Binding of LH and FSH to porcine granulosa cells during follicular maturation

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Summary. The uptake of ^{125}I -labelled LH by equal numbers of granulosa cells from small, medium or large follicles was greater by cells from large follicles. In contrast, granulosa cells obtained from small follicles bound much more ^{125}I -labelled FSH per cell than did cells obtained from medium and large follicles. Competition studies with unlabelled hormones indicated that porcine granulosa cells have specific receptors for LH and FSH. The addition of diethylstilboestrol enhanced the binding of ^{125}I -labelled LH and inhibited the binding of ^{125}I -labelled FSH to granulosa cells harvested from small and medium-sized follicles, but had no effect on those from large follicles.

Introduction

Previous studies have suggested that specific receptors with high affinity for human LH and HCG are present on granulosa cells harvested from pig follicles (Kammerman, Canfield, Kolena & Channing, 1972; Channing & Kammerman, 1973a). However, there is little information about the binding of FSH to granulosa cells: specific binding of iodinated FSH to rat granulosa cells has been demonstrated by autoradiographic techniques (Midgley, 1973; Presl, Pospisil, Figarova & Krabec, 1974) and the numbers of specific FSH receptors on rat granulosa cells have been measured (Nimrod, Erickson & Ryan, 1976).

The present report is of the specific binding of LH and FSH to porcine granulosa cells during follicular maturation.

Materials and Methods

Highly purified human LH (Lot No. 235014) and human FSH (Lot No. 235015) were purchased from Calbiochem, Inc., California.

Iodination of LH and FSH was carried out by the lactoperoxidase method described by Miyachi, Vaitukaitis, Nieschlag & Lipsett (1972), which employed 2 μg FSH, 1 mCi-equivalent of $\text{Na } ^{125}\text{I}$ and 10 ng lactoperoxidase in 60 μl 0.4 M-sodium acetate, pH 5.6. After incubation at room temperature for 30 min, three separate portions of 10 ng H_2O_2 (in 5 μl) were added at 10-min intervals. The reaction was stopped by adding 0.5 ml 0.05 M-dithiothreitol. The labelled hormone was separated from excess $\text{Na } ^{125}\text{I}$ by gel filtration on a Bio-Gel P-60 column. The specific activity of the LH and FSH was consistently in the order of 40–60 $\mu\text{Ci}/\mu\text{g}$. The labelled hormone was stored at 4°C in a 0.14 M-NaCl–0.01 M-sodium phosphate buffer, pH 7.4, containing 0.5% bovine serum albumin and 5 μg sodium ethylmercurithiosalicylate/ml. No change in binding activity was found after 4 weeks of storage.

Granulosa cells were collected as described by Channing (1970) by aspiration from small (1–2 mm), medium (3–5 mm) and large (6–10 mm) follicles from 6–12-month-old pigs. The granulosa cells were centrifuged to separate them from follicular fluid, washed and resuspended in Medium 199. Samples of the cell suspension were taken for cell counting, after which the volume was adjusted with Medium 199 to give a final concentration of 5×10^6 cells/ml.

The granulosa cell suspensions (500 μl) were pipetted into a 10 $\times$ 75 mm test tube containing 50 μl ^{125}I -labelled LH or ^{125}I -labelled FSH solution (1×10^6 ct/min), in Medium 199. The tubes were incubated for 30, 60 and 120 min at 37°C in a shaking incubator and then centrifuged at 2500 *g* for 10 min at 4°C. The supernatant was removed by suction and the cells resuspended and washed three

times with 1 ml Medium 199. The tubes containing the pellets of washed granulosa cells were then counted in an automatic gamma counter. All radioactivities were corrected for minor non-specific binding of iodinated hormones to glass tubes and the incubations were performed in triplicate.

To investigate competition with unlabelled hormone, various doses of LH (Calbiochem LH or LER 960) or FSH (Calbiochem FSH or LER 1366) were added and the effects of unlabelled LH and FSH on the binding of the labelled hormones to granulosa cells from medium-sized follicles were plotted on a logarithm-logit paper.

To detect the effect of oestrogen on the binding of LH and FSH, 10–100 µg diethylstilboestrol (Nutritional Biochemicals, Corp., Cleveland, Ohio) were added to the tubes containing 500 µl granulosa cell suspension. The tubes were incubated for 120 min at 37°C in a shaking incubator. After centrifugation the supernatant was removed by suction. The cells were resuspended in Medium 199 and the binding of ^{125}I -labelled LH and FSH was tested as described.

Results

The appearances of haematoxylin and eosin stained smears of the granulosa cells obtained from small, medium and large follicles did not differ significantly. The mean ($\pm$ S.E.M.) cell diameters of the individual granulosa cells harvested from the three sizes of follicles were also similar (small, 13.6 ± 0.6 µm; medium, 14.1 ± 0.8 µm; large, 13.9 ± 0.6 µm).

Incubation of granulosa cells from the three types of follicles resulted in a significant amount of labelled hormone being bound. The binding increased gradually from 30 to 120 min. The granulosa cells collected from large follicles bound much more ^{125}I -labelled LH per cell than did cells collected from small or medium-sized follicles ($P < 0.01$) (Table 1). In contrast, the granulosa cells from small follicles bound much more ^{125}I -labelled FSH per cell than did cells obtained from medium or large-sized follicles ($P < 0.01$; Table 1). These differences in hormone binding cannot be attributed to differences in cell size, because the diameters of the granulosa cells were similar, whatever their source.

