Effects of Luteinizing Hormone on Phosphoinositide Metabolism in Rat Granulosa Cells*

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Rat granulosa cells isolated from mature Graafian follicles were incubated with luteinizing hormone under various conditions in order to follow the synthesis and degradation of phospholipids. During acute incubations, luteinizing hormone provoked rapid and concentration-dependent increases in the incorporation of 32P04 into phosphatidic acid, phosphatidylinositol, and the polyphosphoinositides. Similarly, luteinizing hormone provoked increases in labeling of phosphatidylinositol and the polyphosphoinositides when granulosa cells were incubated with myo-[2-3H]inositol. When granulosa cells were prelabeled with 32P04 in order to label phosphatidylinositol to constant specific radioactivity (4 h), luteinizing hormone treatment significantly increased 32P-phosphatidylinositol levels (23%). Comparable increases (27%) in the cellular concentrations of phosphatidylinositol were observed in response to luteinizing hormone. In pulse-chase experiments employing 32P04- or [3H]inositol-prelabeled cells, luteinizing hormone did not alter phospholipid degradation. In addition, luteinizing hormone did not stimulate degradation of polyphosphoinositides.

These results demonstrate that: (a) luteinizing hormone has selective effects on phospholipid metabolism in rat granulosa cells which involve phosphatidic acid, phosphatidylinositol, and the polyphosphoinositides, (b) luteinizing hormone increases cellular levels of phosphatidylglycerol and presumably phosphatidic acid and the polyphosphoinositides, and (c) luteinizing hormone does not increase phospholipid degradation. Our findings suggest that luteinizing hormone provokes increases in de novo synthesis of phosphatidylinositol in rat granulosa cells. These changes in phospholipid metabolism may be important for steroidogenesis and other enzymatic processes during treatment with luteinizing hormone.

We have previously reported that LH* increases the levels of PI in isolated rat granulosa cells (1). Although the increases observed in phospholipid mass were rather small, approximately 17%, and were associated with greater (70%) increases in 32P04 incorporation into PI during acute labeling experiments, we proposed that the effect of LH may simply involve an increase in de novo PI synthesis. The possibility existed, however, that both de novo synthesis and turnover of PI (accelerated PI hydrolysis to form diacylglycerol followed by resynthesis of PI from PA) were occurring simultaneously.

Turnover of PI is the most commonly reported process when increased levels of radioactivity in PI are observed following receptor activation. However, it is difficult to distinguish between turnover and de novo synthesis of PI when examining the incorporation of radioactive precursors. In addition, the ability to observe PI turnover may be hampered by multiple metabolic pools of PI (hormone-insensitive versus hormone-sensitive pools) (2). Another mechanism for PI labeling has been recently described. In many hormone-sensitive tissues, polyphosphoinositide degradation occurs as the initial event following receptor activation; i.e. depletion of polyphosphoinositides temporarily precedes increases in PI labeling (3, 4). It has been suggested that under these conditions PI is converted to polyphosphoinositides and that labeling of PI simply reflects replenishment of the reservoir of PI (5).

In this report we provide the initial evidence that, in addition to increasing the synthesis of PI, LH also increases polyphosphoinositide metabolism in rat granulosa cells. This study shows that the effects of LH on polyphosphoinositide metabolism in rat granulosa cells are not due to increased turnover or degradation of PI or the polyphosphoinositides.

MATERIALS AND METHODS

Pregnant mare's serum gonadotropin and ovine LH (NIH-o19) were kindly supplied by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health. [1,2,6,7-3H]Progesterone (90 Ci/mmol) and myo-[2-3H]inositol (10 mCi/mmol) were purchased from New England Nuclear. 32PO4 (carrier free, 285 Ci/mg) was purchased from ICN Chemical and Radioisotope Division (Irving, CA). Phospholipids were obtained from Sigma. The antisera for the progestosterone assay was obtained from Dr. Gordon Niswender (Colorado State University, Fort Collins, CO).

