Secretory and cytosolic phospholipase A₂ activities and expression are regulated by oxytocin and estradiol during labor

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Abstract

The release of arachidonic acid from membrane glycerophospholipids through the action of phospholipases (PLs) is the first step in the biosynthesis of prostaglandins (PGs). In reproductive tissues, the most important PLs are cytosolic PLA₂ (cPLA₂) and types IIA and V of the secretory isoform (sPLA₂). The aim of this work was to investigate the role of ovarian steroid hormones and oxytocin (OT) in the regulation of rat uterine PLA₂ activity and expression during pregnancy and labor. The activity of sPLA₂ increased near labor, whereas cPLA₂ activity augmented towards the end of gestation. The levels of sPLA₂ IIA and cPLA₂ mRNA showed an increase before labor ($P < 0.05$, day 21), whereas sPLA₂ V mRNA was not regulated during pregnancy. The administration of atosiban (synthetic OT antagonist) together with tamoxifen (antagonist of estrogen receptors) was able to decrease cytosolic and secretory PLA₂ activities, diminish the expression of sPLA₂ IIA and cPLA₂, as well as decrease PGF_{2 α} production before the onset of labor ($P < 0.01$). The ovarian steroid did not affect PLA₂ during pregnancy. Collectively, these findings indicate that in the rat uterus, both 17 β -estradiol and OT could be regulating the activity and the expression of the secretory and the cytosolic isoforms of PLA₂, thus controlling PGF_{2 α} synthesis prior to the onset of labor.

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Introduction

Phospholipase A₂ (PLA₂) has long been recognized as a family of enzymes that hydrolyze the sn-2 acyl ester bond of cell membrane phospholipids, thus releasing free fatty acids, and lysophospholipids. Their most significant function is to initiate the arachidonic acid (AA) cascade by liberating these acids from membrane phospholipids leading to production of prostaglandins (PGs). These lipid mediators have been implicated in the development of pregnancy and the onset of parturition (Olson *et al.* 1992, Lopez Bernal *et al.* 1993). As pregnancy advances, PG increase in the rat uterus leads to enhanced contractility (Ribeiro *et al.* 2003, Farina *et al.* 2004). Then, the regulation of PG synthesis in the uterus could be a key factor to control the length of pregnancy.

Multiple isoforms of PLA₂ have been identified in a range of tissues. These isoforms include the secretory and the non-secretory cytosolic isozymes (Rice 1995a). The secretory types of the PLA₂ (sPLA₂) are low-molecular-weight enzymes that require millimolar concentrations of Ca²⁺ to be active. Up to now, five mammalian sPLA₂

isoforms (types I, IIA, IIC, V, and X) have been identified. Group IIA sPLA₂ is induced in many cell types (Nakano *et al.* 1990, Arbibe *et al.* 1997). Abundant messages for type V sPLA₂ were detected in macrophages (Balboa *et al.* 1996) and in a few cells sPLA₂ V appears to substitute sPLA₂ IIA (Murakami *et al.* 1998). The high-molecular-weight cytosolic PLA₂ (cPLA₂) needs micromolar concentrations of Ca²⁺ to translocate to the cell membrane and contains no disulphide bonds. Current evidence suggests that two sPLA₂ isozymes, types IIA and V, and cPLA₂ type IV are functionally coupled with cyclooxygenase (COX). COX converts AA to the intermediate PG precursor PGH₂. Liberation of AA by means of the PL and conversion of this lipid into PGs represent the two crucial rate-limiting steps in the PG biosynthetic pathway (Murakami *et al.* 1998, 1999).

Recently, Brant *et al.* (2006) observed that sPLA₂ and cPLA₂ proteins are expressed in the rat uterus at mid (day 10) and late (day 20) pregnancy. Further evidence suggests that this enzyme could play a role in membrane remodeling but not in AA release (Murakami *et al.* 1998). Although sPLA₂ types IIA and V and cPLA₂ were identified

in human gestational tissues (Lappas & Rice 2004), it is still unknown which of these enzymes are responsible for the increase in PG synthesis observed at the onset of labor.

