ANDROGENIC MODULATION OF PROGESTERONE METABOLISM BY RAT GRANULOSA CELLS IN CULTURE

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ABSTRACT

Effects of androgens on progesterone accumulation, utilization of exogenous progesterone and accumulation of [4-^{14}C]progesterone metabolites by rat granulosa cells in culture were studied. Androgen increased progesterone accumulation in cultures without exogenous progesterone and slowed the overall decline of progesterone concentration in cultures supplemented with exogenous progesterone. Both aromatizable testosterone and nonaromatizable 5α-dihydrotestosterone decreased [4-^{14}C]progesterone utilization by granulosa cells by 12 to 30%. This effect was observed irrespective of whether the cells were continuously exposed to androgens or only pre-exposed. In the same experiments, androgens decreased conversion of radiolabeled progesterone to 20α-hydroxy-4-pregnen-3-one by 11 to 50% and to 5α-pregnane-3α,20α-diol by 26 to 49%. Accumulation of 3α-hydroxy-5α-pregnan-20-one was not altered in 3 h incubations and was increased by up to 43% in 24 h incubations by androgen treatment. It is suggested that androgens alter progesterone catabolism by granulosa cells by decreasing 20α-hydroxysteroid dehydrogenase activity and that this effect may contribute to overall stimulatory action of androgens on progesterone accumulation.

INTRODUCTION

Androgens, alone and in synergism with follicle-stimulating hormone (FSH), were demonstrated to stimulate progesterone accumulation by granulosa cell cultures in rat (1,2,3,4) and in humans (5). The capability of androgens to stimulate progesterone accumulation beyond maximum FSH-saturated levels is not due to the mediation at the step of cAMP production (6). Thus, C_{19} steroids may act independently of FSH and possibly on different steps of progesterone biosynthesis and/or catabolism. Androgens, as well as FSH, are believed to stimulate progesterone production by enhancing the activity of cholesterol side-chain cleavage but do not affect the transport of cholesterol to mitochondria

(7), while FSH alone, but not androgens, increases conversion of pregnenolone to progesterone by stimulation of 3β-hydroxysteroid dehydrogenase /Δ^5-Δ^4 isomerase activity (8,9).

To our knowledge, studies on the effects of androgens on progesterone catabolism are quite limited. Even though androgens do not compete with progesterone for 5α-reductase activity (10), the involvement of androgens in other steps of progesterone catabolism cannot be ruled out. In the present study we examined effects of androgens on progesterone utilization and on accumulation of progesterone metabolites by rat granulosa cells in cultures.

MATERIALS AND METHODS

Ovarian granulosa cells were obtained from immature, estrogen-treated Sprague-Dawley rats as described previously (11). Cells were washed and incubated in Eagle's Minimum Essential Medium (MEM) supplemented with 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. Incubations volumes were adjusted to 1 ml and contained $2x10^5$ granulosa cells each. Incubations were carried out in 12x75 mm polystyrene culture tubes (Falcon) at 37°C under an atmosphere of 5% CO_2 in air. Testosterone (T), 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one, DHT), progesterone (P_4), and radiolabeled [4-^{14}C]progesterone (S.A. 56 mCi/mmol, Amersham Corp.) were added to the culture media at 0.5 μM concentrations as specified in each experiment. Incubations were terminated by centrifuging the cells at 300 x g for 5 min. The media were collected and the protein content of the cells was determined by the method of Lowry et al (12).

The media were extracted with five volumes of diethyl ether; extracts were evaporated in water bath at 35°C under nitrogen and redissolved in absolute ethanol. Extracts of the samples without radiolabeled steroids were analyzed for progesterone content by specific radioimmunoassay. Extracts containing radiolabeled steroids were supplemented with the following steroids serving as internal standards: 0.5-1 μg of progesterone, 20α-hydroxy-4-pregnen-3-one, 5α-pregnane-3,20-dione, 20α-hydroxy-5α-pregnan-20-one, and 5α-pregnane-3α,20α-diol, all obtained from Sigma. These extracts were subjected to thin-layer chromatography (TLC) separation on silica gel plastic sheets (Merck) in chloroform:acetone (4:1, vol:vol) system. Radioactive progesterone and its metabolites were detected by radioautography following exposure of chromatograms to no-screen medical x-ray film (Kodak). Subsequently, the chromatograms were sprayed with sulfuric acid-ethanol (1:1, vol:vol) and charred for 15 min at 120°C to visualize the standard steroids.

