Failure of 5α-Dihydrotestosterone to Block Androgen Sterilization in the Female Rat (36155)

RENEE ULRICH, ARTHUR YUWILER, AND EDWARD GELLER
(Introduced by C. H. Sawyer)

Neurobiochemistry Laboratory, T-85, Veterans Administration Hospital (Psychosocial Medicine), Los Angeles, California 90073; and Department of Psychiatry, University of California, Center for Health Sciences, Los Angeles, California 90024

Testosterone is converted to dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) by many of the target tissues of testosterone (1, 2); and this metabolite seems to be at least as potent as testosterone itself in some biological tests (3). In the prostate gland, 5α-reductase, the enzyme involved in this conversion, is found mainly in microsomal and mitochondrial fractions (4). Once formed, dihydrotestosterone is bound to protein in the cytosol and the entire complex then moves into the nucleus—the apparent site of hormone action (5).

Testosterone can be converted to dihydrotestosterone by minced preparations of either rat hypothalamus or pituitaries, presumably by action of 5α-reductase (6); and it is therefore possible that testosterone may be reduced in some neural sites before exerting its effect on the central nervous system. Testosterone propionate acts on the basal hypothalamus of the newborn female rat to "masculinize" the brain so that the cyclic pattern of the sex hormone secretions is abolished (7). The present experiments investigate the possibility that dihydrotestosterone could be the active form of this hormonal action.

Methods and Materials. Female Long-Evans rats, from our own colony, were injected subcutaneously with either 100 μg of testosterone propionate (TP), 100 μg of 5α-dihydrotestosterone (DHT), 100 μg of TP and 1.25 mg of DHT, or oil only. All hormones were dissolved in corn oil and the injection volume was 0.05 ml. The animals were injected before 5 days of age and were maintained in an environmentally controlled room with the lights on for 12 hr/day. Rats were weaned at 21 days of age, checked for vaginal opening daily (starting at day 30) and the vaginal smears were taken for 10 days, starting at 65 days of age. Animals were weighed and sacrificed at approximately 90 days of age. Ovaries and anterior pituitary were removed, cleaned, and weighed.

Results and Discussion. Dihydrotestosterone, unlike testosterone propionate, did not produce sterilization when injected into the newborn female rat (Table I). The time of vaginal opening was comparable in rats injected with 100 μg of TP, 100 μg of DHT, or oil only. TP-injected females had a significantly higher percentage of estrous vaginal smears than either oil-injected or DHT-injected rats. Similarly, the ovarian weight of TP-injected rats was significantly less than the ovarian weights of either of the other two groups. Macroscopic examination revealed that the ovaries of the controls and the DHT-injected rats contained both follicles and new and old corpora lutea; those of the TP-injected females contained follicles only. Anterior pituitary weights were comparable in all three groups.

Furthermore, DHT, administered simultaneously with TP, did not block the sterilizing activity of testosterone (Table II). Animals injected with both hormones and rats injected with TP only had comparable body and ovarian weights, the mean ages at vaginal opening were comparable and percentages of estrous vaginal smears were the same.

Our data confirm the reports of Luttge and

TABLE I. Does Dihydrotestosterone Produce Sterility?

Group	Body wt (g)	Age at vaginal opening (Days)	Percentage estrus smears	Ovarian wt (mg)	Anterior pituitary wt (mg)
1. Oil	220.7 ± 9.02[a]	33.6 ± 0.96	27.3 ± 4.07	77.0 ± 3.13	7.40 ± 0.69
2. 100 μg of testosterone propionate	225.9 ± 6.12	35.2 ± 1.19	66.7 ± 9.86[c]	43.3 ± 8.71[d]	7.14 ± 0.37
3. 100 μg of dihydrotestosterone	201.8 ± 7.25[b]	34.4 ± 1.06	25.0 ± 7.77	80.1 ± 3.16	6.41 ± 1.00

[a] Mean ± SE.
[b] $p < .05$ in comparison to Group 2.
[c] $p < .01$ and $p < 0.025$ in comparison to Groups 1 and 3, respectively.
[d] $p < .005$ in comparison to Groups 1 and 3.