During pregnancy, uterine $\text{PGF}_{2\alpha}$ production is suppressed to maintain uterus quiescence and in some species to prevent luteal regression (Horton & Poyser 1976). Uterine $\text{PGF}_{2\alpha}$ production increases abruptly in rat uterus at day 22 of pregnancy, immediately before the onset of labor (Farina *et al.* 2004). We have previously reported that during pregnancy, COX-1 and COX-2 expression is augmented at the end of gestation. The exogenous administration of progesterone (P) at the end of pregnancy significantly inhibits uterine $\text{PGF}_{2\alpha}$ production as well as COX-2 protein expression. Furthermore, it increases the activity of PG dehydrogenase, the enzyme responsible for initial inactivation of several PGs.

Increased PLA_2 activity during late gestation could be due to unidentified factors that augment at this time (Rice 1995b). One possible candidate is estrogen, an ovarian hormone whose concentration is elevated in late gestation (day 21). Besides, it has been shown that estrogen exposure enhances PLA_2 production (Dey *et al.* 1982, Bonney 1985).

Oxytocin (OT) is another potential factor that could be contributing to modulating PLA_2 level. OT is the most potent uterotonic agent known so far and is currently used to induce labor (Chard 1989). Lefebvre *et al.* (1992) showed that during gestation OT mRNA increases at term in the rat uterus and may act primarily as a local mediator rather than as a circulating hormone. OT binds to a cell-surface membrane receptor that activates a complex intracellular signaling pathway that ultimately leads to the activation of PLA_2 (Lee & Silvia 1994). The OT receptor (OTR) has been cloned, and the levels of OTR mRNA increase abruptly in the rat myometrium during labor (Rozen *et al.* 1995). Nevertheless, the precise mechanism underlying the progressive increase in PLA_2 activity and expression in the pregnant uterus before the onset of labor is still unknown. In this context, we hypothesized that steroid hormones and OT would affect the activity of uterine PLA_2 involved in the liberation of AA at the time of labor. To examine this possibility, we will consider the effect of 1) RU-486, a selective antagonist of P receptors (PRs); 2) tamoxifen, an inhibitor of estrogen receptors; and 3) atosiban, a synthetic antagonist that blocks OT receptors, on the activity and the mRNA expression of sPLA IIA and V and cPLA in the uterus.

Materials and Methods

Drugs and chemicals

Progesterone, $\text{PGF}_{2\alpha}$ and $\text{PGF}_{2\alpha}$ antiserum were purchased from Sigma Chemical. Estradiol antiserum and mifepristone (RU-486) were kindly provided by G D Niswender (Colorado State University, Fort Collins, CO, USA). Tamoxifen was kindly provided by Gador Laboratory (Buenos Aires,

Argentina). Atosiban was purchased from Ferring Pharmaceuticals (Ferring AB). $(5,6,8,9,11,12,14,15(n)-^3\text{H})\text{-PGF}_{2\alpha}$ (160 Ci/mmol, 200 $\mu\text{Ci/ml}$) was obtained from Amersham Corporation. RNA extraction, RT and PCR reagents were obtained from Invitrogen Life Technology. All other chemicals were of analytical grade.

Animals

Time-mated pregnant rats of the Wistar strain (200–230 g body weight) were used. Rats were maintained on a 14 h light:10 h darkness schedule and received a supply of animal chow and water *ad libitum*. The experimental procedures reported here were approved by the Animal Care Committee of the Center Pharmacological and Botanical Studies of the National Research Council (CEFYBO – CONICET) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council 1996). Rats were treated as previously described (Farina *et al.* 2004). Briefly, pregnant animals ($n=6$ for each state) were killed on different days of gestation (5, 13, 17, 21, and 22) as well as one day *post partum*. Spontaneous term labor occurs on the night of the 22nd day (day 1: day sperm plug was observed).

