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# ENDOMETRIAL PHOSPHOLIPASE A2 ENZYMES AND THEIR REGULATION BY STEROID HORMONES

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**Summary**—The presence of two phospholipase A2 (PLA2) enzymes, designated PLA2(i) and PLA2(ii), has been demonstrated in human endometrium. These enzymes differ with respect to pH and calcium requirements, location within the tissue and regulation by steroid hormones. Phospholipase A2(i) is calcium dependent, optimally active at pH 7.5–9.0 and present mainly in the glandular component of the endometrium. Changes in activity occur during the menstrual cycle which are indicative of regulation by ovarian steroids. Conversely, PLA2(ii) is calcium independent, optimally active at pH 7.0 and located predominantly in the stromal layer. Wide variation in PLA2(ii) activity was found between individual subjects and there was no relationship with the stage of the menstrual cycle. Activity was, however, much higher in pathological endometrium and in endometrium from subjects with severe dysmenorrhoea. Triton X-100 activated PLA2(i) but not PLA2(ii). In cultured explants of endometrium, both enzymes were inhibited by progesterone whereas oestradiol and dexamethasone had no effect. However, progesterone priming followed by treatment with oestradiol caused a 2-fold stimulation of PLA2(i) but not PLA2(ii). Phospholipase A2 is favoured as the rate-limiting step in the generation of arachidonic acid for prostaglandin synthesis. However, our studies so far do not support a direct relationship between PLA2 and endometrial concentrations of prostaglandins, which implies that other important regulatory steps are involved. Other enzymes which are potentially capable of mobilizing arachidonic acid should also be investigated.

## INTRODUCTION

The rate-limiting step in the synthesis of prostaglandins (PGs) is considered to be the enzyme phospholipase A2 (PLA2) which liberates arachidonic acid from membrane-bound phospholipids. Free arachidonic acid is not usually stored within the cell but is either re-esterified into phospholipids or further metabolized to a range of products which include the prostaglandins PGE2 and PGF2$\alpha$. Prostaglandins are found in high concentrations in human endometrium, particularly in secretory and menstrual phase tissue [1]. It is the presence of these high concentrations of PGs in menstrual endometrium and menstrual fluid which has in part provided support for a role for PGs in menstruation [2]. The association is further emphasized by the evidence of abnormal prostaglandin production in association with dysfunctional uterine bleeding [3, 4] and dysmenorrhoea [2].

Studies with animals and with human tissue have shown that steroid hormones regulate endometrial prostaglandin production. Oestradiol, particularly following progesterone priming, increases PG output from the uterus of ovariectomized animals [5–7] and from human endometrium treated *in vivo* [8] and *in vitro* [9]. It is generally assumed that steroid hormones control endometrial prostaglandin production via modulation of PG synthetase. However, phospholipase enzymes, notably PLA2, may also be regulated by steroid hormones. Oestradiol has been shown to stimulate PLA2 activity in the endometrium of ovariectomized rats *in vivo* [10]. In this study administration of progesterone alone inhibited PLA2 activity but treatment with progesterone followed by oestradiol caused a stimulation of PLA2 activity above that of oestradiol alone. Glucocorticoids also inhibited PLA activity, which is of interest with respect to the action of glucocorticoids on PLA2 in other tissues [11]. A relationship between PLA2 activity and ovarian steroids has also been demonstrated by Downing and Poyser [12], who showed that in the guinea-pig the activity of endometrial PLA2 was higher on day 16 of the oestrous cycle than on day 7. The increase followed the rise in plasma oestradiol concentrations which occurs on day 10. Further to these findings we have recently demonstrated the presence of a calcium-dependent PLA2 in human endometrium and shown that there are cyclical changes in activity which are dependent on the stage of the menstrual cycle [13]. Phospholipase A2 activity was low in the early proliferative phase, increasing towards mid-cycle to reach a maximum in early secretory phase tissue and thereafter declining in the presence of luteal phase concentrations of progesterone. This pattern of enzyme activity is indicative of the influence of steroid hormones and suggests that oestradiol stimulates and progesterone inhibits endometrial PLA2 activity. The maximum activity found in the early secretory phase may depend on a critical concentration of both steroids.

A direct relationship between endometrial PLA2 activity and the endometrial tissue concentrations of PGs reported by other workers[1] has not been demonstrated by our studies so far [13]. Since PLA2 enzymes differ with respect to calcium and pH

requirements we were prompted to use these indices to detect other PLA2 enzymes which could be important in the control of menstruation. The measurement of PLA2 activity in the presence and absence of calcium over a wide pH range as reported below has demonstrated the presence of a second PLA2 enzyme in endometrium. The present paper is dedicated to presenting evidence for the existence of two PLA2 enzymes in human endometrium and to reporting the influence of ovarian steroids on both enzymes.

