) as evidenced by the leftward shift in the dose-response curves (Fig. 3). The E_{max} for CCh was unaltered at term while a 50% increase occurred for OT ($P<0.05$; Fig. 3). It

is therefore interesting to note that total myometrial InsP responses correlated well with contractile activity of the circular muscle, thereby suggesting that the important changes in CCh and OT responses between pregnancy and term occur in this muscle layer.

Quantification of rat myometrial PLC β 1 and PLC β 3 during pregnancy

In contrast to OTR, the density of myometrial PLC-coupled M₃ receptor is unchanged during the course of pregnancy in the rat (Lajat *et al.* 1996). The increased uterine sensitivity to CCh might thus be related to changes occurring downstream of mRs. We recently showed that the amount of myometrial PLC β 1 and PLC β 3 is greatly increased in term rats versus day 15 of pregnancy (Mhaouty-Kodja *et al.* 2004). Interestingly, the level of their cognate Gq α protein is also increased at the end of pregnancy as we have previously reported (Cohen-Tannoudji *et al.* 1995). To verify whether the pattern of myometrial PLC β expression correlates with that of Gq α protein, we therefore extended our immunodetection analysis of PLC β isozymes to different days of pregnancy. Since PLC β 1 and PLC β 3 are highly enriched in the cytosolic compartment of pregnant and term rat myometrium (Mhaouty-Kodja *et al.* 2004) all the subsequent studies focused on the cytosolic expression of both enzymes.

By using specific antibodies, we detected 150 kDa signals corresponding to PLC β 1 and PLC β 3 in pregnant and term rat myometrium as previously reported (Mhaouty-Kodja *et al.* 2004). Figure 4 shows that the level of both proteins was very low on days 12 and 15 of pregnancy and increased by 300% at term ($P<0.05$). An interesting finding was that the up-regulation of PLC β 1 and PLC β 3 was maximal from day 21 of pregnancy (Fig. 4). It thus appears that, as for many contraction-associated proteins, the up-regulation of PLC β isozymes takes place before term, during uterine preparation to labour. Since this period is characterized by important changes in the steroid hormone environment, we assessed whether progesterone and/or oestradiol modulate the expression of rat myometrial PLC β 1 and PLC β 3.

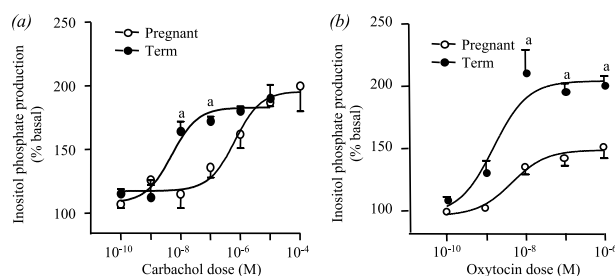


Figure 3 InsP production of myometrial strips from rats on day 15 of pregnancy and at term in response to (a) CCh and (b) OT. Results are expressed as percent of basal InsP production and are means \pm S.E.M. of four to five independent experiments. ^a $P<0.05$ versus the same concentration on day 15 of pregnancy.

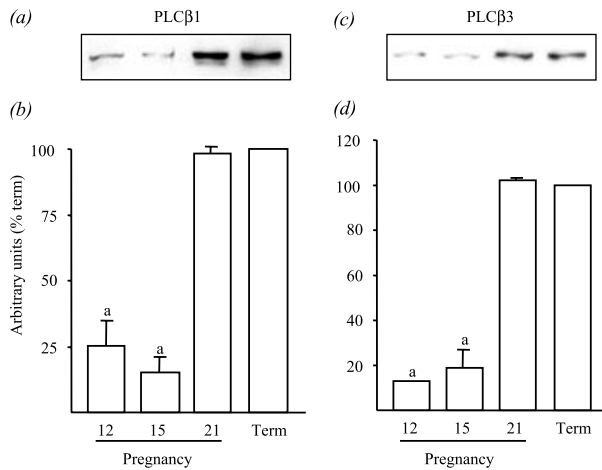


Figure 4 Quantification of amounts of rat myometrial PLCβ1 and PLCβ3. (a and c) Representative Western blots for 150 kDa PLCβ1 and PLCβ3 on days 12, 15 and 21 of pregnancy and at term. (b and d) Quantitative data are expressed as percent of term. Values are means \pm S.E.M. of three to four independent experiments. ^a $P < 0.05$ versus term or day 21 of pregnancy.