Radioactive metabolites were identified on a preliminary basis by aligning and trans-illuminating the chromatograms with their corresponding radioautograms. Radioactive zones were cut from chromatograms and radioassayed in a LKB 1217 Rackbeta liquid scintillation counter. The recovery of radioactivity was always greater than 90%. Some chromatograms were not subjected to charring. Radiolabeled steroids were eluted from these chromatograms and their identification confirmed by recrystallization to constant specific activity (13) or by two-dimensional TLC in benzene:methanol (9:1, vol:vol) and in ethyl acetate systems.

The experimental data were statistically evaluated by analysis of variance followed by Duncan's New Multiple Range Test (14) or, where appropriate, by Student's t-test.

RESULTS

Androgen Effect on Progesterone Accumulation and Utilization

Granulosa cells were preincubated for 24 h in the presence or absence of T (0.5 μM), washed and incubated again with or without T (0.5 μM) for 6, 12 and 24 h. Additionally, some incubations were supplemented with P_4 (0.5 μM). Accumulation of endogenous progesterone is presented in Table IA. For all time intervals studied, progesterone concentrations of T-treated incubations were 3-4 fold higher than those of control incubations; concentration differences were 0.96, 1.11 and 1.28 nM for 6, 12 and 24 h incubations, respectively. However, within the studied time intervals, progesterone concentrations did not change for control incubations and increased only slightly for testosterone treated incubations.

Concentrations of progesterone in the incubations supplemented with exogenous P_4 decreased rapidly throughout the experiment for both control and T-treated incubations (Table IB). For each time interval, progesterone concentrations of T-treated incubations were significantly higher than those of control incubations; the differences were 20.7, 18.3 and 21.9 nM for 6, 12 and 24 h incubations, respectively. Thus, the testosterone-induced changes of progesterone concentration in incubations supplemented with exogenous progesterone were approximately twenty-fold higher than in incubations with only endogenous progesterone.

TABLE I

EFFECT OF TESTOSTERONE ON PROGESTERONE ACCUMULATION AND UTILIZATION BY RAT GRANULOSA CELLS*

Culture Conditions	Progesterone Concentration (nM) 6h	12 h	24 h
A No Exogenous Progesterone			
Control	0.38 ± 0.03	0.42 ± 0.02	0.34 ± 0.01
Testosterone (0.5 μM)	1.34[b] ± 0.12	1.53[c] ± 0.06	1.62[c] ± 0.04
B Supplemented with Exogenous Progesterone (0.5 μM)			
Control	176.6 ± 3.0	88.9 ± 4.1	26.6 ± 1.8
Testosterone (0.5 μM)	197.3[a] ± 7.9	107.2[a] ± 4.3	48.5[c] ± 1.5

*Cells were preincubated in the absence or in the presence of testosterone for 24 h, washed and reincubated in the absence or presence of testosterone and in the absence (A) or in the presence (B) of exogenous progesterone for 6, 12 and 24 h. Each value represents the mean (±SEM) from triplicate incubations. The means significantly different from the controls are denoted as follows: a, $p<0.05$; b, $p<0.01$; c, $p<0.001$.

Androgen Effect on [4-^{14}C]Progesterone

Granulosa cells were preincubated for 24 h in the absence or in the presence of 0.5 μM of T or DHT, washed and reincubated with the same concentrations of androgens and additionally with [4-^{14}C]progesterone (0.5 μM) for 3 and 24 h. Utilization of radiolabeled progesterone (Fig. 1) was significantly decreased by androgens:testosterone produced 26 and 25% decrease while 5α-dihydrotestosterone caused 25 and 12% decrease at 3 and 24 h incubations, respectively.

Granulosa cells metabolized progesterone to three major products: (i) 20α-hydroxy-4-pregnen-3-one and (ii) 3α-hydroxy-5α-pregnan-20-one,

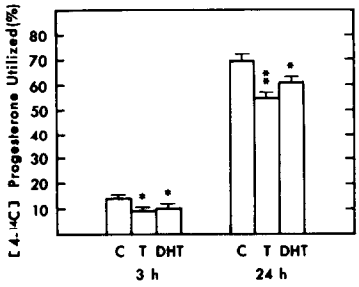

Fig. 1: Effect of androgens on utilization of radiolabeled progesterone by rat granulosa cells. Cells were preincubated in the absence (C) or in the presence of testosterone (T) or 5α-dihydrotestosterone (DHT) at 0.5 μM concentrations for 24 h, washed and reincubated with the same androgens and, additionally, with [4-^{14}C]progesterone (0.5 μM) for 3 and 24 h. Each bar represents the mean (±SEM) from triplicate incubation. The means significantly different from the control ones are denoted as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

which were identified by recrystallization to constant specific activity (Table II); and (iii) 5α-pregnane-3α,20α-diol, which was tentatively identified on the basis of its mobility in various chromatographic systems (see Material and Methods).