Immature Sprague-Dawley rats (Charles River) were injected subcutaneously with 20 IU pregnant mare's serum gonadotropin between 0800 and 1200 h on day 27 or 28 and were killed 48 h later. Ovaries were removed, trimmed, and placed in Medium 199 (GIBCO) containing 25 mM Hepes and 1% bovine serum albumin. This modified medium was used in all subsequent incubations. The ovaries were incubated at room temperature for 15 min in medium containing 6.8 mM EGTA followed by a 5-min incubation in medium containing 5 mM EGTA plus 0.5 mM succrose as described by Campbell (6) with minor modifications (7). Granulosa cells were harvested by puncturing follicles with a needle and expressing the cells with gentle pressure. Approximately 4 x 10^5 viable cells/ovary were obtained. Viabal-

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‡ The abbreviations used are: LH, luteinizing hormone; PI, phosphatidylinositol; PA, phosphatidic acid; DPI, phosphatidylinositol 4-phosphate; LPI, lyso phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; LPC, lyso phosphatidylcholine; TPI, phosphatidylinositol 4,5-bisphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N′,N″,N‴-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ACTH, adrenocorticotropic hormone.
Stimulatory effect of LH on 32P incorporation into phospholipids by rat granulosa cells

Isolated rat granulosa cells were incubated for 60 min in medium containing 50 μCi of 32P04 in the absence (control) or presence of LH (1 μg/ml). Data are shown as mean ± S.E. after expression as a percentage of the average total 32P-phospholipid levels in each experiment. The difference (d) between control and LH samples was determined in each experiment and is shown as the mean ± S.E. The number of experiments is shown in parentheses. Statistical significance was determined using the t test for paired comparisons.

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Control</th>
<th>LH</th>
<th>P (LH versus control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of total 32P-phospholipid</td>
<td>d</td>
<td>% (n)</td>
</tr>
<tr>
<td>DPI</td>
<td>1.76 ± 0.22</td>
<td>2.14 ± 0.25</td>
<td>0.38 ± 0.10</td>
</tr>
<tr>
<td>PA</td>
<td>1.72 ± 0.23</td>
<td>2.16 ± 0.30</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>PS + LPI + LPC</td>
<td>1.41 ± 0.25</td>
<td>1.77 ± 0.37</td>
<td>0.36 ± 0.18</td>
</tr>
<tr>
<td>PI</td>
<td>4.35 ± 0.33</td>
<td>7.67 ± 0.50</td>
<td>3.32 ± 0.28</td>
</tr>
<tr>
<td>PC + PE + PG</td>
<td>89.98 ± 0.71</td>
<td>85.57 ± 0.95</td>
<td>4.41 ± 0.35</td>
</tr>
</tbody>
</table>

NS, not statistically significant.

RESULTS

Effect of LH on 32P Incorporation into Phospholipid by Rat Granulosa Cells—The distribution of 32P in phospholipids in acute incubations (60 min) is given in Table I. Most of the radioactivity (approximately 90%) was found in the area of the chromatogram which contained PC + PE + PG. Together, PE + PG contained approximately 5% of the total radioactivity. In control cells, approximately 4.4% of the total counts were found in PI; 1.4% in the fraction containing PS + LPI + LPC; 1.7% in PA; and 1.8% in DPI. Addition of LH (1 μg/ml) provoked the following increases over a 60-min period: PI, 76%; PA, 26% DPI, 22%. LH treatment did not increase 32P04 incorporation into other phospholipids. The decrease in the percentage of total 32P-phospholipid in the PE + PG fraction simply reflected the increases in PI, PA, and DPI.

