Regulation of quail oviduct phospholipase A₂ activity by estradiol

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The phospholipase A₂ (PLA₂) activity was measured in the oviduct of immature and estradiol benzoate (EB)-treated quails. The pH profiles demonstrate the presence of two PLA₂ isomers in the avian oviduct: a neutral isomer, optimally active at pH 7–7.5 and calcium independent, responsible for most of the hydrolytic activity in the immature oviduct and poorly stimulated by estradiol; and an alkaline isomer, optimally active at pH 8–9.5 and calcium dependent, with little activity in the immature tissue but markedly stimulated by EB. After EB injection, PLA₂ activation occurs at first during the prereplicative period of oviduct cells (+172% at 6 h), it is dose dependent from 0.01 to 1 mg/kg EB and can be prevented by cycloheximide together with ornithine decarboxylase activation. Moreover, estradiol was inactive on cell-free extracts of immature oviducts. These results suggest that EB increases PLA₂ activity through gene activation and de novo protein synthesis. The correlation between the early stimulation of PLA₂ activity and the proliferation of oviduct cells is discussed.

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Phospholipase A₂ (PLA₂) is considered as the rate limiting enzyme in the release of arachidonic acid from membrane phospholipids (1). The arachidonic acid released is not stored within the cell but is either re-esterified into lipid stores or converted into bioactive substances such as prostaglandins, thromboxanes and leukotrienes (2). It has been demonstrated in several species that eicosanoids play a number of physiological roles in reproduction. For example, prostaglandins have been implicated in the initiation of menstruation (3) and are involved in increased vascular permeability and decidualization in preparation for blastocyst implantation (4). Leukotrienes also have been implicated in the preimplantation period (5) and a supportive role for thromboxane in estradiol-induced uterine growth has been proposed in the rat (6).

Our knowledge of the diversity of forms and functions of PLA₂ has increased in the last few years and recently a nomenclature for these enzymes, based on their structure and sequence, has been proposed. Two major groups are described: the extracellular or low molecular mass (14–18 kD) forms, called sPLA₂ because of their secretory distribution; and the intracellular or high molecular mass (31–110 kD) forms, called cPLA₂ because of their cytosolic origin (7). The 14–18 kD enzymes have been classified using evolutionary relationships into type I (mammalian pancreatic, cobra venom, etc.), type II (mammalian platelets, human synovial fluid, viper venom, etc.) and type III (bee venom, etc.); the 31–110 kD PLA₂ are designed as type IV (8). The PLA₂ appears as a pluripotent enzyme playing many roles in normal and pathological cellular functions. Recent data have involved directly a secreted low-molecular-weight PLA₂ in the control of fibroblast (9) and chondrocyte (10) proliferation.

Numerous studies have shown that steroid hormones regulate eicosanoid production, particularly prostaglandins, in the endometrial tissue (11, 12). This modulation was attributed at first to an effect on the prostaglandin synthase pathway. However, regulation of PLA₂ activity by steroid hormones has been demonstrated in rat uterus (13), where chronic estrogen treatment stimulates PLA₂ activity and progesterone pretreatment followed by estrogens inhibits this effect. Further studies have described the presence of two PLA₂ enzymes in human endometrium (14–17): one is calcium dependent, optimally active at pH 7.5–9.0 and present mainly in the glandular component of the endometrium; the other is calcium independent, optimally active at pH 7.0 and located predominantly in the stromal layer. Both enzymes are inhibited by progesterone, whereas estradiol alone was inactive. However, progesterone pretreatment followed by treatment with estradiol caused a twofold stimulation of the calcium-dependent enzyme but not the calcium-independent enzyme. Thus, estrogen can act by modulating multiple forms of PLA₂ enzymes that are regulated by pH and calcium requirements.