Time-mated rats were divided into five groups:

- (1) Rats on day 12 of gestation (when the levels of serum progesterone are high) were injected i.p. with 300 μl RU-486 (antagonist of progesterone, 2.5 mg/rat) or vehicle (ethanol 30% v/v) and were killed 24 h after the injection (day 13 of pregnancy).
- (2) Rats on days 20 and 21 (when the serum progesterone levels are low) were injected s.c. with 200 μl progesterone (2 mg/rat) or vehicle (sesame oil) every 12 h and were killed on day 22, before the onset of labor.
- (3) Rats on day 16 of pregnancy were primed with 100 μl of 17β -estradiol (E_2 , 1 $\mu\text{g/rat}$ i.p.) or vehicle (ethanol 30% v/v). On day 17 of gestation, animals received 50 μl E_2 (5 $\mu\text{g/rat}$ i.p.) or vehicle and were killed 6 h later.
- (4) Rats from day 15 to 20 or 21 of gestation were treated with 200 μl tamoxifen (200 $\mu\text{g/rat}$ per day) or vehicle (sesame oil) every 24 h and were killed on day 21 or 22 respectively.
- (5) Rats on day 21 of pregnancy were treated with atosiban (a synthetic OT antagonist, 75 $\mu\text{g/rat}$, 100 μl) or vehicle (NaCl 0.9%), and were injected every 3 h (four doses in all) and killed 12 h after the first dose at 10 pm (day 21.5 of pregnancy).

All animals were anesthetized with ether and the blood was drawn from the heart. The serum was stored (-20°C) for steroid analysis. The uterus, containing both myometrium and endometrium, was cleaned of fat, placenta, fetuses, and blood vessels. Then, tissues were stored (-70°C) for PLA_2 activity assays or extracted for PCR analysis.

Estradiol RIA

E₂ was measured in serum samples. Briefly, blood was allowed to clot and centrifuged at 3000 g for 10 min. Serum was extracted twice with 2 ml diethyl ether and estradiol concentrations were determined by RIA. Estradiol antiserum showed low cross-reactivity: 1% for progesterone and testosterone, <5% for 17 α estradiol and estriol, and 10% for estrone. Antiserum was provided by Dr G D Niswender (Colorado State University, Fort Collins, CO, USA). The sensitivity was 5–10 pg per tube and 2–5 μ l serum were assayed routinely. Results were expressed as serum estradiol (ng/ml).

Measurement of sPLA₂ and cPLA₂ activity

Secretory and cytosolic PLA₂ activities measurements were performed with a commercially available assay system (Cayman Chemical Company, Ellsworth Rd, Ann Arbor, MI, USA) exactly as recommended by the manufacturer. 1,2-dithio analog of diheptanoyl phosphatidylcholine was the substrate used for sPLA₂ activity, as it serves as a substrate for most PLA₂ except cPLA₂ type V. Substrate hydrolysis was quantified by measuring the absorbance at 405 nm at different time points and sPLA₂ activity was calculated.

Cytosolic PLA₂ activity was measured in tissue homogenates using arachidonoyl thio-PC as substrate. To avoid any measurement corresponding to iPLA₂ activity, bromoenol lactone (an iPLA₂ specific inhibitor) was added to the samples prior to assaying according to the manufacturer. The data were expressed as nmol/min per ml.

cDNA synthesis and RT-PCR

Total RNA was isolated from rat uteri on different days of pregnancy using Trizol reagent (Invitrogen Life Technologies). RNA was quantified by measuring the absorbance at 260 nm. According to the manufacturer's instructions, first-strand cDNA was synthesized from 2 μ g total RNA, using Moloney murine leukemia virus reverse transcriptase and random primers (Invitrogen) in the presence of recombinant RNase inhibitor. After first-strand synthesis, PCR was performed with specific intron-spanning primers (Invitrogen Custom Primers; Table 1) based on the sequences of PLA₂ and β -actin reported previously (Ohara *et al.* 1986, Dieter *et al.* 2002). Except for the amplification of sPLA₂ type V that requires Platinum Taq DNA polymerase (Invitrogen), all other amplifications were performed using Taq DNA polymerase (Invitrogen). The amplification parameters were as follows: 94 °C for 5 min (initial denaturation), and 30 cycles of 94 °C for 40 s, 52 °C 2 min, 72 °C 1 min, 72 °C 5 min for β -actin; 94 °C for 1 min (initial denaturation), and 34 cycles of 94 °C for 30 s, 57 °C 40 s, 72 °C 2 min for sPLA₂ type IIA; 94 °C for 2 min (initial denaturation), and 34 cycles of 94 °C for 1 min, 62 °C 45 s (touchdown –0.1 °C/cycle), 72 °C 1 min for sPLA₂ type V; and 96 °C