The effects of progesterone and oestradiol on rat myometrial PLCβ1 and PLCβ3 expression

To determine whether sex steroids regulate the expression of rat myometrial PLCβ1 and PLCβ3, pregnant rats received subcutaneous injections of progesterone or oestradiol. The days of steroid administration were chosen on the basis of our previous studies where myometrial concentrations of progesterone and oestradiol were determined during the course of pregnancy (Elwady-Mérézak *et al.* 1994, Cohen-Tannoudji *et al.* 1995). Progesterone and oestradiol were thus delivered to rats when their myometrial endogenous levels are low, i.e. day 21 of pregnancy for progesterone and day 15 of pregnancy for oestradiol.

Immunoblotting studies illustrated in Fig. 5 show that progesterone failed to alter the level of rat myometrial PLCβ1 and PLCβ3, which remained similar to that determined on day 21 of pregnancy ($98 \pm 2\%$ and $107 \pm 18\%$ of day 21 in progesterone-treated rats for PLCβ1 and PLCβ3 respectively). In contrast, oestradiol administration increased the amount of PLCβ1 to a level similar to that observed on day 21 of pregnancy as shown in Fig. 6a and b (+260% above day 15 of pregnancy in oestradiol-treated rats; $P < 0.05$). Such treatment also increased the level of PLCβ3 (+200% above day 15 of pregnancy in oestradiol-treated rats; $P < 0.05$) (Fig. 6c and d). In control rats receiving oil, the levels of PLCβ1 and PLCβ3 did not change from the values determined on day 15 of pregnancy.

These data revealed, for the first time, that the expression of rat myometrial PLCβ1 and PLCβ3 is under the control of oestradiol. The oestradiol-dependent up-

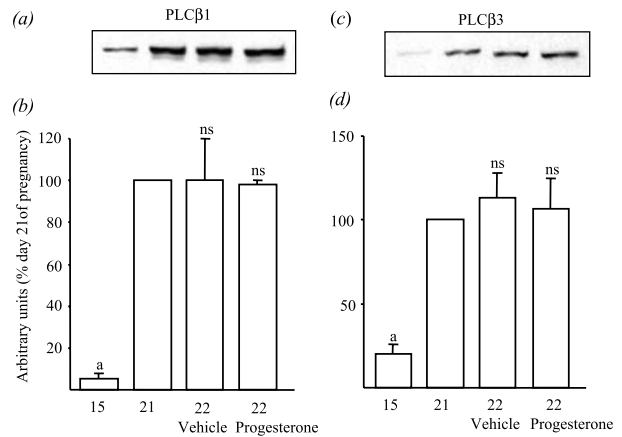


Figure 5 The effect of progesterone on rat myometrial PLCβ1 and PLCβ3 expression. (a and c) Representative Western blots for PLCβ1 and PLCβ3 on days 15, 21 or 22 of pregnancy after administration of progesterone or vehicle. (b and d) Quantitative data are expressed as percent of day 21 of pregnancy. Values are means \pm S.E.M. of three independent experiments. ^a $P < 0.001$ versus day 21 of pregnancy. ns, not significant.

regulation of both enzymes at the end of pregnancy, together with the increased amount of Gqα protein, could underlie the enhanced uterine sensitivity to CCh and OT near term.

Discussion

The present study reports the first comparison of CCh- and OT-induced uterine activity and myometrial InsP production between pregnant and term rats. Our results

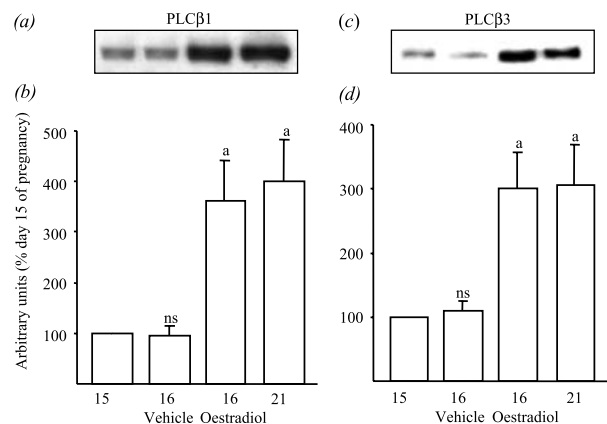


Figure 6 The effect of oestradiol on rat myometrial PLCβ1 and PLCβ3 expression. (a and c) Representative Western blots for PLCβ1 and PLCβ3 on days 15, 21 or 16 of pregnancy after administration of oestradiol or vehicle. (b and d) Quantitative data are expressed as percent of day 15 of pregnancy. Values are means \pm S.E.M. of four experiments. ^a $P < 0.05$ versus day 15 of pregnancy. ns, not significant.

