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DIFFERENTIAL ACTIONS OF FSH AND ANDROGENS ON PROGESTERONE CATABOLISM BY RAT GRANULOSA CELLS[1]

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SUMMARY: Rat granulosa cells were preincubated with follicle-stimulating hormone (FSH, 1 μg/ml) and/or androgens (testosterone and 5α-dihydrotestosterone, 0.5 μM) and subsequent [4-^{14}C]progesterone metabolism by these cells was studied. Granulosa cells metabolized radiolabeled progesterone to three major products 20α-hydroxy-4-pregnen-3-one, 3α-hydroxy-5α-pregnan-20-one, and 5α-pregnane-3α,20α-diol. Androgens, but not FSH, decreased overall progesterone utilization. Both FSH and androgens decreased conversion of progesterone to 20α-hydroxy-4-pregnen-3-one. The accumulation of 5α-pregnane-3α,20α-diol was increased by FSH, decreased by testosterone, and not altered by 5α-dihydrotestosterone. The accumulation of 3α-hydroxy-5α-pregnan-20-one was significantly increased by both FSH and androgens. Present results indicate that FSH as well as androgens modulate progesterone metabolism by rat granulosa cells. FSH appears to increase 5α-reductase activity and/or decrease 20α-hydroxysteroid dehydrogenase activity while androgens decrease only 20α-hydroxysteroid dehydrogenase activity.

Follicle-stimulating hormone (FSH) and androgens were shown to increase progesterone accumulation by ovarian granulosa cells in rat (1,2,3,4); pig (5); and human (6). Both FSH and testosterone were reported to enhance the activity of mitochondrial cholesterol side-chain cleavage enzymes (7) and thus to stimulate biosynthesis of progesterone. Synergism between FSH and androgens (1,2,6) suggests that at least some of the actions of these hormones are independent and possibly are exerted on different steps of progesterone production and/or catabolism. FSH, but not androgens, was found to stimulate 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase activity and to stimulate conversion of pregnenolone to progesterone (8,9).

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However, little is known about the modulation of progesterone catabolism by either FSH or androgens. It has been reported that androgens do not compete with progesterone for 5α-reductase activity (10); nonetheless, the involvement of androgens in other steps of progesterone catabolism cannot be ruled out. A recent study suggests that androgens decrease progesterone catabolism by inhibition of 20α-hydroxysteroid dehydrogenase (20α-HSD) activity with no effect on 5α-reductase activity (11). The present study was designed to determine whether FSH alters progesterone catabolism and, if so, to compare FSH effects with androgenic effects.

MATERIALS AND METHODS

Ovarian granulosa cells were obtained from estrogen-treated Sprague-Dawley rats as described previously (12). All incubations were carried out in Eagle's Minimum Essential Medium (MEM) containing non-essential amino acids (0.1 mM), L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (250 ng/ml) - all obtained from GIBCO. Non-labeled steroids were obtained from Sigma Chemical Co., St. Louis, MO. [4-^{14}C]Progesterone was obtained from Amersham Co. (56 mCi/mmol). Granulosa cells (2.5×10^5 cells/ml) were incubated in 12x75 mm polystyrene culture tubes (Falcon) at 37°C under an atmosphere of 5% CO_2 in air. During the initial 24 h incubation period, granulosa cells were cultured with or without 0.5 μM testosterone (T), 0.5 μM 5α-dihydrotestosterone (DHT) and/or 1 μg/ml ovine follicle-stimulating hormone (NIAMDD-oFSH-15). Following this incubation, the cells were washed in fresh medium and incubated for another 3 h in the presence of 0.5 μM [4-^{14}C] progesterone. The incubation media were harvested and the protein content of the cells was determined by the methods of Lowry (13). The media were extracted twice with five volumes of diethyl ether; extracts were evaporated in a water bath at 35°C under nitrogen, reconstituted in absoute ethanol and supplemented with the following steroids serving as internal standards: 0.5 μg of progesterone; 20α-hydroxy-4-pregnen-3-one, 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnan-3-one, 3α-hydroxy-5α-pregnan-20-one, and 5α-pregnane-3α,20α-diol. These extracts were separated by thin layer chromatography on silica-coated plates (MERCK) in chloroform:acetone (4:1, v:v) system. The developed chromatograms were sprayed with sulfuric acid: ethanol (1:1, v:v) and internal standards visualized by charring for 15 min at 120°C.