for 5 min (initial denaturation), and 29 cycles of 95 °C for 30 s, 60 °C 1 min (touchdown –0.1 °C/cycle), 72 °C 1 min, 72 °C 5 min for cPLA₂ type IV.

Aliquots of 10 μ l of the PCR were loaded onto a 2% agarose gel. After ethidium bromide staining, bands were visualized on a transilluminator and image analysis was performed by densitometric scanning of the gels using an Image J (National Institutes of Health) Program. Data were expressed as the relative amount of PLA₂ isoforms versus β -actin mRNA (control).

PGF_{2 α} determination

PGF_{2 α} was quantified as previously described (Farina *et al.* 2004) using rabbit antiserum (Sigma Chemical Co). Briefly, uteri were removed and then rinsed thoroughly in cold Krebs–Ringer bicarbonate (KRB) buffer before PGF_{2 α} determination by RIA. Tissues were incubated in a KRB buffer for 60 min at 37 °C. Total protein content was determined by the Bradford (1976) method. Supernatants were acidified to pH 3 with 1 M HCl and PGs were extracted twice with 2 ml ethyl acetate. Sensitivity of the assay was 5–10 pg/ml and cross-reactivity was less than 0.1% with other PGs. Intra- and inter-assay variations were each <8.0%. Results were expressed as ng PGF_{2 α} /h per mg protein.

Statistical analysis

Statistical analysis was performed using the InStat Program (Graph Pad Software, San Diego, CA, USA). Comparisons between values of groups were performed using one-way ANOVA. Significance was determined using Tukey's multiple comparison tests for unequal replicates.

All values presented in this study are mean $\pm$ S.E.M. Differences between means were considered significant at $P < 0.05$.

Results

Uterine PLA₂ activity

We measured sPLA₂ and cPLA₂ activities in the rat uterus during pregnancy (day 5, 13, 17, 21, and 22) and one day after parturition. These studies showed that uterine sPLA₂ activity remained low at early and mid gestation. On day 21 (end of gestation), sPLA₂ activity increased sharply and declined towards day 22 of pregnancy, just before the onset of labor (Fig. 1A). We wanted to determine if there was a temporal relationship between uterine PLA₂ activity and steroid hormone serum levels. We have previously reported that progesterone peaked on day 13 of gestation and diminished at the end of pregnancy (Farina *et al.* 2004). Thus, in the present study, we measured E₂ serum levels at different days of pregnancy (5, 13, 21, and 22) and *post partum*. Figure 1A shows that serum estradiol concentration remained low through early and mid gestation,

Table 1 Oligonucleotide primers used for amplification of phospholipase A₂ isoforms from rat uterus.

	Primers sequences 5'3'	Product (pb)
β-actin (21)	Sense: GTGGGGCGCCCCAGGCACCA Antisense: CTCCTTAATGTCACGCACGATTTTC	610
sPLA ₂ type IIA (21)	Sense: GGGAATTCAGCCTTCTGGAGTTTGGGCAAA Antisense: CTGCTAAGCTTCAGCAACTGGGCGTCTT	360
sPLA ₂ type V (21)	Sense: CCCTAAGGATGGCACTGATTGG Antisense: CCGGTCACAAGCACAAAGCC	172
cPLA ₂ (22)	Sense: AAGGCCAAGTGACACCAGCC Antisense: GAAACAGAGCAACGAGATGGG	452

increased at day 21 of gestation, and began to decline at day 22 until 24 h after parturition. On the other hand, cPLA₂ activity rose at mid gestation and remained high until one day after parturition (Fig. 1B).

