Tissue specific compartmental analysis of gonadotropin stimulation of ovarian ornithine decarboxylase

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Abstract. Luteinizing hormone is known to stimulate the enzyme ornithine decarboxylase in the ovary. Highly purified human follicle stimulating hormone that is devoid of significant biologically active luteinizing hormone can also induce ornithine decarboxylase activity in intact immature rats with a time course of induction similar to that reported for luteinizing hormone. A maximum of 8–10-fold stimulation above controls was observed 4 h following intravenous administration of human follicle stimulating hormone. This stimulation followed a strict dose response relationship. Ovine luteinizing hormone and human chorionic gonadotropin always induced more ovarian ornithine decarboxylase activity than that achieved by maximally effective doses of follicle stimulating hormone. This could not be attributed solely to the ability of specific cell population to respond to the respective gonadotropins. Although granulosa cells contained little receptor for luteinizing hormone/human chorionic gonadotropin and the residual tissue contained little receptor for follicle stimulating hormone, each tissue responded to these gonadotropins in a manner suggestive of the mediation by one or more diffusible factors. A relationship between gonadotropin induced 3'5'-cyclic adenosine monophosphate (cyclic adenosine monophosphate) concentration and ornithine decarboxylase activity suggests that the mediation of gonadotropin stimulated ovarian ornithine decarboxylase is not solely through cyclic adenosine monophosphate, indicating the presence of other factors in the induction of gonadotropin increased ornithine decarboxylase activity.

Keywords. Ovarian ornithine decarboxylase; ovarian compartment; receptors; cAMP; gonadotropins.

Introduction

Mammalian ornithine decarboxylase (ODC) (EC 4·1·1·17. L-ornithine carboxylyase), catalyzes the decarboxylation of L-ornithine to putrescine and carbon dioxide. This is the first and rate limiting reaction in polyamine biosynthesis (Morris and Fillingame, 1974).

Enhanced elaboration of polyamines associated with increased macromolecular biogenesis has been repeatedly observed. A large and rapid increase in ODC activity is a characteristic early event in several hormone stimulated target tissues, including ovarian ODC stimulated by human chorionic gonadotropin (hCG) and luteinizing hormone (LH) (Kaye et al., 1973). Whether or not follicle stimulating hormone (FSH), can stimulate ovarian ODC in vivo has not been completely resolved (Sheela Rani and Moudgal, 1979).

Abbreviations used: ODC, Ornithine decarboxylase; hCG, human chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle stimulating hormone; hFSH, human FSH; PLP, pyridoxal-5'-phosphate; DTT, dithiothreitol.
In this report, using highly purified human FSH (hFSH) with insignificant contamination of biologically active LH, we demonstrate that hFSH can stimulate ODC in a time and dose dependent manner in intact immature female rats and that this response depends to a large extent on the route of administration. Further, it appears that much greater consideration must be given to differential responsiveness of ovarian compartments. Additional evidence indicates that gonadotropin stimulation of ODC may at least in part, involve a secondary intracellular mediator which is not cAMP.

Materials and methods

Intact immature female rats obtained from Holtzman, Madison, Wisconsin, USA were maintained on pelleted food and water ad libitum. hFSH, oLH and hCG (Roussel Corp., 2950 IU/mg) in 0.1% gelatin/0.01 M phosphate buffer containing 0.14 M NaCl, pH 7 (PBS) were administered either subcutaneously or intravenously. 17-β-Estradiol in propylene glycol was administered subcutaneously.

Pyridoxal 5’ phosphate (PLP), dithiothreitol (DTT), DL-ornithine and 17-β-estradiol were obtained from Sigma Chemicals Co., St. Louis, Missouri, USA. [1-14C]-Ornithine monohydrochloride (52.8 mCi/mmol) and hyamine hydroxide were obtained from New England Nuclear, Boston, USA. Glass tubes (16 × 100 mm), and stoppers fitted with center wells were obtained from Kontes Glass Co., New Jersey, USA. All other chemicals used were of analytical grade.

Granulosa cell expression

The ovaries were trimmed of extra ovarian tissue and placed in cold PBS. Granulosa cells were isolated in PBS by applying gentle pressure to the ovaries with a blunt spatula. The rest of the ovary was designated residual tissue (Zeleznik et al., 1974). This tissue contained theca, interstitial cells and non-expressed granulosa cells. The expressed granulosa cells and the residual tissue were washed twice with PBS to remove follicular and interstitial fluid, respectively.