## EXPERIMENTAL

### *Clinical material*

Endometrial tissue was obtained in the operating theatre from women who were undergoing curettage or hysterectomy for conditions unassociated with malignancy. Unless otherwise stated, only women with regular cycles and no endometrial pathology were included in the study. The tissue was dated by histological examination and where applicable assigned to the appropriate phase of the menstrual cycle as indicated in the figure legends.

Endometrial tissue was transported to the laboratory on ice in Hanks Balanced Salt Solution (HBSS) and either used immediately or stored at −20°C until required. Samples were analysed within 1 week of collection although storage for at least 1 month at −20°C had no effect on enzyme activity.

### *Preparation of endometrial tissue homogenate*

The tissue was washed in saline, weighed and homogenized (using a Polytron homogenizer; Kinematica, GmbH, Lucerne, Switzerland) at a concentration of 200 mg/ml in 0.25 M sucrose and then centrifuged at 600 **g** for 10 min. The supernatant was decanted and retained for PLA2 studies.

### *Preparation of stromal and glandular components*

Endometrial tissue was washed in saline, chopped using a McIlwain chopper and then digested for 1 h at 37°C with 0.25% collagenase (Type I, Sigma Chemical Co. Ltd, Poole, Dorset) containing 1 mg/ml deoxyribonuclease I (Sigma). The digest was then diluted with HBSS to neutralize the effect of collagenase and filtered through 100 μm mesh to remove any undigested fragments of tissue. Glands and stromal cells were separated by filtration (38 μm mesh) and the glands backwashed from the sieve with HBSS. The separated components were harvested by gentle centrifugation, any contaminating red blood cells being removed from the stromal cells by further centrifugation. The cell pellets were then resuspended in 0.25 M sucrose prior to storage at −20°C for subsequent assay. At the time of assay, cell preparations were sonicated and used without further centrifugation.

### *Culture of endometrial tissue*

The method of culture followed that described by Abel and Baird[9]. Under sterile conditions the tissue was washed in saline to remove blood and cut into fragments of approximately 1 $mm^3$ using a McIlwain chopper. Tissue fragments were placed on lens paper resting upon stainless steel grids in polystyrene culture dishes (3.5 cm diameter) containing 2 ml Ham's F10 culture medium supplemented with 5% charcoal-stripped foetal calf serum, 2 mM glutamine, 10 U/ml nystatin (all obtained from Gibco), 10 μg/ml insulin (Sigma), 25 mg/ml streptomycin sulphate (Evans Medical Ltd., Greenford, Middlesex) and 7.5 mg/ml sodium benzypenicillin (Glaxo). The tissue was incubated at 37°C in a humidified atmosphere of 95% air–5% $CO_2$ for a total period of 72 h which comprised a preincubation period of 24 h followed by two 24-h periods of treatment with steroid hormones. Oestradiol-17β and progesterone were added to the incubation medium in 10 μl ethanol while untreated controls received 10 μl ethanol without added steroids. At the end of the culture period the tissue was homogenized in 0.25 M sucrose and stored at −20°C for assay of PLA2 enzymes.

### *Phospholipase A2 assay*

Phospholipase A2 was measured according to the method previously described[13] with slight modifications. The substrate used was 100,000 d.p.m. 1-palmitoyl 2[1-$^{14}$C]oleoyl phosphatidylcholine (Amersham International plc, Bucks, England) together with unlabelled 1-palmitoyl-2-oleoyl phosphatidylcholine (Sigma) prepared directly in the incubation tube in 0.1 M buffer (as specified below). The final concentration of substrate used was 254 μM. The incubate contained 100 μl buffer, 50 μl enzyme preparation (approximately 200 μg protein) and 50 μl water containing Triton, calcium or EGTA as appropriate to the experiment. The period of incubation was usually 1 h at 37°C. [$^3$H]Oleic acid (20,000 c.p.m; Amersham) was presented in the incubation mixture to monitor procedural loses. The reaction was terminated by the addition of 2 ml chloroform–methanol (2:1, v/v). Extraction of oleic acid was completed as previously described[13].

The data were expressed as pmol oleic acid released/mg protein per min or per h as appropriate. The protein concentration of the enzyme preparation was determined according to the method of Lowry *et al.*[14]. Menstrual cycle and tissue culture data were analysed by Student's *t*-test.