Table 1. The binding of ^{125}I -labelled LH and ^{125}I -labelled FSH to porcine granulosa cells harvested from follicles of different sizes

	Time of incubation (min)					
	LH binding			FSH binding		
	30	60	120	30	60	120
Small	7533 $\pm$ 521	9122 $\pm$ 632	9522 $\pm$ 531	1428 $\pm$ 258	20833 $\pm$ 461	28008 $\pm$ 642
Medium	15521 $\pm$ 732	18103 $\pm$ 1212	30122 $\pm$ 822	5677 $\pm$ 270	8211 $\pm$ 573	10541 $\pm$ 362
Large	55012 $\pm$ 821	65502 $\pm$ 1252	72306 $\pm$ 1921	5103 $\pm$ 167	6512 $\pm$ 296	9147 $\pm$ 249

Values are mean $\pm$ S.E.M. (ct/min/ 5×10^6 cells).

Unlabelled LH competed with ^{125}I -labelled LH for binding sites of granulosa cells obtained from medium-sized follicles in a graded manner, whereas unlabelled FSH did not (Text-fig. 1a). Unlabelled FSH also inhibited the binding of ^{125}I -labelled FSH to granulosa cells in a graded manner, but unlabelled LH did not (Text-fig. 1b).

Table 2. The effect of diethylstilboestrol on the binding of ^{125}I -labelled LH and FSH to porcine granulosa cells from medium-sized follicles

	Dose of diethylstilboestrol (µg)				
	0	10	20	50	100
LH binding	4012 $\pm$ 186	4185 $\pm$ 116	4514 $\pm$ 523	7721 $\pm$ 162	7572 $\pm$ 193
FSH binding	9075 $\pm$ 262	7682 $\pm$ 103	3425 $\pm$ 292	1382 $\pm$ 76	1508 $\pm$ 173

Values are mean $\pm$ S.E.M. (ct/min/ 5×10^6 cells).

Table 3. Effect of 50 µg diethylstilboestrol on the binding of ^{125}I -labelled LH and FSH to porcine granulosa cells from follicles of different sizes

Follicles	LH binding	FSH binding
Small		
Control	1482 ± 121	9692 ± 278
Experimental	5196 ± 276	4442 ± 176
Medium		
Control	4463 ± 305	8476 ± 682
Experimental	7697 ± 174	4822 ± 186
Large		
Control	6582 ± 282	5982 ± 286
Experimental	7012 ± 191	6142 ± 212

Values are mean ± S.E.M. (ct/min/5 × 10⁶ cells).

The effects of adding diethylstilboestrol to the incubation of granulosa cells from medium-sized pig follicles with labelled hormones are shown in Table 2. The two high doses caused a significant increase in LH binding ($P < 0.01$). When 50 µg diethylstilboestrol were added to incubations of granulosa cells (Table 3), the binding of labelled LH was increased with cells from small and medium-sized follicles but was not affected with cells from large follicles ($P < 0.01$). FSH binding was decreased with cells from small and medium-sized follicles ($P < 0.01$).

Text-fig. 1. The effects of LH and FSH on the binding of (a) ^{125}I -labelled LH and (b) ^{125}I -labelled FSH to porcine granulosa cells from medium-sized follicles.

Discussion

Specific receptors with high affinity for human LH and HCG have been demonstrated in a variety of testis and ovary preparations (Lee & Ryan, 1973; Dufau, Charreau & Catt, 1973; Hanour & Saxena, 1974). Granulosa cells harvested from porcine follicles have been particularly useful in studying

gonadotrophin-receptor interactions (Kammerman *et al.*, 1972; Channing & Kammerman, 1973a) because they represent a uniform population of intact cells of a single type, obtainable without excessive trauma from a target organ which contains many types of cells. These granulosa cells have been shown to bind specifically radioiodinated HCG and probably to have the same receptor for both HCG and LH (Kammerman *et al.*, 1972; Channing & Kammerman, 1973b).

The present finding that granulosa cells harvested from large follicles bound much more ^{125}I -labelled LH per cell than did cells harvested from small or medium-sized follicles, although the cells themselves are of the same size, is consistent with previous reports (Channing & Kammerman, 1973a). This increased gonadotrophin binding may explain why LH is capable of stimulating cyclic AMP production and progesterone only in the mature follicle cell (Channing & Kammerman, 1973a). The increased binding represents an increase in the number of binding sites on granulosa cells during follicle maturation (Kammerman & Ross, 1975).

Our study has shown that porcine granulosa cells also have binding sites for FSH, and cells collected from small follicles bound much more ^{125}I -labelled FSH per cell than did cells from medium or large follicles. The competition studies with unlabelled hormones revealed that porcine granulosa cells have specific receptors for LH and FSH, and the binding characteristics of labelled FSH and LH to the granulosa cells from the three follicle types are in sharp contrast.

The concentration of oestrogen in follicular fluid is known to increase during follicular maturation (Sanyal, Berger, Thompson, Taymor & Horne, 1974; McNatty, Hunter, McNeilly & Sawers, 1975). Oestrogen has also been reported to stimulate synthesis of progesterone by porcine granulosa cells in tissue culture (Goldenberg, Bridson & Kohler, 1972). Our experiments with diethylstilboestrol showed enhanced binding of labelled LH and suppressed binding of labelled FSH to granulosa cells harvested from small and medium-sized pig follicles. In contrast, Richards (1975) and Richards & Midgley (1976) found that oestrogen stimulated FSH binding, but not LH binding, in rats. This difference might be a real specific difference or due to variation in the experimental systems.

From these studies, it is evident that there are two specific receptors for LH and FSH on porcine granulosa cells. Furthermore, there is a marked difference between the binding of ^{125}I -labelled LH and ^{125}I -labelled FSH to granulosa cells collected from the three sizes of follicle. Although the reason for this marked difference, other than degrees of follicular maturation, is not evident, our results suggest that oestrogen might play an important role in the binding of LH and FSH to granulosa cells.

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