Expression of uterine PLA₂ isoforms during pregnancy

RT-PCR analysis was conducted to determine the expression patterns of rat uterine PLA₂ enzymes at

early, mid, and late gestation. Figure 2 shows that sPLA₂ types IIA and V, as well as cPLA₂ enzyme, were clearly detected on all the analyzed days. sPLA₂ type IIA changed its expression along gestation and was significantly increased on days 21 ($P<0.001$) and 22 ($P<0.05$) of pregnancy, before the onset of labor (Fig. 2B). Similar results were found when cPLA₂ mRNA was determined which was significantly

Figure 1 (A) Uterine sPLA₂ activity (bars) and serum estradiol concentration (solid triangles) during pregnancy (day 5, 13, 21, and 22) and 24 h post partum. Rat uterus was homogenized and sPLA₂ activity was determined (see Materials and Methods). Each bar represents the mean $\pm$ s.e.m. from three different experiments with $n=3$. 'a' $P<0.001$ versus day 5, 13, and post labor (PL); 'b' $P<0.01$ versus day 5, 13, and post labor (PL). Blood was collected from gravid rat at specific gestational days during pregnancy ($n=4$ rats per point). The lane represents the mean $\pm$ s.e.m. plasma estradiol concentration. (B) Uterine cPLA₂ activity during pregnancy and 24 h post labor (PL). Each bar represents the mean $\pm$ s.e.m. from three different experiments with $n=3$. Significant differences, compared with day 5 of pregnancy, are represented (a: $P<0.001$, ANOVA).

Figure 2 Changes in relative sPLA₂ types IIA and V and cPLA₂ mRNA levels in the uterus during pregnancy and labor. Total RNA was extracted and subjected to RT-PCR for sPLA₂ II and V, cPLA₂, and β-actin. (A) Representative RT-PCR gels were stained with ethidium bromide and exposed to u.v. (B) Densitometric analysis of uterine mRNA. Each lane represents the mean $\pm$ s.e.m. of sPLA₂ type IIA (open diamond), sPLA₂ type V (open square), and cPLA₂ (solid triangle) mRNAs normalized to β-actin from three separate experiments. Uterine sPLA₂ type IIA: 'a' $P<0.001$ versus day 5, 13, and PL; 'c' $P<0.05$ versus day 22. Uterine cPLA₂: 'c' $P<0.05$ versus all day analyzed.

increased on day 21 ($P < 0.05$). On the other hand, sPLA₂ type V mRNA was detected as a worthless band of 172 bases (Table 1). No changes in sPLA₂ type V mRNA expression were observed in all the analyzed days during pregnancy or *post partum*.

Effects of steroid hormones on uterine PLA₂ activity and expression

The temporal association between the increase in sPLA₂ type IIA expression and activity with the augmentation in estradiol and the decrease in progesterone levels suggested that the ovarian steroids (estrogen and progesterone) could be modulating PLA₂ activity and/or expression in the rat uterus during pregnancy. To further ascertain the role of steroid hormones on the modulation of PLA₂ during pregnancy, rats were treated as follows: 1) administration of mifepristone (RU-486, an antiprogesterone) or E₂ at mid gestation and 2) administration of progesterone or tamoxifen (an antiestrogen) at late gestation. Neither of these treatments was able to modify uterine mRNA expression of types IIA and V sPLA₂ and cPLA₂ (Fig. 3). Additionally, these treatments did not modulate sPLA₂ (Fig. 4A and C) and cPLA₂ (Fig. 4B and D) activities in the rat uterus.