## RESULTS

### *pH Profiles*

Phospholipase A2 activity was measured in endometrium over the pH range 4–10 in the

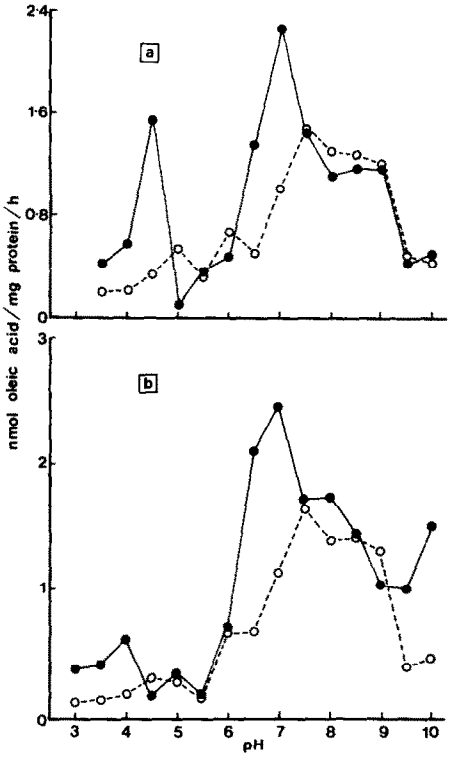


Fig. 1. The effect of pH on phospholipase A2 activity in (a) mid-secretory (b) late-secretory phase endometrium in the presence (open circles, dotted lines) and absence (closed circles, solid lines) of 5 mM calcium. Calcium was replaced by 0.5 mM EGTA in calcium-free incubation medium. Each point represents the average of duplicate estimations. The buffers used to cover the range were: sodium acetate–acetic acid (pH 4–5.5), sodium phosphate (pH 6–7), Tris–HCl (pH 7.5–9) and carbonate–bicarbonate (pH 9.5–10). The incubation medium contained 0.1% Triton. Enzyme activity is expressed as nmol oleic acid released/mg protein per h.

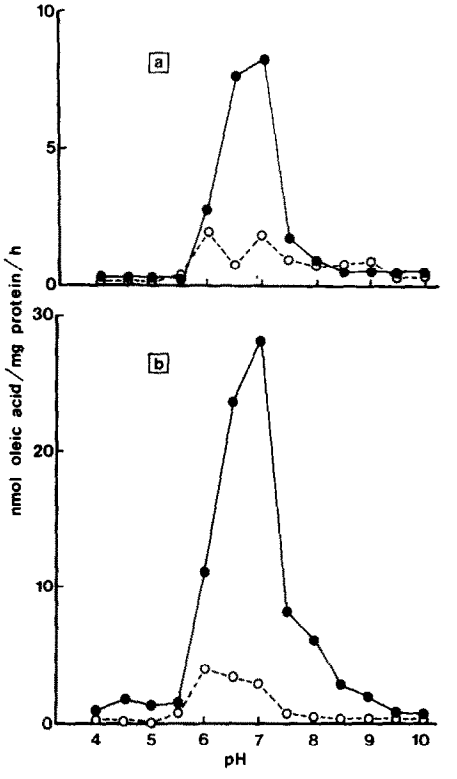


Fig. 2. The effect of pH on phospholipase A2 activity in (a) early-secretory endometrium with atypical hyperplasia (b) endometrium with small inactive glands in a decidualized stroma, in the presence (open circles, dotted lines) and absence (closed circles, solid lines) of 5 mM calcium. Calcium was replaced by 0.5 mM EGTA in calcium-free incubation medium. Each point represents the average of duplicate estimations. The buffers used to cover the range were: sodium acetate–acetic acid (pH 4–5.5), sodium phosphate (pH 6–7), Tris–HCl (pH 7.5–5.9) and carbonate–bicarbonate (pH 9.5–10). The incubation medium contained 0.1% Triton. Enzyme activity is expressed as nmol oleic acid released/mg protein per h.

presence and absence of 5 mM calcium. Calcium was replaced by 0.5 mM EGTA in calcium-free media. Two profiles are presented in Fig. 1, which demonstrates the calcium and pH requirements for PLA2 enzymes in (a) mid-secretory (b) late-secretory endometrium. In the presence of 5 mM calcium, PLA2 activity was at a maximum at pH 7.5–8.5 (1.5 ng/mg protein per h). In the absence of calcium, in both mid- and late-secretory endometrium, the major peak of PLA2 activity occurred at pH 7. Under these conditions PLA2 activity was double that measured in the presence of calcium at pH 8. Evidence of a lysosomal PLA2 particularly in mid-secretory endometrium was indicated by a peak of activity at pH 4.5 in calcium-free conditions.

A relationship between endometrial pathology and PLA2 activity is suggested by two pH profiles shown in Fig. 2. Profile (a) represents PLA2 activity in early secretory endometrium with areas of atypical hyperplasia while profile (b) demonstrates PLA2 activity in endometrium with small inactive glands in a decidualized stroma. Both subjects had complained of menorrhagia. Phospholipase A2 activity in the absence of calcium was maximal at pH 7 but in these tissues reached (a) 3-fold and (b) 30-fold that measured under the same conditions in normal secretory phase tissue. Activity measured in the presence of 5 mM calcium was within the range measured for normal proliferative phase tissue, namely 0.75 and 0.4 nmol/mg protein per h in (a) and (b) respectively.

