Hormonal control of ovarian phospholipase A<sub>2</sub> activity in rats

R. C. Bonney<sup>1</sup> and C. A. Wilson<sup>2*</sup>

<sup>1</sup>Unit of Metabolic Medicine, St Mary's Hospital Medical School, London W2 1PG, UK; and  
<sup>2</sup>Department of Obstetrics and Gynaecology, St George's Hospital Medical School,  
London SW17 0RE, UK

The ovulatory effect of LH is mediated by prostaglandins, the synthesis of which involves prostaglandin synthetase and phospholipase A<sub>2</sub> (PLA<sub>2</sub>). It is known that the activity of the former is regulated by LH in the ovary and the objective of this study was to establish the presence of PLA<sub>2</sub> in the rat ovary and to determine whether LH has a role in regulating its activity. We demonstrated the presence of a calcium-dependent PLA<sub>2</sub> in rat ovaries and showed that, in adult rats, the activity of this enzyme rises significantly on the evening of pro-oestrus (at the expected time of the preovulatory LH surge) and remains high over the evening hours, declining by the next morning. Furthermore, pregnant mares' serum gonadotrophin (PMSG), which when injected into 28-day-old rats induces an LH surge 54 h later, caused at the same time, a rise in ovarian PLA<sub>2</sub> activity. Administration of 5 µg oestradiol benzoate S.C. to 25-day-old rats stimulated ovarian PLA<sub>2</sub> activity 54 h later, at the time of the oestrogen-induced LH surge. Human chorionic gonadotrophin (hCG) had no effect on circulating concentrations of LH, FSH or oestradiol but stimulated ovarian PLA<sub>2</sub> activity 2 h later. Gonadotrophin-releasing hormone (GnRH) given to 25-day-old rats (either untreated or primed with 0.5 mg oestradiol benzoate) had no effect on ovarian PLA<sub>2</sub> activity but the resulting LH surge may have been too transient. Our findings indicate that LH is probably the main regulator of ovarian PLA<sub>2</sub> activity in rats and that the preovulatory LH surge induces ovarian prostaglandin production not only by enhancing prostaglandin synthetase but also PLA<sub>2</sub> activity.

Introduction

The mid-cycle LH surge is essential for the occurrence of ovulation and is accompanied by a rise in prostaglandin concentrations and prostaglandin synthetase activity within the ovary which remain high until the time of ovulation, some 12 h later (Le Maire et al., 1975; Bauminger and Lindner, 1975).

Experimental evidence indicates that prostaglandins mediate the ovulatory effect of LH. Thus, exogenous LH increases prostaglandin concentrations in the ovary in vitro (Bauminger and Lindner, 1975) and in granulosa cells in vitro (Clark et al., 1978) and inhibition of prostaglandin synthesis (using indomethacin) just before the expected endogenous prostaglandin rise inhibits ovulation. This effect can be reversed, in rats, by treatment with exogenous PGE<sub>2</sub> (Armstrong and Grimw 1972; Lindner et al., 1980; Poyser, 1987).

LH can stimulate the expression of prostaglandin synthetase activity in granulosa cells of pre-ovulatory follicles (Clark et al., 1978; Hedin et al., 1987; Sirois and Richards, 1992; Sirois et al., 1992) and presumably this is the cause of increased concentrations of prostaglandins. However, the rate-limiting step for prostaglandin synthesis is the availability of the prostaglandin precursor arachidonic acid which is released from membrane-bound phospholipid stores by the enzyme phospholipase A<sub>2</sub> (PLA<sub>2</sub>) (Irvine, 1982). It seems likely, therefore, that PLA<sub>2</sub> activity may also rise over the pre-ovulatory period (Bauminger and Lindner, 1975; Poyser, 1987). As far as we know, the possibility that LH induces ovarian PLA<sub>2</sub> activity has not been investigated, nor has the alternative consideration that the preovulatory rise in oestradiol might affect ovarian PLA<sub>2</sub>; this seems feasible since oestradiol can stimulate PLA<sub>2</sub> in the uterine endometrium (Dey et al., 1982; Bonney et al., 1987).

