Preferential esterification of arachidonic acid into ethanolamine phospholipids in epithelial cells from ovine endometrium

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In sheep, the pulsatile release of prostaglandin $F_{2\alpha}$ by the endometrium is necessary to achieve luteolysis which occurs at the end of the oestrous cycle. The production of prostaglandins is known to depend upon the availability of arachidonic acid, the fatty acid precursor of prostaglandin biosynthesis. Consequently, the mechanisms controlling intracellular amounts of arachidonate may be involved in the regulation of prostaglandin synthesis. Since arachidonic acid is mostly found in phospholipids and the endometrial epithelium is the primary source of prostaglandin $F_{2\alpha}$ during luteolysis, the fate of arachidonic acid when incorporated into epithelial cells from the ovine uterus was investigated. Endometrial epithelial cells isolated from cyclic ewes at day 15 after oestrus were cultured in the presence of [3 H]arachidonic acid. Incorporation and distribution of the radiolabelled arachidonic acid into the various phospholipid classes were examined using HPLC. We observed that ethanolamine glycerophospholipids contained 61% of the total tritiated arachidonic acid incorporated into cellular lipids, whereas phosphatidylinositols, phosphatidylcholines and phosphatidylserines contained 17%, 13% and 4.7%, respectively. In addition, the radioactivity measured within phosphatidylethanolamines was preferentially detected in the 1-alkenyl-2-acyl (44%) forms of ethanolamine phospholipids, also called plasmalogens. The kinetic study of arachidonic acid uptake into ethanolamine phospholipids showed that arachidonic acid was rapidly esterified into the diacyl forms and then uptake decreased, whereas the incorporation increased continuously into the plasmalogen forms for at least 24 h. These results demonstrate that the primary pool of esterified arachidonic acid is found in ethanolamine plasmalogens of epithelial cells from the ovine endometrium. The high arachidonate content of ethanolamine plasmalogens suggests that these phospholipids play a crucial role in the control of arachidonic acid availability and ultimately in the regulation of prostaglandin synthesis.

Introduction

In sheep, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is produced by the uterine endometrium during luteolysis which occurs on days 13–16 after oestrus (McCracken *et al.*, 1972; Goding, 1974). At this time, large amounts of $PGF_{2\alpha}$ are released in a pulsatile manner under luteal oxytocin stimulation (Zarco *et al.*, 1988; review by Flint *et al.*, 1990). The amounts of $PGF_{2\alpha}$ secreted into the utero-ovarian venous plasma increase from 1 ng ml^{-1} before luteolysis (days 0–12 after oestrus) to more than tenfold at luteolysis (DeWitt, 1991). Consequently large amounts of arachidonic acid, the metabolic precursor of prostaglandins, are required by the cell to supply the high production of $PGF_{2\alpha}$ observed at the onset of luteolysis.

There is a very small quantity of unesterified arachidonic acid in the cell that is ready to enter the synthesis of prostaglandins. Arachidonic acid is generally found esterified at the *sn*-2 position of glycerophospholipids (Irvine, 1982). Thus, its availability depends mainly on two factors: the arachidonate

content in membrane phospholipids and its release after phospholipid hydrolysis.

Phospholipase A_2 is the principal enzyme involved in the release of arachidonic acid from phospholipids. Consequently, the regulation of phospholipase A_2 activity plays a key role in prostaglandin biosynthesis (Van den Bosch, 1980). In the ovine endometrium, the modulation of phospholipase A_2 activity can account for the differential pattern of $PGF_{2\alpha}$ secretion observed between oestrous cycle and pregnancy. Indeed, it has been shown that endometrial phospholipase A_2 activity was lowered as early as day 14 of pregnancy compared with its activity during the oestrous cycle (Tamby *et al.*, 1993).

The distribution of arachidonate in membrane lipids of ovine endometrial explants was described by Lewis *et al.* (1992), who showed that half of the arachidonic acid was incorporated into neutral lipids and half into phospholipids. Although the limitations of thin layer chromatography did not allow observation of all classes and sub-classes of phospholipid, Lewis *et al.* suggested that triglycerides, phosphatidylcholines and phosphatidylinositols constitute the main arachidonate stores for prostaglandin synthesis. Furthermore, an

Received 13 October 1995.

arachidonate-releasing pathway from phosphoinositides has been proposed as responsible for the increase in PGF_{2α} secretion after stimulation of the endometrium by oxytocin during luteolysis (Flint *et al.*, 1986). Such a mechanism requires phospholipase C activity followed by diacyl- and monoacyl-glycerol lipase activities. This suggests that phosphatidyl-inositols would be the preferential source of arachidonate for prostaglandin biosynthesis. However, the distribution of arachidonic acid in phospholipids has yet to be determined in sheep endometrium.