TABLE II. Can Dihydrotestosterone Block Androgen Sterilization?[a]

Group	Body wt (g)	Age at vaginal opening (days)	Percentage estrus smears	Ovarian wt (mg)
100 μg of TP	245.9 ± 10.4[b]	34.1 ± 1.62	44.4 ± 10.2	39.7 ± 3.48
100 μg of TP + 1.25 mg of DHT	259.9 ± 7.07	33.7 ± 1.33	48.0 ± 16.0	52.2 ± 6.10

[a] TP = testosterone propionate; DHT = dihydrotestosterone.
[b] Mean ± SE.

Whalen (8) and Naftolin *et al.* (9) that DHT lacks the masculinizing action of TP when administered to rats before they are 5 days old. Since DHT also fails to prevent the sterilization action of TP, one can speculate that DHT either is not taken up by the same brain sites as TP or that, if it is taken up by such sites, it is readily displaced by TP. Even if DHT and TP both bind to similar neural sites, their effect on these sites must differ.

These results add to a growing body of evidence on the effects of DHT on peripheral and central (neural) targets of androgen. Although testosterone is hydroxylated to DHT by at least some of its traditional peripheral targets—prostate (5) and epididymis (10)—and can be hormonally active in this form (3), the DHT fails to mimic the classical central actions of testosterone on mating behavior (11, 12) and androgen sterilization.

Summary. Female rats were injected subcutaneously with either 100 μg of testosterone propionate (TP), 100 μg of 5α-dihydrotestosterone (DHT), 100 μg of TP and 1.25 mg of DHT, or oil prior to 5 days of age. Percent estrous smears was significantly higher and ovarian weight was significantly lower in TP-injected rats in comparison to rats injected with oil or DHT. Injection of both DHT and TP failed to eradicate these changes. Thus, DHT did not produce the persistent estrus syndrome seen in the TP-injected rats and, furthermore, DHT did not prevent this action of TP upon the reproductive system.

This study was supported by a grant from the U.S. Public Health Service. Dihydrotestosterone was graciously supplied by Syntex de México. The authors thank Raymond Wallace, Cynthia Wang, Bennie Bennett, and Selma Plotkin for their technical assistance.

1. Bruchovsky, N., and Wilson, J. D., J. Biol. Chem. **243**, 2012 (1968).

2. Balieu, E., Lasnitzki, I., and Robel, P., Nature (London) **219**, 1155 (1968).

3. Dorfman, R. I., *in* "Methods in Hormone Research" (R. I. Dorfman, ed.), p. 235. Academic Press, New York (1966).

4. Shimazaki, J., Kurihara, H., Ito, Y., and Shida, K., Gunma J. Med. Sci. **14**, 326 (1965).

5. Fang, S., Anderson, K. M., and Liao, S., J. Biol. Chem. **244**, 6584 (1969).

6. Jaffe, R. B., Steroids **14**, 483 (1969).

7. Gorski, R. A., J. Reprod. Fert., Suppl. **1**, 67 (1966).

8. Luttge, W. G., and Whalen, R. E., Horm. Behav. **1**, 265 (1970).

9. Naftolin, F., Munck, A., and Brown-Grant, K., Abstr. Meet. Endocrine Soc., 53rd **1971**, 129.

10. Inano, H., Machino, A., and Tamaoki, B., Endocrinology **84**, 997 (1969).

11. Beyer, C., McDonald, P., and Vidal, N., Endocrinology **86**, 939 (1970).

12. McDonald, P., Beyer, C., Newton, F., Brien, B., Baker, R., Tan, H. S., Sampson, C., Kitching P., Greenhill, R., and Pritchard, D., Nature (London) **227**, 964 (1970).

Received Sept. 1, 1971. P.S.E.B.M., 1972, Vol. 139.