The bird oviduct has been studied extensively to investigate cell proliferation and cell differentiation in
response to steroid hormones, but no data are available for \( \text{PLA}_2 \) enzymes in this tissue or their hormonal regulation. In previous studies we have shown that estradiol stimulates indirectly the proliferation of quail oviduct epithelial cells (18) and that cAMP regulatory systems are involved in this indirect mechanism (19, 20). We have demonstrated also that dexamethasone inhibits estrogen-induced fluid inhibition and oviduct epithelial cell proliferation, whereas it stimulates particularly ovalbumin production (21, 22). It is noteworthy that glucocorticoids have been shown recently to inhibit 14-kD \( \text{PLA}_2 \) production (23). In more recent works we have reported that in natural or estradiol-induced development of the quail oviduct, and more specifically during the period of intense cellular proliferation, significant changes occurred in the fatty acid composition of oviduct phospholipids: the proportion of 20:4(n-6) and 22:4(n-6) decreased, whereas those of 18:2(n-6) and 22:6(n-3) increased (24). Moreover, injection of estradiol benzoate to immature quails stimulated oviduct \( \text{PLA}_2 \) activity while tamoxifen, a non-steroidal antiestrogen, significantly reduced both basal and estrogen-induced \( \text{PLA}_2 \) activities (25). Taken together, these results suggest a relationship between \( \text{PLA}_2 \) activity and estrogen-induced epithelial cell proliferation.

In the present study we have investigated the characteristics of the enzyme (pH and calcium requirements, substrate specificity) in the immature oviduct, the kinetics of its activation by estrogens in primary stimulation or after repeated injections and the relationship between \( \text{PLA}_2 \) activation and oviduct cell proliferation.

Materials and methods

**Chemicals**

\( \text{L-3-Phosphatidylcholine, 1-stearoyl-2-[1}^{14}\text{C]arachidonyl (1.85–2.2 GBq/mmol, 50–60 mCi/mmol; Ref. CFA 655) and 1,2-di[1}^{14}\text{C]oleoyl (3.7–4.4 GBq/mmol; 100–120 mCi/mmol; Ref. CFA 695) were purchased from Amersham International. L-[1}^{14}\text{C]ornithine (1.48–2.2 GBq/mmol; 40–60 mCi/mmol; Ref. NEC 710) was purchased from NEN (Du Pont de Nemours, Les Ulis, France). Estradiol benzoate (EB) was obtained from Roussel Uclaf (Benzoygysteryl, 5 mg/ml). Bisbenzimide H 33258 was obtained from Fluka (Switzerland) and other reagents and standards from Sigma (St Louis, MO, USA). All solvents used were of analytical grade.**

**Animals and tissue preparation**

Immature female quails (Coturnix coturnix japonica), 18–21 days old, were obtained from “La Caille des Dombes” (Saint André de Corcy, France). Standard chow (UAR 115, UAR Villemoisson sur Orge, France) and water were provided ad libitum. Animals were raised in groups at a constant temperature (23°C) in a daily cycle of light and darkness (14 h light).

Estradiol benzoate (0.01–1 mg/kg) dissolved in olive oil was injected im in a total volume of 0.1 ml. Control animals received the vehicle alone. Treatments varied according to the group studied (one to three injections) and are described in the Results section. Animals were sacrificed by decapitation at different times after the last injection. The oviduct was removed quickly, cleaned of connective tissue, weighed and homogenized immediately in ice-cold 0.1 mol/l TRIS-HCl buffer (pH 8.0) containing 5 mmol/l CaCl2 and 0.25 mol/l sucrose (1 ml for 100 mg of tissue) using a polytron homogenizer for 2 min at 4°C. The DNA content of the homogenate was measured according to the method of Labarca and Paigen (26). The homogenate then was centrifuged at 800 g for 10 min at 4°C and the \( \text{PLA}_2 \) activity was measured on the resulting supernatant.

**Phospholipase \( \text{A}_2 \) assay**

Phospholipase \( \text{A}_2 \) activity was measured on the low-speed supernatant of oviduct homogenate using an assay based on the liberation of arachidonic acid from 1-stearoyl-\( [1}^{14}\text{C] arachidonyl phosphatidylcho-}

line as described previously (25). The assay, composed of 100 \( \mu l \) of the supernatant defined above and 100 \( \mu l \) of the substrate solution, was incubated at 37°C. The substrate solution (final concentration 260 \( \mu l \) mol/l) contained unlabeled \( \text{L-\alpha-phosphatidylcho-
}