As the presence of PLA<sub>2</sub> activity in the rat ovary has not as yet been reported, we established an assay for ovarian PLA<sub>2</sub> and determined the characteristics of the enzyme. The assay was then used to measure ovarian PLA<sub>2</sub> activity throughout the oestrous cycle, relating enzyme activity to hormonal changes and experiments were then performed to establish whether LH or oestradiol or both are responsible for the changes in PLA<sub>2</sub> activity in the ovary.

Materials and Methods

Animals and treatments

<sup>Adult rats</sup>. Female Wistar rats, 12–16 weeks old, weighing 220–260 g, were kept in a fixed lighting regimen of 12 h light: 12 h dark (lights on 07.00 h), five animals per cage with food and water ad libitum. All the rats were ovariectomized under

*Correspondence.
Received 16 November 1992.
halothane (Fluothane, ICI Pharmaceuticals Ltd, Macclesfield) and nitrous oxide anaesthesia at definite periods during the day, i.e. 09.30–10.30 h, 13.30–14.30 h, 18.30–19.30 h or 23.30–0.30 h, designated as 10, 14, 19, and 24.00 h, respectively. Vaginal smears were taken just before the operation and according to the proportion of cells present, the rats were subdivided into pro-oestrus (mainly epithelial cells, and confirmed by a fluid-filled uterus), oestrus (mainly cornified cells and confirmed by a swollen oviduct) and dioestrus (mainly leucocytes). As daily smears were not taken before the day of ovariectomy, it was not possible to distinguish between the first and second day of dioestrus.

On removal of the ovaries from the body, the attached adipose tissue was rapidly and carefully removed and the ovaries were snap frozen in liquid nitrogen and then stored at −80°C until assayed for PLA₂ activity.

**Immature rats.** Female Wistar rats were kept from day 21 post partum (day of weaning) in groups of five to ten, in the same conditions as the adults. Pregnant mares’ serum gonadotrophin (PMSG; Folligon; Intervet Ltd, Cambridge) was injected s.c. (5 IU in 0.1 ml saline) on day 28 at 12.00 h. Groups of rats were decapitated at 16.00 h, 18.30 h and 24.00 h on day 30 or 10.00 h on day 31. Saline-treated control groups were killed at 12.00 h on day 28 and on day 30 at 18.30 h. Blood was collected from the trunk and centrifuged at 400 g for 10 min; the resulting plasma was stored at −20°C until it was assayed for LH and oestradiol. The ovaries were removed, frozen and stored as above. Further groups of 25-day-old female rats were treated with either 10 IU hCG per rat (Chorion, Svs Ltd, Lewes) given s.c. in 0.1 ml saline, 500 ng GnRH per rat (HRF Ayerst, Gondorelin; Ayerst Labs Ltd, Andover) given i.p. in 0.1 ml saline or 0.5 μg or 5 μg oestradiol benzoate per rat (Sigma Chemical Co. Ltd, Poole) given s.c. in 0.1 ml corn oil. One group received 0.5 μg oestradiol benzoate on day 24 followed by 500 ng GnRH on day 25.

**Hormone assays**

**Gonadotrophins assays.** Plasma LH and FSH concentrations were determined by radioimmunoassay using reagents kindly provided by the National Hormone and Pituitary Program (Baltimore, Maryland) using NIDDK-rLH-RP2 and NIDDK-NFSH-RP2 as the standards and NIDDK-rLH-S10 and NIDDK-nFSH-S11 as the antibodies. Intra- and interassay coefficients of variance were 9.5% and 8%, respectively, and the sensitivity of the assay was 10 pg per tube for LH, and for FSH the intra- and interassay coefficients of variance were 12.5% and 10.5%, respectively and the sensitivity of the assay was 2 ng per tube.

A Gamma-B direct oestradiol double-antibody radioimmunoassay kit was used (IDS Ltd, Bolda, Tyne and Wear). The inter-assay coefficient of variance was 5–7% over the concentration range assayed and the sensitivity was 1.5 pg ml⁻¹. Of the natural hormones only 17α-oestradiol showed crossreactivity (1.4%).

**Phospholipase A₂ assay**

**Preparation of tissue homogenate.** Each ovary was homogenized (Polytron homogenizer: Kinematica GmbH, Lucerne) for 20 s in 0.25 mol sucrose l⁻¹ (100 mg tissue ml⁻¹) and the homogenate was then centrifuged at 600 g for 5 min. The supernatant was decanted for immediate assay of PLA₂. The protein concentration of the supernatant was determined by the method of Lowry et al. (1951).

