Effect of dihydrotestosterone on follicular development, ovulation and reproductive capacity of mice

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Summary. A single injection of a 5 α -dihydrotestosterone (DHT) was administered to cyclic female mice on the day of metoestrus. DHT (1 mg) prolonged the dioestrous stage of the cycle by 24 h and there were 50% fewer large (normal) follicles than in oil-injected controls. Degenerating ova were observed in the oviduct of 70% of the DHT-treated mice and there was a significant reduction in the numbers of females becoming pregnant and bearing normal fetuses.

Introduction

Oestrogens are known to induce proliferation of granulosa cells (Pencharz, 1940; Paesi, 1952; Payne & Hellbaum, 1955; Goldenberg, Vaitukitis & Ross, 1972). Although the presence of testosterone receptors in the cytosol of granulosa cells has been reported (Schreiber, Reid & Ross, 1976), the role of androgens in follicular development is still not clear. Hillier & Ross (1979) have demonstrated that injection of testosterone induced degeneration of small follicles in immature rats, but the animals used were hypophysectomized and primed with oestrogen. These changes therefore need to be compared with events during a normal follicular cycle (Richards, 1979). A study was therefore undertaken with cyclic mice to observe the effects of dihydrotestosterone, a non-aromatizable androgen, on follicular development, ovulation and subsequent fertility.

Materials and Methods

The androgen, 5 α -androstane-17 β -ol-3-one (5 α -dihydrotestosterone: DHT), was obtained from Sigma Chemical Co. (St Louis, Missouri).

Normal cyclic mice of the Swiss strain, bred randomly in our Institute's colony were used for the study. The animals were maintained in air-conditioned rooms with a 14 h light/24 h schedule, and they were fed a standard laboratory diet and water *ad libitum*. The stage of the oestrous cycle was checked by assessment of daily vaginal smears.

Experiment 1

The absence of large preovulatory follicles in the rodent ovary during oestrus and metoestrus has been reported by Hirshfield & Midgley (1978). DHT was therefore injected on the day of metoestrus to examine the effect on the development of large follicles.

On the day of metoestrus mice were injected subcutaneously with 100 μ g or 1 mg DHT in 0.1 ml olive oil. Controls were injected with 0.1 ml olive oil. Each group consisted of 7–8 mice.

The animals were killed 24 h after the injection. The ovaries were removed, cleaned, weighed to the nearest 0.1 mg and fixed in 10% buffered formalin. Serial paraffin-wax sections were stained with Heidenhein's azan stain (Preece, 1959). Follicles in each section were classified as small, medium and large on the basis of the number of granulosa cells in the largest section of the follicle (Pedersen & Peters, 1968). The small follicles were those containing ≤ 20 granulosa cells (Type 3a). The medium and large follicles were further classified as normal or atretic, atretic follicles being those containing > 2 granulosa cells with pycnotic nuclei (Hirshfield & Midgley, 1978).

Experiment 2

On the day of metoestrus each of 20 cyclic mice were injected s.c. with 1 mg DHT in 0.1 ml olive oil and 20 controls were injected with 0.1 ml olive oil. Vaginal smears were examined daily. Ten animals from each group were killed on the first day of oestrus. The ovaries were fixed and the oviducts were examined under the microscope for the presence of ova.

The other 10 animals of each group were mated with known fertile males on the day of pro-oestrus and checked for the presence of a copulatory plug on the following day (Day 1 of pregnancy). The mice were killed on Day 19 of pregnancy and the numbers of corpora lutea, fetuses and resorption sites were counted.

Results

Experiment 1

As shown in Table 1, there was a significant reduction in relative ovarian weight within 24 h of treatment with 1 mg DHT and a 50% reduction in the number of normal large follicles. The increase in the number of large atretic follicles was not significant. The follicle numbers in the other categories were similar in all the treatment groups.

Table 1. The effect of a single injection of DHT on ovarian weight and number of follicles in mice

Treatment (no. of mice)	Body wt (g)	Ovarian wt (mg/100 g body wt)	Large follicles		Medium follicles		Small follicles
			Normal	Atretic	Normal	Atretic	
Olive oil, 0.1 ml (8)	17.3 ± 0.69	42.43 ± 1.55	7.25 ± 0.75	9.00 ± 1.13	13.34 ± 1.34	2.00 ± 0.53	132.8 ± 6.5
DHT, 100 μ g (7)	17.6 ± 0.12	42.00 ± 1.66	6.57 ± 0.72	7.14 ± 0.96	14.00 ± 3.02	3.71 ± 0.68	127.0 ± 8.2
DHT, 1 mg (7)	17.8 ± 0.65	35.25* ± 1.70	3.43* ± 0.57	10.29 ± 2.84	18.3 ± 2.91	1.43 ± 0.72	138.2 ± 9.4

Values are mean $\pm$ s.e.m.