The preliminary identification of the radioactive metabolites was achieved by aligning and transilluminating the chromatograms with their corresponding radioautograms. Final identification by recrystallization to constant specific activity was carried out on non-charred metabolites eluted from the part of the chromatograms. Radioactive zones were cut out and radioassayed in a LKB 1207 Rackbeta liquid scintillation counter.

The experimental data were evaluated statistically by analysis of variance followed by Duncan's New Multiple Range Test (14).

RESULTS

The overall utilization of [4-^{14}C]progesterone by rat granulosa cells following the preincubation with FSH and/or androgens is presented in Figure 1. Preincubation with androgens, both in the presence and absence of

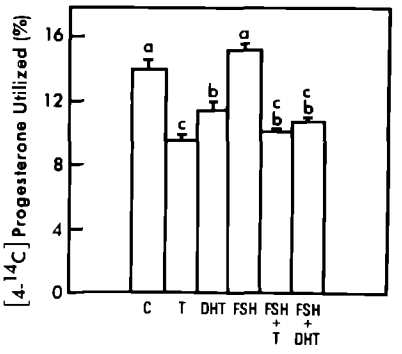

Fig. 1: Effect of FSH and androgens on [4-^{14}C]progesterone utilization by rat granulosa cells. Cells (2.5x10 cells/ml) were preincubated in the absence (C) or in the presence of testosterone (T, 0.5 μM), 5α-dihydrotestosterone (DHT, 0.5 μM) and/or FSH (1 μg/ml) for 24 h, washed and reincubated with [4-^{14}C]progesterone (0.5 μM) for 3 h. Each bar represents the mean (±SEM) from five incubations. The means with no subscripts in common are significantly different ($p<0.05$).

FSH, decreased significantly the utilization of progesterone (18-31%), while preincubation with FSH alone had no effect.

Progesterone was metabolized primarily to three products: 20α-hydroxy-4-pregnen-3-one, 5α-pregnane-3α,20α-diol, and 3α-hydroxy-5α-pregnan-20-one. These steroids were identified on the basis of their R_f values and by recrystallization to constant specific activity. Total radioactivity of these compounds contributed to more than 95% of the radioactivity of all progesterone products extracted from the culture media.

Preincubation with FSH alone slightly decreased conversion of progesterone to 20α-hydroxy-4-pregnen-3-one (12%), while preincubation with androgens, either alone or with FSH, caused a dramatic decrease ranging from 39 to 54% (Figure 2). The conversion of progesterone to 3α-hydroxy-5α-pregnan-20-one was increased significantly following pre-exposure to FSH and androgens whether administered separately or together: androgens alone caused a 24 to 41% increase, FSH alone caused a 59% increase, and FSH plus androgens caused a 58 to 69% increase. Following FSH treatment, the conversion of progesterone to 5α-pregnane-3α,20α-diol was increased by 51%. This effect was absent in incubations treated with FSH and androgens together. In the incubations pretreated with androgens alone, progesterone conversion

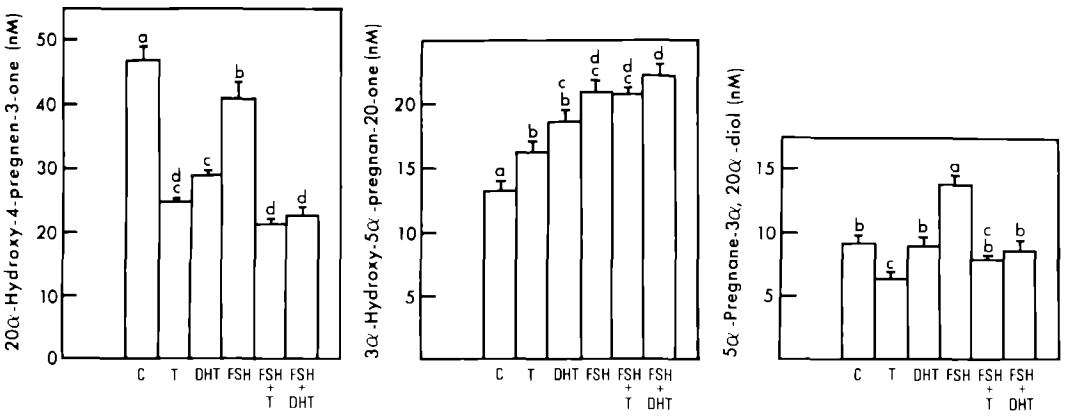

Fig. 2: Effect of FSH and androgens on conversion of radiolabeled progesterone to its metabolites. Experiment description is presented in Figure 1.