Effect of atosiban and tamoxifen on PLA₂ expression and activity in rat uterus

Although the precise factors that participate in the onset of labor have not been fully described, it is well recognized that OT and ovarian steroids play a major role. The presence of sPLA₂ types IIA and V and cPLA₂ mRNA was detected in the uterus on the night of day 21 of gestation (day 21.5). The administration of separate tamoxifen or atosiban did not provoke any significant difference in the mRNA expression of the analyzed isoforms (Fig. 5). However, the administration of tamoxifen together with atosiban significantly inhibited mRNA expression of sPLA₂ IIA (Fig. 5) and the sPLA₂ activity (Fig. 6A, $P < 0.01$).

Moreover, cPLA₂ activity was significantly decreased following the treatment with atosiban ($P < 0.01$) or tamoxifen together with atosiban (Fig. 6B, $P < 0.001$). The mRNA expression of cPLA₂ was inhibited ($P < 0.05$) only when we administrated atosiban together with tamoxifen (Fig. 5B).

Effect of atosiban and tamoxifen on PGF_{2α} synthesis in rat uterus

PGs are the main mediators of uterine contractility prior to the onset of labor. Besides the regulation of PLA₂ activity, we wanted to study if estradiol or OT were

Figure 3 Expression of sPLA₂ types IIA and V and cPLA₂ mRNA from uterine of rats treated with RU-486 (2.5 mg/rat), progesterone (P, 2 mg/rat), 17β-estradiol (E₂, 5 μg/rat), or tamoxifen (TAM, 200 μg/rat). Total RNA was extracted and subjected to RT-PCR for sPLA₂ II and V, cPLA₂, and β-actin. (A) Representative agarose gels were stained with ethidium bromide and exposed to u.v. (B) Densitometric analysis of uterine mRNAs. Each lane represents the mean $\pm$ S.E.M. of sPLA₂ type IIA (open diamond), sPLA₂ type V (open square), and cPLA₂ (solid triangle) mRNAs normalized to β-actin from three separate experiments. There is no significant difference in the expression of PLA₂ mRNAs between each treatment (ANOVA).

regulating the synthesis of PGs before the onset of labor. Thus, rats were treated with the specific inhibitors tamoxifen, atosiban, or both, and PGF_{2α} synthesis was determined in the uterine strips. Figure 7 shows that PGF_{2α} synthesis was markedly diminished ($P < 0.01$) only in the uterine tissue from rats treated with tamoxifen together with atosiban on day 21 of pregnancy. There was no significant difference between the controls and the groups treated with tamoxifen or atosiban.

Figure 4 Uterine sPLA₂ and cPLA₂ activities in rats treated with 1) mifepristone (RU-486) or 17 β -estradiol (E₂) at mid gestation (A and C) and 2) progesterone (P) or tamoxifen (TAM) at late gestation (B and D). Each bar represents the mean $\pm$ S.E.M. from two different experiments with $n=4$. There is no significant difference in the activities of secretory and cytosolic PLA₂s according to each treatment (ANOVA).

Discussion

The role of the PGs (catabolic products of glycerophospholipids) as second messengers has been studied extensively over recent decades. The formation of these second messengers is mediated through the action of specific PL, including PLA₁, A₂, C, and D. PLA₂ isozymes have been identified in human gestational tissues (Freed *et al.* 1997), and there is differential expression of these enzymes in tissues obtained at preterm and term labor (Lappas & Rice 2004). This work is the first to identify the presence of two secretory PLA₂ isoenzymes (type IIA and V) and cPLA₂ mRNA in the rat uterus at early (day 5), mid (day 13), and late (days 21 and 22) gestation, as well as one day after parturition. Consistent with the possible involvement of these isoenzymes in the onset and/or progression of labor and delivery, we demonstrated that sPLA₂ IIA and cPLA₂ gene expression are developmentally regulated, with mRNA levels increasing significantly on day 21, just before the onset of labor. This result suggests that these enzymes might be some of the factors associated with increased PG synthesis near the onset of labor. In contrast, we were unable to detect any changes in the expression of sPLA₂ V mRNA with the progress of gestation or in association with labor. Although some authors did not find any association between delivery and changes in group IIA sPLA₂ expression (Bennet *et al.* 1994, Aitken *et al.* 1996, Freed *et al.* 1997), others have identified an increase in sPLA₂ protein expression in rat uterus (Murakami *et al.* 1999) and human myometrium (Slater *et al.* 2004) in late gestation.