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>32P-Phospholipid (cpm)</th>
<th>Phospholipid phosphorus (P)</th>
<th>Specific radioactivity (cpm × 10^4/μg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylinositol</td>
<td>29.5 ± 7.5</td>
<td>382 ± 63</td>
<td>7.7 ± 1.8</td>
</tr>
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<td>LH (1 μg/ml)</td>
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<td>472 ± 86 (123)</td>
<td>11.2 ± 2.1 (146)</td>
</tr>
<tr>
<td>LH minus control</td>
<td>24° ± 5.4</td>
<td>90° ± 29</td>
<td>3.5° ± 0.66</td>
</tr>
<tr>
<td>Phosphatidylcholine plus phosphatidylethanolamine plus phosphatidylglycerol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>654 ± 159</td>
<td>3139 ± 715</td>
<td>21.5 ± 4.7</td>
</tr>
<tr>
<td>LH (1 μg/ml)</td>
<td>628 ± 153 (96)</td>
<td>3218 ± 674 (103)</td>
<td>19.6 ± 3.8 (91)</td>
</tr>
<tr>
<td>LH minus control</td>
<td>-26 ± 11.7</td>
<td>79 ± 128</td>
<td>-1.9 ± 1.02</td>
</tr>
</tbody>
</table>

* p < 0.05 versus control, paired t test.

Effect of LH on phospholipid synthesis by rat granulosa cells

Rat granulosa cells were incubated for 60 min in medium with 50 μCi of 32P04 in the absence (control) or presence of LH (1 μg/ml). After extraction and purification by thin-layer chromatography, phospholipid phosphorus was measured by colorimetry and 32P incorporation determined by scintillation counting. Results are normalized to reflect levels in 10^7 cells. Data are shown as means ± S.E. (n = 5). The numbers in parentheses represent the percentage of the control responses.

<table>
<thead>
<tr>
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* p < 0.05 versus control, paired t test.
TABLE III

Stimulatory effect of LH on incorporation of 32P04 and myo-[2-3H]inositol into polyphosphoinositides by isolated rat granulosa cells

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>32P-Phospholipid</th>
<th>3H-Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPI</td>
<td>TPI</td>
</tr>
<tr>
<td>Control</td>
<td>4903 ± 50</td>
<td>8355 ± 182</td>
</tr>
<tr>
<td>LH 1 μg/ml</td>
<td>5813 ± 1940</td>
<td>10182 ± 4168</td>
</tr>
</tbody>
</table>

*Rat granulosa cells were incubated in medium containing 50 μCi of 32P04 or 10 μCi of myo-[2-3H]inositol for 60 min in the presence or absence of LH (1 μg/ml). Phospholipids were extracted and purified by successive unidimensional thin-layer chromatography, as described under "Materials and Methods."

*p < 0.05, t test, (mean ± S.E., n = 3).

Table II shows the effects of LH on phospholipid synthesis in rat granulosa cells. In addition to increasing 32P04 incorporation into PI, LH also significantly increased the tissue concentrations of PI (23%). In these experiments in which granulosa cells were acutely incubated with media containing 32P04, the levels of polyphosphoinositides were unchanged by LH treatment. Under control conditions, in the presence of LH, LH stimulated significant increases in [32P]PI levels after 10 min of incubation and further increases were noticed throughout the incubation period.

A similar time course for PI labeling was observed when isolated granulosa cells were incubated with myo-[2-3H]inositol (Fig. 2). Time-dependent increases in [3H]PI levels were observed under control conditions. Treatment with LH increased [3H]PI levels at each time point examined (36% increase after 15 min of incubation and 92% and 159% increase after 60 and 120 min, respectively). The time course for LH-induced PI labeling was well correlated with LH-induced progesterone accumulation.

In order to further characterize the time course of LH action on PA and PI labeling, experiments were conducted to examine the early responses to LH. The response to LH was examined; (a) in cells which were not prelabeled (i.e. LH and 32P04 were added simultaneously at the beginning of the incubations) and (b) in cells which were prelabeled for 60 min (to label phospholipids with rapid turnover rates as extensively as possible) and subsequently treated with control medium or LH. As shown in the lower panel of Fig. 3, control labeling of PA increased very rapidly and reached a plateau after 60 min of incubation. The initial rate of control PI labeling (upper panel) was much less than PA. However, after the initial lag, PI levels increased rapidly and continued to increase throughout the 120-min incubation period.