Since the endometrial epithelium is the major source of uterine PGF_{2α} and the contribution from stroma cells is only minor (Findlay, 1983; O *et al.*, 1983; Charpigny *et al.*, 1991; Danet-Desnoyers *et al.*, 1994), it is of primary importance to assess arachidonic acid reservoirs in epithelial cells without stromal interference. The aim of this study was to investigate the distribution of arachidonic acid in the membrane lipids of endometrial epithelial cells collected from ewes on day 15 after oestrus. Primary cultures of epithelial cells, incorporation of radioactive fatty acids and HPLC separation were used to identify the different classes of phospholipid that contain arachidonic acid.

Materials and Methods

Media and reagents

Intravaginal sponges of 17α-acetoxy-9α-fluoro-11β-hydroxyprogesterone and pregnant mares' serum gonadotrophin (PMSG) were purchased from Intervet S.A. (Angers, France). Media, that is, Hank's, Earle's and Ham-F12, as well as Hepes, penicillin and streptomycin were purchased from Seromed (Intermed SA, Noisy-le-Grand). Nystatin was obtained from Serva (Bio-Whittaker, Fontenay-sous-Bois). Insulin was the Actrapid HM from Novo Nordisk (Boulogne-Billancourt). Ultrosor SF (lyophilized serum substitute, steroid free) was purchased from IBF (Villeneuve-la-Garenne) and was reconstituted with Earle's medium beforehand. Collagenase and hyaluronidase, BSA, unlabelled arachidonic acid and all standard phospholipids were obtained from Sigma (Saint Quentin Fallavier). Solvents (HPLC grade) used for chromatography were purchased from Prolabo (Paris).

Animals

Six ewes ('Préalpes du sud' breed) were induced into oestrus using the protocol described by Robinson (1965) and Shelton (1965). Briefly, ewes were treated for 14 days with intravaginal sponges containing 300 mg 17α-acetoxy-9α-fluoro-11β-hydroxyprogesterone. After removal of sponges at day 14, ewes were injected i.m. with 500 iu PMSG. Oestrus occurred within 48 h after injection and was checked for with a vasectomized ram. The day of oestrus was considered as day 0 of the oestrous cycle.

Preparation of epithelial aggregates from ovine endometrium

The method of preparation was that previously used by Danet-Desnoyers *et al.* (1994). Ewes were slaughtered on day

15 of the oestrous cycle. The uterine horns were removed and the endometrium (including caruncular and intercaruncular regions) was dissected within 15 min of death. The endometrium from each ewe was processed separately as follows. The endometrium was washed in calcium- and magnesium-free Hank's balanced salt solution and weighed. An average of 25 g (wet mass) of endometrium was minced and the resulting paste was diluted in five volumes of Earle's medium containing 15 mmol Hepes ml⁻¹, 150 iu collagenase ml⁻¹, 150 iu hyaluronidase ml⁻¹ and 2% (w/v) BSA. The enzymatic digestion was performed at 37°C for 90 min with gentle agitation. Then, the dispersed tissue was centrifuged at 500 g for 5 min and the pellet resuspended in 25 volumes of Earle's medium. After several cycles of washing and decantation, the suspension was filtered through gauze to remove non-digested tissue and conjunctive fibres. The filtrate was then passed through a 30 μm nylon mesh (Scrynel). The flow-through fraction consisted of individual epithelial cells, fibroblasts and blood cells. The retained fraction was essentially formed of aggregates of epithelial cells from the glandular part of the epithelium. These aggregates were collected by rinsing the nylon mesh with 50 ml Earle's medium. The suspension of aggregates was then centrifuged at 500 g for 5 min and the pellet resuspended directly into the culture medium. The final concentration of epithelial aggregates was approximately equivalent to 5.1 × 10⁵ cells ml⁻¹ medium.

