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Effect of Androgens on the Ovarian Morphology of the Hypophysectomized Rat (41528)

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Abstract. Experiments were designed to study the short-term effects of androgens on ovarian follicular morphology in immature, hypophysectomized rats. Rats were given 1 mg diethylstilbestrol (DES) daily for 5 days. On the fifth day, they were treated with 5-α-dihydrotestosterone (DHT), testosterone (T), and flutamide metabolite, α,α,α-trifluoro-2-methyl-4′-nitro-*m*-lactotoluidide (FM) alone and in combination and killed 24 hr later. One group of rats received DES alone. Ovarian serial sections (5 μm) were observed in each treatment for the presence of pyknotic nuclei as an index of atresia. Diameter of the follicles was measured, a percentage of atretic follicles was calculated, and the data were analyzed for all size follicles, follicles 150 μm or less (small), and follicles measuring over 150 μm (large). The results show that DHT and DHT plus T increased follicular atresia when all follicle sizes were considered. None of the treatments significantly changed follicular atresia in small follicles. In large follicles, both DHT and T increased follicular atresia. Furthermore, FM prevented T-induced atresia while by itself FM had no effect on follicular morphology. That the FM decreased the action of T in promoting atresia adds support to the conclusion that T promotes atresia by inhibiting the follicular growth promoting action of DES in the ovary. Also, this study shows that DHT promotes follicular atresia.

Follicular atresia is the degenerative process by which the ovarian follicle loses its integrity and the oocyte is lost from the follicle other than by ovulation. It is a naturally occurring phenomena in all mammals and is the major means by which eggs are lost from the ovary. The endogenous factors promoting follicular atresia are unknown. However, that testosterone promotes follicular atresia by inhibiting the growth-promoting action of estrogen on follicles has been reported (1–5). Those studies primarily tested the long-term effects of testosterone in estrogen-primed, hypophysectomized rats while to our knowledge none investigated 5-α-dihydrotestosterone (DHT), the active form of testosterone carrying out intracellular function in male accessory sex glands (6).

In the following study, histological examination of ovaries was used to assess the progress and extent of follicular atresia 24 hr following administration of androgens (testosterone and DHT) to hypophysectomized female rats primed with diethylstilbestrol (DES).

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Materials and Methods. *Animals.* Female Sprague–Dawley rats were hypophysectomized at age 22 days by Charles River Breeding Laboratories and arrived 2 days later. They were housed in air-conditioned rooms, illuminated between 0600 and 2000 hr. Purina Lab Chow and tap water were available *ad libitum.*

Materials. Testosterone, DHT, and DES were purchased from Sigma Chemical Company, St. Louis, Missouri. A flutamide metabolite (α,α,α-trifluoro-2-methyl-4′-nitro-*m*-lactotoluidide) hence referred to as FM was a gift from Dr. R. O. Neri, Schering Corporation, Bloomfield, New Jersey.

Steroid hormones were dissolved in sesame seed oil while FM was dissolved in 48% ethanol. All drugs (1.0 mg) were administered subcutaneously, in 0.1-ml volume.

Treatment of animals. Beginning at age 25 days (3 days following hypophysectomy) all rats received 1 mg of DES daily for 4 days. On the fifth day, rats were assigned to six different groups and treated as follows: Group 1, DES; group 2, DES and testosterone; group 3, DES and DHT; group 4, DES, DHT, and testosterone; group 5, DES and

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FM; and group 6, DES, FM, and testosterone. Rats were sacrificed by decapitation 24 hr following treatments. The ovaries were immediately removed, trimmed free of fat and mesentary, and were promptly placed in a fixative for histological studies.