**Assay procedure.** The measurement of PLA₂ activity was based on a method previously reported for endometrium (Bonney, 1985; Bonney et al., 1987) and myometrium (Bonney and Wong 1988), but was modified to incorporate the conditions required by the rat ovary enzyme (see optimization of the assay). The substrate used was 70 000 d.p.m. 1-palmitoyl-2-[¹⁴C]oleoyl phosphatidylcholine (Amersham International plc, Bucks) together with unlabelled 1-palmitoyl-2-oleoyl phosphatidylcholine (Sigma Chemical Co. Ltd, Poole) to a final concentration of 625 μmol l⁻¹. The substrate (prepared in ethanol: toluene, 1:1 v:v) was added directly into 10 mm × 100 mm glass tubes together with 10 000 c.p.m. [³H]oleic acid to monitor procedural losses. It was evaporated to dryness under a stream of nitrogen and then reconstituted, by vigorous vortexing in 100 μl Tris–HCl buffer (0.2 mol l⁻¹, pH 8.5), containing 4 mmol calcium l⁻¹ and 0.4% Triton-X100. The enzyme preparation (150–400 μg protein in 100 μl) was added and the tubes were incubated in a shaking water bath for 1 h at 37°C. Substrate blanks were routinely included in each assay, although these were negligible (as were boiled tissue blanks). The reaction was terminated by the addition of 1 ml methanol. The extraction of lipids was completed by the addition of 1 ml chloroform and phase separation was obtained by the addition of 1 ml KCl (2 mol l⁻¹)/EDTA (5 mmol l⁻¹). The chloroform layer was removed and oleic acid isolated by thin layer chromatography using the system petroleum ether (40:60°C fraction): diethyl ether:acetic acid (80:20:2 v:v:v). The oleic acid band was visualized with iodine vapour, scraped into scintillation vials containing 10 ml Ecoscent (National Diagnostics, Aylesbury, Bucks) and counted in a Beckman LS7500 scintillation counter. Data were expressed as nmol oleic acid released mg⁻¹ protein h⁻¹.

**Optimization of the assay.** In all cases, assays were performed in duplicate and each experiment was repeated three or four times.

The linearity of the assay with respect to incubation time was ascertained over 0–120 min under standard assay conditions.

Similarly, linearity with respect to protein concentration was determined by varying the amount of enzyme preparation used within the range 100–1700 μg protein per tube.

The relationship between enzyme activity and substrate concentration was determined using a range of substrate concentrations between 0.25 and 400 nmol per tube (31.25 μmol–2 mmol l⁻¹ final concentration).

The effect of detergent on the activity of rat ovarian PLA₂ was determined by varying the concentration of Triton-X100 in the incubation medium. The final concentration range used was 0–8% and the assay conditions were as described above.

The amount of calcium added to the incubation medium was varied over the range 0–8 mmol l⁻¹ (final concentration). Tubes without calcium contained 0.5 mmol EGTA l⁻¹ and the lowest concentration of calcium used was 0.25 mmol l⁻¹. Otherwise, standard assay conditions were used.
Fig. 1. Time course of PLA₂ activity as measured by the release of oleic acid from 1-palmitoyl-2-oleoyl-phosphatidylcholine. Enzyme activity is expressed as nmol oleic acid released mg⁻¹ protein.

The pH requirement of the enzyme was investigated over the pH range 4–10 using standard assay conditions and the following buffer systems: sodium acetate–acetic acid, pH 4.0–4.5; sodium phosphate, pH 6.0–7.0; Tris–HCl, pH 7.5–9.0; sodium carbonate–bicarbonate, pH 9.5–10.0.

Statistical analysis

Comparison of the data was made by one-way analysis of variance followed by Gabriel’s multiple comparison test for unequal groups. Linear contrasts for predefined contrasts or Scheffe’s test for apparently large contrasts were carried out where relevant. Ad hoc comparisons were made using Student’s t test.

Results

Characteristics of the rat ovary PLA₂ enzyme

The results of the three or four repeat experiments performed to characterize various aspects of the enzyme were virtually identical and the figures therefore show the mean of the duplicates obtained in one of the experiments.