* Significantly different from control values, $P < 0.01$ (t test).

Experiment 2

On the first day of oestrus, most of the DHT-treated animals had degenerating ova in the oviducts (Table 2). Degenerating ova had no attached cumulus cells and some showed fragmentation of the cytoplasm. Normal as well as degenerating ova were located in the oviduct of 3 mice. In the tests of reproductive capacity of DHT-treated females, control and treated mice mated readily but the numbers of females becoming and remaining pregnant with live fetuses were affected by the DHT treatment (Table 3). The numbers of CL indicated that ovulation rate had been affected ($P < 0.01$, χ^2).

Table 2. Effect of a single injection of DHT on ovulation in cyclic mice

Treatment	No. of mice	No. of mice with normal ova at oestrus	Mean $\pm$ s.e.m. no. of normal ova/mouse	No. of animals with abnormal ova(%)	Total no. of degenerating/normal ova
Olive oil, 0.1 ml	10	10	8.5 $\pm$ 0.27	0	0/85
DHT, 1 mg	10	3	4.9 1.43*	7†	26/49‡

* Significantly different from control value, $P < 0.02$ (t test).

† 3 mice had only abnormal ova.

‡ Significantly different from control value, $P < 0.01$ (χ^2).**Table 3.** Effect of a single injection of DHT on reproductive capacity of mice

Treatment	No. of mice	No. of mice			Mean $\pm$ s.e.m. no. of normal fetuses	Total no. of normal fetuses/total no. of CL (%)
		With normal fetuses and no resorption sites on Day 19 of pregnancy	With resorption sites	Not pregnant		
Olive oil, 0.1 ml	10	10	0	0	7.6 $\pm$ 0.56	76/79 (96)
DHT, 1 mg	10	2	2†	6‡	2.5* $\pm$ 1.10	25/33 (76)

* Significantly different from control value, $P < 0.001$ (t test).

† One resorption site and live fetuses in each female.

‡ 2 females showed dioestrous smears for 14 days, the other 4 had normal 4-day cycles.

Discussion

The 50% reduction in numbers of large normal follicles within 24 h of the injection of DHT is in agreement with the results of Payne & Runser (1958) and Hillier & Ross (1979) who demonstrated that testosterone propionate induced degeneration of ovarian follicles, although their observations (on hypophysectomized immature rats) related only to small follicles.

We observed an equal number of non-atretic and atretic large follicles in the ovaries of control mice. This is in agreement with the observations of Byskov (1974) who reported the ratio of non-atretic to atretic antral follicles to be between 1:1 and 1:3 in mice. Although there was no significant change in the normal and atretic medium-sized follicles in DHT-treated mice compared with controls, the reduction in the number of large follicles in the treated group was accompanied by an increase (not significant statistically) in the number of large atretic and medium-sized normal follicles, and the total number of medium-sized and large follicles in the control and DHT-treated mice were comparable (31.59 and 33.45 respectively).

Injection of DHT prolonged the dioestrous stage of the oestrous cycle by 24 h in all the animals and also decreased the number of normal ova in the oviduct on the day of oestrus. In adult rats, injection of dehydroepiandrosterone (DHA) caused ovarian failure and polycystic ovarian changes within a day of treatment (Parker & Mahesh, 1976). Gnodde, van Dielen & Van Look (1979) have also observed that administration of androstenedione, a weak androgen, to rats prolonged the dioestrous stage and caused a reduction in the number of ova in the oviducts; they did not, however, report whether the ova were abnormal. Androstenedione is a precursor for oestrogen synthesis, while DHT is a non-aromatizable androgen. This and also the species difference may account for the different result in the present study. The reason for the

degeneration is unknown, but could be due to reduced protein synthesis in the follicle (Siracusa, Whittingham, Molinaro & Vivarelli, 1978).

Injection of DHT in cyclic mice reduced the ovarian weight and inhibited follicular development within 24 h. DHT may have a direct action on the ovary; testosterone receptors are known to be present in the cytosol of the granulosa cells (Schreiber *et al.*, 1976).

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