Histology. Ovaries were fixed in Bouin's solution for 2 hr and dehydrated using graded solutions of alcohol and they were stored in 70% ethanol. Ovaries were processed by an Autotechnicon and embedded in paraffin. Serial sections (5 μm) were mounted on slides and stained with hematoxylin and eosin. Every other mounted section was examined at a magnification of 430× for evidence of follicular atresia. At least 14 sections were observed for each ovary. Follicular atresia was defined by the presence of one or more pyknotic granulosa cells. Pyknosis (thickening, degeneration of a cell in which the nucleus shrinks in size and the chromatin condenses to a solid structureless mass) was determined by darkly stained spots appearing within the follicle (7). The number of pyknotic nuclei appearing in each follicle was recorded. Follicles scored healthy were devoid of these spots.

An ocular micrometer was employed to measure the largest cross-sectional diameter (including theca) of both healthy and atretic follicles. Follicles counted were those containing a discernible ovum and at least one layer of granulosa cells.

Statistical analysis. Means and standard errors were calculated for percentage atretic follicles of all sizes, percentage atretic small (150 μm or less) follicles, and percentage atretic large (>150 μm) follicles. The data were statistically evaluated by analysis of variance and the differences between groups were tested by the Student/Newman–Keuls test (8).

Results. The percentage atretic follicles (all sizes) was calculated for each ovary in order to compare the extent of atresia induced by various treatments (Table I). The analysis of variance revealed that there was a significant ($P < 0.05$) treatment effect. Analysis by the Student/Newman–Keuls test indicated that treatment with DHT or DHT plus testosterone significantly ($P < 0.1$) increased atresia when compared to treatment with DES alone. These data suggest that DHT, an intracellularly active androgen in male accessory sex glands, increases follicular atresia in DES-primed immature hypophysectomized rats 24 hr after its administration. Testosterone treatment did not significantly increase atresia when compared to the DES control group. These findings are consistent with previous studies (4) which found no significant increase in follicular atresia 24 hr following testosterone administration.

Follicles were divided into two groups, small (≤150 μm) and large follicles (>150 μm), in order to study the responses of dif-

TABLE I. EFFECT OF ANDROGENS ON ATRESIA OF OVARIAN FOLLICLES

Treatment[a]	No. of ovaries	Percentage atretic follicles[b]		
		All sizes	150 μm or less	Larger than 150 μm
DES alone	8	63 ± 6.2[c]	27 ± 12.4	83 ± 4.2
T	8	63 ± 8.0	31 ± 9.6	93 ± 3.9
DHT	6	72 ± 8.8	34 ± 10.9	96 ± 3.1
DHT + T	7	72 ± 4.0	30 ± 5.9	95 ± 2.2
FM	7	66 ± 5.9	24 ± 8.6	85 ± 3.1
FM + T	8	65 ± 4.3	33 ± 3.9	85 ± 3.5

[a] Immature, hypophysectomized rats were injected daily for 4 days with 1 mg of diethylstilbestrol (DES). On the fifth day, rats were assigned to six different treatment groups. One group received 1 mg DES alone while the others, in addition to DES, received either 1 mg testosterone (T), 1 mg 5-α-dihydrotesterone (DHT) or 1 mg flutamide metabolite (FM) alone or in combination as indicated. Twenty-four hours following the treatment, animals were sacrificed. Histological sections of ovaries (5 μm thick) were observed at 430× magnification for the presence of pyknotic nuclei in the granulosa cells.

[b] Percentage atretic follicles were calculated for all size follicles and also within follicles measuring 150 μm or less and follicles larger than 150 μm in size.

[c] Mean percentage ± SEM.

ferent size follicles to the various treatments. Analysis of variance revealed that there is no significant added variance component among groups for mean percent atresia of small follicles (Table I). That testosterone did not increase the percentage of atretic small follicles is in agreement with a previous study (3) where they found that testosterone, presumably secreted by the ovary in response to administration of human chorionic gonadotrophin (hCG), failed to promote atresia in follicles less than 200 μm in diameter.