Primary culture of epithelial cells

The culture medium consisted of Ham-F12 containing 5 μg insulin ml⁻¹, 0.1 U nystatin ml⁻¹, 0.1 iu penicillin ml⁻¹, 0.1 μg streptomycin ml⁻¹ and 1% (v/v) Ultrosor SF. Endometrial cells from each ewe were cultured separately. Epithelial cell aggregates were plated into six-well plates (35 mm in diameter, 2 ml per well). The plates were incubated at 38°C in a humidified atmosphere of 95% air and 5% CO₂. The culture medium was changed after the first 48 h of incubation and then was renewed every 48 h. Confluent monolayers of uterine epithelial cells were obtained after 6 days in culture. Each monolayer contained approximately 5.5 × 10⁵ cells in 10 cm² well at confluence. During the culture period, the purity of cell monolayers was checked by phase-contrast light microscopy. On the basis of morphological differences between epithelial and fibroblastic cells, contamination of the cell monolayers by stromal fibroblasts was estimated to be less than 3%. The confluent cells obtained by this method were previously characterized by immunocytochemistry (Guillomot *et al.*, 1988). The cells reacted positively with an anti-cytokeratin 18 monoclonal antibody (clone KS18.174, Progen/Clinisciences, Montrouge) and negatively with an anti-vimentin monoclonal antibody (clone NCL Vim V9, Novo Castra/Tebu, Le Perray-en-Yvelines), confirming their epithelial nature.

Radiolabelling of cellular lipids with [³H]arachidonic acid

At day 6 of culture, the medium was removed and replaced with culture medium containing 0.33 μCi [³H]arachidonic acid ml⁻¹ (2 ml per well, specific activity 213.9 Ci mmol⁻¹, Amersham, Les Ulis, France) plus 0.01 μg unlabelled

arachidonic acid ml^{-1} (2 ml per well). The cells were then incubated for between 30 min and 24 h. At the end of the incubation period, the radioactive medium was discarded and the cells were rinsed with culture medium containing 1 mg BSA ml^{-1} to trap the remaining arachidonic acid that was not incorporated into the cells.

Lipid extraction

Cellular lipids were extracted using a modified method of Bligh and Dyer (1959). Each cell monolayer was extracted separately at room temperature. Cells were scraped in 1 ml methanol:0.1% acetic acid (70:30) using a rubber policeman and were poured into a glass tube. Then 1.5 ml methanol, 0.3 ml distilled water and 0.2 ml KCl (3 mol l^{-1}) were added. The tubes were vortexed and 1 ml chloroform was added. The tubes were stirred to form a single phase. Then, 1 ml of chloroform plus 1 ml of water were added and the tubes were vigorously vortexed. After a centrifugation at 110 *g* for 10 min, the mixture separated into two phases. The lower phase (chloroform phase) was decanted into new glass tubes. Two millilitres of chloroform was added to the remaining upper phase (aqueous phase) for a second extraction. After vortexing and centrifuging the mixture at 110 *g* for 10 min, the resulting chloroform phase was decanted and pooled with the first one. The pooled chloroform phases were washed with 1 volume of chloroform:methanol:water (3:48:47), vortexed and centrifuged at 110 *g* for 15 min. The final chloroform phase was decanted in new glass tubes and the chloroform was evaporated at 40°C under a stream of N_2 . The dry residue was solubilized in chloroform:methanol (2:1) and stored at -20°C until HPLC analysis.

Chromatographic analysis of lipid extracts

Chromatographic analysis of lipids was conducted according to the method described by Dugan *et al.* (1986) with modifications. The separation was performed on a Zorbax silica column (150 mm $\times$ 4.6 mm i.d., 5 μm particle size) equipped with a guard column of the same nature (18 mm $\times$ 3.9 mm i.d.). Both columns were held at 34°C and the solvents were kept at 25°C with continuous stirring. Columns were equilibrated at a flow rate of 2 ml min^{-1} . The equilibration phase consisted of 45% solvent A (*n*-hexane:propanol-2 (3:2)) and 55% solvent B (*n*-hexane:propanol-2 (3:2) containing 5.5% water).