In cells incubated without prelabeling, LH significantly increased (40% increase) PA labeling within 2 min of incu-
activity after 60 min of incubation. Incorporation of \(^{32}\)P into DPI was significantly increased into DPI within 10 min of incubation in the presence of LH using either labeling protocol (data not shown).

Concentration Dependence of the LH-induced Stimulation of Phospholipid Metabolism—To further evaluate the response of isolated rat granulosa cells to LH, studies were performed to determine the lowest effective concentration of LH which would produce an increase in phospholipid labeling. Fig. 4 shows that granulosa cells responded to LH in a concentration-dependent manner in their ability to increase \(^{32}\)P incorporation into PI, PA, and DPI. Significant increases in \(^{32}\)P incorporation into PI, PA, and DPI occurred at concentrations of 5–50 ng/ml of LH and maximal responses occurred at concentrations of 500–1000 ng/ml of LH. No significant changes were observed in the PC + PE + PG fraction at any concentration of LH.

Fig. 5 shows the dose-response curves for LH-induced progesterone accumulation and \(^{32}\)P incorporation into PI. Maximal progesterone accumulation was observed at concentrations of LH greater than 50 ng/ml whereas maximal \(^{32}\)P-PI labeling was observed at concentrations of LH greater than 500 ng/ml. The ED\(_{50}\) values for LH-induced increases in progesterone accumulation and \(^{32}\)P-PI labeling were 2.9

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**FIG. 3.** Temporal profile of \(^{32}\)P incorporation into phosphatidylinositol and phosphatic acid in response to LH. Isolated rat granulosa cells were incubated for up to 90 min in medium containing \(^{32}\)P, LH (1 \(\mu\)g/ml) or control medium was added (arrows) to samples at time zero or after 60 min of prelabeling and the incubations were continued for 30 min. The data are shown in the mean ± S.E., \(n = 3\). The inset shows the data after expression as the mean ± S.E. of the percentage increase over control values. \(\ast, p < 0.05\).

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**FIG. 4.** Stimulation of \(^{32}\)P incorporation into phospholipids by increasing concentrations of LH. Isolated rat granulosa cells were incubated for 60 min in medium containing \(^{32}\)P, with LH (0–5000 ng/ml). The data are expressed as mean ± S.E. (\(n = 3\)). \(\ast, p < 0.05\) versus control, denotes the lowest concentrations of LH which provoked a significant increase in \(^{32}\)P incorporation.

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**FIG. 5.** Stimulation of \(^{32}\)P incorporation into phosphatidylinositol and progesterone accumulation by increasing concentrations of LH. Isolated rat granulosa cells were incubated for 60 min in medium containing \(^{32}\)P, with LH (0–5000 ng/ml). The data shown are a composite of three separate experiments. Each data point represents the average response from duplicate or triplicate samples obtained in one experiment after expression as a percentage of the maximal response observed in the experiment.
that preincubation of isolated rat granulosa cells with 32P04 and 49.3 ng/ml, respectively.

Triplicate incubations. The presence of LH (1 pg/ml). Data represent the means of phosphoinositols after prelabeling isolated rat granulosa cells for 270.

Phospholipid determinations or were further incubated for 60 min in the absence (CTL) or presence of LH (1 μg/ml). Data represent the means ± S.E. from triplicate incubations (*, p < 0.05 versus CTL).

Effects of LH on Prelabeled Phospholipids—Fig. 6 shows that preincubation of isolated rat granulosa cells with 32P04 for 2 h was sufficient to label PA and DPI to constant specific radioactivity (i.e. further incubation under control conditions in the presence of 32P04 did not result in additional labeling). However, 32P levels in PI and the fraction containing PC + PE + PG continued to increase throughout the incubation period (180 min). Treatment of the prelabeled cells with LH resulted in significant increases (37%) in both DPI and PA. LH also stimulated 32P04 incorporation into PI (33% increase), but not PC + PE + PG. LH also significantly stimulated progesterone accumulation in these incubations (nanograms/2 × 10^6 cells; control, 12.4 ± 0.2; LH, 62.5 ± 0.6).