Several years ago, Dey *et al.* (1982) showed that in hypophysectomized rats uterine PLA₂ activity is under the influence of steroids hormones and Cox *et al.* (1982) showed that PLA₂ activity was markedly increased in the rat uterus by E₂ treatment during early pregnancy. Such assays estimate the effect of steroid hormones on net enzymatic activity and do not discriminate between individual PLA₂ isoenzymes. A decade passed by and Farrugia *et al.* (1993) showed that type II PLA₂ is responsible for up to 80% of the total PLA₂ enzymatic activity present in placental tissues and that it represents the major component of PLA₂ activity in human fetal membranes at term (Aitken *et al.* 1993). Recently, Brant *et al.* (2006) showed a significant increase in rat uterine calcium-independent PLA₂ activity with the progression of labor. In the present work, we demonstrated a gestation-related increase of both the secretory and the cytosolic PLA₂ activities in the rat uterus. Secretory PLA₂ increased at late pregnancy similar to E₂ levels. These data suggest that endogenous estradiol could be modulating the expression and/or activity of sPLA₂ during pregnancy. A substantial augmentation of cPLA₂ activity was observed at mid gestation and this activity remained increased until labor. A possible explanation for the increment in cPLA₂ activity at mid gestation is an effect of a variety of cytokines and

Figure 5 Expression of RT-PCR products using specific primers for sPLA₂ types IIA and V, cPLA₂, and β-actin mRNAs. In all cases, experiments were repeated twice using cDNA from four different animals. (A) Representative agarose gels were stained with ethidium bromide and exposed to u.v. (B) Each bar represents the mean $\pm$ S.E.M. of sPLA₂ type IIA and cPLA₂ mRNAs normalized to β-actin. 'c' $P < 0.05$ versus day 21.5 (night of day 21). There is no significant difference in the expression of PLA₂ type V mRNAs between each treatment.

mitogens previously shown to induce activation and increase the synthesis of this enzyme in different cell types (Clark *et al.* 1995). Transforming growth factor-β1 is highly expressed in rat decidual cells at mid pregnancy (Shooner *et al.* 2005), and it has been shown that it increases the levels of cPLA₂ protein and activity (Brown *et al.* 2000). It is well recognized that cPLA₂ is a central regulator of stimulus-coupled cellular AA release, whereas the role of sPLA₂ in the metabolism of AA has remained ambiguous. Nevertheless, different studies have suggested that sPLA₂ IIA, or related sPLA₂ isozymes, could participate in immediate and delayed phases of cellular AA release and PG generation (Pfeilschifter *et al.* 1993, Akiba *et al.* 2001). However, little is known regarding how the expression and the activity of uterine PLA₂ enzymes change during pregnancy. The observed increases in PLA₂ expression and activity prior to delivery might be the previous step for the increase of PG synthesis at the time of labor. This issue is relevant, particularly in the rat model as uterine-derived PGs are responsible for ovarian luteolysis, which initiates the sequence of biochemical events that lead to labor.

The uterus is one of the target organs of the steroid hormones, progesterone and estradiol. To ascertain

Figure 6 Uterine secretory (A) and cytosolic (B) PLA₂ activities in rats treated with atosiban (AT), tamoxifen (TAM), or both on day 21 at night (21.5; see Materials and Methods). Each bar represents the mean $\pm$ S.E.M. from two different experiments with $n = 4$. (A) 'b' $P < 0.01$ versus day 21.5; (B) 'a' $P < 0.001$ and 'b' $P < 0.01$ versus day 21.5.

whether endogenous estradiol and progesterone were involved in the increased expression and/or activity of uterine PLA₂s, the effects of tamoxifen, an antiestrogenic agent, and RU-486, an antiprogesterone, were examined. Our observations suggest that steroid hormones do not regulate the expression and the activity of uterine PLA₂s during pregnancy and labor.