Lipid extracts previously stored at -20°C in chloroform:methanol (2:1) were evaporated at 40°C under a stream of N_2 . The dry residues were dissolved in 100 μl HPLC equilibration phase (45% solvent A:55% solvent B). An aliquot of each sample was taken and radioactivity was measured on a β counter. Samples were then injected into the HPLC system for analysis. Five minutes after injection, a gradient from 55%–100% solvent B was developed over 15 min. Solvent B was maintained at 100% for 4 min to complete the separation. Solvents were then returned to a ratio 45:55 over 2 min and the columns were allowed to re-equilibrate for 10 min. The eluted ^3H -labelled compounds were detected using an 'on line' β counter (Flo/One A515, Packard). Standard phospholipids were detected at 206 nm using a UV detector (model 490,

Waters). Phospholipids were identified by comparison with the retention times of standard molecules that were processed in the same way as the samples. Fractions corresponding to the radioactive peaks were collected.

Further analysis of the collected fractions was performed by thin layer chromatography using the method described by Gilfillan *et al.* (1983) to confirm the nature of the phospholipids. The standard phospholipids were phosphatidylethanolamine, phosphatidic acid, phosphatidylinositol, phosphatidylserine and phosphatidylcholine.

Throughout this study, every step of analysis was performed separately for each cell monolayer. For each animal, the reported values represented the average of five cell cultures from the same ewe. Finally, the results were expressed as the mean $\pm$ SEM of six ewes.

HPLC chromatography of ethanolamine plasmalogens

The method described by Dugan *et al.* (1986) was followed with slight modifications. The same HPLC system and columns as for phospholipid separation were used for the separation of plasmalogens. Columns were held at 42°C and were equilibrated with 84% solvent C (*n*-hexane:propanol-2 (98.8:1.2)) and 16% solvent D (propanol-2: H_2O (93:7)). A flow rate of 1.5 ml min^{-1} was maintained throughout the chromatography.

The fractions containing ethanolamine glycerophospholipids collected in the previous step of chromatography were evaporated at 40°C under a stream of N_2 and the dry residues were dissolved in 84% solvent C:16% solvent D. The sample was injected into the HPLC system and eluted in isocratic condition (84% C:16% D). The separation was completed in 30 min. The columns were washed with 100% solvent D for 15 min after every five separations to keep the best resolution. The subclasses of ethanolamine phospholipids were identified by comparison with the retention times observed for standard phosphatidylethanolamine consisting of 60% plasmalogens and 40% diacyl phosphatidylethanolamine. The identity of plasmalogens in both the standard and samples was confirmed by acid treatment of the phosphatidylethanolamine followed by HPLC analysis. The treatment with acid (0.1 mol HCl l^{-1} for 1 h) results in hydrolysis of the ether link in position 1 of plasmalogens without affecting the ester bonds of diacyl phospholipids. Such acid treatment caused a complete disappearance of the peak corresponding to 1-alkenyl-2-acyl phosphatidylethanolamine and concurrently generated a large peak at the retention time of lysophosphatidylethanolamine.

Results

Time-course of free [^3H]arachidonic acid incorporation into cellular lipids

The radioactive arachidonic acid added to the culture medium of endometrial epithelial cells was rapidly incorporated into lipids (Fig. 1). After 3 h of incubation, [^3H]arachidonic acid uptake reached 50% of its maximum value, as determined by the radioactivity measured in the total lipid extract. Maximal

Fig. 1. Time-course of [^3H]arachidonic acid incorporation into epithelial cells from ovine endometrium as measured by the radioactivity detected in total lipid extracts (mean d.p.m. $\pm$ SEM, $n = 6$).

incorporation of [^3H]arachidonic acid was reached by 9 h of culture and was maintained for at least 24 h.

Distribution of arachidonic acid among phospholipid classes

HPLC analysis of a total lipid extract from epithelial cells incubated with [^3H]arachidonic acid is shown (Fig. 2a). Six peaks of radioactivity were observed in the chromatogram with the respective retention times (R_t) of 1.6, 4.0, 7.9, 10.5, 12.1 and 21.8 min. The first peak ($R_t = 1.6$ min) corresponded to the neutral lipids. It was mainly composed of mono-, di- and tri-glycerides. The peaks with R_t 4.0, 7.9, 12.1 and 21.8 min were identified, by comparison with the R_t of standard compounds (Fig. 2b), as distinct phospholipid classes, respectively: phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and phosphatidylcholine. The phosphatidic acid which normally eluted between phosphatidylethanolamine and phosphatidylinositol was not detected in the radioactivity chromatograms. The peak at R_t 10.5 min was not detected in all samples and was identified by TLC as a lysophospholipid originating from phosphatidylethanolamine.