The pH profiles illustrated in Figs 1 and 2 demonstrate the presence of two PLA enzymes in human endometrium with different pH and calcium requirements. These enzymes have been designated PLA2(i), a calcium-dependent enzyme with optimum activity at pH 8 and PLA2(ii) a calcium-independent enzyme with optimum activity at pH 7. This nomenclature has been adopted below.

*Calcium requirements of endometrial PLA2 enzymes*

Figure 3 illustrates the effect of increasing concentrations of calcium on PLA2 activity measured in endometrial homogenate at pH 8 (PLA2(i)) and pH 7

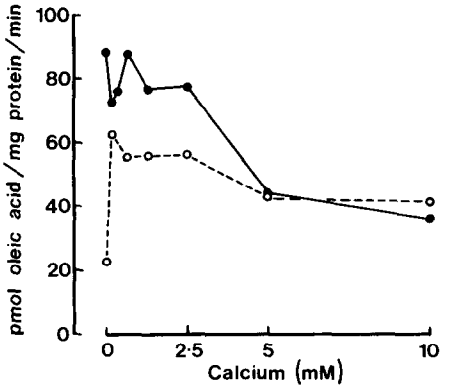


Fig. 3. The effect of increasing concentrations of calcium on phospholipase A2 activity in endometrium. Phospholipase A2 activity was measured at pH 7 (closed circles, solid lines) and at pH 8 (open circles, dotted lines). Calcium was replaced by 0.5 mM EGTA in calcium-free incubation medium. Each point represents the average of duplicate estimations. The incubation medium contained 0.1% Triton. Enzyme activity is expressed as pmol oleic acid released/mg protein per min.

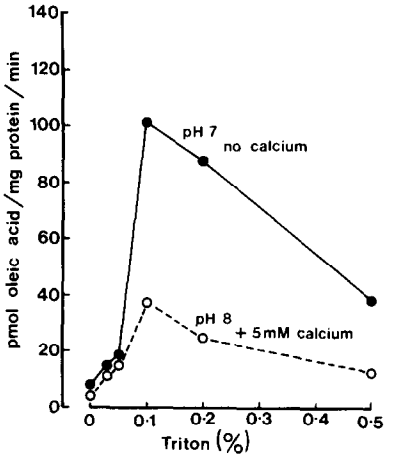


Fig. 4. The effect of Triton X-100 on phospholipase A2 activity in endometrium measured at pH 7 in the absence of calcium (closed circles, solid lines) and at pH 8 in the presence of 5 mM calcium (open circles, dotted lines). Calcium was replaced by 0.5 mM EGTA in calcium-free medium. Each point represents the average of duplicate estimations. Enzyme activity is expressed as pmol oleic acid released/mg protein per min.

(PLA2(ii)). Phospholipase A2(i) in the absence of calcium was only 40% of that measured in the presence of 0.625–2.5 mM calcium. At 5 and 10 mM calcium concentrations PLA2(i) activity was slightly reduced. On the other hand, PLA2(ii) activity was maximal in the absence of calcium and decreased with increasing calcium concentrations to (at 10 mM calcium) 40% of the activity measured in calcium-free conditions.

*The importance of Triton X-100 in the expression of PLA2 activity*

The effect of the detergent Triton X-100 on the activity of endometrial PLA2 enzymes is shown in Fig. 4. Both phospholipases were maximally active in the presence of 0.1% Triton. Increasing the concentration of Triton to 0.5% caused a marked decrease in PLA2 activity to 25% (PLA2(i)) and 40% (PLA2(ii)) of the maximum measured. Phospholipase A2 activity was also decreased in the presence of concentrations of Triton of less than 0.1% and in the absence of Triton, activity was only 2–5% of the maximum measured.

*Phospholipase A2 enzymes in the endometrium during the menstrual cycle*

Phospholipase A2 enzymes PLA2(i) and PLA2(11) were measured in endometrium obtained from women at different stages of the menstrual cycle. The profile of PLA2(i) activity measured in the presence of 5 mM calcium at pH 8 is presented in Fig. 5. There was a significant 4-fold increase in PLA2(i) activity in early/mid-secretory phase tissue compared to that of proliferative phase tissue ($P < 0.001$, $t = 11.49$, df 15). Similarly, the activity of PLA2(i) was 3-fold higher in late-secretory phase endometrium than in proliferative phase tissue ($P < 0.001$, $t = 10.44$, df 15). The mean PLA2(i) activity of late-secretory phase tissue was lower than that of the early/mid-secretory phase (40.6 ± 7.2 and 34.7 ± 3.8 pmol/mg protein per min respectively, $n = 7$) but the difference was not significant ($t = 1.92$, df 12). Menstrual phase tissue had the lowest PLA2(i) activity (mean value 3.4 ($n = 2$) compared with 11.8 ± 4.2, pmol/mg protein per min for proliferative phase tissue ($n = 10$)). However, the availability of only two menstrual phase tissue samples precluded any statistical comparison.