The conversion of [4-^{14}C]progesterone to 20α-hydroxy-4-pregnen-3-one was reduced by 35 and 50% in 3 h incubations (Fig. 2) and by 24 and 11% in 24 h incubations (Fig. 3) by T and DHT treatments, respectively. Similarly, the conversion to 5α-pregnane-3α,20α-diol was reduced by 32 and 33% in 3 h incubations and by 29 and 26% in 24 h incubations by T and DHT, respectively. Conversion to 3α-hydroxy-5α-pregnan-20-one was not altered by either androgen in 3 h incubations; however, in 24 h incubations, the accumulation of this metabolite increased by 32% for T treatment and 43% for DHT treatment.

In order to determine whether the continuous presence of androgens was required to detect changes of progesterone metabolism,

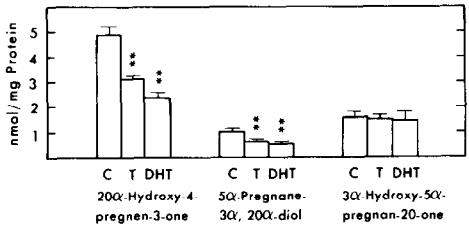

Fig. 2: Effect of androgens on conversion of radiolabeled progesterone to its metabolites during 3 h incubation. Experiment description is presented in Fig. 1.

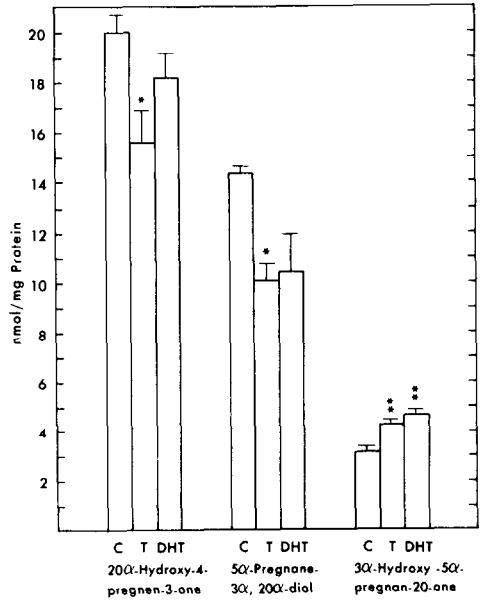

Fig. 3: Effect of androgens on conversion of radiolabeled progesterone to its metabolites during 24 h incubation. Experiment description is presented in Fig. 1.

TABLE II

RADIOCHEMICAL IDENTIFICATION OF [4-^{14}C]PROGESTERONE METABOLITES BY RECRYSTALLIZATION

Compound	Carrier (mg)	Recrystallized from	Specific activity (dpm/mg) after recrystallization number			
			0	1	2	3
20α-Hydroxy-4-pregnen-3-one	10.6	acetone/hexane	1,890	2,050	1,950	1,910
3α-Hydroxy-5α-pregnan-20-one	5.0	acetone/hexane	2,560	2,650	2,640	2,580

granulosa cells were preincubated for 24 h with or without androgens, washed thoroughly and reincubated for 3 h with [4-^{14}C]progesterone but without androgens. Pre-exposure to T and DHT decreased utilization of radiolabeled progesterone by about 30% (Fig. 4). Accumulation of 20α-hydroxy-4-pregnen-3-one decreased by 36 and 29% and accumulation of 5α-pregnane-3α,20α-diol decreased by 46 and 49% following the pre-exposure to T and DHT, respectively (Fig. 5). Accumulation of 3α-hydroxy-5α-pregnan-20-one was not altered by either androgen.

DISCUSSION

To our knowledge, this study provides evidence for the first time that androgens alter progesterone catabolism by ovarian granulosa cells. The present results may help to explain the mechanisms of stimulatory action of androgens on progesterone accumulation.