Other groups obtained similar results. It has been reported that E₂ has no effect on PLA₂ expression in sheep (Di *et al.* 1999), rat (Ospina *et al.* 2002), and baboons (Rosenthal *et al.* 2001). In contrast, others found that estradiol or different combinations of steroid treatments promote an increase in PLA₂ protein levels (Rupnow *et al.* 2002) and activity (Fayard *et al.* 1994, Periwai *et al.* 1996). These discrepancies could be due to

Figure 7 Effect of atosiban (AT), tamoxifen (TAM), or both on PGF_{2α} synthesis in the rat uterus at late pregnancy. Each bar represents the mean $\pm$ S.E.M. of two experiments with $n=4$. 'b' $P<0.01$ versus day 21.5 alone.

differences in the strains of the rats used, differences in the dose and timetable of hormones administration, as well as differences in the tissue processing.

Several lines of evidence suggest a role for the OT/OTR system in the regulation of parturition. The concentration of OT in plasma and the expression of uterine OTR increase before parturition. Infusions of OT stimulate myometrial contractions to a magnitude indistinguishable from normal labor and the administration of antagonists of OTR can disrupt the pattern of normal labor (Mitchell & Schmid 2001). Finally, OT stimulates PGF_{2α} synthesis possibly by increasing cPLA₂ activity (Burns *et al.* 2000). Estrogen appears to be the major regulator of OT synthesis. In the rat, the administration of an estrogen receptor antagonist during late pregnancy reduced uterine OT mRNA and protein causing a significant delay in parturition (Fang *et al.* 1996). In addition, estrogen stimulates the synthesis of OTR (Soloff 1975). Thus, it is plausible that the expression and activity of uterine PLA₂s was induced by both estradiol and OT. In this work, we have shown that plasma estradiol concentrations significantly increased on day 21 of gestation, but the administration of tamoxifen, an antiestrogen, did not modify the time-dependent-induced cPLA₂ activity during gestation. On the other hand, a synthetic OT antagonist, atosiban, was able to diminish cPLA₂ activity. We have also demonstrated that the co-administration of atosiban with tamoxifen during day 21 of pregnancy significantly inhibited sPLA₂ and cPLA₂ activities before the onset of labor and also diminished the mRNA expression of sPLA₂ IIA and cPLA₂.

Changes in uterine PGs production appear to be involved in the regulation of myometrial activity during pregnancy and labor. We have recently demonstrated that uterine PGF_{2α} synthesis increased abruptly on day 22, just before the onset of labor (Farina *et al.* 2004).

Although the contribution of PLA₂ isoenzymes in the progress of parturition has been previously demonstrated

by other investigators, there are still no reports about which factors could be modulating the activity and/or the expression of these isoenzymes during gestation and at the time of labor. The primary stimulus to the onset of labor is believed to be an increase in E:P ratio in maternal circulation (Challis & Lye 1994). Our data suggest that this endocrine axis, while necessary, is not sufficient to bring about the increased activity of phospholipases A₂. In the present study, we demonstrated for the first time that not only the E:P ratio modulates labor, but also the presence of OT together with E₂ are crucial for the regulation of uterine PLA₂ activity and expression prior to labor. In addition, we showed that this regulation over PLA₂ isoenzymes was reflected in the synthesis of PGF_{2α}, the ultimate and more potent mediator of myometrial contractions during delivery.

OT acting through its receptor is involved in the myometrial hyperactivity of preterm labor, and atosiban has now been registered in many countries for the treatment of preterm labor. The effect of this drug on uterine PLA₂ activity and subsequently on PGF₂ synthesis could be part of its mechanism of action (Akerlund 2006).

In conclusion, our results suggest that a specific modulation of both the secretory and non-secretory PLA₂s by estradiol and OT might be implicated in the increase of PGs synthesis before the onset of labor. These observations reveal that both hormones are essential for the upregulation of uterine phospholipase expression and activity. More studies are necessary to elucidate if a combination of tamoxifen and atosiban could be used to prevent preterm delivery.

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