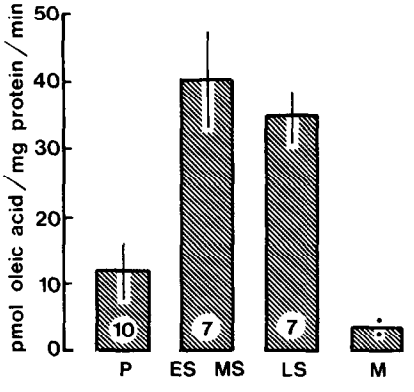


Fig. 5. Phospholipase A2 activity in endometrium with respect to the stage of the menstrual cycle. Samples were assayed at pH 8 in the presence of 5 mM calcium. Values are mean ± SD pmol/mg protein per min. The value for each individual subject was determined in duplicate and the number of subjects per group is indicated at the base of each bar. Where only two observations were available the individual values are indicated. Abbreviations: P, proliferative phase (days 4–14); ES/MS, early/mid-secretory phase (2–4 days post ovulation); LS, late-secretory phase (11–14 days post ovulation); M, menstrual phase. Significant differences (Student's $t$-test): P vs ES/MS and P vs LS, $P < 0.001$.

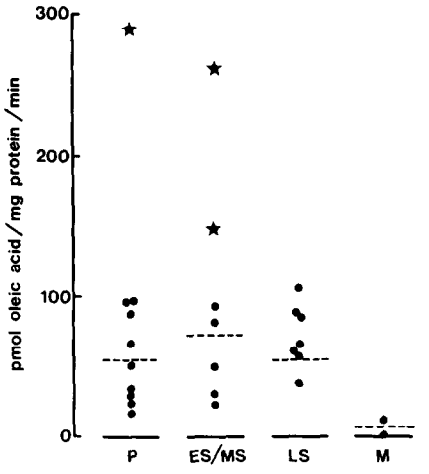


Fig. 6. Phospholipase A2 activity in the endometrium with respect to the stage of the menstrual cycle. Samples (from Fig. 5) were assayed at pH 7 in the absence of calcium in medium containing 0.5 mM EGTA. The data are presented as individual observations (each an average of duplicate estimations). The mean of each group, excluding samples represented by a star symbol, is indicated by a horizontal bar. The mean values for each group were not significantly different. Samples represented by a star were from subjects who had complained of severe dysmenorrhoea.

The data obtained for PLA2(ii) activity (measured at pH 7 in calcium-free medium) is the same tissue samples are presented in Fig. 6 as individual observations. There was a wide variation in the values obtained for each group (range: 16.0–288.8, 22.7–262.5, 38.1–106.2 pmol/mg protein per min for proliferative phase, and early/mid- and late-secretory phases respectively) and no relationship to the stage of the menstrual cycle. The mean value calculated for each stage of the cycle and indicated by a horizontal dotted line excludes three high values represented by a star symbol. These samples of endometrium were obtained from patients who had complained of severe dysmenorrhoea. All three had exceptionally high PLA2(ii) activity (148.6, 262.5, 288.8 pmol/mg protein per min) although the activity of PLA2(i) in the same samples was not increased. Mean PLA2(ii) values ±SD, excluding subjects with dysmenorrhoea, were $55.3 \pm 30.6$, $n = 9$, $71.9 \pm 22.9$, $n = 7$, and $55.5 \pm 32.3$, $n = 5$ pmol/mg protein per min for proliferative phase and early/mid- and late-secretory phases respectively). Menstrual phase tissue has lower PLA2(ii) activity (0.1 and 11.1 pmol/mg protein per min) than tissues from other stages of the cycle but, as stated above, the number of samples was limiting.