Preparation and assay of ODC

ODC activity was extracted and assayed according to Kaye et al. (1973). Briefly, the ovaries, granulosa cells or residual tissue were homogenized in a Potter-Elvejhem homogenizer in isotonic sucrose (0.25 M), containing 25 mM Tris-HCl (pH 7.4), 5 mM DTT, 0.1 mM EDTA and 1 μM PLP. The homogenate was centrifuged for 30 min at 30,000 g in a Sorvall refrigerated centrifuge. The supernatant was used as an enzyme source. The standard enzyme assay contained 50 mM Tris-HCl (pH 7.4), 0.1 mM PLP, 5 mM DTT, 0.5 mM DL-ornithine containing 0.2 μCi of label and 0.1 ml of enzyme (50–500 μg protein) in a total volume of 500 μl. The enzyme was assayed at 37°C. The reaction was terminated by the injection of 1 ml of 10% (w/v) TCA. The reaction was linear upto 2 h between a protein concentration of 10–2,000 μg. Protein was estimated by the method of Lowry et al. (1951). The content of the gonadotropin receptors in the various tissues was estimated according
to published procedure (Richards et al., 1976). Concentrations of cAMP in the granulosa cells and the residual tissue were measured by radioimmunoassay (Richards et al., 1979).

Results

Time course of changes in ODC activity

Changes in the activity of ODC in granulosa cells and residual tissue in response to a single subcutaneous injection of oLH in intact immature rats are depicted in figure 1. The enzyme activity increased rapidly in both the granulosa cell and residual tissue compartment in response to 30 µg oLH. A maximum increase occurred at 4 h following hormone administration and then declined rapidly. On the basis of this and the results of replicate experiments, most subsequent studies were terminated at 4h. When, 2 µg of FSH was administered subcutaneously and enzyme activity measured in granulosa and residual tissues, no increase in enzyme activity was observed.

Figure 1. Time course of ovarian ODC stimulation in response to oLH and hFSH. 24 day old intact immature rats were administered either a single injection of oLH (30 µg, subcutaneously) or hFSH (2 µg, subcutaneously). The enzyme activity in the residual tissue and dispersed granulosa cells were estimated as detailed in the text. (●), ODC activity in the residual tissue; (O), granulosa in response to oLH; (▲), residual; (△), granulosa cell in response to hFSH. Values are mean of two closely agreeing values.

Effects of FSH, hCG, estradiol and FSH + estradiol ODC activity

Figure 2A depicts changes in ODC activity in whole ovaries of intact immature rats treated with various hormones. A single subcutaneous injection of hFSH (2 µg),
failed to stimulate ODC activity, while a single injection of 17-β-estradiol (500 µg) decreased the basal activity. A combination of both these hormones also failed to stimulate the enzyme activity. However, intravenous administration of oFSH or hFSH to intact immature rats caused a dramatic increase in ovarian ODC activity (figure 2B). A 15-fold increase in activity was observed when 6 µg of oFSH was administered and a further 5-fold increase in this (30 µg) dose did not result in any further increase in the enzyme activity. When 10IU of hCG was given intravenously, enzyme activity increased to 3-fold higher than that obtained with a maximally effective dose of hFSH (6 µg).

**Time course of ODC activity**

The changes in response to intravenous hFSH with time are depicted in figure 3. Intravenous injection of 5 µg hFSH stimulated ODC in a time dependent manner. A significant increase over the saline treated control occurred as early as 1 h following the hormonal injection. Maximal stimulation occurred 4 h following hormone treatment. By 6 h the activity started to decline and enzyme activity could not be distinguished from saline treated controls by 12 h.

**Dose response relationships**

The effects of increasing dosage of hFSH on ODC activity measured 4 h after intravenous administration are shown in figure 4. As little as 1 µg of hFSH increased enzyme activity significantly. The ODC activity increased with hormone dosage. However, it caused no further increase in enzyme activity.
Ovarian ornithine decarboxylase

Figure 3. Time course of ovarian ODC stimulation by hFSH. Details are as described in the text.

Figure 4. Dose dependent increase in ODC in response to hFSH. Animals were sacrificed 4h following the hormone administration. Values are expressed as mean ± SEM (n = 4).