For large follicles, there was a significant ($P < 0.01$) treatment effect (Table I). As determined by the Student/Newman–Keuls test, the DHT, testosterone, and DHT plus testosterone treatments induced a significantly ($P < 0.05$) higher atresia than treatment with DES, FM, and FM plus testosterone. Testosterone, DHT, and DHT plus testosterone treatments were not significantly different from each other. These data suggest that both testosterone and DHT are equally effective in promoting atresia in large follicles and commence to do so within 24 hr of their administration. In large follicles, FM suppressed the action of testosterone since the FM plus testosterone group had a significantly lower percentage of atretic follicles than the testosterone group (Table I).

That androgens promote atresia in large follicles is in accordance with a previous study (3) which found an increase in the percentage of atretic follicles measuring over 200 μm in diameter when hCG was administered to DES-primed, immature, hypophysectomized rats. In that study, hCG presumably promoted the production of androgens by the theca and these in turn promoted atresia.

In these short-term studies, both DHT and testosterone promoted atresia in large follicles, but DHT appeared more effective in promoting atresia when all size follicles were considered.

Discussion. Previous investigations on follicular atresia have dealt primarily with long-term administration of the androgen testosterone (1, 2, 5). In the present short-term study atresia of large follicles was brought about by testosterone and DHT (Table I). However, neither androgen was effective in promoting atresia in small follicles. Overall, DHT was more effective than testosterone in promoting atresia when all sizes of follicles were considered.

The presence of cytosol and nuclear androgen binding proteins, which have characteristics of receptors, in the ovary have been reported (19). Therefore, it is assumed that testosterone acts through intracellular mechanisms in bringing about atresia of follicles. The cytosol protein binds DHT with slightly less affinity than testosterone. The k_d for DHT is estimated at 9×10^{-9} M, while that of T is calculated at 2.4×10^{-9} M (9). This suggests that DHT mediates its effect on follicles by binding to a variation of the testosterone receptor or that once bound to the testosterone receptor, DHT is able to promote a more extensive atretic response than testosterone when all follicle sizes are considered.

Follicular atresia in hypophysectomized rats with ovine luteinizing hormone is associated with a loss of estradiol receptor in granulosa cells (10). Furthermore, cytosol replenishment and nuclear retention of the ovarian estradiol receptor is reduced 24 hr after testosterone treatment (4). A mechanism by which testosterone promotes atresia in the estrogen-primed rat (following the translocation of the testosterone–receptor complex) may be through the negative effect of testosterone on the estradiol receptor since estradiol is involved in delaying follicular atresia (11). DHT may be involved in a similar mechanism using the same testosterone cytosol receptor or a variation of it.

Both DHT and testosterone promote progesterone production in granulosa cell cultures obtained from preantral follicles (12). However, androgens do not promote progesterone production in granulosa cell cultures unless supplemented with follicle-stimulating hormone (13). Since follicle-stimulating hormone is presumably not present in the hypophysectomized rat, it is assumed that progesterone is not synthesized when these animals are given DHT or testosterone. Furthermore, when progesterone is administered to estrogen-primed, immature, hypophysectomized rats, it fails to significantly reduce the cytosol or nuclear estrogen receptor (4). It is then unlikely that testosterone

and DHT inhibition of follicular growth in estrogen-primed ovaries is mediated through progesterone.

In the present work, the data reveal that the FM was effective in inhibiting testosterone action in the rat ovary and that the FM, when given alone, had no effect on follicular growth (Table I). This finding is in agreement with another study (3) where flutamide administered along with hCG to estrogen-primed hypophysectomized rats reduced atresia presumably by inhibiting the action of testosterone produced by the ovary in response to hCG. Flutamide and its metabolite inhibit the formation of a nuclear protein–androgen complex (14). Further, these antiandrogens do not affect the metabolic conversion by the rat ventral prostate of testosterone to its active metabolite dihydrotestosterone or to any of the other metabolic products (14). Since the ovary and prostate receptors have similar properties (15), it is assumed that the mechanism of action of the FM in the female is similar to that in the male. Our results also suggest that FM lacks androgenic and antiestrogenic activity since treatment with FM alone did not differ from that of the controls (Table I). That DHT and testosterone can experimentally induce follicular atresia supports the possibility that these hormones may be involved in the mechanism of follicular atresia.

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