line-\( \beta \)-arachidonyl-\( \gamma \)-stearoyl and about 10\(^{6}\) cpm/ml of labeled substrate (CFA 655) in 0.1 mol/l TRIS-HCl buffer (pH 8.0) and 0.1% Triton X100 (the mol% Triton X100 was kept constant in all experiments). A maximal reaction velocity was observed with this substrate concentration and the release of arachidonic acid was linear with time for up to 90 min. With an incubation time of 60 min, which was used mostly, the release of arachidonic acid increased linearly with tissue concentration in the assay from 10 to 250 mg/l (not shown). The reaction was stopped by the addition of 2 ml of chloroform–methanol (2:1, v/v) containing 0.02% butylated hydroxy toluene (BHT) as an antioxidant and 100 \( \mu g \) of oleic acid as a carrier. The extraction of lipids was completed by the addition of 0.2 ml of 2 mol/l KCl plus 0.5 mmol/l EDTA. After centrifugation (900 g for 5 min), the chloroform layer was removed by aspiration. A second extraction with 1 ml of chloroform was realized and the pooled chloroform extracts were evaporated to dryness under a vacuum.

Lipid classes were separated using anion exchange chromatography columns (Amersham, Ref. RPN 1917). The dry lipid extract was dissolved in 1.5 ml of chloroform and applied to the top of the column washed previously with 5 ml of chloroform. Neutral lipids were eluted with 5 ml of chloroform–isopropanol (2:1, v/v), free fatty acids (with labeled arachidonic acid) were eluted with 5 ml of diethyl ether containing
2% acetic acid and more polar lipids were eluted with 5 ml of methanol. Eluates were collected in scintillation vials, evaporated to dryness and their radioactivity measured in a scintillation counter (Packard, Tri-Carb 3225). Procedural losses occurring during the isolation of the reaction products were determined from internal standards; more than 85% of the radioactivity was recovered at the end of the process. The PLA₂ activity was determined from the radioactivity found in the free fatty acid fraction (second eluate). Because rapid changes occur in the proliferation of quail oviduct cells (hyperplasia) and in the synthesis and accumulation of specific proteins such as ovalbumin (hypertrophy) during natural maturation or after EB injection, as demonstrated previously (19, 21, 27, 28), PLA₂ activity values measured in the low-speed supernatant of oviduct homogenates were normalized to the DNA content of the whole homogenate and expressed as pmol arachidonic acid released·min⁻¹·mg⁻¹ DNA.

For determination of the optimum pH and calcium requirements, the cell homogenate was prepared in sucrose (0.25 mol/l) and the supernatant (50 µl) then was buffered with 100 µl of the following buffers (0.2 mol/l): sodium acetate–acetic acid (pH 5–5.5) or sodium phosphate (pH 6–7) or TRIS·HCl (pH 7.5–9) or sodium carbonate–bicarbonate (pH 9.5) and 50 µl of either CaCl₂ or EGTA solutions.

Ornithine decarboxylase (ODC) assay

Ornithine decarboxylase (EC: 4.1.1.17) is the first and rate-limiting enzyme in polyamine biosynthesis and changes in ODC activity reflect the effects of the growth stimulus on protein biosynthesis (29). The ODC activity was used as a marker of the inhibitory effect of cycloheximide on protein synthesis and was measured in the cytosol (1 h at 105 000 g) by a technique derived from those described by Farrar et al. (30) and Otani et al. (31). Briefly, the radioactive ¹⁴CO₂ formed in the presence of pyridoxal phosphate. EDTA, dithiothreitol and L-[¹⁴C]ornithine was collected on GF/C filters and measured by liquid scintillation. Under the conditions used, the decarboxylation reaction was linear with time and enzyme quantity. Background decarboxylation, not attributable to ODC, determined in the presence of D,L-α-difluoromethyl ornithine (DFMO), a specific inhibitor of ODC, never exceeded 5%.

Statistical analysis

Data are expressed as means ± S.E.M. Overall comparisons were made with the Kruskal–Wallis rank test after testing the homogeneity of dispersion with the squared rank test (32) and an experimental error rate of 5%. When the homogeneity of means was rejected, a comparison of individual groups was performed by the Nemenyi joint rank test (33). The large sample approximation (33) was used when the number of replicates in the two treatments were unequal. Only the comparisons between two groups discussed in the text were indicated in the figures.

Results

Characterization and properties of basal and estrogen-induced oviductal PLA₂ activities

The PLA₂ activity was measured in the oviduct of control and EB-treated quails over the pH range 5–10 in the presence or absence of 5 mmol/l calcium. Calcium was replaced by 0.5 mmol/l EGTA in calcium-free media. The pH profiles of PLA₂ activity in control oviduct illustrated in Fig. 1A. demonstrate the presence of two PLA₂ fractions with different pH and calcium requirements. The main fraction is active at pH 7–7.5, independent of the presence or absence of calcium. A smaller fraction is active at pH 9–10 in the presence of 5 mmol/l calcium: its hydrolytic activity was reduced significantly in the presence of EGTA in the incubation medium (~60% at pH 9.5).

