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STEROID HORMONES MODULATE PROLACTIN BINDING BY CULTURED PORCINE GRANULOSA CELLS

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SUMMARY: We have studied the effects of the gonadal steroids - testosterone, 17β-estradiol, progesterone, and 5α-dihydrotestosterone on the prolactin-binding activity of porcine granulosa cells maintained in monolayer culture. Testosterone, estradiol, and progesterone all significantly enhanced prolactin binding (55%, 107%, and 112% above control, respectively). In contrast, the non-aromatizable androgen, 5α-dihydrotestosterone, caused an insignificant suppression of prolactin binding. The anti-androgen, cyproterone acetate, did not influence prolactin binding when used alone, and did not inhibit the effects of testosterone. These data suggest that the stimulatory effects of testosterone may require aromatization to estradiol.

INTRODUCTION

Previous studies from this laboratory have demonstrated specific binding sites ("receptors") for prolactin on freshly isolated porcine granulosa cells and corpora lutea (1,2). This binding activity appears to be under physiological control *in vivo*, since the number of receptors/cell changes substantially during the reproductive cycle and pregnancy (1,2). In the current study, we have examined a potential role for ovarian steroids as regulators of prolactin receptor activity by assessing the effect of these hormones on prolactin binding to porcine granulosa cells maintained in monolayer culture.

MATERIALS AND METHODS

Materials: Ovine prolactin (oPRL) (NIH-S11 and NIH-S12) was provided by the Hormone Distribution Office, NIAMDD. Cyproterone acetate was a gift of Schering AG, Berlin. All other steroids were purchased from Sigma Chemical Corp. Tissue culture medium and fetal calf serum were from Flow Laboratories; bovine serum albumin, fraction V, from Miles Laboratories; gentamicin from the Schering Corporation; amphotericin B and mycostatin from Squibb. Carrier free Na[^{125}I] was purchased from New England Nuclear. Lidocaine was obtained in sterile solution from Astra Pharmaceuticals.

Culture Techniques: Porcine granulosa cells were isolated from small (1-3 mm) porcine follicles as previously described (1). Approximately 10^7 cells were inoculated into 100 mm culture dishes (Falcon) containing Medium 199 with Hanks salts, a bicarbonate buffer system, 10% fetal calf serum, gentamicin (50 µg/ml), amphotericin B (2.5 µg/ml), and mycostatin (50 U/ml).

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The cultures were maintained in monolayer for 8 days at 37 C in a 95% air-5% CO_2 atmosphere. The medium was changed every 48 hr. Steroid hormones were added in absolute ethanol (0.5% v/v); control cultures received an equivalent volume of ethanol.

oPRL Binding Assay: The binding of oPRL to cultured granulosa cells was assessed by the methods previously used with freshly isolated granulosa cells (1) with a few exceptions. Initial studies showed that cells removed from culture plates by a 20-30 min incubation with the local anesthetic lidocaine (3.4 mM) (3), exhibited binding characteristics which were superior to those of cells assayed directly on culture plates or dispersed with trypsin, EDTA, or mechanical scraping. After dispersion with lidocaine, cells from 20-30 control and hormone-treated cultures were pooled, washed twice in medium 199, hepes buffer, pH 7.6, 1% bovine serum albumin, and aliquoted into plastic assay tubes containing the same medium and [^{125}I]iodo-oPRL (50,000 cpm or 0.6 ng) in the presence or absence of saturating concentrations of oPRL (2 µg/ml). Preparation and validation of tracer hormone, incubation conditions and computation of specific binding have been described in detail (1). Four to six measurements of specific binding expressed per mg DNA (4) were made for control and hormone-treated cells in each experiment, and the significance of differences computed using Student's *t* test. The overall significance of a treatment effect was assessed using a chi-squared statistic derived from significance values for a series of individual experiments (5). For comparison, data from different experiments has been normalized as percent control (Table I).