*Localization of PLA2 enzymes in glandular and stromal compartments of endometrium*

Endometrial tissue was separated into stromal and glandular components as described above. Phospholipase A2 activity was measured in both stromal and glandular tissues at pH 8 and pH 7 in the presence and absence of 5 mM calcium respectively. Under these conditions stromal cell activity was 184.1 and 34.3 pmol/mg protein per min for PLA2(ii) and PLA2(i) respectively. The activity of PLA2 in glands obtained from the same tissue was 20.2 and 85.0 pmol/mg protein per min for PLA2(ii) and PLA2(i) respectively. These data suggest that PLA2(i) is predominant in glandular endometrium whereas PLA2(ii) is present mainly in stromal tissue. Further evidence for the presence of two separate enzymes is presented in Fig. 7, which demonstrates (a) the calcium requirements of the enzymes in the two tissues and (b) the response to activation by Triton. Figure 7(a) clearly demonstrates the inhibitory effect of calcium on stromal cell PLA2 activity at pH 7. Maximum activity was measured in the absence of calcium and was reduced to 17% in

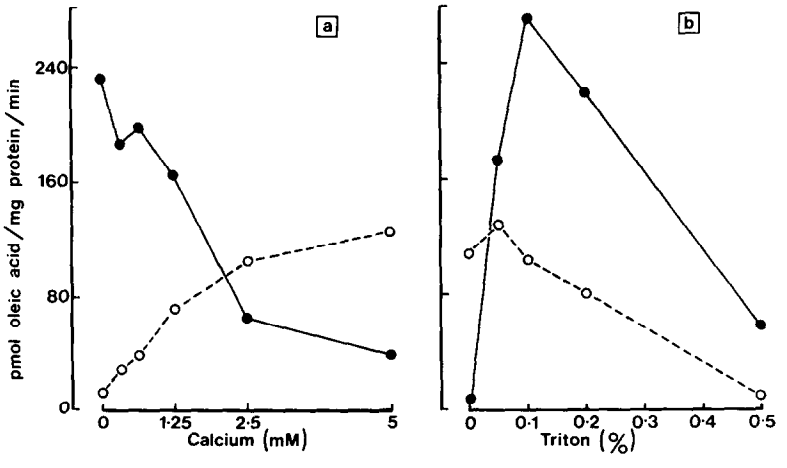


Fig. 7. The effect of (a) calcium (b) Triton X-100 on phospholipase A2 activity in endometrial stromal cells (closed circles, solid lines) and endometrial glands (open circles, dotted lines). Each value is expressed as pmol oleic acid/mg protein per min. Glandular phospholipase A2 activity was measured at pH 8 and stromal phospholipase A2 activity at pH 7 in the presence of (a) increasing concentrations of calcium in medium containing 0.1% Triton (b) increasing concentrations of Triton in medium containing either 5 mM calcium (glands) or 0.5 mM EGTA (stromal cells).

the presence of 5 mM calcium. On the other hand, activity of the glandular enzyme was maximal at 5 mM calcium and decreased when concentrations of calcium were reduced. Only 10% of the maximum activity was measured in the absence of calcium.

The presence of Triton was obligatory for the measurement of phospholipase A2(ii) in stromal cells (Fig. 1b). Maximum activity was measured in the presence of 0.1% Triton. When the concentration of Triton was increased to 0.5% there was a marked decrease in enzyme activity to 22% of that measured in the presence of 0.1% Triton. The glandular enzyme responded differently to the presence of Triton in the incubation medium. Activity was at a maximum with 0.05% Triton and declined in the presence of 0.1–0.5% Triton (10% of the activity measured in the presence of 0.05% Triton). However, in the absence of Triton there was only a slight decrease (16%) in activity with respect to the maximum measured in the presence of 0.05% Triton.

*Tissue culture studies*

The effect of oestradiol (20 nmol/dish), progesterone (200 nmol/dish) and dexamethasone (20 nmol/dish) on PLA2 activity in cultured explants of proliferative phase endometrium is shown in Fig. 9. Treatment with oestradiol for 2 days had no effect on either PLA2(i) or PLA2(ii) activity whereas treatment with progesterone for 2 days significantly reduced the activity of both enzymes by approximately 50% [$P < 0.02$ and $P < 0.05$ for PLA2(i) and PLA2(ii) respectively]. Oestradiol (day 1) followed by progesterone (day 2) had no effect on either enzyme. However, progesterone (day 1) followed by oestradiol (day 2) stimulated a significant (2-fold) increase ($P < 0.05$) in PLA2(i) but not PLA2(ii) activity. Dexamethasone had no effect on either enzyme.