Compartmentalization of gonadotropin stimulated ovarian ODC and cAMP

Figure 5 summarizes the results of measuring hCG/hFSH stimulated ODC activity along with the cAMP content in separated granulosa cells and residual tissue compartments. Since granulosa cells obtained from individual animals were not sufficient to measure ODC activity, the enzyme activity was measured in granulosa cells
Figure 5. Gonadotropin stimulated ODC and cAMP in 24 day intact immature rats. ODC activity (□), and cAMP concentrations (■) were measured 4 and 1 h following hormonal administration (peak period) respectively. ODC activity was measured as detailed in the text. Values are mean of duplicates. cAMP values are mean ± SEM (n = 4).

Pooled from 8 ovaries. The typical results are depicted in the figure 5. In untreated or saline treated control animals, the specific activity of ODC in granulosa cells was similar to that in residual tissue. The administration of 5 µg of hFSH caused a 6-fold stimulation in ODC activity in both the granulosa cells and residual tissue at 4 h. Under the same conditions, 10 IU of hCG increased the activity of the enzyme 17-fold in granulosa cells and 25-fold in the residual compartment. Concentrations of cAMP were measured at the time of maximum stimulation, 60 min following the hormone treatment. Following 5 µg of hFSH (iv) administration cAMP increased 14-fold over the control in the granulosa cells, but only 3-fold in the residual compartment. Administration of hCG (10 IU) also increased the concentration of cAMP 7-fold in the granulosa cells and 4-fold in the residual tissue.

Effects of db-cAMP and theophylline on ODC activity

Table 1 summarizes the stimulation of ovarian ODC activity obtained with combined db-cAMP and theophylline, a phosphodiesterase inhibitor. Theophylline alone had no effect on basal enzyme activity. Simultaneous injection of db-cAMP and theophylline (1 mg) stimulated ODC. However, the increase was only 3-fold over the control levels. Higher doses of db-cAMP and 1-methyl-3-isobutyl-xanthine, another phosphodiesterase inhibitor or theophylline were toxic to the animals.

Table 1. Stimulation of ovarian ODC in 24 day old intact rats by theophylline, dibulryl cAMP and hCG.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity (nmol of 14CO2 liberated/mg protein/h)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>2±0.2</td>
</tr>
<tr>
<td>hCG (10 IU, iv)</td>
<td>99±8.5</td>
</tr>
<tr>
<td>db-cAMP (5 mg) + theophylline (1 mg)</td>
<td>6.2±2.3</td>
</tr>
<tr>
<td>Theophylline (1 mg)</td>
<td>22±0.6</td>
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Ovarian ODC activity was measured 4h following intravenous injection. Results are expressed as mean ± SEM (n = 4).
Ovarian ornithine decarboxylase

Compartmentalization of gonadotropin receptors

Table 2 depicts the specific binding of $^{125}$I-hCG or $^{125}$I-FSH to the granulosa cells or residual tissue used for ODC assay. Granulosa cells contained most of the FSH receptor, while residual tissue contained less than 10% of the total FSH receptor. Conversely, residual tissue contained 92% of the hCG receptor, while granulosa cells contained only 8% of the total hCG receptor activity. This distribution of gonadotropin receptor activity agrees well with previous reports (Richards, 1979).

### Table 2. Distribution of gonadotropin receptor in the ovary of intact immature rats.

<table>
<thead>
<tr>
<th></th>
<th>cpm/µg DNA</th>
<th>Ratio</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Granulosa</td>
<td>Residual</td>
<td>R/G</td>
</tr>
<tr>
<td>$^{125}$I-FSH</td>
<td>652±77</td>
<td>62.5±7.5</td>
<td>0.0958</td>
</tr>
<tr>
<td>$^{125}$I-hCG</td>
<td>65±12</td>
<td>80.25±25</td>
<td>12.29</td>
</tr>
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</table>

For the measurement of binding, $^{125}$I-labelled hCG and $^{125}$I-labelled hFSH, were used under saturating conditions in the presence and absence of excess unlabelled hormone. The difference represents specific binding. Values are mean ± SEM (n=4).