The pH profiles of PLA₂ activity in EB-treated oviduct are illustrated in Fig. 1B. The presence of the two PLA₂ fractions detected in control oviduct is still obvious in the EB-treated oviduct. The relative changes of PLA₂ activity induced by the hormonal treatment are represented in Fig. 1C. Estrogen treatment increased much more the activity of the alkaline fraction (about threelfold) than that of the neutral fraction (about 1.5-fold). Moreover, the increase of enzyme activity at a given pH was not significantly different in the presence of EGTA or Ca²⁺, which suggests that estradiol did not affect the PLA₂ sensitivity to Ca²⁺.

It is noteworthy that the maximal increase of enzyme activity after estrogen treatment occurred at pH 8–9 (Fig. 1C), while the alkaline isoform was optimally active at pH 9–10 in the immature oviduct (Fig. 1A). These results suggest that EB shifted the optimal activity of the alkaline fraction to a more neutral pH.

The effects of increasing calcium concentrations on PLA₂ activity, measured in oviduct homogenate of EB-treated birds at pH 7 and pH 9, are illustrated in Fig. 2. While at pH 7 the enzyme activity showed no significant change in the presence of free calcium over the range 0.1 µmol/l–10 mmol/l at pH 9 the enzyme activity was stimulated by millimolar concentrations of calcium, demonstrating that the alkaline fraction was calcium dependent.

To characterize further the estradiol-activated PLA₂, we investigated the sn-2 acyl chain selectivity by measuring the hydrolysis of phosphatidylcholine containing either [L-¹⁴C]arachidonoyl or [L-¹⁴C]oleoyl at the sn-2 position. Oleate was released to the same extent as arachidonate (not shown).
Fig. 1. Effects of pH and calcium on oviduct phospholipase A₂ (PLA₂) activity of control (A) and estradiol benzoate (EB)-treated quails (B, C). Twenty-one-day-old immature quails received three daily injections of EB (1 mg/kg, im) and were killed 24 h after the last injection: control animals received only the vehicle. The PLA₂ activity was measured in oviduct homogenates at different pH, either in the presence of 5 mmol/l calcium or 0.5 mmol/l EGTA. Each point is the mean ± SEM of at least six independent values. In (C), the enzyme activity of the EB-treated group is expressed as a percentage of the corresponding control value.

Kinetics of enzyme activation by EB
The time course of enzyme activation was determined after one or three daily injections of EB; PLA₂ activity was measured under optimal conditions determined previously (pH 8 in the presence of 5 mmol/l calcium: Fig. 3).

After a single injection of 1 mg/kg EB to immature quails, a first surge of PLA₂ activity was observed between 0 and 12 h. The PLA₂ activity was increased significantly at 3 h (+63%), was maximally active at 24 h (+476%), remained high up to 40 h and then declined to the control value at 72 h.

After three daily injections of EB (Fig. 3B), the PLA₂ activity was increased significantly 1 h after the last injection (+70%) and maximal activation was observed at 6 h (+179%). The PLA₂ activity remained higher than in control immature quails 24 h or 30 h after the third EB injection (+350% and +220%, respectively).

Relation between estrogen-induced PLA₂ activity and cell proliferation
The effects of one injection of different doses of EB on oviduct growth and PLA₂ activity are summarized in Table 1. The different parameters are measured 6 h after the hormone injection, i.e. in the middle of the prereplicative period, and again at 24 h after the injection, when the increase in DNA content is at its maximum (19).