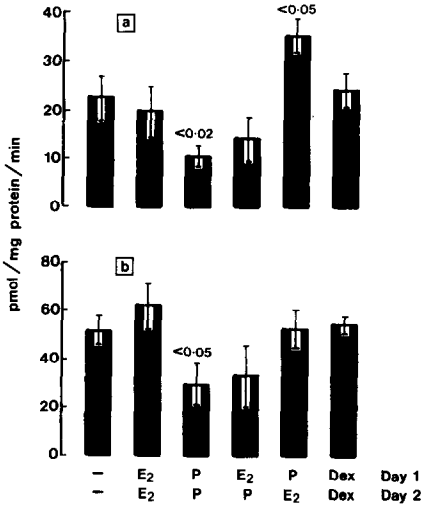


Fig. 8. The effect of oestradiol-17$\beta$ (20 nmol/dish), progesterone (200 nmol/dish) and dexamethasone (20 nmol/dish) on cultured explants of proliferative phase endometrium with respect to (a) phospholipase A2(i) (b) phospholipase A2(ii) activity. Each vertical bar represents the mean ± SD ($n = 3$) activity for each treatment, expressed as pmol/mg protein per min. Abbreviations: E2, oestradiol 17$\beta$; P, progesterone; Dex, dexamethasone. Day 1 and Day 2 treatments were as indicated on the horizontal axis. Significant differences, treatment vs control were: $P < 0.02$ and $< 0.05$ for PLA2(i) and PLA2(ii) respectively; $P$ followed by E2, $< 0.05$, for PLA2(i).

## DISCUSSION

We have previously reported the presence of a PLA2 enzyme in human endometrium which is calcium dependent with a pH optimum of 8–9. The activity of this enzyme was shown to vary cyclically under the influence of ovarian steroids [13]. Maximum activity was measured in early-secretory phase tissue 2–4 days post ovulation and declined progressively through the secretory phase to a low level at menstruation. This pattern of activity does not accord with the general finding that the capacity of the endometrium to synthesize PGs increases from midcycle onwards and reaches maximum potential at the time of menstruation [1, 15, 16]. There may be several explanations for the lack of a direct relationship between the activity of the rats-limiting enzyme and the PG-synthesizing capacity of the endometrium. As yet, no single study has provided measurements of both parameters at the same time and it is possible that the PG synthetase complex responds differently to ovarian steroids than does PLA2. In the present study, however, we elected to investigate the likelihood that other PLA2 enzymes with different pH and calcium requirements may be present in endometrium, which could be more important in the control of menstruation.

The pH profiles presented in this paper demonstrate the presence of two PLA2 enzymes, one which is maximally active pH 7.5–9.0 in the presence of calcium (PLA2(i)) as described previously [13] and another which has a pH optimum of 7.0 and is inhibited by calcium (PLA2(ii)). It was of interest to note that the activity of PLA2(ii) but not PLA2(i) was markedly higher in pathological tissue. Studies are presently in progress in this laboratory to investigate the significance of this observation.

Measurement of both phospholipases in samples of endometrium obtained from women at different stages of the menstrual cycle also showed striking differences between the two enzymes. The data obtained for PLA2(i) compared closely with those previously reported and referred to above [13]. In the present study, the mean activity of proliferative phase tissue was 11.8 pmol/mg protein per min and increased to a mean of 40.6 pmol/mg protein per min in early/mid-secretory phase tissue. These values are in close agreement with those of our earlier study. In contrast, the activity of PLA2(ii), which was often but not consistently higher than that of PLA2(i), showed wide variation within each group and did not

change with the stage of the cycle. Menstrual phase activity was low under both conditions of assay although the limited number of samples in this group restricts interpretation of the data.

This menstrual cycle study has therefore provided further evidence for the presence of two separate PLA2 enzymes and suggests that while one is regulated by steroid hormones, the other is not. There is, however, still no clear indication of a direct relationship between the activity of either PLA2 enzyme and PG production, which implies that other regulatory steps must be involved.

An important finding from this study is that the two enzymes appear to be located at different sites within the endometrium. Stromal tissue was shown to contain predominantly PLA2(ii) while PLA2(i) was present mainly in glandular tissue. It is the activity of the stromal enzyme (PLA2(ii)) which appears to be increased in pathological endometrium and in menstrual disorders such as dysmenorrhoea. Yet in these tissues the activity of PLA2(i) is not abnormally increased. Women with dysmenorrhoea have been shown to have much higher endometrial concentrations of prostaglandins [2] which could be associated with high PLA2(ii) activity. In women with polycystic ovarian disease, endometrial PLA2(ii) activity is also much higher (unpublished data) and it is noteworthy that in these women the endometrium is thicker than in normal women [17]. The activity of PLA2(i) increases during the proliferative phase as the endometrial glands develop and under the influence of increasing concentrations of oestradiol, reaches a maximum in the early-secretory phase. During the late-secretory phase progesterone appear to have an inhibitory effect and PLA2(i) activity declines to proliferative phase values at menstruation. Other endometrial enzymes of glandular origin have similar profiles of activity; for example, PG dehydrogenase which catalyses the metabolism of PGs [18] and 17$\beta$-hydroxysteroid dehydrogenase which interconverts oestradiol-17$\beta$ and oestrone [19].