showed that the potency of CCh to induce both uterine contraction and InsP production increases at term. At this time, it is interesting to note that the EC₅₀ value calculated for CCh-induced uterine contraction was similar to that reported in cyclic oestrous rats (Houdeau *et al.* 2003), i.e. following oestrogen impregnation. Furthermore, it was observed that administration of oestradiol to non-pregnant rats resulted in a sharp decrease in the EC₅₀ value for CCh-induced uterine contraction (Abdalla *et al.* 2004), similar to that observed in our study between pregnancy and term. Because an abrupt increase in plasma and myometrial oestrogen occurred at the onset of parturition, we suggest that the mR signalling pathway is influenced by changes in the hormonal status between day 15 of pregnancy and term. The higher potency of CCh to increase InsP accumulation and uterine contraction thus occurs when the uterus is under oestrogen dominance. In contrast to a previous report (Lajat *et al.* 1996), we found no major changes in the ability of mRs to trigger a maximal InsP production between pregnancy and term. This discrepancy is probably due to the presently observed enhancement (+72%) in basal levels of myometrial InsP accumulation at term, which was taken into account in our studies to determine the specific effects of CCh. Moreover, our contraction studies confirmed that no changes in the E_{max} for CCh occur between pregnancy and term. In similar experimental conditions, we found that both uterine sensitivity and maximal response to OT were increased at term, which is in agreement with previous reports (Riemer *et al.* 1986).

Rat myometrial M₃ receptor and OTR are differently sensitive to sex steroids. Indeed, while OTR is down-regulated by progesterone during pregnancy and up-regulated by oestradiol near term (Larcher *et al.* 1995), the density of rat myometrial M₃ subtype remains unchanged throughout pregnancy (Lajat *et al.* 1996). Furthermore, neither ovariectomy nor oestradiol treatment affected the density of rat myometrial M₃ subtype (Choppin *et al.* 1999, Abdalla *et al.* 2004). The increased potency of CCh to induce InsP production and uterine contraction at term is therefore probably related to changes occurring downstream of the M₃ receptor, at the level of G protein and/or PLC β enzymes. In many physiological and cellular models, M₃ receptor was shown to couple to both PLC β 1 and PLC β 3 via activation of the Gq α protein (Kim *et al.* 1997, Piiper *et al.* 1997, Strassheim & Williams 2000). Interestingly, we have recently shown that among the three PLC β s (PLC β 1, PLC β 3 and PLC β 4) predominantly expressed in pregnant rat myometrium PLC β 4 is increased at mid-pregnancy while PLC β 1 and PLC β 3 are up-regulated at term (Mhaouty-Kodja *et al.* 2004). Furthermore, the pattern of PLC β 1 and PLC β 3 expression correlates well with that of their cognate Gq α protein. Indeed, we have previously reported a 120% increase in the amount of rat myometrial Gq α protein between day 12 of pregnancy and term (Cohen-Tannoudji *et al.* 1995). In

addition, as for PLC β 1 and PLC β 3, the amount of Gq α protein is maximal from day 21 of pregnancy (Cohen-Tannoudji *et al.* 1995).

From all these data we can conclude that the increased uterine sensitivity to CCh depends on changes occurring downstream of the mR while uterine hyper-responsiveness to OT involves up-regulation at both the OTR and post-OTR levels. First, this provides an explanation for the selective increase of the maximal response to OT stimulation at term. Secondly, it suggests that, for a given receptor, amelioration of affinity towards agonists requires an increased availability of G protein and effectors, whereas an enhancement of maximal response requires a further increase of receptor expression level.

The current study provides the first evidence that PLC β 1 and PLC β 3 are targets of oestradiol. Indeed, up to date, only corticoids were reported to regulate the expression of PLC β enzymes (Dwivedi & Pandey 1999, Cueille *et al.* 2003). Furthermore, although oestradiol was suggested to facilitate norepinephrine-, CCh- and OT-induced PLC activation at a post-receptor level in the myometrium (Riemer *et al.* 1987, Phaneuf *et al.* 1995, Abdalla *et al.* 2000, 2004), no studies have addressed its direct effect on PLC β expression. Our findings indicated that administration of oestradiol to rats triggers the expression of myometrial PLC β 1 and PLC β 3 to a level normally observed between day 21 of pregnancy and term. This treatment thus anticipates the PLC β 1 and PLC β 3 increase that occurs at the end of pregnancy, probably following the enhancement of the oestrogen:progesterone ratio. Our results also indicated that, under our treatment conditions, myometrial PLC β 1 and PLC β 3 are insensitive to progesterone. Using a similar protocol, we have previously reported that progesterone significantly reduces the expression of rat myometrial Gq α (Cohen-Tannoudji *et al.* 1995).

