MODULATION OF PROGESTIN SECRETION IN OVARIAN CELLS BY 17β-HYDROXY-5α-ANDROSTAN-3-ONE (DIHYDROTESTOSTERONE): A DIRECT DEMONSTRATION IN MONOLAYER CULTURE

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SUMMARY: These studies with a monolayer system of porcine granulosa cells provide a direct demonstration of the ability of androgen to stimulate progestin secretion by ovarian cells. A preferential action of the more potent androgens, dihydrotestosterone and testosterone, was shown but only dihydrotestosterone demonstrated the capacity to stimulate progestin secretion throughout the culture period. Estradiol 17-β markedly depressed progestin synthesis. The results suggest a modulatory role for androgens in the development of full steroidogenic potential by ovarian granulosa cells during follicular development.

INTRODUCTION

The role of androgens in ovarian follicle development has been variously considered to be either mitogenic or anti-mitogenic in character. For example, testosterone[3] (T) administration to intact young female rats enhances follicle size (1), and genetic studies of mice carrying the X-linked testicular feminization mutation suggest a physiological role for androgen in the preservation of primordial follicles (2). On the other hand, the administration of testosterone, or of gonadotropin capable of increasing the local concentration of androgen in the ovary, results in a marked reduction in ovarian

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3. Nomenclature and abbreviations utilized: 17β-hydroxy-5α-Androstan-3-one = Dihydrotestosterone (DHT); 17β-hydroxy-4-Androstene-3-one = Testosterone (T); 4-Pregnene-3, 20-dione = Progesterone (P); 1,3,5(10)-estratriene-3, 17β-diol = Estradiol 17β ($E_2\beta$); 3β-hydroxy-5-Androstene-17-one = dehydroepiandrosterone (DHEA).

weight and degenerative changes in the ovarian follicles of estrogen-treated, hypophysectomized immature rats (3,4,5).

Specific biochemical modes of androgen action in follicle development have not been described. The present study was designed to investigate the influence of dihydrotestosterone (DHT) upon the steroidogenic capability of one follicular cell type, the isolated granulosa cell.

MATERIALS AND METHODS

Porcine ovaries were collected at a local slaughterhouse as previously described (6). Moderately differentiated granulosa cells were used exclusively in this study and are defined as those harvested from 3-5 mm ovarian follicles present in the late luteal phase of the estrous cycle. The cells were collected by aspiration (7) and centrifuged from the follicular fluid at 26 x g. The cell pellet was resuspended in culture medium (90% Hams F_{12} nutrient medium: 10% fetal calf serum containing 100 U/ml penicillin, 100 µg/ml streptomycin and 2.5 µg/ml amphotericin B) and aliquots containing 5-10 x 10^6 cells were added to 35 mm petri dishes (Falcon Plastics). Cultures were maintained at 37° C in a humidified atmosphere of 5% CO_2 in air for 4 days in 2 ml of control or experimental medium which was changed every 48 hours.

Steroids were obtained commercially and added to the cultures in 10 µl of ethanol. Progestin secretion was determined by radioimmunoassay (8) using [^{3}H]-progesterone as the tracer; 10 µl of medium diluted 1:1 with saline were assayed in duplicate without extraction (9). The antiserum used (#S-49) was obtained from Dr. G. E. Abraham, Torrance, California. The cross-reactivities of DHT and T in this sytem (relative to mass of progesterone required for 50% inhibition of radio-ligand binding) were 0.0003 and 0.0001 respectively, which produced some displacement in the progestin assay when microgram quantities of these androgens were tested in culture. All determinations were corrected for this contribution. Total progestin was quantitated in lieu of specifically isolating progesterone prior to assay since the vast majority of the progestin

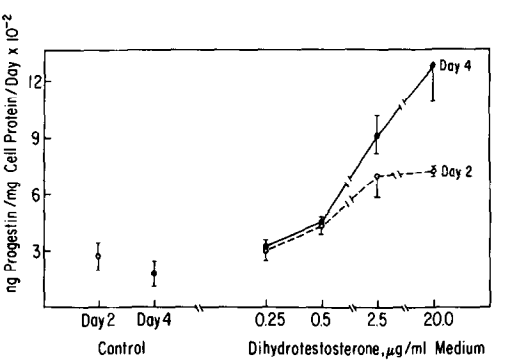

Figure 1. Dose-response relationship between dihydrotestosterone and progestin secretion in porcine granulosa cell monolayers. Open and solid circles represent progestin secretion during 2 and 4 days of culture, respectively. Brackets and hemi-brackets represent ± SEM. Each point represents the mean of 4 cultures.

secreted by isolated porcine granulosa cells is progesterone (6,12). Secretion was normalized on the basis of cell protein per culture (10). Student's t test was used in the data analysis (11).