The nature of phospholipids identified by HPLC was verified using TLC. This was particularly important for the peaks with retention times ranging from 5 to 15 min for which there could be ambiguities. This method confirmed the identity of all the phospholipids separated by HPLC and particularly phosphatidylinositol and phosphatidylserine (results not shown).

Esterification of arachidonic acid into phospholipids

After incubation for 24 h in the presence of [^3H]arachidonic acid, up to 96% of the total radioactivity was found in the different phospholipid classes (Table 1). Neutral lipids contained less than 4% of the total radioactivity. Similar results were observed at 6 or 9 h of incubation.

Among the four classes of phospholipid which significantly esterified [^3H]arachidonic acid in the epithelial cells, the phosphatidylethanolamines contained the highest part of the radioactivity detected. After 24 h of culture in the presence

of [^3H]arachidonic acid, more than 61% of the total [^3H]arachidonic acid incorporated into the phospholipids was found in the phosphatidylethanolamines. Phosphatidylinositol and phosphatidylcholine species incorporated approximately 17% and 13% of [^3H]arachidonic acid, respectively. Phosphatidylserine incorporated less [^3H]arachidonic acid than did the other classes (4.7%).

Preferential esterification of arachidonic acid into ethanolamine plasmalogens

Fractionation of phosphatidylethanolamines by a second step of HPLC gave two major peaks with retention times corresponding to plasmalogens (first peak) and diacyl phosphatidylethanolamines (second peak) as previously established by Dugan *et al.* (1986) (Fig. 3). Acid treatment of the first peak confirmed that it was the 1-alkenyl-2-acyl form of ethanolamine glycerophosphates, that is, ethanolamine plasmalogens (data not shown). The second peak mainly consisted of 1,2-diacyl phosphatidylethanolamines. The 1-alkyl-2-acyl phosphatidylethanolamine eluted with the diacyl form.

The ethanolamine plasmalogens constituted the major class of phospholipid that incorporated [^3H]arachidonic acid in cultured epithelial cells from ovine endometrium (see Table 1). After 24 h of incubation, 44% of the radioactive arachidonic acid was detected in the ethanolamine plasmalogens.

Time-dependent incorporation of [^3H]arachidonic acid into ethanolamine glycerophospholipids

The 1-alkenyl-2-acyl and 1,2-diacyl forms incorporated [^3H]arachidonic acid in a time-dependent manner when the epithelial cells were incubated for 24 h (Fig. 4). A differential pattern of labelling was observed for the two sub-classes. After 2 h of [^3H]arachidonic acid incorporation, 78% of the total radioactivity was detected within the diacyl forms. Maximum labelling of the diacyl forms was reached in 6 h and then radioactivity decreased progressively. In contrast, incorporation of [^3H]arachidonic acid into ethanolamine plasmalogens was less rapid than for the diacyl forms since 22% of the radioactivity was detected into plasmalogens after 2 h of incubation. As the incubation progressed, incorporation in plasmalogens increased and became greater than in the diacyl forms. Moreover, [^3H]arachidonic acid incorporation did not reach a maximum as no apparent saturation was observed within 24 h of incubation. At the end of the 24 h incubation time, plasmalogens represented 69% of the radiolabelled phosphatidylethanolamines, and the diacyl forms represented 31%.

Discussion

The aim of this study was to investigate the fate of arachidonic acid in the epithelial cells from ovine endometrium. It was shown previously that endometrial epithelium produces and metabolizes more prostaglandin-F than does stroma (Findlay, 1983; O *et al.*, 1983). In addition, primary cultures of epithelial and stromal cells from bovine and ovine endometrium

Fig. 2. HPLC analysis of (a) total lipid extract from ovine endometrial epithelial cells compared with (b) the standard phospholipids. SF: solvent front; NL: neutral lipids; PE: phosphatidylethanolamine; PA: phosphatidic acid; PI: phosphatidylinositol; LPE: lysophosphatidylethanolamine; PS: phosphatidylserine; PC: phosphatidylcholine.