Oleic acid was released from 1-palmitoyl-2-oleoyl phosphatidylcholine in a time-dependent linear manner over the 120 min incubation investigated (Fig. 1). There was also a direct relationship between the amount of ovarian homogenate used and the quantity of oleic acid released for up to 400 µg protein per assay tube (Fig. 2). The incubation period selected for the assay of samples was therefore 1 h and the amount of ovarian homogenate used was restricted to the linear range.

Phospholipase A₂ activity increased with increasing substrate concentration up to 100 nmol phosphatidylcholine per tube (500 µmol l⁻¹), remained linear over the range of 100–200 nmol per tube, but then declined as the concentration of substrate was further increased to 400 nmol per tube (Fig. 3).

The addition of low concentrations of Triton-X100 (0.25–1%) to the incubation medium increased the activity of the enzyme (Fig. 4), but there was no absolute requirement for the addition of detergent, as in the absence of Triton-X100, enzyme activity was only slightly reduced and higher concentrations of Triton-X100 (2–8%) had an inhibitory effect on enzyme activity. The enzyme had an absolute requirement for calcium (Fig. 5), as exclusion of calcium from the incubation medium completely prevented substrate hydrolysis. Maximal enzyme activity was observed at a calcium concentration of 2 mmol l⁻¹. However, higher concentrations of calcium (8 mmol l⁻¹) were inhibitory. The optimum pH range for maximum PLA₂ activity was...
expressed

Fig. 4. The effect of Triton-X100 on PLA$_2$ activity. Enzyme activity is expressed as nmol oleic acid released mg$^{-1}$ protein h$^{-1}$.

Fig. 5. Phospholipase A$_2$ activity with respect to calcium concentration. Enzyme activity is expressed as nmol oleic acid released mg$^{-1}$ protein h$^{-1}$.

8.5–9.0, that is, in the alkaline range but with a sharp decline in activity at pH 9.5 (Fig. 6).

**Changes in ovarian PLA$_2$ activity over the rat oestrous cycle**

At 19.00 h at pro-oestrus (the time of the pre-ovulatory LH surge in our rats (MacKenzie et al., 1988)) there was a significant increase in PLA$_2$ activity compared with all the other days and times ($P < 0.05$ by Gabriel’s test) (Table 1). As this rise had been hypothesized, a predefined linear contrast could be made and this was highly significant ($P < 0.0001$).

The low level of activity noted at 14.00 h at oestrus was only significantly lower than the values measured at 19.00 h and 24.00 h at pro-oestrus ($P < 0.05$ by Gabriel’s test), although

ad hoc comparisons using the Student’s $t$ test indicated activity was lower at this time than at 10.00 h ($P < 0.01$) and 19.00 h ($P < 0.001$) at oestrus and the other two days at 14.00 h ($P < 0.05$).

There was an apparent diurnal rhythm in PLA$_2$ activity in that all the values at 10.00 h were lower than those at 24.00 h, but using Scheffe’s test for apparently large linear contrasts indicated that the differences were not significant.

**Changes in ovarian PLA$_2$ activity in immature rats treated with PMSG**

An LH surge occurred at 18.30 h on day 30 in the treated animals, approximately 54 h after the PMSG injection; FSH concentration also rose but more gradually (Table 2). Oestradiol concentrations were increased at 16.00 h on day 30 and declined over the following hours; this probably represents the descending slope of the oestradiol surge which occurs earlier in the day (approximately 48 h after PMSG treatment; Wilson et al., 1974).

PLA$_2$ activity was also significantly increased at 18.30 h on day 30 in the PMSG-treated animals compared with the saline controls ($P < 0.0001$) and remained high over the evening hours and the next morning after the occurrence of ovulation. The ovarian PLA$_2$ activity measured in the immature rats was approximately four times lower ($P < 0.0001$) than that seen in the adult ovaries, even though circulating concentrations of oestradiol and LH were similar to adult concentrations (Smith et al., 1975; Wilson et al., 1974).