to 5α-pregnane-3α,20α-diol was not altered by 5α-dihydrotestosterone but was decreased by 29% by testosterone.

DISCUSSION

Numerous studies have demonstrated the capabilities of FSH and androgens to alter various enzymatic activities of granulosa cells. It has been shown that androgens stimulate cholesterol side-chain cleavage activity (7), increase aromatase activity (15,16), and increase 17β-hydroxysteroid dehydrogenase activity (17). These effects are directly comparable to the FSH elicited effects, namely stimulation of cholesterol side-chain cleavage activity (7), aromatase activity (18), and 17β-hydroxysteroid dehydrogenase activity (19,20).

In the present study we have demonstrated that FSH as well as androgens may be involved in the regulation of enzymatic activities of yet another aspect of steroidogenesis: metabolism of progesterone. The results obtained in this study show that FSH modulates progesterone metabolism by rat granulosa cells and that this modulation is different than that exerted by androgens. Present data confirm our previous study (11) and show that androgens decrease overall progesterone catabolism by inhibition of 20α-hydroxysteroid dehydrogenase (20α-HSD). This effect was observed both in the absence and presence of FSH as androgens decreased the conversion of

radiolabeled progesterone to 20α-hydroxy-4-pregnen-3-one and 5α-pregnane-3α,20α-diol.

It should be pointed out that 5α-dihydrotestosterone decreased progesterone utilization and conversion of progesterone to 20α-hydroxy-4-pregnen-3-one to a lesser degree than testosterone, both in the absence and presence of FSH. Furthermore, in the absence of FSH, 5α-dihydrotestosterone did not alter significantly the accumulation of 5α-pregnane-3α,20α-diol. These findings are consistant with the findings of others (21) that the stimulatory effect of 5α-dihydrotestosterone on progestin secretion by granulosa cells is one-third of the testosterone-elicited effect. Presumably, the lower androgenic activity of 5α-dihydrotestosterone may be due to its rapid catabolism.

Although FSH had no effect on overall progesterone utilization, it did alter significantly the accumulation of all detected progesterone metabolites: decreased 20α-hydroxy-4-pregnen-3-one, increased 5α-pregnane-3α,20α-diol, and increased 3α-hydroxy-5α-pregnan-20-one. The results obtained suggest that FSH may act independently of androgens to modulate enzymatic activities involved in progesterone metabolism (Figure 3) by increasing 5α-reductase activity and/or decreasing 20α-HSD activity. Such effects may be of physiological significance, since it has been demonstrated that some progesterone metabolites are physiologically active while others are regarded, at least currently, as true inactive catabolites. 20α-Hydroxy-4-pregnen-3-one, 5α-pregnane-3,20-dione, and 3α-hydroxy-5α-pregnan-20-one were shown to be potent regulators of gonadotropin release

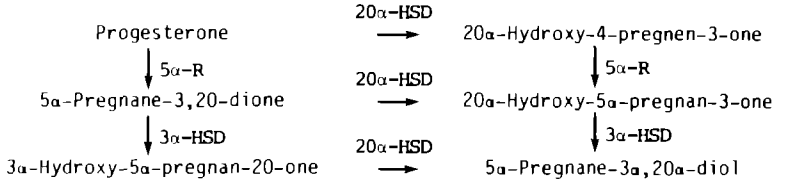

Fig. 3: Pathways of progesterone catabolism by rat granulosa cells. Enzymatic activities denoted as follows: 20α-HSD, 20α-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; 3α-HSD, 3α-hydroxysteroid dehydrogenase.

(22,23), while 20α-hydroxy-5α-pregnan-3-one and 5α-pregnane-3α,20α-diol were demonstrated to have only a minor influence on gonadotropin release (23).

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