Table 1. Distribution of arachidonic acid among the different classes of phospholipid in ovine endometrial epithelial cells after 24 h incubation

	Phosphatidylethanolamine		Phosphatidylinositol	Phosphatidylserine	Phosphatidylcholine
	1-alkenyl-2-acyl phosphatidylethanolamine	1,2-diacyl phosphatidylethanolamine			
Neutral lipids					
	44 ± 1.9	17.1 ± 2.1	17.2 ± 3.8	4.7 ± 3.1	13.2 ± 1.3

Values represent the percentage of radioactivity contained in the peaks: mean ± SEM, $n = 6$.

indicated that epithelial cells predominantly secreted $\text{PGF}_{2\alpha}$ compared with stromal cells (Fortier *et al.*, 1988; Cherny and Findlay, 1990; Danet-Desnoyers *et al.*, 1994). In the study reported here, epithelial cells released two to ten times more $\text{PGF}_{2\alpha}$ than did stromal cells (Charpigny *et al.*, 1991). Thus uterine epithelial cells are a useful model to study the metabolism of arachidonic acid.

We have shown that free arachidonic acid was rapidly incorporated into lipids of epithelial cells under normal culture conditions. Most arachidonic acid incorporation into lipids occurred after incubation for 6–8 h in the presence of [^3H]arachidonic acid. In human endometrium, Korte *et al.* (1983) and Bonney *et al.* (1991) have reported a maximum incorporation of arachidonic acid into phospholipids *in vitro*

Fig. 3. HPLC separation of ethanolamine phospholipids from ovine endometrial epithelial cells into 1-alkenyl-2-acyl phosphatidylethanolamine (alkenylPE) and 1,2-diacyl phosphatidylethanolamine (diacylPE).

Fig. 4. Time-course of incorporation of [^3H]arachidonic acid into (Δ) ethanolamine plasmalogens and ($\bullet$) diacyl phosphatidylethanolamines of ovine endometrial epithelial cells, as measured by the radioactivity in each peak separated by isocratic HPLC (mean d.p.m. $\pm$ SEM, $n = 6$).

after incubation for 4–8 h for both epithelial and stromal cells.

The present results show that arachidonic acid was mostly incorporated into phosphatidylethanolamines, phosphatidylinositols and phosphatidylcholines. In addition, we demonstrated that arachidonic acid was predominantly esterified in a particular form of phospholipid called ethanolamine plasmalogen. Surprisingly, the existence of plasmalogens had not been shown before in the ovine endometrium. This result suggests that plasmalogens play an important role in the regulation of arachidonic acid incorporation and metabolism in this cell type.

Most authors working on uterine phospholipids have shown that arachidonic acid is incorporated mostly into glycerophospholipids in various cell types (for review, see Irvine, 1982). Leaver and Poyser (1981) found that 93% of the total arachidonate was esterified into phospholipids of the guinea-pig uterus. In human endometrium, Bonney *et al.* (1991)

showed that 2–4% of arachidonic acid was esterified in the triglycerides, and proposed that the remaining 96% was incorporated into phospholipids of glandular cells *in vitro*. Our study is consistent with these results since more than 96% of the incorporated arachidonate was in the phospholipids, whereas the neutral lipids contained very small quantities of arachidonate. In contrast, Lewis *et al.* (1992) reported that in the ovine endometrium, 50% of arachidonic acid was incorporated into polar lipids; the other 50% was esterified into neutral lipids. However, it must be noted that these authors used endometrial explants consisting of both epithelial and stromal cells. Consequently, the qualitative differences in arachidonic acid uptake were probably due to the stromal component of the endometrium, although this point needs to be clarified. Nevertheless, it seems likely that phospholipids are the main store of arachidonic acid in a large variety of cells including the epithelial cells from ovine endometrium.

In the study reported here, it was established that, in epithelial cells from ovine endometrium, the phosphatidylinositols contained 17% of the total bound [^3H]arachidonic acid. This proportion is higher than those reported in ovine endometrium by Lewis *et al.* (1992) and Flint *et al.* (1986), who found 11% and 5.6%, respectively. However, these authors measured the overall arachidonic acid incorporation into phosphatidylinositols of endometrial explants (epithelial and stromal tissues). The result reported here specifies the incorporation of arachidonic acid into the epithelial cells of endometrium. The large amounts of arachidonate in phosphatidylinositols of the epithelium reinforces the hypothesis proposed by Flint *et al.* (1986) that phosphoinositides are a significant source of arachidonic acid for uterine prostaglandin synthesis.