Estradiol benzoate at 0.01 mg/kg had no effect on the oviduct growth (wt weight and DNA content) at 6 h and 24 h or on PLA₂ activity at 6 h. However, PLA₂ activity was increased significantly at 24 h, which shows the high sensitivity of this parameter to low EB doses. Estradiol benzoate at 0.1 mg/kg increased the oviduct wet weight at 6 h (+54% and +114%, respectively) without significant change in the DNA content. Hyperplasia occurred later and both weight and DNA content were increased at 24 h. As expected, the PLA₂ activity was increased significantly 6 h after the injection of 0.1 or 1 mg/kg EB (+90% and +162%,

Fig. 2. Effects of calcium on phospholipase A₂ (PLA₂) activity in estradiol benzoate (EB)-treated quails. Twenty-one-day-old immature quails received three daily injections (1 mg/kg) of EB and were killed 24 h after the last injection. The PLA₂ activity was measured in the oviduct homogenate at pH 7 and pH 9 in the presence of increasing concentrations of calcium. The results from a representative experiment are shown. Similar results were obtained in three different experiments.
were tested. 17β-Estradiol (E2-17β) significantly increased the oviduct weight and PLA2 activity at 6 h (+63% and +138%, respectively). At 24 h all the parameters, including the oviduct DNA content (+59%), were increased. 17α-Estradiol (E2-17α) had no effect at 6 h. Twenty-four hours after the injection, E2-17α increased the oviduct wet weight, but less than E2-17β or EB (-51% and -71%, respectively), and did not affect significantly the oviduct DNA content or the PLA2 activity.

The enzyme sensitivity to estradiol in vitro also was determined. Samples of cell-free extracts of immature quail oviducts were incubated in the presence of various concentrations of E2-17β (10^{-11}-10^{-7} mol/l). The hormone was dissolved in ethanol and added to the assay mixture, the final ethanol concentration being 0.01% in control and hormone-treated samples. No significant variation of PLA2 activity was observed whatever the concentration of E2-17β used (not shown).

The PLA2 activity also was measured in the oviduct of non-treated quails at the beginning of natural sexual development. The results, presented in Table 2, show that enzyme activity increased rapidly during this period of intense proliferation of oviduct cells.

**Discussion**

Regulation of uterine PLA2 activity by steroid hormones was first demonstrated by Dej and co-workers (13) in mature hypophysectomized females rats. Chronic estrogen treatment greatly stimulated uterine PLA2 activity. This effect was reduced by progesterone pretreatment or dexamethasone cotreatment. A relationship with ovarian steroids was indicated further by Downing and Poyser (34), who measured PLA2 activity in guinea-pig endometrium on days 7 and 16 of the estrus cycle and noted an increase in activity on day 16, in relation with the rise in circulating estradiol after day 10. Further studies in human endometrium during the menstrual cycle (14-17) demonstrate the presence of two PLA2 enzymes. One of these enzymes, which is calcium dependent and optimally active at pH 7.5-9.0, is present mainly in the endometrial glands and its activity changes during the cycle, indicative of a regulation by steroid hormones. The other PLA2 enzyme, which is calcium independent and optimally active at pH 7.0, is located predominantly in the stromal layer. In cultured explants of endometrium, both enzymes were inhibited by progesterone, whereas estradiol and dexamethasone had no effect. All these studies concern fully developed and differentiated tissues and little is known about PLA2 enzymes in immature tissue and their regulation by estradiol in primary stimulation.

The pH profiles presented in this paper demonstrate the presence of two PLA2 isoforms in the avian oviduct.
Table 1. Effect of estrogens on the oviduct growth and phospholipase A₂ (PLA₂) and ornithine decarboxylase (ODC) activities.