RESULTS

Data from all the experiments are compiled in Table I. The stimulatory effect of testosterone and 17β-estradiol on prolactin binding to cultured granulosa cells were observed in each of several experiments. Although 17β-estradiol appears to be a more potent stimulator of prolactin binding than testosterone overall, two experiments directly comparing the two hormones showed no significant differences (data not shown). Since granulosa cells are capable of aromatizing testosterone (6), the testosterone effect could have been exerted directly or after conversion to estradiol. To address this question, we performed two experiments with the non-aromatizable androgen, 5α-dihydrotestosterone. This steroid failed to show stimulation of binding. Instead, a modest, insignificant, inhibitory effect was suggested. Further, the anti-androgen, cyproterone acetate, failed to alter prolactin binding activity when used alone or in combination with testosterone. Collectively, these experiments suggest that testosterone effects in this system are exerted indirectly, after aromatization to estradiol.

Progesterone also exerts a stimulatory effect on prolactin binding which is equivalent quantitatively to that encountered with testosterone or estradiol.

TABLE I. Effect of Steroid Treatment on Prolactin Binding by Cultured Granulosa Cells

	Specific PRL binding (% control ± SEM)	No. of Experiments	*p (treatment vs. control)
Testosterone (500 ng/ml)	155 ± 42	10	<.01
Estradiol (500 ng/ml)	207 ± 87	3	<.01
Dihydrotestosterone (500 ng/ml)	61 ± 27	2	>.5
Cyproterone acetate (5 µg/ml)	103 ± 64	4	>.9
Progesterone (500 ng/ml)	212 ± 73	6	<.01
Testosterone vs. Cyproterone acetate + Testosterone	108 ± 73	4	>.9

*see Materials and Methods

DISCUSSION

Previous studies from this laboratory have shown that the prolactin receptor concentration of freshly isolated porcine granulosa cells and luteal tissue varies with the physiological state of the ovary. The number of specific prolactin binding sites/cell is high in cells from immature follicles, declines with follicular maturation and increases again in functioning corpora lutea (1,2). Pregnancy occasions a further increase in the prolactin-receptor concentration of luteal tissue (2). These observations suggested hormonal control of prolactin receptors, and ovarian steroids seemed logical candidates. We have found high concentrations of 17β-estradiol, testosterone, dihydrotestosterone, and progesterone in follicular fluid, the physiological milieu of granulosa cells (7). Receptors for estrogens (8), androgens (9), and progestins (10) have been described in granulosa or luteal cells, and a number of putative regulatory functions have been assigned to each (11,12). In particular, estradiol, along with FSH, has been implicated in the control of prolactin receptors in rat granulosa cells *in vivo* (13). However, to our knowledge, the current studies constitute the first *in vitro* demonstration of the modulation of ovarian prolactin receptors by steroid hormones.

Such direct in vitro observations avoid complex hormonal interactions which regulate prolactin binding in vivo. For example, the estrogen induction of hepatic lactogen receptors in vivo involves stimulation of the secretion of prolactin which induces its own receptor (14). Even with cultured cells, however, it is pertinent to inquire whether the steroids administered alter binding themselves or via active metabolites. As discussed in the Results section, the available evidence suggests that testosterone increases prolactin binding after conversion to estradiol. Similar pathways have been invoked to explain some of the actions of exogenous testosterone on the rat ovary in vivo (15). In contrast, the effects of progesterone are unlikely to be accounted for by conversion to estrogens. Granulosa cells are deficient in 17α-hydroxylase (16), and require androgens derived from thecal elements to synthesize estrogens (17). Consequently, progesterone probably exerts a direct effect on prolactin binding by the granulosa cell.

Using monolayer cultures similar to those employed here to study prolactin binding activity, we have noted that estradiol and prolactin interact synergistically to stimulate progesterone secretion by porcine granulosa cells (Veldhuis and Hammond, unpublished). These observations suggest that the changes in receptor concentrations described here may have physiological relevance.

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