The main result of the present study is that ethanolamine plasmalogens are the primary site of arachidonic acid incorporation into uterine epithelial cells. Plasmalogens have not been described previously in either ovine endometrium (Lewis *et al.*, 1992) or in guinea-pig uterus (Leaver and Poyser, 1981; Ning and Poyser, 1984). This was probably due to

methodological aspects. In contrast to HPLC, TLC, which was used in these other studies, is not a suitable method for the separation of alkenyl and diacyl forms of phosphatidylethanolamines.

In the guinea-pig uterus, arachidonic acid was found mostly in phosphatidylethanolamines and phosphatidylcholines (Leaver and Poyser, 1981). In contrast to our results, Lewis *et al.* (1992) did not observe large amounts of arachidonic acid in phosphatidylethanolamines from ovine endometrium. They reported that phosphatidylcholine and phosphatidylinositol were the major sites of arachidonic acid storage. These conflicting observations could be explained by the differences in *in vitro* models: endometrial explants were used by Lewis *et al.* (1992) and endometrial epithelial cells in the present work.

Ethanolamine glycerophosphate synthesis can occur via three distinct pathways: (i) the CDP-ethanolamine pathway via desaturation of the 1-alkyl-2-acyl glycerophosphoethanolamine; (ii) the decarboxylation of serine glycerophospholipids and (iii) a calcium-dependent base exchange of ethanolamine with pre-existing phospholipids (reviewed by Garg and Haerdi, 1993). The continuous increase of radioactivity in the plasmalogens and the concomitant decrease in the diacyl/alkylacyl forms suggest that the synthesis of ethanolamine plasmalogens occurs preferentially via the desaturation pathway.

Our results suggest that ethanolamine plasmalogens may be an important source of arachidonic acid. Although putative functions have been attributed to plasmalogens in numerous cell types, their biological significance remains unclear (for review, see Garg and Haerdi, 1993). Among the potential roles assigned to plasmalogens, the roles that are relevant to the synthesis of prostaglandins, that is, the regulation of intracellular arachidonic acid concentrations, should be mentioned. Plasmalogens are considered as intracellular stores of unsaturated fatty acids, especially for the (n-3) series, as arachidonic acid (Cook *et al.*, 1991). For this reason, they are likely to be involved in the availability of arachidonic acid for the synthesis of prostanoids. In rabbit vascular smooth muscle stimulated by angiotensin-II, Ford and Gross (1989) reported that 60% of the arachidonic acid released from ethanolamine glycerophospholipids came from the plasmalogen moiety, suggesting their importance in the regulation of the arachidonic acid pool.

Ethanolamine plasmalogens appear to represent the primary storage form for the bulk of arachidonic acid needed to produce high concentrations of prostaglandins. It was recently demonstrated that hydrolysis of plasmalogens was directed by a membrane-associated Ca^{2+} -independent phospholipase A_2 . This enzyme is distinct from cytosolic Ca^{2+} -dependent phospholipases and has a high specificity for plasmalogens (Ford *et al.*, 1991). Such a phospholipase A_2 could participate in the direct release of arachidonic acid from plasmalogen stores, emphasizing its regulatory role in the intracellular availability of free arachidonic acid.

We have previously reported that an inhibitor of endometrial phospholipase A_2 causes a reduction in $\text{PGF}_{2\alpha}$ production by the ovine uterus during early pregnancy (Tamby *et al.*, 1993). Furthermore, we observed an increase in arachidonic acid content of ethanolamine plasmalogens in epithelial cells from early pregnant ewes, compared with nonpregnant animals

(J. Ph. Tamby, G. Charpigny and P. Reinaud, unpublished). It can be hypothesized that inhibition of the plasmalogen-specific phospholipase A_2 could occur during the early stages of pregnancy and cause a retention of arachidonate within plasmalogens. Although this remains to be demonstrated, these observations suggest that there may be a direct relationship between phospholipase A_2 activity and the arachidonic acid content of plasmalogens in epithelial cells of ovine uterus.

The structural role of plasmalogens could be important in a reproductive context. Because of their atypical structure, plasmalogens contribute to the cell membrane organization (Lohner *et al.*, 1991). As the uterus stretches extensively during pregnancy, plasmalogens could play a major role in the membrane fluidity and rearrangement, although this hypothesis has not yet been tested.

This work was supported by a fellowship from the Association pour la Recherche sur le Cancer and a grant from the E.C. (Biotech Research Program no. BIO2CT920067). We gratefully thank G. Danet-Desnoyers and M. Guillomot for their helpful suggestions.

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