Together, these data add a supplemental step in the regulation of rat myometrial InsP production and thus uterine contraction by sex steroids. It appears then that progesterone and oestradiol not only regulate the expression of receptors like OTR and FP specifically but also act on transduction entities common to different receptors, i.e. Gq α protein and PLC β enzymes. During pregnancy, down-regulation of Gq α by progesterone (Cohen-Tannoudji *et al.* 1995) associated with low levels of PLC β 1 and PLC β 3 (Mhaouty-Kodja *et al.* 2004, the present study) may participate in the prevention of uterine activation. At term, the increased expression of Gq α and PLC β 1 and PLC β 3 due to progesterone withdrawal and oestrogen increase respectively may underlie the enhancement of uterine responsiveness to uterotonic factors. In myometrium, the expression of many proteins including OTR, connexin-43, cyclo-oxygenase 2 and Gi2 α protein was shown to be regulated at the transcriptional level by oestradiol (Larcher *et al.* 1995, Lefebvre *et al.* 1995, Cohen-Tannoudji *et al.* 1995, Wu *et al.* 1997). Whether

or not oestradiol controls the expression of PLC β 1 and PLC β 3 at the transcriptional or post-transcriptional level remains to be determined. Analysis of the recently cloned promoter sequence of human PLC β 1 (Peruzzi *et al.* 2002) and rat PLC β 3 (Kang *et al.* 1997) did not reveal the presence of any known oestrogen-response element. However, it is now clearly established that oestrogens can also control the expression of target genes indirectly through association of their receptors with transcription factors such as Sp1 or AP-1 (Klinge 2001). Interestingly, multiple binding sites for these factors were identified in the 5'-promotor regions of PLC β 1 and PLC β 3 (Kang *et al.* 1997, Peruzzi *et al.* 2002).

Our contraction studies have shown that the sensitivity of the circular but not the longitudinal muscle to CCh and OT increases at term. It is of interest that InsP accumulation measured in the whole myometrium also rises at this time, thereby suggesting that the underlying changes in the expression of OTR, Gq α , PLC β 1 and PLC β 3 occur mainly in the circular muscle. In keeping with this hypothesis, the regulation of OTR by progesterone appears to be restricted to the circular muscle (El Alj *et al.* 1993). Indeed, this muscle layer contains fivefold less OTR (Kitazawa *et al.* 2001) and is less sensitive to OT than the longitudinal muscle at mid-pregnancy (Mhaouty-Kodja *et al.* 2004). In addition, rat α 1-ARs, which are selectively expressed in the circular muscle (Legrand *et al.* 1991, Mhaouty-Kodja *et al.* 2004), are also less potent to induce Gq α -mediated PLC activation during pregnancy (Limon-Boulez *et al.* 1997). We therefore propose that, during pregnancy, progesterone maintains quiescence of the uterine circular muscle through direct down-regulation of the PLC signalling molecules (OTR and Gq α). In the longitudinal muscle, progesterone might act indirectly on the PLC system through sensitization of the β -adrenergic signalling pathway (Vivat *et al.* 1992, Elwardy-Mérézak *et al.* 1994). Activation of rat β -ARs, which are selectively localized in this muscle layer (Legrand *et al.* 1991), significantly decreases myometrial InsP production through both cAMP-dependent and -independent mechanisms (Khac *et al.* 1996, Dodge *et al.* 1999, Mhaouty-Kodja *et al.* 2004). At the end of pregnancy, progesterone withdrawal and the subsequent β -AR desensitization (Cohen-Tannoudji *et al.* 1991) probably relieve the PLC system from inhibitory constraints in the longitudinal muscle. Concomitantly, the increase in oestradiol concentrations enhances transcription of the transduction entities involved in the PLC signalling pathway (OTR, PLC β 1 and PLC β 3) in the circular muscle. Further studies are required to verify this hypothesis in order to enhance our understanding of the regulatory mechanisms underlying the contractile responses of the longitudinal and circular muscles of the uterus.

In summary, we report that the potency of CCh and OT to induce rat uterine contraction and myometrial InsP production increases at term. Interestingly, this correlates

well with the up-regulation of myometrial PLC β 1 and PLC β 3 at this time. This could, together with the previously reported up-regulation of Gq α protein, participate in switching the rat uterus from a quiescent to an active state. The expression of both PLC β 1 and PLC β 3 is under the positive control of oestradiol and seems to be insensitive to progesterone. We also report that changes in uterine responsiveness to CCh and OT occur mainly in the circular muscle, thereby suggesting differences in the mechanisms underlying the regulation of the PLC system during the course of pregnancy between the longitudinal and circular muscles.

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