<table>
<thead>
<tr>
<th>Treatment (doses in mg/kg)</th>
<th>Oviduct weight (mg)</th>
<th>DNA (µg/oviduct)</th>
<th>PLA₂ activity (pmol arachidonic acid released·min⁻¹·mg⁻¹ DNA)</th>
<th>ODC activity (pmol CO₂/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 12)</td>
<td>14.3 ± 1.6</td>
<td>172 ± 21</td>
<td>29 ± 4.1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>6 h</td>
<td>EB 0.01 (N = 8)</td>
<td>16.4 ± 1.8</td>
<td>171 ± 19</td>
<td>38 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>EB 0.1 (N = 6)</td>
<td>22.1 ± 2.1b</td>
<td>194 ± 23</td>
<td>55 ± 6.7b</td>
</tr>
<tr>
<td></td>
<td>EB 1 (N = 5)</td>
<td>30.7 ± 2.4b</td>
<td>187 ± 21</td>
<td>76 ± 8.1b</td>
</tr>
<tr>
<td></td>
<td>Cyclo 5 (N = 6)</td>
<td>12.4 ± 1.2</td>
<td>166 ± 18</td>
<td>29 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>EB + Cyclo 5 (N = 6)</td>
<td>14.5 ± 2.0c</td>
<td>159 ± 17</td>
<td>43 ± 5.3c</td>
</tr>
<tr>
<td></td>
<td>E₂-17β 1 (N = 6)</td>
<td>23.3 ± 2.6b</td>
<td>181 ± 16</td>
<td>69 ± 7.4b</td>
</tr>
<tr>
<td></td>
<td>E₂-17α 1 (N = 6)</td>
<td>17.1 ± 3.1</td>
<td>164 ± 22</td>
<td>26 ± 5.0d</td>
</tr>
<tr>
<td>24 h</td>
<td>EB 0.01 (N = 6)</td>
<td>17.2 ± 1.9</td>
<td>192 ± 21</td>
<td>54 ± 7.2b</td>
</tr>
<tr>
<td></td>
<td>EB 0.1 (N = 5)</td>
<td>32.4 ± 6.2b</td>
<td>227 ± 24b</td>
<td>70 ± 6.4b</td>
</tr>
<tr>
<td></td>
<td>EB 1 (N = 5)</td>
<td>57.4 ± 4.3b</td>
<td>280 ± 31b</td>
<td>139 ± 9.4b</td>
</tr>
<tr>
<td></td>
<td>E₂-17β 1 (N = 6)</td>
<td>39.6 ± 4.2b</td>
<td>273 ± 28b</td>
<td>109 ± 9.1b</td>
</tr>
<tr>
<td></td>
<td>E₂-17α 1 (N = 6)</td>
<td>26.7 ± 3.1d</td>
<td>189 ± 18d</td>
<td>28 ± 4.9d</td>
</tr>
</tbody>
</table>

*Twenty-one-day-old immature quails received one injection of estradiol benzoate (EB, 0.01–1 mg/kg), 17β-estradiol (E₂-17β, 1 mg/kg) or 17α-estradiol (E₂-17α, 1 mg/kg) and were killed 6 h or 24 h after the injection. Two other groups received one injection of cycloheximide (5 mg/kg) either alone or concurrently with 1 mg/kg EB. Control animals received only the vehicle. The PLA₂ activity was measured on oviduct homogenate at pH 8, in the presence of 5 mmol/l calcium. Each value is the mean of N independent determinations ± sm.

aSignificant difference with the control group (p < 0.05).
bSignificant difference with 6-h EB 1 group (p < 0.05).
cSignificant difference with the corresponding EB-17β group (p < 0.05).

one, optimally active at pH 7–7.5 and calcium independent, is responsible for most of the hydrolytic activity in the immature oviduct and poorly stimulated by estradiol; the other, calcium dependent and optimally active at pH 9–10, shows little activity in the immature tissue. Estradiol markedly stimulates this alkaline fraction and apparently shifts its optimal activity to a more neutral pH. This difference may be representative of changes induced by EB at the enzyme level, such as phosphorylation. The identification of these changes requires further study. With regard to calcium and pH requirements, the PLA₂ isoforms of the avian oviduct are similar to those described in human endometrium (17).

Earlier studies in our laboratory showed that a single injection of EB to immature quails induced a proliferative response of the oviduct cells: DNA synthesis increased after a lag period of about 12 h, peaked at 24 h and then decreased (19). The present study shows that EB treatment increases PLA₂ activity during the prereplicative (0–12 h) period of oviduct cells. Maximal activation (+172%) was observed at 6 h. This effect was prevented by the administration of cycloheximide and almost the same inhibition was observed for ODC activity, suggesting that EB increases PLA₂ activity through the classical nuclear receptor–gene activation pathway and de novo protein synthesis. The absence of effect of estradiol added directly in vitro to the supernatant of oviduct homogenates and previous results from our laboratory showing that the antiestrogen tamoxifen suppresses PLA₂ activation by EB (25) support this mechanism. A direct action of steroid sulfates on endometrial PLA₂ activity has been reported (16) but only at very high concentrations (10⁻⁴ mol/l).