Progesterone accumulation in granulosa cell cultures is the net result of two processes: progesterone production and catabolism. In this study these processes appeared to reach the state of equilibrium

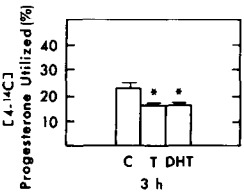

Fig. 4: Effect of androgens on utilization of radiolabeled progesterone by rat granulosa cells. Cells were preincubated in the absence (C) or in the presence of testosterone (T) or 5α-dihydrotestosterone (DHT) at 0.5 μM concentrations for 24 h, washed and reincubated with [4-^{14}C]progesterone (0.5 μM) but without androgens for 3 h.

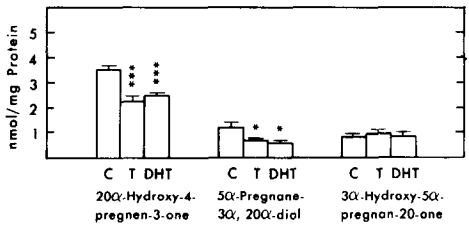

Fig. 5: Effect of androgens on conversion of radiolabeled progesterone to its metabolites during 3 h incubation. Experiment description is presented in Fig. 4.

within six hours of incubation. Exposure of the cells to androgen resulted in a shift of the equilibrium towards higher progesterone accumulation; thus, androgen increased progesterone production and/or decreased its catabolism. When the cell cultures were supplemented with exogenous progesterone, catabolism prevailed during the following 24 h. The overall decrease of progesterone levels was not only slowed by the androgen treatment but also the differences of progesterone concentrations in the presence or absence of the androgen were twenty-fold higher in progesterone supplemented cultures than in cultures not supplemented

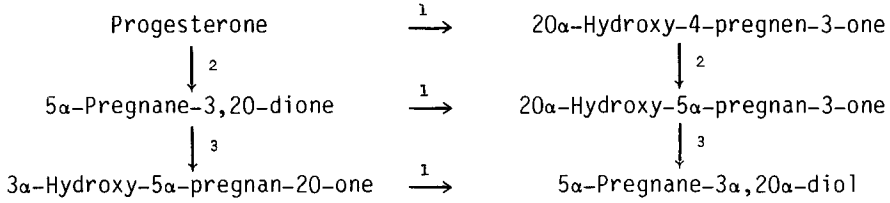

Fig. 6: Pathways of progesterone catabolism by rat granulosa cells. Enzymatic activities denoted as follows: 1, 20α-hydroxysteroid dehydrogenase; 2, 5α-reductase; 3, 3α-hydroxysteroid dehydrogenase.

with progesterone. These results would be difficult to explain solely by the effect of androgens on progesterone producton and thus seem to indicate that androgens may decrease progesterone catabolism and consequently increase its accumulation. Further evidence obtained from this study indicates that androgens decreased the utilization of radiolabeled progesterone and the conversion to 20α-hydroxy-4-pregnen-3-one and 5α-pregnane-3α,20α-diol. These results do not appear to be due merely to the dilution effect caused by the increased production of endogenous progesterone since the conversion of [4-^{14}C]progesterone to 3α-hydroxy-5α-pregnan-20-one remained unchanged in 3 h incubations and increased in 24 h incubations of androgen-treated cultures. Thus, it appears that androgens decrease the activity of 20α-hydroxysteroid dehydrogenase (20α-HSD) but do not affect the activities of 5α-reductase and 3α-hydroxysteroid dehydrogenase. Increased accumulation of 3α-hydroxy-5α-pregnan-20-one may be a secondary effect of the decrease 20α-HSD activity (Fig. 6).

The androgenic modulation of progesterone metabolism cannot be attributed to estrogens since both aromatizable testosterone and non-aromatizable 5α-dihydrotestosterone caused comparable decreases of progesterone utilization and 20α-HSD activity. The observation that only pre-exposure to androgens, and not their continuous presence, is required to

alter progesterone catabolism corroborates the previous observations that androgens act via specific receptors in rat ovarian tissue (15,16).

The present results indicate that inhibition of 20α-HSD may represent another mechanism regulating progesterone accumulation by granulosa cells, in addition to the previously described stimulation of cholesterol side-chain cleavage activity (7). This mechanism also allows regulation of the ratio of concentrations of both major progestins elaborated by the ovaries: progesterone and 20α-hydroxy-4-pregnen-3-one.

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