**Changes in ovarian PLA$_2$ activity in immature rats treated with oestradiol benzoate**

As would be expected, the exogenous oestradiol benzoate (3 $\mu$g) given to 25-day-old female rats greatly increased the circulating concentration of oestradiol as noted at both 24 and
Table 1. Changes in ovarian PLA₂ activity (nmol oleic acid released mg⁻¹ protein min⁻¹) over the days of the rat oestrous cycle

<table>
<thead>
<tr>
<th>Day of the cycle</th>
<th>10.00</th>
<th>14.00</th>
<th>Time (h)</th>
<th>19.00</th>
<th>24.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-oestrous</td>
<td>17.20 ± 4.30 (6)</td>
<td>20.08 ± 3.86 (7)</td>
<td>32.94 ± 3.48 (9)*</td>
<td>25.9 ± 3.79 (7)</td>
<td></td>
</tr>
<tr>
<td>Oestrus</td>
<td>19.1 ± 2.30 (3)</td>
<td>9.66 ± 1.09 (7)</td>
<td>21.10 ± 1.70 (12)</td>
<td>23.29 ± 3.51 (10)</td>
<td></td>
</tr>
<tr>
<td>Dioestrous*</td>
<td>18.34 ± 0.76 (8)</td>
<td>16.85 ± 2.23 (16)</td>
<td>21.05 ± 2.41 (9)</td>
<td>23.66 ± 3.41 (8)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM. Figures in parentheses indicate number of animals in the group.

*P < 0.0001 (Linear-predefined contrast and Gabriel's test).

Table 2. Changes in ovarian PLA₂ activity and hormone plasma concentrations in female rats treated with 5 iu PMSG on day 28 at 12.00 h

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>28</th>
<th>30</th>
<th>30</th>
<th>30</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>12.00†</td>
<td>16.00</td>
<td>18.30</td>
<td>23.00</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Changes after PMSG

| LH (ng ml⁻¹) | 2.60 ± 1.04 (4) | 10.2 ± 1.5 (6) | 64.8 ± 11.6 (5)** | 13.9 ± 4.5 (6) | — |
| FSH (µg ml⁻¹) | 3.86 ± 0.92 (4) | 4.08 ± 0.92 (6) | 6.62 ± 0.67 (3) | 14.3 ± 3.05 (8)* | — |
| Oestradiol (pg ml⁻¹) | 9.0 ± 0.87 (4) | 46.2 ± 5.3 (6)** | 13.8 ± 3.3 (3) | 13.8 ± 1.6 (7) | — |
| PLA₂ activity (nmol oleic acid mg⁻¹ protein min⁻¹) | 2.37 ± 0.28 (4) | 4.0 ± 0.62 (6) | 7.25 ± 0.1 (10)** | 9.6 ± 1.0 (8)** | 9.68 ± 0.65 (7)** |

Changes after saline

| LH (ng ml⁻¹) | 2.60 ± 1.04 (4) | — | 2.62 ± 0.18 (6) | — | — |
| PLA₂ (nmol oleic acid mg⁻¹ protein min⁻¹) | 2.37 ± 0.28 (4) | — | 1.62 ± 0.24 (6) | — | — |

Results are expressed as means ± SEM. Figures in parentheses indicate numbers of animals in the group.

ANOVA tests:

LH: F = 19.92 (df 3.17) P < 0.0001.
FSH: F = 4.89 (df 3.17) P < 0.01.
Oestradiol: F = 25.28 (df 3.16) P < 0.0001.
PLA₂: F = 21.70 (df 4.30) P < 0.0001.

*P < 0.05 compared with all other groups (FSH).
**P < 0.001 compared with all other groups (PLA₂).
***P < 0.0001 compared with all other groups (oestradiol and LH).
†Results shown for day 28 noon obtained from saline-treated rats killed immediately after treatment and at the same time as the other groups received 5 iu PMSG.

54 h after injection (Table 3). At 24 h, ovarian PLA₂ activity was slightly, but not significantly increased and at 54 h, both LH and PLA₂ activity (but not FSH) were significantly raised (P < 0.01 for LH; P < 0.0001 for PLA₂) (Table 3). When the lower dose of oestradiol benzoate (0.5 µg) was administered, neither LH nor PLA₂ increased 54 h after the treatment.