Effect of LH on Depletion of Prelabeled Phosphoinositides—Since many investigators have reported that the incorporation of 32P04 into PI represents an increased rate of PI turnover (initial degradation, presumably via PI-specific phospholipase C, followed by phosphorylation of diacylglycerol by diglyceride kinase to form PA and subsequent resynthesis of PI), the following experiments were conducted to determine whether or not the effect of LH on the labeling of PI could be reflected by increased turnover of PI. Fig. 7 shows the results of pulse-chase experiments in which granulosa cell phospholipids were prelabeled with 32P04 for 60 min, chased for 60 min, and incubated in the absence (control) or presence of LH for up to 60 min. During the last 60 min of incubation under control conditions, 32P levels in PA and DPI declined, while 32P levels in PI and PC + PE + PG were relatively unchanged. LH treatment did not increase or decrease the loss of 32P from PA, DPI, PI, or PC + PE + PG indicating that LH has no effect on increasing or decreasing the rate of phospholipid degradation. Thus, after prelabeling and removing the source of radioactive precursors, LH did not increase (or decrease) the turnover of PI, PA, and DPI. LH did, however, increase mately 4 h and remained relatively constant thereafter. When LH was added to granulosa cells prelabeled for 270 min, a significant increase (27%) in [32P]PA levels was observed. Under these conditions, LH increased [32P]PA levels by 28% over controls. Since LH-induced increases in cellular PI levels (mass) were virtually the same (see above), these results strongly suggest that the LH-induced increases in cellular PI labeling are reflective of increased synthesis of PI. LH also significantly increased progesterone accumulation in these incubations (nanograms/2 × 10^6 cells; control, 12.4 ± 0.2; LH, 62.5 ± 0.6).

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progestosterone accumulation in these experiments (nanograms/2 x 10^6 cells at 60 min; control, 6.3 ± 1.4; LH, 36.2 ± 1.5).

In other pulse-chase experiments, isolated rat granulosa cells were prelabeled for 60 min with myo-[2-3H]inositol, washed three times, and chased for 30 min in the presence or absence of LH. While LH provoked its usual increases in progestosterone accumulation (nanograms/2 x 10^6 cells; control, 4.1 ± 0.5; LH, 26 ± 7; mean ± S.E., n = 3), LH did not stimulate depletion of prelabeled [3H]PI (99.9 ± 4.9% of control, mean ± S.E., n = 3).

Although no depletion of PI was observed in cells prelabeled under control conditions, it is possible that the effect of LH involves a hormone-sensitive pool of PI. To investigate this possibility, isolated granulosa cells were prelabeled with medium containing 32PO_4, in the absence (control) or presence of LH, then chased for 60 min in the absence of hormone or isotope and finally incubated in medium without 32PO_4, in the presence or absence of LH for up to 60 min. Although LH provoked its usual increase in PI levels during the prelabeling period, LH did not stimulate depletion of 32P-PI from cells prelabeled with or without LH (data not shown).

Recently, hormone-induced hydrolysis of phosphoinositides rather than PI has been proposed as the initial event in the action of some agents following receptor activation (3, 4). In tissues where agonist-induced phosphoinositide hydrolysis occurs, depletion of polyphosphoinositides was found to temporally precede increases in the labeling of PI. Phosphatidylinositol labeling may therefore represent a compensatory mechanism for the replenishment of polyphosphoinositides. We investigated this possibility in rat granulosa cells which were prelabeled for 2 h (sufficient time for TPI, DPI, and PA to reach constant specific radioactivity). In incubations lasting 90 s, LH had no effect on levels of prelabeled DPI or TPI (98 ± 2 and 101 ± 4% of control, respectively; mean ± S.E., n = 3). Similarly, no depletion of [32P] polyphosphoinositide levels were observed in incubations lasting from 1 to 5 min (data not shown). On the other hand, we have shown that treatment of prelabeled granulosa cells for 90 s with another agonist, gonadotropin-releasing hormone, caused DPI and TPI levels to decrease significantly (10).