Discussion

Published results indicate that FSH is not an inducer of ODC in the mammalian ovary in vivo (Osterman and Hammond, 1977; Sheela Rani and Moudgal, 1979; Veldhuis et al., 1981; White and Ojeda, 1981). In accord with this we found that a single subcutaneous injection of purified FSH, that had been treated with chymotrypsin to remove LH contamination, failed to stimulate ovarian ODC when measured 1, 2, 4 or 6 h following the hormone administration (figures 1, 2A). This precludes a shift in the time course of enzyme stimulation. The dose of hFSH used was at least twice the amount needed to induce and promote near maximal follicular development and to increase the incorporation of tritiated thymidine into DNA (Rao et al., 1978). A combination of estradiol and FSH also failed to increase ODC. However, a single intravenous administration of FSH increased the ovarian ODC activity in a time and dose dependent manner. The ODC response followed a strict time course and peaked at 4 h, similar to the results observed with other systems (Kaye et al., 1973). The response showed dose dependency over the range of 1–10 µg. Thus, the inability of other investigators to demonstrate that FSH can stimulate ovarian ODC in vivo, may be due to the use of the subcutaneous route with an insufficient dose. Although plasma concentrations of FSH after injection by these two routes were not compared in our study, it is certain that higher concentrations would have been reached following intravenous administration at least shortly after injection. This is the most likely explanation of the results, and suggests that FSH can act acutely to stimulate ODC following brief, but sufficiently high elevations of plasma concentrations.

Although granulosa cells in antral preovulatory follicles of the rat ovary contain
large numbers of LH/hCG receptor sites and LH responsive adenylate cyclase, granulosa cells in preantral follicles contain very few hCG receptor sites and little or no LH responsive adenylate cyclase (Richards et al., 1979). It was surprising to find that administration of LH to immature rats with preantral follicles led to marked increase in ODC activity in isolated granulosa cells (figure 1). To understand these results more fully, we attempted to determine which of the following alternative explanations could be responsible for the increased activity: (i) stimulation by a contaminant, most likely FSH, (ii) contamination of granulosa cells by co-expressed LH-responsive cells, (iii) indirect stimulation by a product derived from LH-responsive cells and (iv) unusual responsivity of granulosa cell ODC activity to LH stimulation.

The first possibility, that the stimulation of ODC in granulosa cells from preantral follicles was due to a contaminant, most likely FSH, seems unlikely. The oLH used in these studies is a highly purified preparation (LER 1733, 1:64 × NIH-LH-Sl. FSH contamination 0:04 × NIH-FSH-Sl). Thus, the amount of contaminating FSH injected with the oLH was far less than that found necessary for inducing ODC activity. Further, hCG also stimulated ODC activity in granulosa cells from preantral follicles and this hormone is relatively free of FSH activity.

The second possibility that expressed granulosa cells are contaminated by co-expressed LH-responsive cells, can almost be discarded on the basis of the binding studies. Thus, the specific activity of LH/hCG receptor sites in the expressed granulosa cell preparation considered on a per cell basis (CPM/µg DNA) was only 8% of the activity in the residual tissue. For this explanation to be valid, the contaminating LH/hCG receptor positive cells should have an ODC inducible system that is about 7 times more responsive to hCG than the ODC responsive system present in the rest of the residual cells (figure 5).

Data are not at hand to rule out either of the last two possibilities. LH/hCG receptor activity present in the granulosa cell preparation may be associated with a sub-population of cells that are unusually responsive to receptor site occupation by hCG. The possibility that the granulosa cells are actually being stimulated indirectly by an extra-cellular factor derived from hCG responsive cells, also can not be ruled out. Prostaglandins, catecholamines, polyamines, cyclic nucleotides and other peptide factors in follicular fluid are all candidates for such a mediator. The lack of response to very large amounts of estradiol does make this steroid an unlikely candidate.

The apparent lack of correlation amongst gonadotropin receptor distribution, ODC and cAMP activities in granulosa and residual tissue, respectively, may suggests differences in the coupling efficiency of hormone receptor and adenylase cyclase activation in these two compartments.

The submaximal doses of hCG and oLH used in this study were more effective in stimulating ODC activity than all the doses of FSH tested including those that which were maximally effective. This was true for the whole ovary, the granulosa cells and the residual tissue. In spite of this, FSH was more effective than hCG in stimulating granulosa cell accumulation of cAMP (figure 5). These relative effects on cAMP and ODC are extremely difficult to reconcile with the postulates that cAMP might act as the exclusive second messenger for gonadotropin stimulation of ODC (Johnson and Sashida, 1977; Osterman et al., 1978). Indeed, the results support the suggestion that gonadotropin stimulation of ODC is a cAMP independent response (figure 5). Thus,
the mechanism utilized by gonadotropins in stimulating ODC and the factors involved remains to be identified.

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References