Changes in ovarian PLA₂ in immature rats after increased gonadotrophin activity

Administration of 500 ng GnRH per rat caused a rise in plasma LH, 40 min later, which declined thereafter as noted at 6 and 24 h after injection (Table 4). Plasma oestradiol, FSH and ovarian PLA₂ activity were not affected by the GnRH treatment. When the rats were pretreated with 0.5 µg oestradiol benzoate 24 h before the GnRH, the rise in LH seen 40 min after the latter was greatly enhanced and FSH concentrations also rose slightly, but PLA₂ activity was not raised but was depressed (P < 0.01) (see Table 4). The rise in LH induced by the GnRH may have been too transient to affect the ovary and so another group of rats was treated with 10 iu HCG, which has the same action as LH but a longer half-life (Parlow and Ward 1961). This treatment did not alter endogenous LH, FSH or oestradiol concentrations, but at 2 h after treatment, there was a significant increase in PLA₂ activity, which continued to rise, as noted at 6 and 24 h (Table 5).
Table 3. Changes in ovarian PLA₂ activity and hormone plasma concentrations in female rats treated with oestradiol benzoate on day 25

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 h</th>
<th>24 h after 5 µg OB</th>
<th>54 h after 5 µg OB</th>
<th>54 h after 0.5 µg OB</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (ng ml⁻¹)</td>
<td>3.30 ± 0.47 (6)</td>
<td>4.62 ± 1.8 (4)</td>
<td>25.4 ± 6.03 (5)**</td>
<td>2.55 ± 0.84 (6)</td>
</tr>
<tr>
<td>FSH (µg ml⁻¹)</td>
<td>6.32 ± 1.16 (6)</td>
<td>6.28 ± 0.70 (5)</td>
<td>9.06 ± 2.6 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Oestradiol (pg ml⁻¹)</td>
<td>10.6 ± 1.5 (6)</td>
<td>&gt;300 (5)**</td>
<td>189 ± 64.0 (4)**</td>
<td>20.7 ± 1.42 (6)</td>
</tr>
<tr>
<td>PLA₂ activity (nmol oleic acid mg⁻¹ protein min⁻¹)</td>
<td>2.2 ± 0.14 (6)</td>
<td>3.5 ± 0.04 (5)</td>
<td>9.1 ± 1.76 (5)**</td>
<td>1.5 ± 0.12 (6)</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM. Figures in parentheses indicate number of animals in the group.

ANOVA tests:
LH: F = 12.08 (df 3,17) P < 0.02.
FSH: F = 0.82 (df 2,14) P < 0.46.
Oestradiol: F = 35.82 (df 3,17) P < 0.0001.
PLA₂: F = 17.89 (df 3,18) P < 0.0001.
**P < 0.01 compared with all other groups (LH).
***P < 0.0001 compared with value at 0 h and 0.5 µg oestradiol benzoate group (PLA₂) (Gabriel's test).

Table 4. Changes in ovarian PLA₂ activity and hormone plasma concentrations in female rats treated with 500 ng GnRH per rat on day 25

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 h</th>
<th>40 min*</th>
<th>Time after injection of GnRH</th>
<th>6 h</th>
<th>24 h</th>
<th>40 min*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (ng ml⁻¹)</td>
<td>2.55 ± 0.72 (6)</td>
<td>68.5 ± 21.0 (5)*</td>
<td>18.5 ± 1.65 (7)</td>
<td>11.11 ± 5.1 (5)</td>
<td>97.0 ± 37.1 (5)**</td>
<td></td>
</tr>
<tr>
<td>FSH (µg ml⁻¹)</td>
<td>6.15 ± 0.95 (6)</td>
<td>6.8 ± 1.5 (4)</td>
<td>6.33 ± 1.87 (5)</td>
<td>5.8 ± 1.52 (5)</td>
<td>11.5 ± 3.32 (5)</td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pg ml⁻¹)</td>
<td>10.6 ± 1.5 (6)</td>
<td>14.00 ± 1.11 (5)</td>
<td>9.96 ± 0.61 (7)</td>
<td>8.42 ± 0.59 (5)</td>
<td>35.2 ± 11.97 (5)**</td>
<td></td>
</tr>
<tr>
<td>PLA₂ activity (nmol oleic acid mg⁻¹ protein min⁻¹)</td>
<td>2.20 ± 0.14 (6)</td>
<td>2.50 ± 0.18 (5)</td>
<td>2.40 ± 0.19 (7)</td>
<td>1.90 ± 0.31 (5)</td>
<td>1.30 ± 0.09 (5)**</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM. Figures in parentheses indicate number of animals in the group. *Primed with 0.5 µg oestradiol benzoate, 24 h before GnRH injection.