**DISCUSSION**

The present results extend our previous observations on phospholipid metabolism in rat granulosa cells (1) and provide the initial observation that LH provokes significant increases in polyphosphoinositide metabolism in rat granulosa cells. In response to LH, there are relatively rapid and dose-dependent increases in the incorporation of 32PO_4 into PA, PI, and the polyphosphoinositides, while the incorporation of 32PO_4 into other phospholipids is unaffected. In addition, the effects of LH on 32PO_4 and [3H]inositol incorporation into PI, DPI, and TPI are similar. In conjunction with increases in the incorporation of radioactive precursors, LH provokes a small increase in cellular PI levels, but does not alter cellular levels of PC + PE + PG.

It seems unlikely that the present results could be explained by hormone-induced alterations in the specific radioactivity of ATP (the precursor providing the 32PO_4 found in PA, which is the precursor for the phosphoinositides). First, LH-induced increases in the labeling of PI, DPI, and TPI were also observed with another precursor, myo-[2-3H]inositol. Second, after labeling PI to constant specific radioactivity LH-induced increases in [32P]PI levels were similar to the increases observed in cellular PI levels. Third, an increase in ATP specific radioactivity would have to be localized to a discrete pool(s) of ATP since the labeling of PC, which contains approximately 85% of total 32P-phospholipid (PI contains 4-8%), is also dependent on a [32P]ATP precursor pool and was unaffected by LH. If an increase in ATP specific radioactivity occurs in response to LH it must be small (<5% of total) and contained within a specific cellular compartment(s).

The present results indicate that the LH-induced increases in 32PO_4 or [3H]inositol-labeled phospholipids can be best explained by an increase in phospholipid synthesis. Phospholipid degradation rates (as observed in pulse-chase experiments) were essentially unchanged by LH treatment. In other pulse-chase experiments, LH had no effect on 32P-phospholipid levels from cells prelabeled in either the presence or absence of LH. Thus, it seems that the action of LH does not involve the turnover (breakdown and resynthesis) of a discrete hormone-sensitive pool of PI. Similarly, no depletion of prelabeled polyphosphoinositides were observed under any experimental conditions. The action of LH on labeling of PI, therefore, appears to be unrelated to hydrolysis and turnover of PI or the polyphosphoinositides.

In the present study we have employed 32P measurement to estimate PI mass after prelabeling to constant specific radioactivity. The effect of LH on PI levels after prelabeling granulosa cells for 270 min (27% increase over controls, Table I) was similar to the effect on PI levels observed with colorimetric determination of phospholipid-phosphorus (23% increase over controls, Table II). That the observed increases in 32PO_4 incorporation can be attributed to increased phospholipid synthesis is supported by our observations that PI and polyphosphoinositide degradation did not appear to increase, even at very early times of LH treatment. We therefore believe that LH primarily stimulates a de novo phospholipid synthesis effect, and does not appear to influence PI or polyphosphoinositide hydrolysis and turnover.

The mechanism whereby LH provokes increases in rat granulosa cell phosphoinositide metabolism is presently uncertain. In rat ovarian tissues, LH is known to stimulate glucose uptake and glycolysis (11, 12) which may increase the availability of precursors for PA synthesis. Increased activity of glycerol-3-phosphate acyltransferase may also increase cellular levels of PA. Although it is not known whether or not LH stimulates this enzyme, insulin, another hormone which also provokes phosphoinositide synthesis (13, 14), increases the activity of this enzyme in adipose tissue (15) and liver (16). Alternatively, LH may increase phosphoinositide synthesis by inhibiting PA degradation. This mechanism seems unlikely, however, since inhibition of PA phosphatase would reduce diacylglycerol availability for the synthesis of PC and PE (quantitatively the major phospholipids in granulosa cells) and reductions in the net levels or labeling of these phospholipids were not observed in cells treated with LH (Table II). Although a direct effect of LH on polyphosphoinositide synthesis cannot be ruled out (17), the synthesis of polyphosphoinositides probably occurs as a result of the increased levels of PI.