RESULTS

On the basis of the dose-response curve shown in Figure 1, a DHT concentration of 2.5 μg/ml was used in subsequent studies. At this level DHT significantly stimulated ($p \leq 0.05$) progestin secretion at 2 and/or 4 days of culture in 8 of 9 experiments.

The specificity of the effect is illustrated in Figure 2. Of the androgens tested, only DHT significantly stimulated progestin secretion at both 2 and 4 days of culture; T exhibited a stimulatory effect only at 4 days. Dehydroepiandrosterone (DHEA) significantly decreased progestin secretion during the initial 48 hr and although in the experiment represented (Fig 2) it was stimulatory during the next 48 hr, this was not a consistent finding. Estradiol-17β ($E_2\beta$) significantly diminished progestin secretion during both culture periods. Further experiments (data not shown) corroborated these results. Dose-response studies with $E_2\beta$ and DHEA confirmed their inhibitory effects and T at 2.5 μg/ml significantly stimulated progestin secretion at 4 days of culture in 2 of 3 additional studies. The interpretation of the effects of T and DHEA is difficult, however, since these androgens are aromatized to estrogen by porcine granulosa cells (12, and unpublished observations).

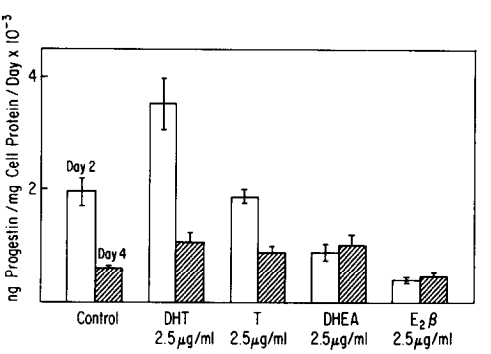

Figure 2. Effect of dihydrotestosterone (DHT), testosterone (T), dehydroepiandrosterone (DHEA), and estradiol 17β ($E_2\beta$) upon progestin secretion by porcine granulosa cell monolayers at 2 (open bars) and 4 (hatched bars) days of culture. Brackets and hemi-brackets represent ± SEM. Each bar represents the mean of 4 cultures. See text for details.

A comparison of the control groups in Figs 1 and 2 illustrates two different types of secretory patterns noted, i.e., maintenance (Fig 1) vs. a significant loss (Fig 2) of steroidogenic capability with time in culture. These patterns were not qualitatively changed by DHT in the experiments represented although that capability has been noted on several occasions (unpublished observations). The cause(s) of these different secretory patterns may be related to variation in the degree of steroidogenic maturation achieved by the cells *in vivo*, even though their selection for study *in vitro* was based upon a rather restrictive anatomical criterion, i.e., 3-5 mm follicles.

DHT did not alter cellular growth rate *in vitro* based upon average total protein per culture after 4 days: controls and DHT-treated cultures averaged 73 ± 5 μg (S.E.M.) and 76 ± 5 μg (S.E.M.), respectively (9 experiments, pooled data; n = 41 for both comparisons). No consistently observable morphological differences between the two groups were noted by light-microscopic examination of living cultures.

DISCUSSION

These studies establish that isolated granulosa cells grown in culture are responsive to androgens. The ability of DHT to stimulate progestin secretion in this system suggests the possibility that androgens may be regulators of steroidogenic capability during follicle development. A physiological role for androgen action in these cells is futher supported by the recent demonstration of a high affinity androgen binding protein in rat granulosa cell cytosol (13).

Since only moderately differentiated cells were utilized in this study, it is not yet apparent whether cells of all developmental stages will exhibit DHT-stimulated progestin secretion. Estrogens, for example, do not act uniformly during granulosa cell maturation since, in contrast to the results obtained here with moderately differentiated cells, Goldenberg *et al* found that $E_2\beta$ and diethylstilbestrol stimulated progestin synthesis by more mature porcine granulosa cells (9).

The fact that DHT and $E_2\beta$ exhibited opposite effects upon progestin secretion in this system suggests that physiologically important interactive processes may remain to be elucidated which are presumably operative in vivo. Both estrogen and androgen are present in very high concentrations in follicular fluid (14). These interrelationships as well as the cellular mechanism(s) by which DHT modulates progestin secretion in ovarian cells await further study.

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