ANOVA tests:
LH: F = 5.57 (df 4,23) P < 0.003.
FSH: F = 1.45 (df 4,20) P < 0.25.
Oestradiol: F = 4.95 (df 4,23) P < 0.0035.
PLA₂: F = 5.81 (df 4,23) P < 0.0025.
*P < 0.05 compared with 0, 6 and 24 h (LH).
**P < 0.01 compared with 0 h (LH); compared with all other groups (oestradiol and PLA₂) (Gabriel's test).

Discussion

The presence of PLA₂ in the rat ovary, not hitherto reported, has been demonstrated in the study reported here. The enzyme possessed the characteristics of a classic calcium-dependent membrane-bound PLA₂ (van den Bosch, 1980). The optimum pH for enzyme activity was shown to be in the alkaline range (pH 8.5–9.0) and omitting calcium from the incubation medium resulted in complete loss of activity. Furthermore, a high concentration of calcium (2 mmol l⁻¹) was required to generate a maximum response. These characteristics contrast with those of the recently identified cytosolic PLA₂ enzymes which require only micromolar concentrations of calcium for activation (Kramer et al., 1991; Tremblay et al., 1992) and with the PLA₂ of human myometrium and endometrial stromal tissue, which is calcium independent and optimally active at neutral pH (Bonney et al., 1987; Bonney and Wong, 1988).

In the normal physiological situation, follicular rupture takes place only after the occurrence of the preovulatory LH surge. There is good evidence that LH induces the synthesis of prostaglandins from both thecal and granulosa cells and, depending on the species, either PGF₂₀ (rats) or PGF₂₁α (rabbits, pigs, monkeys) then cause the rupture of the thecal wall (see Poyser, 1987).

LH stimulates ovarian prostaglandin synthesis by enhancing the expression of prostaglandin synthetase (Bauminger and
Lindner, 1975; Sirois et al., 1992). The prostaglandin synthetase in the rat ovary is a specific isoform of the enzyme, selectively responsive to gonadotrophins and found only in the granulosa cells of preovulatory follicles (Sirois and Richards, 1992; Sirois et al., 1992). This enzyme acts on the prostaglandin precursor, arachidonic acid, converting it to PGG2 and PGH2. However, as mentioned earlier, the rate-limiting step for prostaglandin synthesis is the availability of arachidonic acid and it is possible that LH might also act to enhance PLA2 activity. Thyroid-stimulating hormone can increase PLA2 activity and hence prostaglandin production in the thyroid (Haye et al., 1973) and gonadotrophins may have a similar effect in the ovary. Clark et al. (1978) argued against this, as they found that labelled arachidonic acid was not released from cultured granulosa cells after addition of LH. However, they did not measure PLA2 activity directly, and, in vivo, another agent such as oestradiol may be involved.

Measurements of ovarian PLA2 activity during the rat oestrous cycle revealed that, as hypothesized, there was a significant rise at pro-oestrus at the time of the preovulatory LH surge and just after the preovulatory oestraldiol surge. The high activity was maintained over the evening hours up to at least midnight, but had declined on the morning of the next day. A similar result was obtained in immature rats injected with PMSG. This treatment induces the ovary to secrete oestradiol which then stimulates the release of an endogenous LH surge approximately 54 h after the injection, and this in turn causes ovulation 12 h later (Wilson et al., 1974). Both LH concentrations and PLA2 activity were slightly (but not significantly) higher just before the pre-ovulatory LH surge than before treatment on day 28 at 12.00 h, but the PLA2 activity then rose nearly twofold (P < 0.001) at the time of the LH surge at 18.30 h and activity remained high over the evening hours and the next morning. Thus both animal models undergoing ovulation showed a similar pattern of changes in ovarian PLA2, in both cases associated either with the prior rise in oestraldiol secretion or a concomitant rise in LH and lasting over the night-time period. The main differences between the two models were that (i) PLA2 activity was much lower in the immature rats even though the rise in steroid and gonadotrophin concentrations was similar to those seen in the adult and (ii) the PLA2 rise was maintained overnight into the next day after ovulation had occurred in the immature rats, while it had declined on the morning of oestrus in the adults.