The action of hormones on ovarian phospholipid metabolism has received very little attention and some potentially contradicting observations have been made. In rat granulosa cells isolated from mature Graafian follicles (1) and in bovine luteal cells (9), LH increases PI metabolism. However, in previous studies using rat luteal cells prepared from highly luteinized rat ovaries, Strauss et al. (18) reported that LH had no effect on PI metabolism. We have recently obtained similar results in our laboratory. It may be that PI metabolism is already maximally stimulated in these rat corpora lutea and is unresponsive to exogenous gonadotropin. Alternatively, PI metabolism in the rat corpus luteum may not be regulated...
by LH but by another tropic hormone(s). Indeed, prolactin and estrogen play very important roles in the development of the rat corpus luteum (19), whereas LH is the major tropic hormone regulating bovine corpus luteum function (20, 21). It is also interesting to note that PI metabolism in both rat granulosa cells (22, 23) and rat luteal cells (24, 25) is stimulated by other hormones (e.g. gonadotropin-releasing hormone and prostaglandin F₂α). Clearly, further studies are required to define the effects of hormones on ovarian phospholipid metabolism.

The importance of increases in phospholipid metabolism after LH treatment is presently unknown. A strong correlation between the synthesis of phospholipids in the phosphatidate-phosphoinositide pathway and steroidogenesis exists for ACTH action in the rat adrenal (26, 27). In isolated bovine luteal cells (9), increases in PI metabolism are temporally and dose-related to LH-induced progesterone accumulation. In rat Leydig cells (28), LH also stimulates the synthesis of phosphoinositides and steroidogenesis. In rat granulosa cells we have observed that time courses for [3H]inositol incorporation into PIP and phosphoinositides were well correlated. LH also provokes rapid increases in the labeling of PA (<2 min). In addition, incubation of granulosa cells with exogenous PA or PI increases progesterone accumulation (1). However, from the data presented in this paper it is clear that the dose-response relationships for LH-induced PI labeling and progesterone accumulation are quite different. At low concentrations of LH, increases in progesterone occurred without measurable increases in 32P04 incorporation into PA or PI. Similar discrepancies have been reported between steroidogenesis and cyclic AMP accumulation during LH dose-response studies (29). Interestingly, we have observed that exogenous cyclic nucleotides and cholera toxin (at concentrations which greatly increase progesterone accumulation) do not measurably increase PI metabolism in rat granulosa (30) and bovine luteal cells (9), however, positive effects of 8-bromo-CAMP have been reported in rat Leydig cells (28). In contrast to studies in the rat adrenal (31, 32), cholesterol side-chain cleavage is only slightly enhanced by exogenous DFI in mitochondria preparations from rat corpora lutea (33, 34). The latter two observations raise the question of whether LH-induced increases in phosphoinositides can adequately explain the steroidogenic action of LH. Nonetheless, the increase in phosphoinositides is but one manifestation of the generalized increase in de novo PA synthesis and other components of this response may be more closely related to the activation of steroidogenesis.

The action of LH on phospholipid metabolism may be important for the activation of ovarian protein kinase C (35, 36). While PS is slightly more effective, both PA and PI activate protein kinase C in bovine luteal and rat ovarian cytosol preparations (35). Diacylglycerol also increases protein kinase C activity and reduces the calcium and phospholipid concentrations required for enzyme activation. Although it is presently unknown whether LH increases diacylglycerol levels, other hormones (ACTH and insulin) which provoke de novo synthesis of phospholipid also increase diacylglycerol levels in their target tissues (14, 37). Of considerable interest are the recent observations that the tumor-promoting phorbol esters, which mimic the stimulatory action of diacylglycerol on protein kinase C, also provoke progesterone and prostaglandin synthesis (38) in rat granulosa cells isolated from preovulatory follicles. Thus increased levels of phospholipids and their metabolites could be of great importance in the subsequent phosphorylation of proteins which are substrates for protein kinase C. Further work will be needed to test this possibility, and further define the role of phosphoinositide synthesis in LH action.

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