The difference in the calcium requirements of the two enzymes is clearly demonstrated by our studies with glandular and stromal tissue preparations. Glandular PLA2(i) was shown to have an absolute requirement for calcium whereas stromal PLA2(ii) was inhibited by calcium. This distinction is more apparent with isolated endometrial tissue components than with whole tissue homogenates where only a partial dependence of PLA2(i) on calcium could be demonstrated.

Inclusion of the detergent Triton X-100 in the assay system was necessary for the measurement of stromal PLA2 but not glandular PLA2. Again, this is only evident in studies with isolated endometrial tissue components since when using whole tissue homogenates Triton was found to be necessary for the activation of both enzymes. Detergents such as Triton are thought to activate phospholipases by causing conformational membrane changes which expose the active site of the enzyme. However, not all PLA2 enzymes require such activation; for example, the activity of amniotic PLA2 is not affected by Triton [20]. Some PLA2 enzymes are soluble or easily removed from membranes while others are integral membrane proteins and only removed by extraction with detergents or organic solvents. The difference between the two endometrial enzymes with respect to Triton could therefore indicate that they are located in different parts of the cell or cell membrane.

Several points of interest arise from our study with explants of endometrium in culture. As might be anticipated from our menstrual cycle data and from the *in vivo* work of Dey *et al.* [10] with ovariectomized rats, progesterone inhibited PLA2(i) activity. Phospholipase A2(ii) was also inhibited by progesterone, although one might expect from the menstrual cycle data that this enzyme would be unaffected by steroid treatment. The concentrations used in this preliminary study were non-physiological but in another study (data not shown) a range of progesterone doses between 2 and 200 nmol/well also inhibited PLA2(ii) activity. Further work is required to establish a suitable treatment regimen which simulates more effectively the conditions encountered *in vivo*. Oestradiol had no effect on the activity of PLA2 either at the concentration used in this study or at concentrations ranging between 0.02 and 20 nmol/well (data not shown). The absence of a stimulatory effect of oestradiol was surprising since Dey *et al.* in the study quoted above [10] demonstrated a marked stimulation of endometrial PLA2 activity in rats treated with oestradiol implants. It is likely, however, that either the treatment period selected for the explant study was unsuitable or that other mechanisms are involved which are not brought into action in our *in vitro* study. It is noteworthy that in the same experiment PGF2$\alpha$ production was doubled by this treatment (data not shown). Oestradiol does, however, appear to stimulate human endometrial PLA2(i) *in vivo* since the activity increases significantly between early- and late-proliferative phases of the cycle in concert with increased follicular secretion of oestradiol [13].

Pretreatment of endometrial explants with progesterone prior to incubation with oestradiol caused a 2-fold stimulation of PLA2(i) but not of PLA2(ii). This priming action of progesterone recalls the accepted hypothesis that the uterus requires a period of progesterone priming in order to synthesize PGF2$\alpha$ in response to oestradiol [9]. Dey *et al.* also demonstrated that, in the rat, priming with progesterone enhanced the stimulatory effect of oestradiol on endometrial PLA2 activity *in vivo* [10].

Our data obtained from tissue culture studies can again be related to the menstrual cycle where maximal PLA2(i) activity is seen in early secretory phase tissue. Although in the menstrual cycle the

endometrium is not primed with progesterone prior to the oestradiol surge, a critical balance between endometrial tissue concentrations of oestradiol and progesterone may be required to achieve maximum activation of PLA2(i). Notably, PLA2(ii) was not stimulated by oestradiol following progesterone pretreatment. These findings are in accordance with the lack of influence of ovarian steroids on PLA2(ii) activity during the menstrual cycle.

Glucocorticoids such as dexamethasone are known to inhibit PLA2 in several tissues [11] including rat endometrium [10]. It was anticipated, therefore, that dexamethasone would inhibit PLA2 activity in human endometrial explants. In fact, dexamethasone has no effect on the activity of either enzyme, although PGF2$\alpha$ production in this experiment (data not shown) was reduced to 30% of the control value.

We have demonstrated the presence of two PLA2 enzymes in human endometrium (PLA2(i) and PLA2(ii)) which differ with respect to pH and calcium requirements. Although both PLA2(i) and PG synthetase appear to be regulated by ovarian steroids our studies so far throw doubt on a direct relationship between these two enzymes. Other intermediate steps are certainly involved in prostaglandin production. Furthermore, precursor arachidonic acid could arise from a number of intracellular lipid pools; cholesterol esters, phosphatides, mono-, di- and triglycerides might all contain sufficient substrate to support PG biosynthesis. Thus although PLA2 is favoured as the rate-limiting enzyme in PG synthesis other enzymes are potentially capable of mobilizing arachidonic acid for prostaglandin synthesis by the endometrium and should be investigated.

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