The major increase of PLA₂ activity was observed during the proliferative phase (12–40 h) of oviduct cells. The kinetic analysis of PLA₂ activity after three daily injections of EB shows that the first peak was still present and with the same relative magnitude (+179% at 6 h), while the second peak was absent. In quails, natural or estrogen-induced development of the oviduct

Table 2. The phospholipase A₂ (PLA₂) activity in the quail oviduct at the beginning of natural sexual development.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Oviduct weight (mg)</th>
<th>DNA (µg/oviduct)</th>
<th>PLA₂ activity (pmol arachidonic acid released·min⁻¹·mg⁻¹ DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>14.3 ± 1.6</td>
<td>172 ± 21</td>
<td>29 ± 4.1</td>
</tr>
<tr>
<td>24</td>
<td>30.8 ± 2.8</td>
<td>223 ± 23</td>
<td>42 ± 3.6</td>
</tr>
<tr>
<td>27</td>
<td>140.3 ± 11.6</td>
<td>964 ± 74</td>
<td>95 ± 8.9</td>
</tr>
<tr>
<td>29</td>
<td>170.5 ± 13.7</td>
<td>1149 ± 98</td>
<td>170 ± 17.4</td>
</tr>
</tbody>
</table>

*Quails were sacrificed at different ages, as indicated, and sexual development was estimated by the oviduct wet weight. The PLA₂ activity was measured on oviduct homogenate at pH 8 in the presence of 5 mmol/l calcium. Each value is the mean ± sm of at least three independent determinations.
begins with the proliferation of the primitive luminal epithelium: epithelial cells evaginate into subepithelial stroma and form tubular glands (27, 28). Thus, epithelial cells (luminal and evaginated cells), which represent 17% of the total cell population in immature tissue, increase rapidly in number in growing oviducts to account for about 75% of total cells when the oviduct weight is about 400 mg (28), which is near the average weight of the oviduct 24 h after the third injection of EB (356.4 ± 18.4, mean ± SEM, N = 7). By reference to the human endometrium, where the alkaline PLA₂ isoform is located mainly in endometrial glands, we may speculate that the quail oviduct alkaline isoform also is located in the epithelial cells of the immature tissue. Thus the initial increase, which occurs in the absence of cell proliferation, may be representative of the effect of EB on enzyme activity, while the major peak observed between 12 and 40 h after the first injection of EB may be representative of the rapid increase of the epithelial cell population during this period. After the third injection of EB, the ratio of epithelial cells to total cells in the oviduct is much more stable. It should be noted that in non-treated birds, the PLA₂ activity in the oviduct greatly increases at the beginning of natural sexual development (sixfold from 17 to 170 mg oviduct), much more than the proportion of epithelial cells (about threefold; now shown). This result supports a physiological role for estradiol in the regulation of PLA₂ activity because, during the period studied, plasma levels of estradiol increase while progesterone concentration remains low (27). It also suggests that PLA₂ is involved in epithelial cell proliferation and tubular gland formation, which are the two main morphological events of oviduct development during this period (28).

Several other lines of experimental evidence suggest a link between the early increase of PLA₂ activity induced by EB and the proliferation of oviduct epithelial cells. We have reported recently that tamoxifen, a non-steroidal antiestrogen, inhibits both estrogen-induced proliferation and PLA₂ activation in the quail oviduct (25). Moreover, earlier studies in different species have shown that demethasone, a synthetic glucocorticoid that prevents estrogen-induced fluid imbibition and causes a partial inhibition of long-term uterine and oviduct growth responses (21, 35), markedly reduced the PLA₂ activity induced by estrogen (13). More recent data show that glucocorticoids suppress PLA₂ production by blocking mRNA synthesis and post-transcriptional expression (23). In addition, progesterone, when injected concurrently with EB, specifically prevents epithelial cell evagination and tubular gland cell proliferation (36); progesterone also inhibits endometrial PLA₂ activity (13, 17). Finally, recent results from our laboratory reveal that PLA₂ inhibitors, but not inhibitors of eicosanoid biosynthesis, reduce the proliferation of normal rat uterine endometrial cell in culture (37).

The properties of the quail oviduct PLA₂ isoform stimulated by estradiol—optimal pH in the alkaline range, activation by millimolar calcium concentrations and absence of fatty acid specificity at the sn-2 position—are those described for the low-molecular-weight secreted PLA₂. Secreted PLA₂ recently has been involved in the regulation of fibroblast (9, 38) and chondrocyte (10) proliferation. The events triggered by PLA₂ in these cells were mediated by a specific membrane receptor of the enzyme. Further characterization of an estrogen-dependent secreted PLA₂ and identification of specific binding sites in the different cell types of the oviduct are under investigation.

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