The role of oestraldiol and LH in inducing the preovulatory PLA2 rise was investigated further by administering oestraldiol benzoate, hCG and GnRH to immature rats and measuring their effect on ovarian PLA2 activity. In the first experiment when 5 µg oestraldiol benzoate was injected, there was a marked increase in circulating concentrations of oestraldiol and a significant increase in PLA2 activity at 54 h, but not at 24 h, after injection. At 54 h, there was also a significant rise in LH. From these data, however, it is difficult to determine whether the rise in PLA2 activity at 54 h after injection is due solely to the earlier increase in circulating oestraldiol concentrations, to LH or to the sequential action of both hormones. The lower dose of 0.5 µg oestraldiol benzoate did not induce a rise in PLA2 activity, but again it is not known whether this is due to the ineffectiveness of the low concentration used or its inability to induce an LH surge. In fact, this low dose of oestraldiol benzoate caused a slight depression of PLA2 activity and this was seen again as a significant reduction (P < 0.01) when the same dose was followed by GnRH. The effects noted after hCG indicate that perhaps LH is the more important stimulating factor since without increasing plasma oestraldiol concentrations significantly, hCG induced a rise in ovarian PLA2 2 h after its administration with the activity continuing to increase in the 24 h after injection. hCG acts mainly on LH receptors (Lunn and Bell 1968) and can be considered as a long-acting LH preparation (Parlow and Ward 1961). Stimulation of release of endogenous LH using GnRH did not affect PLA2 activity, whether or not the animals were primed with oestrogen 24 h before. Perhaps the GnRH-induced rise in LH was too transient to affect the PLA2. Another possibility is the low responsiveness of the immature follicles in

Table 5. Changes in ovarian PLA2 and hormone plasma concentrations in female rats treated with 10 IU hCG per rat on day 25

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time after injection of hCG (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>LH (ng ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.55 ± 0.72 (6)</td>
</tr>
<tr>
<td>FSH (µg ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.15 ± 0.95 (6)</td>
</tr>
<tr>
<td>Oestradiol (pg ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.60 ± 1.5 (6)</td>
</tr>
<tr>
<td>PLA2 activity</td>
<td></td>
</tr>
<tr>
<td>(nmol oleic acid</td>
<td>2.2 ± 0.14 (6)</td>
</tr>
<tr>
<td>mg⁻¹ protein min⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means ± SEM. Figures in parentheses indicate number of animals in the group.

ANOVA tests:
LH: F = 0.7 (df 3,20) P = 0.56.
FSH: F = 1.3 (df 3,20) P = 0.30.
Oestradiol: F = 3.77 (df 3,20) P < 0.05.
PLA2: F = 37.43 (df 3,21) P = 0.0001.

*P < 0.05 compared with value at 2 h (oestradiol);
**P < 0.001 compared with value at 0 and 24 h (PLA2);
***P < 0.0001 compared with all other groups (PLA2) (Gabriel's test).
this group. However, this is unlikely as the follicles would not have been mature after oestradiol benzoate treatment either. A third possibility is that another factor(s) is required to act in conjunction with LH. It is unlikely to be FSH since this did not increase significantly in the groups treated with oestradiol benzoate or hCG.

The results in this report give no indication of the ovarian compartment in which the increase in PLA₂ activity occurs. Future experiments will involve measuring PLA₂ activity in ovarian cell types to elucidate this. It is possible that interstitial cells are at least one site, since in the group given hCG the rise in PLA₂ was not accompanied by a rise in oestriadiol indicating granulosa cells were not stimulated. It is possible that granulosa cells do respond, but only in the more mature preovulatory follicles in parallel with the LH effect on prostaglandin synthetase.

In conclusion, we have shown that the preovulatory rise in ovarian prostaglandins may be due to an increase, not only in prostaglandin synthetase as shown by others, but also in PLA₂. The increase in activity of both enzymes appears to be induced by LH, whereas FSH and oestradiol, which are also hyper-secreted in the preovulatory period, are probably not involved.

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